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Mid-regional pro-adrenomedullin: A rapid sepsis biomarker for diagnosing spontaneous bacterial peritonitis in cirrhosis

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Abstract

Background: Spontaneous bacterial peritonitis (SBP) is a frequent and life-threatening complication of cirrhosis, contributing to considerable morbidity and mortality.

Methods: A cross-sectional derivation study was conducted to assess the diagnostic accuracy of two sepsis-related calcitonin peptide family biomarkers, mid-regional pro-adrenomedullin (MR-pro-ADM) and procalcitonin, in ascitic fluid for identifying bacteriologically confirmed SBP (BC-SBP). In a subsequent validation study, the diagnostic performance of the 'SBP score' was evaluated in an independent patient cohort using an absolute polymorphonuclear (PMN) leukocyte count threshold of ≥250 cells/mm^3 as the diagnostic benchmark for diagnosing SBP.

Results: In the derivation study, the concentration of MR-pro-ADM in ascitic fluid was significantly higher in patients with BC-SBP compared to those

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without BC-SBP (3.14 nmol/L [IQR, 2.39–6.74] vs. 1.91 nmol/L [IQR, 1.33–2.80]; $p = .0002$). Bayesian ANOVA indicated that MR-pro-ADM was highly discriminative for diagnosing BC-SBP, with a substantial Bayes factor (BFM = 2505), whereas procalcitonin exhibited poor discriminatory performance. Receiver-operating characteristic (ROC) analysis identified an optimal MR-pro-ADM cut-off of ≥2.50 nmol/L for diagnosing BC-SBP, with an area under the ROC curve (AUROC) of 0.746 (95% CI, 0.685–0.801; $p < .0001$). Multivariable logistic regression identified three independent predictors of BC-SBP, which were subsequently incorporated into the 'SBP score' (MR-pro-ADM ≥2.5 nmol/L, absolute PMN count ≥250 cells/mm^3 and Child–Pugh score). In the validation study, the 'SBP score' demonstrated an AUROC of 0.993 (95% CI, 0.929–1.000; $p < .0001$) for diagnosing SBP.

Conclusion: MR-pro-ADM in ascitic fluid emerges as a promising biomarker for SBP diagnosis. Combining MR-pro-ADM with absolute PMN count and Child–Pugh score in the 'SBP score' greatly improves the diagnostic accuracy of SBP.

KEYWORDS

cirrhosis, mid-regional pro-adrenomedullin, rapid assay biomarker, spontaneous bacterial peritonitis

1 | INTRODUCTION

Spontaneous bacterial peritonitis (SBP) is a frequent and severe complication of cirrhosis observed in 29% of cirrhotic patients at admission and up to 70% of those with advanced disease and is defined as an ascitic fluid infection without an evident intra-abdominal, surgically treatable source.[1–3] The diagnosis of SBP is based on an ascitic fluid absolute polymorphonuclear (PMN) neutrophils count ≥250 cells/mm^3 in the absence of an intra-abdominal, surgically treatable source of infection or abdominal malignancy.[4] When SBP is suspected, ascitic fluid culture should be performed and may guide the choice of antibiotic treatment by providing a complete antibiogram within ~24–72 h.[2,5]

From a practical aspect, the ascitic fluid PMN count provides a preliminary diagnosis of SBP, and a positive ascitic fluid culture confirms this.[5,6] Currently, the gold standard for PMN quantification is light microscopy with a manual counting chamber. However, this approach is susceptible to human error and requires a learning curve.[5] The original research, which suggested a PMN count ≥250 cells/mm^3 as the diagnostic threshold for SBP, was published in 1977 and was based on a comparison of 11 patients with SBP versus 57 control cirrhotic patients, using positive ascitic fluid bacterial culture (PAFBC) as an indicator of diagnosis.[7] However, the PMN count largely overlapped between the two subgroups, ranging from 10 to 560 in cirrhotic patients without SBP to 106–5600 in patients with SBP.[7] Based on this first description, all subsequent studies on the topic used the ≥250 PMN cells/mm^3 threshold. Consequently, this measure may lead to misdiagnosis or unnecessary antibiotic prescription, given the need for careful antibiotic stewardship in patients with cirrhosis.[8] On the other hand, when SBP is not diagnosed in a timely fashion, hospital mortality can reach 48%–95%, whereas a 5% rate occurs with rapid management.[9,10] Thus, the use of validated biomarkers in clinical decision-making related to SBP may significantly improve patient outcomes.

