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WJH mainly publishes articles reporting research results and findings obtained in the field of hepatology and covering a wide range of topics including chronic cholestatic liver diseases, cirrhosis and its complications, clinical alcoholic liver disease, drug induced liver disease autoimmune, fatty liver disease, genetic and pediatric liver diseases, hepatocellular carcinoma, hepatic stellate cells and fibrosis, liver immunology, liver regeneration, hepatic surgery, liver transplantation, biliary tract pathophysiology, non-invasive markers of liver fibrosis, viral hepatitis.

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Retrospective Cohort Study

Sensitivity of diagnosis of spontaneous bacterial peritonitis is higher with the automated cell count method

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Abstract

BACKGROUND

Spontaneous bacterial peritonitis (SBP) is one of the most important complications of patients with liver cirrhosis entailing high morbidity and mortality. Making an accurate early diagnosis of this infection is key in the outcome of these patients. The current definition of SBP is based on studies performed more than 40 years ago using a manual technique to count the number of polymorphs in ascitic fluid (AF). There is a lack of data comparing the traditional cell count method with a current automated cell counter. Moreover, current international guidelines do not mention the type of cell count method to be employed and around half of the centers still rely on the traditional manual method.

AIM

To compare the accuracy of polymorph count on AF to diagnose SBP between the

traditional manual cell count method and a modern automated cell counter against SBP cases fulfilling gold standard criteria: Positive AF culture and signs/symptoms of peritonitis.

METHODS

Retrospective analysis including two cohorts: Cross-sectional (cohort 1) and case-control (cohort 2), of patients with decompensated cirrhosis and ascites. Both cell count methods were conducted simultaneously. Positive SBP cases had a pathogenic bacteria isolated on AF and signs/symptoms of peritonitis.

RESULTS

A total of 137 cases with 5 positive-SBP, and 85 cases with 33 positive-SBP were included in cohort 1 and 2, respectively. Positive-SBP cases had worse liver function in both cohorts. The automated method showed higher sensitivity than the manual cell count: 80% *vs* 52%, $P = 0.02$, in cohort 2. Both methods showed very good specificity ($> 95\%$). The best cutoff using the automated cell counter was polymorph $\geq 0.2 \text{ cells} \times 10^9/\text{L}$ (equivalent to 200 cells/mm³) in AF as it has the higher sensitivity keeping a good specificity.

CONCLUSION

The automated cell count method should be preferred over the manual method to diagnose SBP because of its higher sensitivity. SBP definition, using the automated method, as polymorph cell count $\geq 0.2 \text{ cells} \times 10^9/\text{L}$ in AF would need to be considered in patients admitted with decompensated cirrhosis.

Key Words: Spontaneous bacterial peritonitis; Diagnosis; Cirrhosis; Bacterial infection; Automated cell count method; Manual cell count method; Ascitic fluid

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Core Tip: The traditional cutoff recommended by international guidelines (250 polymorphs $\times 10^6/\text{L}$ in ascitic fluid) to diagnose spontaneous bacterial peritonitis (SBP) was set when automated cell counters were not available. There are no data comparing the manual and the automated cell count in patients with cirrhosis against gold standard SBP cases and employing the cell analyzers currently available. This study compares both cells count methods against SBP cases fulfilling gold standard criteria and shows that the automated method has better sensitivity: 80% *vs* 52%, $P = 0.02$ and has a good specificity (96%). It also shows that the most accurate cutoff to diagnose SBP is 0.2 polymorphs $\times 10^9/\text{L}$. Guidelines should recommend the use of one of the modern automated cell counters instead of the manual method.

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INTRODUCTION

Infections constitute one of the most important complications in patients with cirrhosis because of their high frequency and severity[1]. Infections can trigger development of acute-on-chronic liver failure (ACLF)[2,3] and increase mortality 4-fold[4]. SBP is a frequent infection in patients with cirrhosis and ascites; it exacerbates the degree of portal hypertension leading to further decompensation. Almost 10% of patients with ACLF have SBP at or during their admission; mortality in these patients remains high, up to 46% at 28 days[3]. In order to standardize the diagnosis of SBP, international experts met in 2000[5] and recommended diagnosing SBP when the polymorph cell count (PMN) $\geq 250 \text{ cells per mm}^3$ of ascitic fluid (AF), as this cut-off had the greatest sensitivity according to studies available at the time; of note, those studies were carried out between the 70's and early 90's and they all employed the manual cell count method[5]. This definition has been widely accepted and remains the current standard of care[6,7].

The accuracy of the tests employed to diagnose SBP is crucial as it is key to avoid delays in treatment, which could have a devastating impact on the outcome. Delayed paracentesis is associated with double the in-hospital mortality rate [8], and mortality in patients with cirrhosis and septic shock increases 10% every hour of delay in starting antibiotics[9]. At present, four studies have assessed only the correlation between the manual and the automated cell count methods in the diagnosis of SBP[10-13] which showed to be good but did not compare the accuracy of these methods independently against the gold standard which is defined as a combination of clinical and microbiological characteristics[5]. Moreover, the automated cell counters employed on those four studies either no longer exist (ADVIA®; Technicon System H1, Bayer) or have been superseded by new models (Cell-Dyn 3700, Abbott; UF-100®, Sysmex). More recently, two other reports showed the correlation of the cell count between currently available automated cell counters (Sysmex XE-500 and LH750 Beckman Coulter) and the manual method was good, but the methods were again not compared independently against

the gold standard and more importantly, there were no clinical data collected, thus the studies included cases with a variety of causes of ascites, not only cirrhosis and portal hypertension[14,15]. In addition, a survey we conducted among members of the British Association for the Study of the Liver in 2022 showed that 7/14 (50%) centers who responded used either only the manual method or a combination of both methods (unpublished). Therefore, there is a need to assess the accuracy of the manual method and a current model of automated cell counter to diagnose SBP in patients with cirrhosis and portal hypertension related (PHT)-ascites, comparing both methods independently against the gold standard SBP diagnosis. This is the aim of this 5-year long retrospective study.

MATERIALS AND METHODS

Participants

We assessed the accuracy of manual and automated cell count methods on two cohorts of cases with cirrhosis and ascites admitted to University Hospitals Plymouth (UHP) as part of a local audit. All patients with cirrhosis and ascites have AF cell count at admission, and when there is suspicion of infection or undergoing paracentesis[6,7]. All AF samples are sent to Microbiology for culture.

Cohort 1 had a cross-sectional design[16,17] and was composed of all consecutive cases admitted to UHP between December 2017 and February 2019 due to decompensation of cirrhosis. Consecutive cases were identified through AF samples from the Microbiology electronic database. After exclusion criteria were applied, all day-cases having elective large volume paracentesis (LVP) were also excluded.

Cohort 2 had a case-control design[16,17]-specifically, a two-gate design with representative sampling[18]. The cases were identified through AF samples with positive culture, from the Microbiology electronic database, collected from December 2017 to November 2022. After exclusion criteria, those fulfilling positive-SBP criteria were included. The controls were identified on all consecutive AF samples from patients attending for elective LVP between December 2021 and November 2022 from the Day-Case Unit register. After exclusion criteria, those fulfilling strict negative-SBP criteria were included.