Mid-regional pro-adrenomedullin (MR-pro-ADM) and procalcitonin are sepsis-related biomarkers that can be routinely assayed using automated methods with established quality standards, providing results within 30 min.[11–13] These biomarkers are part of the conserved calcitonin peptide family, with *CALCA* (formerly *CALC-I*) encoding procalcitonin and *ADM* (formerly *CALC-IV*) encoding MR-pro-ADM and have been extensively studied as innate immune markers, particularly for identifying septic conditions.[13–19] In this study, we evaluated the utility of these rapid-assay biomarkers in ascitic fluid for diagnosing positive ascitic fluid bacterial culture in the absence of an evident intra-abdominal, surgically treatable source in patients with cirrhosis, a condition defined here as bacteriologically confirmed SBP (BC-SBP). Additionally, we derived and validated the 'SBP score', which demonstrated a highly discriminative area under the receiver-operating

characteristic curve (AUROC) of 0.993 in the validation study for diagnosing SBP in patients with cirrhosis.

2 | METHODS

2.1 | Overview of the study design, study aims and outcomes

2.1.1 | Derivation study

In the derivation study, we conducted a retrospective cross-sectional analysis to evaluate the diagnostic accuracy of mid-regional pro-adrenomedullin (MR-pro-ADM) and procalcitonin as biomarkers for the diagnosis of BC-SBP in patients with cirrhosis. BC-SBP was defined using a composite outcome that incorporated an ascitic PMN neutrophil count of ≥250 cells/mm^3 consistent with international guidelines,[4] along with a positive ascitic fluid culture for a recognized pathogen identified from one or more blood culture bottles, in the absence of an evident intra-abdominal, surgically treatable source. This composite endpoint methodology was employed to ensure a more thorough and comprehensive diagnostic approach to SBP in its typical, homogeneous form. The primary aim of the study was to assess the diagnostic accuracy of MR-pro-ADM and procalcitonin for BC-SBP. The secondary aim was to develop a logistic regression model incorporating significant clinical and biological predictors of BC-SBP to derive the 'SBP score'. All BC-SBP diagnoses were adjudicated by a senior gastroenterologist and a senior microbiologist, both of whom conducted patient chart reviews independently and were blinded to the patients' biomarker data.

2.1.2 | Validation study

In the validation study, we assessed the diagnostic accuracy of the 'SBP score' in an independent cohort of consecutive patients who underwent abdominal paracentesis. The validation study aimed (1) to assess the distribution of MR-pro-ADM levels in ascitic fluid among several groups of patients with cirrhosis (SBP, SBP after antibiotic initiation, hepatocellular carcinoma or portal vein thrombosis without SBP, Cirrhosis without SBP) and those without cirrhosis (peritoneal carcinosis, portal vein thrombosis without underlying cirrhosis and miscellaneous conditions) and (2) to assess the diagnostic accuracy of the SBP score for diagnosing SBP among patients with cirrhosis. The study outcome was a diagnosis of SBP based on an absolute PMN count ≥250 cells/mm^3.[4]

2.2 | Study setting and patient selection criteria for the derivation and validation studies

The derivation study included patients with cirrhosis who were admitted to the University Hospital of Nancy and who underwent abdominal paracentesis. The inclusion criteria were as follows (i) admitted to one of the healthcare facilities of the University Hospital of Nancy between 1 February 2014 and 31 May 2016; (ii) diagnosed with cirrhosis and (iii) had abdominal paracentesis performed during the hospital stay. The exclusion criteria were as follows: (i) hemorrhagic ascitic fluid; (ii) lack of clinical data and (iii) concomitant systemic infection. All tests were performed at the discretion of the physicians in each department as part of the standard evaluation of patients with cirrhosis with ascitic decompensation.

The validation study included consecutive patients who underwent abdominal paracentesis at the University Hospital of Nancy between 1 February 2021 and 30 June 2021. We included patients with cirrhosis and those with non-cirrhotic ascites (peritoneal carcinosis, portal vein thrombosis without underlying cirrhosis, pancreatitis, or chylous ascites) to assess the distribution of the MR-pro-ADM biomarker in the ascitic fluid. We used the same exclusion criteria as in the derivation study.

2.3 | Data collected for the derivation and validation studies

The patients included in the present study were identified using the 'Nancy Biochemical Database', a prospectively maintained electronic database that compiles biochemical data from consecutive patients hospitalized across 67 healthcare departments, including medicine, surgery and obstetrics, at the University Hospital of Nancy.[13,15,20,21] The biochemical and microbiological data are connected to clinical data at the patient level through the electronic health record system of the University Hospital of Nancy. All biological and microbiological data were extracted using the GLIMS laboratory information management system version 8.11.6 (MIPS France SARL, Paris, France). The clinical data were retrieved through electronic chart review using DxCare software (Dedalus France, Le Plessis Robinson, France). The following data were obtained for the study: (i) administrative data, including patient identification number, date of hospital admission, patient healthcare department and date and time of abdominal paracentesis; (ii) demographic data, including age and sex; (iii) clinical data, including aetiology of cirrhosis (alcohol,

nonalcoholic steatohepatitis, hepatitis C virus, hepatitis B virus, autoimmune, other), Child–Pugh score and diuretic use at admission; (iv) blood laboratory findings, including albumin (g/L), total bilirubin (mg/L); prothrombin activity (percentage), C-reactive protein (mg/L) and total proteins (g/L); (v) ascitic fluid biomarkers, including total proteins (g/L) and absolute PMN count (cells/mm^3) and (vi) bacteriological data, including ascitic fluid culture results (positive or negative). Given the retrospective design of the study, French legislation mandates only the assurance of personal data protection.[22] The 'Nancy Biochemical Database' is registered at the French National Commission on Informatics and Liberty, CNIL, under the record No. 1763197v0, which supervises the protection of individuals with regard to the processing of personal data. The Institutional Review Board of the University Hospital of Nancy approved the study (ID: 2020/264) in accordance with the ethical principles of the Declaration of Helsinki.

2.4 | Mid-regional pro-adrenomedullin and procalcitonin assays

In the derivation study, all ascitic fluid samples were tested for MR-pro-ADM and procalcitonin in the Division of Biochemistry, Molecular Biology and Nutrition at the University Hospital of Nancy. Concentrations of MR-pro-ADM (nmol/L) and procalcitonin (ng/mL) in the ascitic fluid were measured using automated B.R.A.H.M.S. KRYPTOR immunofluorescence assays (Thermo Fisher Scientific, Asnières-sur-Seine, France) according to the manufacturer's protocol via a Kryptor Compact Plus Analyser (Thermo Fisher Scientific). The incubation times for MR-pro-ADM and procalcitonin were 29 min and 19 min, respectively. Levels 1 and 2 intra-assay coefficients of variation were 6.32% and 3.83%, respectively, for procalcitonin and 6.70% and 7.60%, respectively, for MR-pro-ADM. For the validation study, all ascitic fluid samples were tested for MR-pro-ADM at the Laboratory of Biochemistry of the Beaujon Hospital (Paris, France) using the same analytical approach as in the derivation study.

2.5 | Ascitic fluid culture

A pair of blood culture bottles (Bactec® Plus Aerobic and Bactec® F Lytic Anaerobic, Becton Dickinson, Le Pont de Claix, France) was inoculated with each patient's ascitic fluid and incubated in a BD Bactec™ 9240 system for at least 5 days. Cultures were considered to be negative if no bacterial growth was detected within the incubation period. Aliquots from positive cultures were taken for direct Gram staining and subcultured on standard solid media for subsequent analysis. Microorganisms were identified by mass spectrometry with a Vitek MS (bioMérieux, Marcy-l'Étoile, France) MALDI-TOF mass spectrometry system or 16S rRNA gene sequencing. Coagulase-negative staphylococci, *Corynebacterium*, *Propionibacterium* and *Bacillus* species were considered contaminants.[23]