The exclusion criteria: Absence of liver cirrhosis, ascites not caused by portal hypertension, presence of permanent ascitic drain, secondary peritonitis, liver transplant, advanced hepatocellular carcinoma (Barcelona Clinic Liver Cancer-C/D), no cell count performed either by manual or automated method, no clinical information available, and fluid sample different from ascites. Repeat samples were excluded as follows: When both cell count methods gave a negative result, only samples taken the same day were excluded; when at least one cell count method gave a positive result, subsequent samples during the same admission were excluded as they would be difficult to interpret.

Positive-SBP criteria: It was the same in both cohorts: (1) Isolation of a pathogenic bacterium on AF; and (2) The presence of at least one clinical sign/symptom of peritonitis, *i.e.* alteration in gastrointestinal motility (vomiting, diarrhoea, or ileus), rebound tenderness, abdominal pain, temperature ≥ 37.5 °C, or shock[5]. Signs/symptoms of peritonitis not documented on the clinical notes were considered absent. The classification of bacteria according to pathogenicity is listed in Table 1. The culture result was not available when the index tests were performed.

Table 1 Type of bacteria according to their pathogenicity in spontaneous bacterial peritonitis

Bacteria	
Pathogenic	<i>Staphylococcus aureus</i>
	<i>Enterococcus faecalis</i>
	<i>Enterococcus faecium</i>
	<i>Escherichia coli</i>
	<i>Klebsiella pneumoniae</i>
	<i>Pseudomonas aeruginosa</i>
	<i>Anaerobes</i>
	<i>Clostridium innocuum</i>
	<i>Streptococcus gallolyticus</i>
	<i>Streptococcus agalactiae</i>
Indeterminate	<i>Sphingomonas paucimobilis</i>

Non-pathogenic	<i>Acinetobacter</i> sp.
	<i>Streptococcus sanguinis</i>
	<i>Streptococcus parasanguinis</i>
	<i>Streptococcus salivarius</i>
	<i>Staphylococcus epidermidis</i>
	<i>Staphylococcus capitis</i>
	<i>Staphylococcus haemolyticus</i>
	<i>Staphylococcus hominis</i>
	<i>Staphylococcus saprophyticus</i>
	<i>Staphylococcus pasteuri</i>
	<i>Staphylococcus pettenkoferi</i>
	<i>Staphylococcus warneri</i>
	<i>Staphylococcus saccharolyticus</i>
	<i>Corynebacterium striatum</i>
	<i>Corynebacterium jeikeium</i>
	<i>Micrococcus luteus</i>
	<i>Dermacoccus nishinomiyaensis</i>
	<i>Kocuria rhizophilia</i>

Negative-SBP criteria: In cohort 1: All remaining cases not fulfilling positive-SBP criteria. In cohort 2: All the following strict criteria had to be fulfilled (to make SBP exclusion as reliable as possible): (1) No admission to hospital within 4 weeks after inclusion- except for another day-case elective paracentesis; (2) Negative AF culture result; (3) No record of any sign/symptom of peritonitis on the medical notes-as described in the definition of positive-SBP; and (4) No antibiotic prescribed -except SBP prophylaxis or rifaximin for HE.

Cell count methods on AF

Manual cell count method: It was performed in the Microbiology Department by a technician employing a modified Fuchs-Rosenthal disposable double-sided cell count chamber, C-Chip DHC-F01. The interobserver coefficient of variability is assessed regularly and its average on white cell count was 0.116 at the time of the study. The result is given in cells $\times 10^6/L$ which is equivalent to cells/mm³. The result was positive when AF PMN ≥ 250 cells $\times 10^6/L$. The result was negative when AF PMN < 250 cells $\times 10^6/L$. There were no indeterminate results.

Automated cell count method: It was performed in the Central Laboratory using the Abbott Alinity HQ high-throughput full blood count analyzer, which uses optical scatter and fluorescence for differential cell count. The Alinity cell counter express the results in cells $\times 10^9/L$. Therefore, 250 cells/mm³ would be equivalent to 0.25 cells $\times 10^9/L$, however the results are expressed with only one decimal, *e.g.* 0.1, 0.2, 0.3, *etc.* To identify the best cutoff to diagnose positive-SBP, receiver operating characteristic (ROC) analysis and 4 \times 4 tables showing the accuracy with different cutoffs were obtained; PMN ≥ 0.2 cells $\times 10^9/L$ (equivalent to 200 cells/mm³) showed the best accuracy, [Figure 1](#) and [Table 2](#). The result was negative when the AF PMN ≤ 0.1 cells $\times 10^9/L$, or when no differential cell count was given and the total white cell count (WCC) ≤ 0.1 cells $\times 10^9/L$, the latter cases were included in the 4 \times 4 tables but not in the ROC analysis. The result was indeterminate when there was no differential cell count and the total WCC ≥ 0.2 cells $\times 10^9/L$, these cases were included in the analysis as negative result to avoid spectrum bias[16].

Statistical analysis

Variables are summarized as frequencies and percentages, mean \pm SD or median and IQR, as appropriate. For comparison of demographic and clinical variables between groups (positive-SBP *vs* negative-SBP) unpaired Student *t* tests for continuous variables with parametric distribution, Mann-Whitney's *U* test for those with non-parametric distribution, and χ^2 or Fisher's exact test for categorical variables was used. In cohort 2, ROC analysis was used to determine optimum cutoffs for the automatic and manual cell count methods. Statistical analyses were performed using IBM SPSS v28.0 and R version 4.2.2. STARD diagrams displayed in [Figure 2](#) and [Figure 3](#).

Table 2 Performance of automated and manual cell count methods in cohort 2 employing different cutoffs, *n* (%)

	SBP		Total
	Positive	Negative	
Manual method cell count			
Result cutoff ≥ 200 cells $\times 10^6$			
Positive	19 (61)	0	19
Negative	12	50 (100)	62
Total	31	50	81
Result cutoff ≥ 250 cells $\times 10^6$			
Positive	18 (58)	0	18
Negative	13	50 (100)	63
Total	31	50	81
Result cutoff ≥ 300 cells $\times 10^6$			
Positive	18 (58)	0	18
Negative	13	50 (100)	63
Total	31	50	81
Automated method cell count			
Result cutoff ≥ 0.1 cells $\times 10^9$ /L			
Positive	24 (89)	26	50
Negative	3	25 (49)	28
Total	27	51	78
Result cutoff ≥ 0.2 cells $\times 10^9$ /L			
Positive	21 (78)	2	50
Negative	6	49 (96)	28
Total	27	51	78
Result cutoff ≥ 0.3 cells $\times 10^9$ /L			
Positive	20 (74)	0	20
Negative	7	51 (100)	58
Total	27	51	78
Result cutoff ≥ 0.4 cells $\times 10^9$ /L			
Positive	19 (70)	0	20
Negative	8	51 (100)	58
Total	27	51	78

SBP: Spontaneous bacterial peritonitis.