2.6 | Absolute PMN neutrophils count

We used the body fluid (BF) module on a Sysmex XN-1000 automated haematology analyser (Sysmex Corporation, Kobe, Japan) to quantify white blood cells (WBCs). Ascitic fluid samples were systematically collected in ethylenediaminetetraacetic acid (EDTA) anticoagulant tubes upon abdominal paracentesis and analysed within 2 h. WBCs were identified and quantified using flow cytometry in a dedicated channel that combined forward scatter (proportional to the size of the cells), side scatter (proportional to the inner complexity of the cells) and fluorescence intensity (proportional to DNA/RNA content) to identify and cluster mononuclear leukocytes (lymphocytes and monocytes) and PMN leukocytes (neutrophils, eosinophils, and basophils). PMN leukocytes were characterized by the combination of high side scatter and low fluorescent marker intensity. High-fluorescence BF cells, such as macrophages and mesothelial cells, were not included in the WBC count. When noncellular particles—such as bacteria or cell debris—were identified, the sample was reexamined by light microscopy. The Sysmex XN1000 BF-automated count has proven to be highly correlated with reference manual counting methods.[24]

2.7 | Post hoc study power and sample size calculation

For the derivation study, we calculated that a total of 209 patients (19 with the composite endpoint and 190 without the composite endpoint) would be required to assess the primary aim using the following criteria: 1:10 ratio of patients to control participants, alpha risk of 0.05, study power of 95% and expected/reference AUROCs of 0.75/0.50.

2.8 | Statistical analysis

All quantitative variables are shown as the median and interquartile range (IQR, 25–75th percentile), and

qualitative variables are expressed as percentages and 95% confidence intervals (95% CIs). The unit of analysis was visit-level data rather than patient-level data since some patients were hospitalized more than once during the study period for abdominal paracentesis.[25] The comparison of MR-pro-ADM and procalcitonin concentrations according to BC-SBP status was performed by the Mann–Whitney *U* test. In addition to the conventional frequentist approach, we used Bayesian ANOVA to compare the distributions of the two studied biomarkers according to BC-SBP status. For each model comparison, we reported the Bayes factor (BF_M), which describes the change from the prior odds to the posterior odds for the model (likelihood odds). We also reported the Bayesian 95% credible interval (95% CrI). The diagnostic accuracies of MR-pro-ADM and procalcitonin were determined by receiver-operating characteristic (ROC) analysis according to DeLong et al.[26] The classification variable used in the ROC analysis was the BC-SBP status. For each ROC analysis, we reported the area under the ROC curve (AUROC) and the associated *p*-value. The optimal diagnostic cut-off was defined using the Youden index J. The bias-corrected and accelerated (BC_a)-bootstrap interval was calculated after 10,000 iterations for the Youden index and its associated values.[27] Other diagnostic accuracy measures included sensitivity, specificity, positive and negative likelihood ratios and positive and negative predictive values. The pairwise AUROC comparison was carried out according to the procedure described by Delong et al.[26] We performed univariate binary logistic regression with Bonferroni adjustment to derive variables potentially associated with the BC-SBP status. All significant findings resulting from univariate analyses were integrated into a binary logistic regression model for multivariable analysis using the BC-SBP status as a dependent variable. All variables with $p < .1$ were included in the model, and variables with $p < .05$ were retained. The results are shown as the regression coefficient, standard error (SE), odds ratio (OR), and 95% CI for each independent predictor and as the percentage of patients correctly classified by the logistic regression model. We assessed model discrimination using ROC analysis and model calibration with the Hosmer and Lemeshow goodness-of-fit test and Nagelkerke R^2 statistics.[28] To derive a risk score for predicting the BC-SBP status, we used the method described by Sullivan et al. by converting the coefficients for independent predictors into a simplified risk score system.[29] We calculated the number of points assigned to each variable by dividing its OR by the smallest OR in the model. Then, we rounded this quotient to the nearest whole number.[30] Finally, we calculated each subject's risk score by summing the points of all the variables to obtain the 'SBP score'. We assessed the optimal cut-off of the SBP score for diagnosing BC-SBP through ROC analysis. Considering the sensitivity and specificity associated with the ROC-defined cut-off of the SBP score, we used a Bayesian approach for assessing the PPV and the NPV and their 95% CI according to an expected prevalence ranging from 1 to 99%, as described by Mercaldo et al.[31] We evaluated the association between the SBP score and the probability of BC-SBP status using binary probit regression. All statistical analyses were conducted with the SAS® 9.4 platform (Cary, NC, USA) and MedCalc v20 (MedCalc Software, Ostend, Belgium) based on a two-sided alpha level of 0.05. For the validation study, an ROC analysis was conducted, following the methodology detailed for the derivation study.

3 | RESULTS

3.1 | Derivation study

Between 1 February 2014 and 31 May 2016, a total of 250 patient visits were recorded. Fourteen patients were excluded from the analysis due to the presence of hemorrhagic ascitic fluid ($n = 4$), insufficient clinical data ($n = 5$) or concomitant infection ($n = 5$), resulting in 236 patient visits included in the final dataset. The descriptive statistics are reported in Table 1. Of the 236 patient visits, 22 had a BC-SBP for the following pathogens: *Escherichia coli* ($n = 10$), *Enterobacter cloacae* ($n = 3$), *Streptococcus mitis/oralis* ($n = 3$), *Salmonella enterica* ($n = 2$), *Actinomyces radingae* ($n = 1$), *Enterococcus faecium* ($n = 1$), *Klebsiella pneumoniae* ($n = 1$) and *Pseudomonas aeruginosa* ($n = 1$).

3.2 | Diagnostic accuracy of ascitic fluid MR-pro-ADM for BC-SBP diagnosis

The MR-pro-ADM concentration was significantly greater in ascitic fluids from patients with BC-SBP than in those from patients without BC-SBP (3.14 nmol/L [IQR, 2.39–6.74] vs. 1.91 nmol/L [IQR, 1.33–2.80]; $p = .0002$). According to the Bayesian ANOVA, MR-pro-ADM was highly discriminant for BC-SBP status. The means and credibility intervals of MR-pro-ADM in ascitic fluids with or without BC-SBP were 4.41 (95% CrI 3.02–5.80) and 2.38 (95% CrI 2.13–2.62), respectively, with a high likelihood ratio ($BF_M = 2505$). According to the ROC analysis, ascitic MR-pro-ADM was significantly associated with BC-SBP, with an AUROC of 0.746 (95% CI, 0.685–0.801; $p < .0001$). The optimal cut-off of ≥2.50 nmol/L had a sensitivity of 76%, a specificity of 69%, a positive predictive value of 20%, and a negative predictive value of 97% for BC-SBP diagnosis (Table 2).

TABLE 1 Descriptive statistics of patient encounters included in the derivation study.

	No BC-SBP ($n=214$)	BC-SBP present ($n=22$)	p-value[a]
Demographics			
Age (years)—median (IQR)[c]	59 (52–65)	57 (44–75)	.99
Male sex—n (%)	154 (72%)	12 (55%)	.99
Aetiology of cirrhosis[b]—n (%)			
Alcohol	169 (79%)	21 (96%)	.99
HCV	32 (15%)	4 (18%)	.99
MASLD	34 (16%)	0 (0%)	.99
HBV	8 (4%)	0 (0%)	.99
Autoimmune	3 (1%)	1 (5%)	.99
Other	17 (8%)	0 (0%)	.99
Cirrhosis stage and diuretics			
Child–Pugh score—median (IQR)[c]	9 (7–10)	10 (10–10)	.0002
Use of diuretics at inclusion—n (%)	162 (76%)	12 (55%)	.99
Laboratory findings, blood—median (IQR)[c]			
Total proteins (g/L)	66.0 (58.0–71.0)	61.0 (57.3–69.5)	.99
C-reactive protein (mg/L)	15.8 (9.2–27.7)	32.3 (9.1–61.6)	.19
Total bilirubin (mg/L)	15.0 (10.0–27.2)	54.0 (41.0–164.0)	<0.0001
Albumin (g/L)	27.5 (24.3–31.5)	24.5 (19.1–29.5)	.03
Prothrombin activity (%)	57.8 (50.7–65.0)	44.3 (35.0–49.0)	<0.0001
Ascitic fluid biomarkers			
Proteins (g/L)—median (IQR)[c]	13.0 (9.0–22.0)	9.5 (7.0–18.0)	.99
Absolute PMN count (cells/mm^3)—median (IQR)[c]	4 (1–17)	22 (6–456)	.38
Absolute PMN count ≥250 cells/mm^3—n (%)	6 (3%)	6 (30%)	<0.0001
Procalcitonin (ng/mL)—median (IQR)[c]	0.15 (0.09–0.36)	0.25 (0.18–0.72)	.99
MR-pro-ADM (nmol/L)—median (IQR)[c]	1.91 (1.33–2.80)	3.14 (2.39–6.74)	.002

Abbreviations: %, percentage; BC-SBP, bacteriologically confirmed spontaneous bacterial peritonitis; HBV, hepatitis B virus; HCV, hepatitis C virus; IQR, interquartile range, 25th–75th percentile; MASLD, metabolic-associated steatotic liver disease; MR-pro-ADM, mid-regional pro-adrenomedullin; PMN, polymorphonuclear neutrophils.