RESULTS

Study populations

Cohort 1 (cross-sectional) was composed of 137 cases from 74 patients admitted with decompensated cirrhosis: 5 positive-SBP cases in 5 patients, and 132 negative-SBP cases in 69 patients. Initially, 484 cases were identified. After applying the exclusion criteria, 215 cases were selected. The most common cause of exclusion was absence of liver cirrhosis ($n = 244$) followed by ascites not related to portal hypertension ($n = 14$). Moreover, 9 duplicate cases and 69 cases who had elective LVP were excluded, leaving 137 cases for analysis, [Figure 4](#). There were only 5 SBP cases in this cohort and thus, the study was expanded to a case-control cohort.

Cohort 2 (case-control) was composed of 85 cases from 46 patients: 33 cases in 32 patients in the positive-SBP group, and 52 cases in 16 patients in the negative-SBP group. Two patients were cases in both groups.

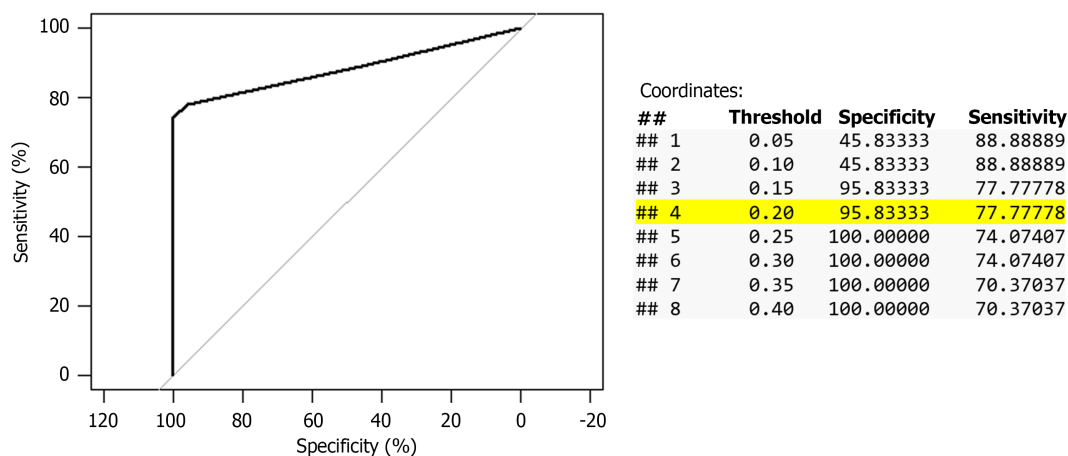


Figure 1 Receiver operating characteristic analysis of automated cell count method in cohort 2. Area under the curve: 88.12%, 95%CI: 78.44%-97.79%.

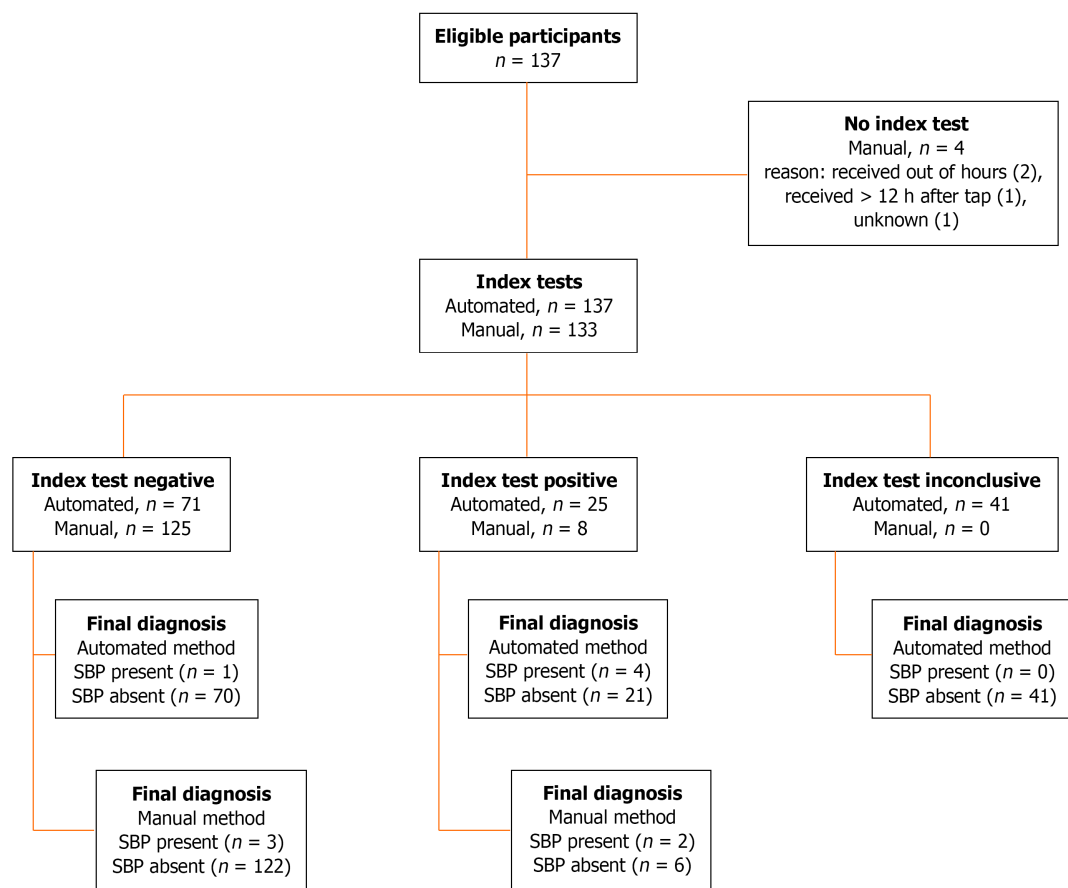


Figure 2 STARD diagram reporting flow of cases through the study in cohort 1. SBP: Spontaneous bacterial peritonitis.

The positive-SBP cases were selected as follows: 237 cases with AF positive culture were identified during the 5-year period. After applying the exclusion criteria, 87 cases were selected. The most common cause of exclusion was the presence of permanent ascitic drain ($n = 42$) followed by oncological ascites ($n = 28$). 50/87 cases had a pathogenic bacteria isolated in the AF. Finally, 33/50 cases having at least one clinical sign/symptom of peritonitis were included, [Figure 5](#). Only one excluded case growing an indeterminate bacterium had positive sign/symptom of peritonitis.

The negative-SBP cases were selected as follows: 78 cases with PHT related ascites attending elective LVP were identified during the 1-year period. After the exclusion criteria were applied, 70 cases were selected. When negative-SBP criteria were applied, 52 cases were finally included ([Figure 6](#)).

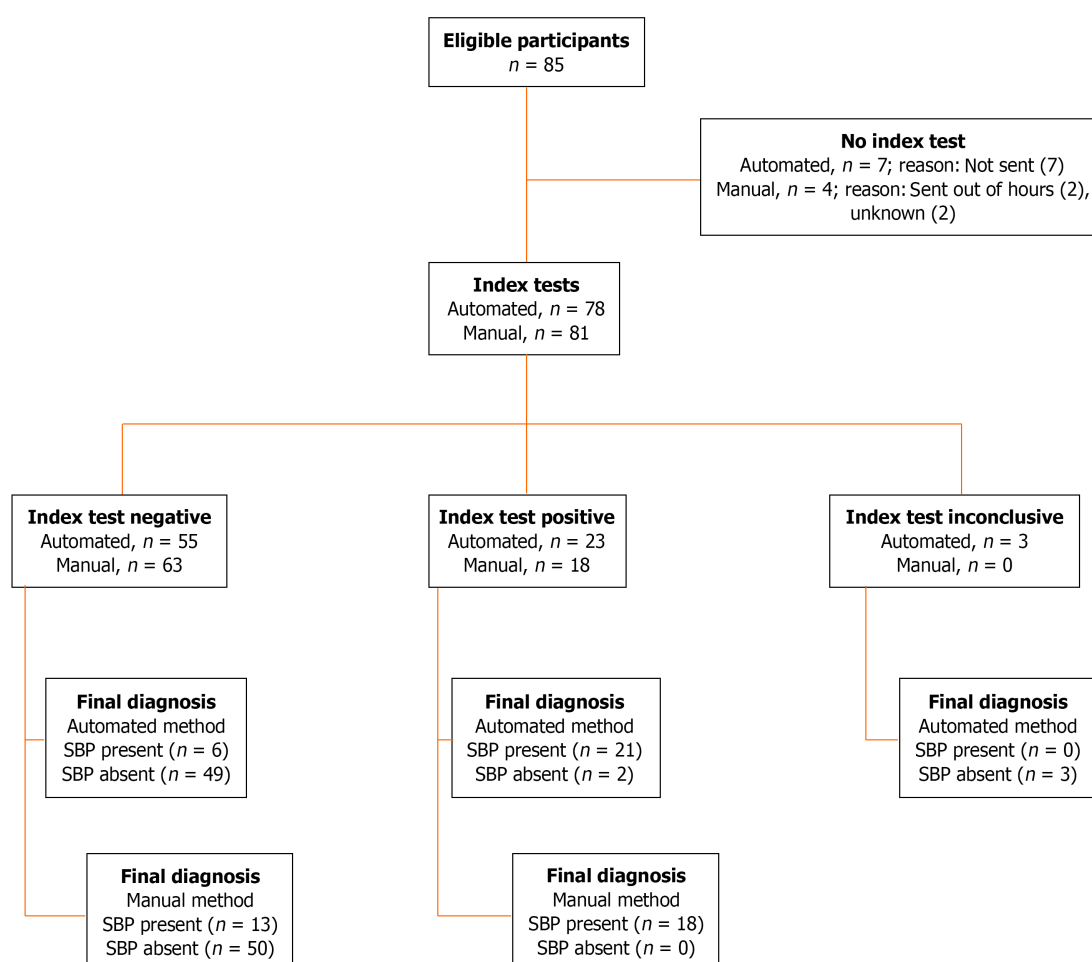


Figure 3 STARD diagram reporting flow of cases through the study in cohort 2.

Clinical and biochemical characteristics of positive-SBP and negative-SBP cases in cohort 1

There was no difference between both groups in relation to sex, age, etiology of cirrhosis, presence of diabetes, use of betablockers, immunosuppression, SBP prophylaxis or rifaximin, main cause of admission or renal function. Cases with SBP had worse liver function: More prolonged international normalized ratio (INR) (2.0 ± 0.5 vs 1.5 ± 0.4 , $P = 0.01$), higher Child-Pugh score (12.0 ± 1.0 vs 10.1 ± 1.7 points, $P = 0.02$), model for end-stage liver disease (MELD) score (25 ± 7 vs 19 ± 7 , $P = 0.06$), and CLIF-C ACLF score (53.5 ± 7.3 vs 43.3 ± 8.4 , $P = 0.02$), Table 3.

Inflammation markers were similar between both groups: C-reactive protein (CRP) levels (59 ± 26 vs 42 ± 40 mg/L, $P = 0.33$), serum leukocyte levels (7.5 ± 2.3 vs 10.2 ± 6.6 cells $\times 10^9/L$, $P = 0.36$), and presence of Systemic Inflammatory Response Syndrome (SIRS) criteria (0 vs 31%, $P = 0.17$). Both cells count methods showed significantly higher ascites polymorph count in positive-SBP cases than negative-SBP cases: 4.76 ± 3.86 vs 0.47 ± 1.89 cells $\times 10^9/L$, $P < 0.001$, using the automated method; and 1226 ± 1706 vs 72 ± 259 cells $\times 10^6/L$, $P < 0.001$, using the manual method, Table 4.

Performance of manual and automated cell count methods in cohort 1

The manual method was performed in 133 (97%) cases and gave differential cell count in all cases. Its sensitivity was 40%, it gave a positive result in 2/5 positive-SBP cases. Its specificity was 95%, it gave a negative result in 122/128 negative-SBP cases. Its positive predictive value (PPV) was 25% (2/8), and its negative predictive value (NPV) was 98% (122/125), Table 5.

The automated method was performed in all 137 (100%) cases, but gave the differential cell count on only 75 (55%) samples; 21 cases without differential cell count had total WCC ≤ 0.1 cells $\times 10^9/L$ and thus gave a negative result. Therefore, indeterminate results were obtained in 41 (30%) cases, which were considered negative for the analysis, Figure 4. Its sensitivity was 80%, it gave a positive result in 4/5 positive-SBP cases. Its specificity was 84%, it gave a negative result in 111/132 negative-SBP cases. Its PPV was 16% (4/25), and its NPV was 99% (111/112), Table 5.

Statistical comparison between the manual and automated cell count methods could not be performed due to the scarcity of positive-SBP cases.

Clinical and biochemical characteristics of positive-SBP and negative-SBP cases in cohort 2

There was no difference between both groups in relation to sex, age, etiology of cirrhosis, presence of diabetes and use of betablockers or immunosuppression. However, positive-SBP cases used rifaximin more frequently (25% vs 2%, $P = 0.002$) and SBP prophylaxis less frequently (16% vs 46%, $P = 0.005$) than negative-SBP cases. The main cause of admission in the

Table 3 Clinical and biochemical characteristics of positive-spontaneous bacterial peritonitis and negative-spontaneous bacterial peritonitis cases in cohort 1, *n* (%) / mean \pm SD

	Negative-SBP (<i>n</i> = 132)	Positive-SBP (<i>n</i> = 5)	<i>P</i> value
Demographic and clinical characteristics			
Male sex	73 (55)	3 (60)	1.0
Age (years)	62.1 \pm 12.9	71.8 \pm 14.2	0.10
Diabetes	23 (21)	2 (40)	0.30
Betablockers	40 (37)	3 (60)	0.61
Immunosuppression	2 (2)	1 (20)	0.13
SBP prophylaxis	26 (24)	0	0.59
Rifaximin	6 (6)	0	1.0
Alcohol-related cirrhosis	94 (71)	3 (60)	0.51
Main cause of admission			
Infection	34 (26)	2 (40)	0.35
Ascites	62 (47)	2 (40)	
Jaundice	9 (7)	1 (20)	
Encephalopathy	9 (7)	0	
UGIB	11 (8)	0	
AKI	2 (1)	0	
Other ¹	5 (4)	0	
Admission in ITU	5 (4)	0	1.0
Shock	2 (2)	0	1.0
Hospital mortality	9/85 (11)	2/5 (40)	0.11
Liver and renal function			
Bilirubin (μ mol/L)	99 \pm 115	188 \pm 208	0.10
Albumin (g/L)	28 \pm 5	29 \pm 4	0.91
INR	1.5 \pm 0.4	2.0 \pm 0.5	0.01
Ascites-Grade 3 ²	84 (64)	2 (40)	0.36
Encephalopathy			
Grade 0-1	91 (69)	3 (60)	0.53
Grade 2-3	41 (31)	2 (40)	
Creatinine (μ mol/L)	84 \pm 47	104 \pm 56	0.36
Sodium (mmol/L)	132 \pm 6	133 \pm 7	0.99
Child-Pugh (score)	10.1 \pm 1.7	12.0 \pm 1.0	0.02
MELD score (points)	19 \pm 7	25 \pm 7	0.06
UKELD score (points)	58 \pm 5	61 \pm 2	0.25
CLIF-C ACLF score (points)	43.3 \pm 8.4	53.5 \pm 7.3	0.02
ACLF grade			
No ACLF	68 (57)	3 (60)	0.66
ACLF grade 1	40 (33)	1 (20)	
ACLF grade 2-3	12 (10)	1 (20)	

¹2 cases with anemia, 2 cases with hyponatremia and falls, and 1 case with hip fracture decompensated with ascites after surgery.

²All other cases had grade-2 ascites.

SBP: Spontaneous bacterial peritonitis; UGIB: Upper gastrointestinal bleeding; AKI: Acute kidney injury; ITU: Intensive therapy unit; INR: International normalized ratio; MELD: Model for end-stage liver disease; UKELD: United Kingdom end-stage liver disease; CLIF-C ACLF: Chronic liver failure consortium acute-on-chronic liver failure.

Table 4 Markers of infection and characteristics of ascitic fluid in positive-spontaneous bacterial peritonitis and negative-spontaneous bacterial peritonitis cases in cohort 1, *n* (%) / mean \pm SD

	Negative-SBP (<i>n</i> = 132)	Positive-SBP (<i>n</i> = 5)	<i>P</i> value
Markers of inflammation			
CRP (mg/L)	42 \pm 40	59 \pm 26	0.33
CRP > 5 mg/L	106 (89)	5 (100)	1.0
Leukocyte count in serum ($\times 10^9$ /L)	10.2 \pm 6.6	7.5 \pm 2.3	0.36
Neutrophil count in serum ($\times 10^9$ /L)	7.6 \pm 5.7	5.8 \pm 1.9	0.50
Temperature ($^{\circ}$ C)	37.0 \pm 0.7	37.5 \pm 0.3	0.14
Heart rate (beats per minute)	91 \pm 15	92 \pm 19	0.87
Respiratory rate (breaths per minute)	18 \pm 4	19 \pm 1	0.74
Presence of SIRS	33/108 (31)	0/5	0.17
MAP (mmHg)	79 \pm 16	89 \pm 10	0.19
Characteristics of the ascitic fluid			
Albumin (g/L)	7.7 \pm 4.9	8.5 \pm 2.1	0.74
Auto. polymorph count (cells $\times 10^9$ /L)	0.47 \pm 1.89	4.76 \pm 3.86	< 0.001
Manual polymorph count (cells $\times 10^6$ /L)	72 \pm 259	1226 \pm 1706	< 0.001

SBP: Spontaneous bacterial peritonitis; CPR: C-reactive protein; SIRS: Systemic inflammatory response syndrome; MAP: Mean arterial pressure.

Table 5 Performance of automated and manual cell count methods in cohort 1, *n* (%)

	Positive-SBP	Negative-SBP	Total
Manual method cell count			
Test result			
Positive	2 (40)	6	8
Negative	3	122 (95)	125
Total	5	128	133
Automated method cell count			
Test result			
Positive	4 (80)	21	25
Negative	1	111 (84)	112
Total	5	132	137

SBP: Spontaneous bacterial peritonitis.

positive-SBP cases was infection (52%) followed by ascites (21%) and jaundice (15%), while it was elective paracentesis in all negative-SBP cases. Positive-SBP cases had worse liver function: Higher bilirubin levels (158 \pm 164 *vs* 42 \pm 27 μ mol/L, *P* < 0.001), more prolonged INR (1.8 \pm 0.7 *vs* 1.3 \pm 0.2, *P* = 0.005), clinical encephalopathy (48% *vs* 0%, *P* < 0.001) and higher Child-Pugh score (11.3 \pm 1.9 *vs* 9.3 \pm 1.3 points, *P* < 0.001); worse renal function: Higher creatinine levels (140 \pm 112 *vs* 69 \pm 18 μ mol/L, *P* < 0.001) and MELD score (21.9 \pm 10.1 *vs* 12.2 \pm 4.0 points, *P* < 0.001); and were more critically ill: Higher rate

Table 6 Clinical and biochemical characteristics of positive-spontaneous bacterial peritonitis and negative-spontaneous bacterial peritonitis cases in cohort 2, *n* (%) / mean \pm SD

	Negative-SBP (<i>n</i> = 52)	Positive-SBP (<i>n</i> = 33)	<i>P</i> value
Demographics and clinical characteristics			
Male sex	21 (40)	17 (52)	0.37
Age (years)	55.0 \pm 13.5	60.4 \pm 14.8	0.08
Alcohol-related cirrhosis	45 (87)	29 (88)	0.16
Diabetes	6 (12)	7 (22)	0.23
Betablockers	16 (31)	13 (41)	0.48
Immunosuppression	0	1 (3)	0.38
SBP prophylaxis	24 (46)	5 (16)	0.005
Rifaximin	1 (2)	8 (25)	0.002
Main cause of admission			
Elective LVP	52 (100)		
Infection		17 (52)	
Ascites		7 (21)	
Jaundice		5 (15)	
Encephalopathy		1 (3)	
UGIB		1 (3)	
AKI		2 (6)	
Admission in ITU	0	7 (21)	< 0.001
Shock	0	7 (22)	< 0.001
Hospital mortality	0/52	14/33 (42)	< 0.001
Liver and renal function			
Bilirubin (μ mol/L)	42 \pm 27	158 \pm 164	< 0.001
Albumin (g/L)	30 \pm 4	29 \pm 5	0.23
INR	1.3 \pm 0.2	1.8 \pm 0.7	< 0.001
Ascites-Grade 3 ¹	49 (94)	20 (61)	< 0.001
Encephalopathy			
Grade 0-1	52 (100)	17 (52)	< 0.001
Grade 2-3	0	16 (48%)	

¹All other cases had grade-2 ascites.

SBP: Spontaneous bacterial peritonitis; LVP: Large volume paracentesis; UGIB: Upper gastrointestinal bleeding; AKI: Acute kidney injury; ITU: Intensive therapy unit; INR: International normalized ratio; MELD: Model for end-stage liver disease; UKELD: United Kingdom end-stage liver disease; CLIF-C ACLF: Chronic liver failure consortium acute-on-chronic liver failure.

of ACLF grade 2-3 (29% *vs* 0%, *P* < 0.001), lower mean arterial pressure (74 \pm 17 *vs* 84 \pm 12 mmHg, *P* = 0.01), shock (22% *vs* 0%, *P* < 0.001), admission to ITU (21% *vs* 0%, *P* < 0.001), and in-hospital mortality (42% *vs* 0%, *P* < 0.001) compared to negative-SBP cases. The cause of death in the positive-SBP cases was ACLF in 9/14 and septic shock in 5/14 cases. More negative-SBP cases had ascites grade-3 because they all attended for LVP (94% *vs* 61%, *P* < 0.001), [Table 6](#).

Positive-SBP cases showed higher degree of inflammation: Higher CRP levels (57 \pm 47 *vs* 22 \pm 17 mg/L, *P* < 0.001), serum leukocyte count (11.1 \pm 5.1 *vs* 7.3 \pm 2.1 cells \times 10⁹/L, *P* < 0.001) and higher prevalence of SIRS (50% *vs* 13%, *P* = 0.004) than negative-SBP cases. Both cells count methods showed higher AF polymorph count in positive-SBP cases than negative-SBP cases: 3.27 \pm 3.68 *vs* 0.06 \pm 0.06 cells \times 10⁹/L, *P* < 0.001 on the automated method; and 2157 \pm 4105 *vs* 20 \pm 22 cells \times 10⁶/L, *P* < 0.001, on the manual method, [Table 7](#).

Performance of manual and automated cell count methods in cohort 2

The manual method was performed in 81/85 (95%) samples and gave differential cell count in all of them. The PMN

Table 7 Markers of infection and characteristics of ascitic fluid in positive-spontaneous bacterial peritonitis and negative-spontaneous bacterial peritonitis cases in cohort 2, *n* (%) / mean \pm SD

	Negative-SBP (<i>n</i> = 52)	Positive-SBP (<i>n</i> = 33)	<i>P</i> value
Markers of inflammation			
CRP (mg/L)	22 \pm 17	57 \pm 47	< 0.001
CRP > 5 mg/L	24 (89)	30 (91)	1.00
Leukocyte count in serum ($\times 10^9$ /L)	7.3 \pm 2.1	11.1 \pm 5.1	< 0.001
Neutrophil count in serum ($\times 10^9$ /L)	5.0 \pm 1.8	8.6 \pm 4.4	< 0.001
Temperature ($^{\circ}$ C)	36.9 \pm 0.3	37.1 \pm 1.0	0.22
Heart rate (beats <i>per</i> minute)	102 \pm 16	104 \pm 20	0.75
Respiratory rate (breaths <i>per</i> minute)	18 \pm 3	21 \pm 5	0.02
Presence of SIRS	4 (13)	14 (50)	0.004
MAP (mmHg)	84 \pm 12	74 \pm 17	0.01
Characteristics of the ascitic fluid			
Albumin (g/L)	8.0 \pm 4.1	7.5 \pm 5.8	0.61
Automated polymorph count (cells $\times 10^9$ /L)	0.06 \pm 0.06	3.27 \pm 3.68	< 0.001
Manual polymorph count (cells $\times 10^6$ /L)	20 \pm 22	2157 \pm 4105	< 0.001

SBP: Spontaneous bacterial peritonitis; CPR: C-reactive protein; SIRS: Systemic inflammatory response syndrome; MAP: mean arterial pressure.

Table 8 Performance of automated and manual cell count methods in cohort 2, *n* (%)

	Positive-SBP	Negative-SBP	Total
Manual method cell count			
Test result			
Positive	18 (58)	0	18
Negative	13	50 (100)	63
Total	31	50	81
Automated method cell count			
Test result			
Positive	21 (78)	2	23
Negative	6	49 (96)	55
Total	27	51	78

SBP: Spontaneous bacterial peritonitis.

Table 9 Performance of automated and manual cell count methods in cases having both methods performed in cohort 2, *n* (%)

	Automated method		Total
	Positive	Negative	
Positive-SBP cases			
Manual method			
Positive	13	0	13 (52)
Negative	7	5	12

Total	20 (80)	5	25
Negative-SBP cases			
Manual method			
Positive	0	0	0
Negative	2	47	49 (100)
Total	0	47 (96)	49

SBP: Spontaneous bacterial peritonitis.

count ranged from 0 to 21280 cells $\times 10^6/L$. Its sensitivity was 58%, it gave positive result in 18/31 positive-SBP cases. Its specificity was 100%, it gave negative result in all 50 negative-SBP cases. Its PPV was 100% (18/18), and its NPV was 79% (50/63), [Table 8](#).

The automated method was performed in 78/85 (92%) samples. It gave the differential cell count on 75 (88%) samples. Three (4%) samples were indeterminate and considered negative result for the analysis. The PMN count ranged from 0.0 to 11.5 cells $\times 10^9$. Its sensitivity was 78%, it gave a positive result in 21/27 positive-SBP cases. Its specificity was 96%, it gave a negative result in 49/55 negative-SBP cases. Its PPV was 91% (21/23), and its NPV was 89% (49/55), [Table 9](#).

To compare the sensitivity and specificity of the manual and automated cell count methods we analyzed only paired samples: 74/85 cases having both methods performed. The sensitivity of the automated method was superior to the manual method, 80% (20/25) *vs* 52% (13/25), $P = 0.02$. The specificity was 100% (49/49) and 96% (47/49) in the manual and automated method, respectively, [Table 9](#).

To address the potential bias that the automated method was more sensitive because it used a lower cutoff, we compared it with the manual method using the same cutoff ≥ 200 PMN $\times 10^6/L$. The sensitivity of the automated method was still superior to the manual method, 80% (20/25) *vs* 56% (14/25), $P = 0.03$. The specificity remained the same, [Table 10](#).

Microbiological characteristics of positive-SBP cases

There were 33 positive-SBP cases in total. The 5 cases in cohort 1 are included in cohort 2. The most common origin was community-acquired, present in 18 (55%), followed by healthcare-associated in 11 (33%) and nosocomial in 4 (12%) cases. Blood or urine cultures were taken in 22 (67%) cases, 8 of them (24%) showed the same bacterium isolated in the AF culture. The most frequent bacterium isolated was *Escherichia coli* in 18 (55%) cases, followed by *Enterococcus faecium* in 8 (24%). With regards to empirical antibiotic therapy (EAT), the most frequently employed was piperacillin/tazobactam in 15 (46%) cases, followed by meropenem in 6 (18%), [Table 11](#). EAT was started before the ascitic tap in 16 cases, and after the ascitic tap in 15 cases, the time to administration was not available in 2 cases. In the latter 15 cases, the EAT was started between 0.8 and 48.6 hours after the ascitic tap was performed. There was a tendency to undergo increased delay in EAT when the ascitic tap gave a (false) negative result: 26.2 ± 19.7 *vs* 12.5 ± 12.8 hours, $P = 0.16$ in the cases with negative ($n = 3$) and with positive result ($n = 11$), respectively, using the automated method; and 27.5 ± 12.2 *vs* 11.1 ± 14.0 hours, $P = 0.055$ in the cases with negative ($n = 5$) and with positive result ($n = 8$), respectively, using the manual method.

DISCUSSION

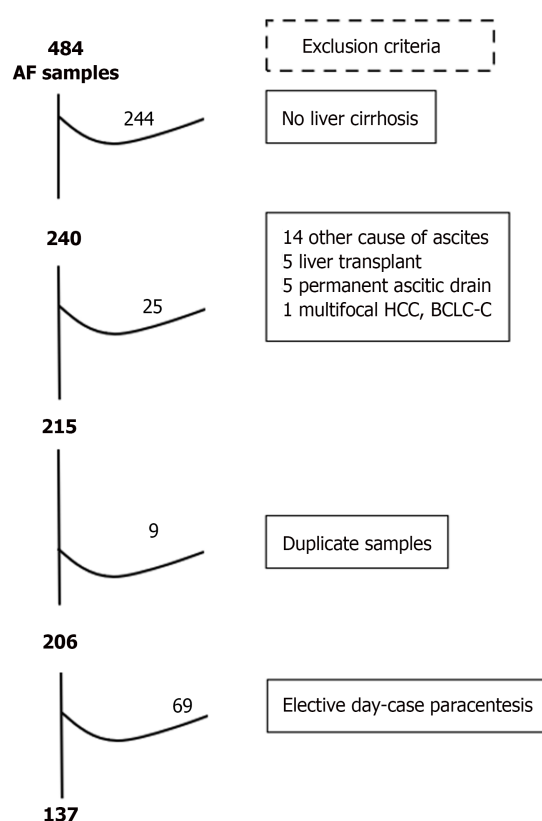
This is the first study comparing the diagnostic accuracy of both types of cells count methods currently employed to diagnose SBP in patients with cirrhosis and ascites: The traditional manual method and a contemporary automated method, against SBP cases defined by gold standard criteria. It shows that the automated method is more sensitive than the manual method, which is clinically very important as increased sensitivity will reduce the number of missed SBP cases and delays in treatment, as suggested by the delay in starting antibiotics seen in cases with false negative cell count results which more than doubles the delay in cases with positive result using the manual method. This difference was less evident with the automated method because running both methods at the same time may have led doctors to prefer the result of the manual over the automated method because it looks falsely more accurate as it is expressed with more decimal figures and in similar units as the traditional definition of SBP. The main limitations of this study are its retrospective design and the small number of positive cases in the cross-sectional study (cohort 1). Thus, the number of positive-SBP cases was only 5 cases (4%) due to the strict criteria used to diagnose SBP and thus, no statistical comparison was possible. In this sense, studies have reported low rates of positive culture in SBP cohorts, around 6% [19] and even in septic shock it is only 36% [20]. We compensated this limitation by subsequently conducting a case-control study (cohort 2) which confirmed the difference in sensitivity was statistically significant.

The sensitivity is assessed in the positive-SBP cases which, in our study, were clinically more unwell, 25% were on rifaximin due to previous hepatic encephalopathy, they showed higher prevalence of septic shock (22%) and hospital mortality (42%), compared to other studies reporting shock in 8%, and 30-day mortality in 26% of SBP cases [19,21]. This difference is explained because all other studies defined SBP according to the cell count result and not to the gold standard criteria and thus, most SBP cases included were culture negative. Moreover, positive AF and blood cultures have been reported as independent predictors of mortality [19,21]. Along these lines, all SBP cases in our cohort grew a

Table 10 Performance of automated and manual cell count methods, both with cutoff PMN ≥ 200 cells/mm³ in cases having both methods performed in cohort 2, *n* (%)

	Automated method		Total
	Positive	Negative	
Positive-SBP cases			
Manual method			
Positive	14	0	14 (56)
Negative	6	5	11
Total	20 (80)	5	25
Negative-SBP cases			
Manual method			
Positive	0	0	0
Negative	2	47	49 (100)
Total	2	47 (96)	49

SBP: Spontaneous bacterial peritonitis.

**Figure 4** Selection of cohort 1 (cross-sectional) cases.

pathogenic bacterium on their AF which was also isolated in either blood or urine cultures in 24% of cases. Therefore, our SBP cases had higher bacterial burden and bloodstream circulation than other cohorts. This can also account for the worse liver function positive-SBP cases had, even when compared to cases admitted with other decompensations, including other types of infections.

The drawback of using the gold standard SBP criteria is that the sensitivity of the cell count methods (index tests) would be higher than when applied to cohorts including the whole spectrum of the disease, i.e. culture negative SBP[16]. However, not using the gold standard SBP criteria would only allow assessment of the correlation between both cell count methods, but no significant comparison of their accuracy could be possible. Furthermore, a “surrogate” gold standard would be needed which would include the cell count result as part of its definition and thus, the sensitivity

Table 11 Microbiological characteristics of positive-spontaneous bacterial peritonitis cases, *n* (%)

	Positive-SBP (<i>n</i> = 33)
Origin of infection	
Community-acquired	18 (55)
Healthcare-associated	11 (33)
Nosocomial	4 (12)
Cultures	
Blood or urine culture taken	22 (67)
Blood or urine culture, same bacteria	8 (24)
Bacteria isolated	
<i>E. coli</i>	18 (55)
<i>Enterococcus faecium</i>	8 (24)
<i>Enterococcus faecalis</i>	3 (9)
<i>Klebsiella pneumoniae</i>	1 (3)
<i>S aureus</i>	1 (3)
<i>Streptococcus agalactiae</i>	1 (3)
<i>Clostridium innocuum</i>	1 (3)
Empirical antibiotic used	
Piperacillin/tazobactam	15 (46)
meropenem	6 (18)
Levofloxacin	4 (12)
Co-amoxiclav	4 (12)
Teicoplanin	2 (6)
Tigecycline	1 (3)
Flucloxacillin	1 (3)

SBP: Spontaneous bacterial peritonitis.

would be falsely increased due to disease verification bias[17]. Hence, SBP gold standard criteria were required despite the potential bias of selecting the most unwell cases. It can also be argued that this subgroup (culture-positive SBP) is the most in need of diagnostic tests with increased sensitivity.

The specificity is assessed in the negative cases and it was very good in both cohorts, although slightly lower in cohort 1, probably because the negative-SBP cases were inpatients with decompensated cirrhosis and ascites who did not fulfil SBP gold standard criteria and thus, they comprised some actual SBP cases such as those with negative culture, asymptomatic (around 14% of SBP are asymptomatic[22]), or when data on signs/symptoms of peritonitis was missing on clinical records. The specificity was higher in cohort 2, likely because negative-SBP cases were strictly selected.

Other disadvantages of the manual cell count method, in addition to its lower sensitivity, are its wide interobserver variability, it is labour intensive/time consuming, could be challenging to get out of hours (contacting technician on-call) and it is more expensive: £7.50 *vs* £1.04 per sample. Moreover, the automated cell counter has the advantage that it is already in place in the laboratory to process blood samples and we used the same mode and working channel to process the AF samples even though the automated analyzer has a specific channel for body fluids, however it could not be employed because there is no External Quality Assessment (EQA) scheme for body fluids in the United Kingdom currently.

Before using the automated cell counter, we ran our own internal quality assessment with repeat testing using the standard blood channel and blood mode. AF generally contains low white cell burden; the lower the cell count the greater the imprecision (deviation index) of the test becomes. We know from full blood count EQA returns that for low white cell count, the error of the mean is reported to one decimal place which means the test cannot be more precise than down to one decimal place. Based on analysis of different cutoffs points, we propose a new definition of SBP based on the results of the automated cell counter, *i.e.*, PMN ≥ 0.2 cells $\times 10^9$ /L instead of the traditional definition based on the manual cell count method as it has the best sensitivity (80%) keeping a very good specificity (96%).

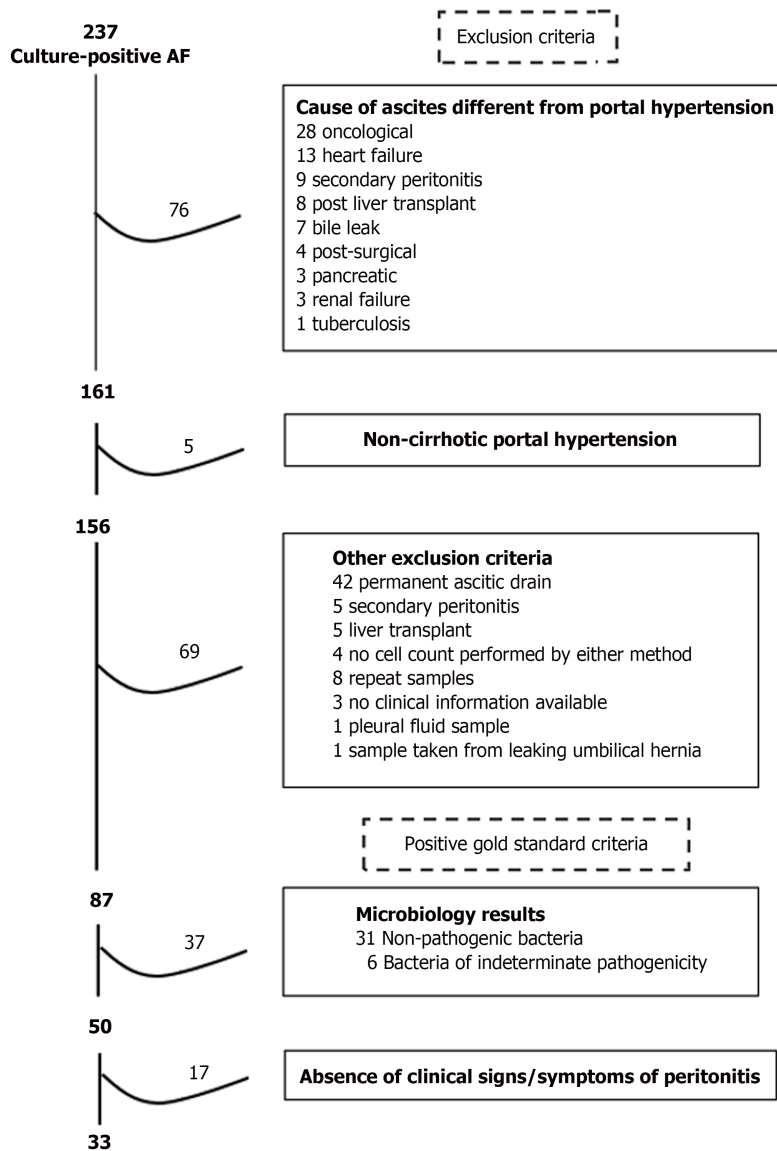


Figure 5 Selection of gold standard positive-spontaneous bacterial peritonitis cases in cohort 2 (case-control).

The automated cell counter performance giving differential cell count was good in cohort 2, composed by culture-positive cases, leaving only 2/79 (3%) samples with indeterminate result. However, when employed in cohort 1, composed by cases with wider spectrum of disease, 22 (16%) cases had indeterminate results, probably because there was not a clear interface between the cell populations due to the presence of debris or apoptosed cells that the analyzer may struggle to identify. This problem may be reduced by employing a body fluid mode which may provide the best fluid/reagent characteristic, thus it would merit further investigation.

Finally, it is important not only to employ an automated cell counter, but a recent model should be selected because the accuracy varies. In a previous audit[23], our group assessed the Sysmex XE2100 model automated cell counter on a cross-sectional cohort of patients with cirrhosis and ascites including 224 cases admitted from November 2015 to June 2016. Automated cell count was performed in 196 (88%) cases, but it gave differential cell count in only 5 (3%) samples, which had total white cell count $> 1.0 \text{ cells} \times 10^9/\text{L}$. That model has been superseded by the XN generation of Sysmex analyzers.

CONCLUSION

In conclusion, although this is a retrospective study, the data found on the case-control cohort supports the automated cell count method, using one of the latest models available, as the preferred cell count method over the manual method to diagnose SBP because of its higher sensitivity which is key when dealing with this potentially lethal complication in patients with advanced cirrhosis and ascites. Moreover, SBP could be defined as $\text{PMN} \geq 0.2 \text{ cells} \times 10^9/\text{L}$ in AF in patients admitted with decompensated cirrhosis.

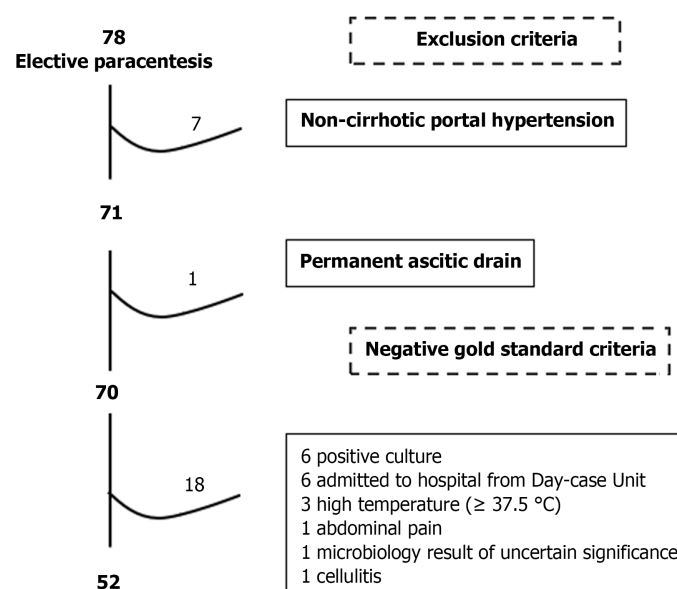


Figure 6 Selection of gold standard negative-spontaneous bacterial peritonitis cases in cohort 2 (case control).

FOOTNOTES

Author contributions: Acevedo-Haro JG, Tilley R, Hosking J, and Thomas W designed the study; Mohamed W, Moodley P, Bendall O, Bennett K, Keelty N, Chan S, and Acevedo-Haro JG participated in data collection; Acevedo-Haro JG, Hosking J, and Tilley R participated in data analysis and interpretation; Acevedo-Haro JG, Tilley R, Thomas W, and Waddy S participated in the writing group.

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Data sharing statement: Not applicable.

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