[a]Chi-squared test or Fisher's exact test, as appropriate, with Bonferroni correction.

[b]Patients can have more than one aetiology.

[c]Mann–Whitney U test with Bonferroni correction.

3.3 | Diagnostic accuracy of the ascitic fluid procalcitonin concentration for BC-SBP diagnosis

The procalcitonin concentration was significantly greater in ascitic fluids from patients with BC-SBP than in those from patients without BC-SBP (0.25 ng/mL [IQR, 0.18–0.72] vs. 0.15 ng/mL [IQR, 0.09–0.36]; $p=.02$). According to the Bayesian ANOVA, procalcitonin levels exhibited poor discrimination for BC-SBP status. The means and CrIs of procalcitonin levels in ascitic fluids with or without BC-SBP were 0.74 (95% CrI 0.25–1.23) and 0.46 (95% CrI 0.32–0.59), respectively, with a low likelihood ratio ($BF_M=0.45$). According to the ROC analysis, ascitic procalcitonin had moderate diagnostic accuracy for BC-SBP, with an AUROC of 0.658 (95% CI, 0.593–0.719; $p=.005$). The optimal cut-off of >0.16 nmol/L had a sensitivity of 82%, a specificity of 54%, a PPV of 16%, and an NPV of 97% for BC-SBP diagnosis (Table 2).

TABLE 2 Receiver-operating characteristic analysis of the ability of MR-pro-ADM and procalcitonin levels to predict bacteriologically confirmed spontaneous bacterial peritonitis in the derivation study.

	MR-pro-ADM (nmol/L)	Procalcitonin (ng/mL)
Area under the ROC curve (AUROC)		
AUROC (95% CI)	0.746 (0.685–0.801)	0.658 (0.593–0.719)
Standard Error[a]	0.053	0.056
z statistic	4.624	2.801
p-value	<.0001	.005
Youden index		
Youden index (J)	0.4524	0.3541
Associated criterion	≥2.50	≥0.16
Criterion values and ROC curve for the associated criteria		
Sensitivity (95% CI)	76% (53–92)	82% (60–95)
Specificity (95% CI)	69% (62–75)	54% (47–61)
Positive likelihood ratio (95% CI)	2.46 (1.80–3.40)	1.76 (1.40–2.30)
Negative likelihood ratio (95% CI)	0.34 (0.20–0.70)	0.34 (0.10–0.80)
Positive predictive value (95% CI)	20% (12–30)	16% (10–24)
Negative predictive value (95% CI)	97% (92–99)	97% (92–99)

Abbreviations: 95% CI, 95% confidence interval; AUROC, area under the receiver-operating characteristic curve; MR-pro-ADM, mid-regional pro-adrenomedullin.

[a]Binomial exact method.

3.4 | Predictors of BC-SBP according to univariate analysis

According to univariate analysis, three variables were significantly associated with BC-SBP diagnosis: ascitic absolute PMN count ≥250 cells/mm^3 (OR, 14.57; 95% CI, 4.16–51.09; $p < .0001$), ascitic MR-pro-ADM ≥2.50 nmol/L (OR, 7.14; 95% CI, 2.51–20.32; $p = .004$) and Child–Pugh score (OR, 1.71; 95% CI, 1.30–2.26; $p = .0002$) (Tables 1 and 3).

3.5 | Independent predictors of BC-SBP according to multivariable analysis: The 'SBP score'

According to the multivariable logistic regression analysis, three factors were independently associated with SBP: absolute PMN count ≥250 cells/mm^3 (OR, 14.38; 95% CI, 3.17–65.21; $p = .0005$), MR-pro-ADM ≥2.5 nmol/L (OR, 7.25; 95% CI, 2.04–25.79; $p = .002$), and Child–Pugh score (OR, 1.61; 95% CI, 1.15–2.26; $p = .006$). The logistic regression model had good discrimination, with an AUROC of 0.859 (95% CI, 0.804–0.903) (Table 3).

We derived the 'SBP score' from the logistic regression equation as follows: 'SBP score' = (absolute PMN count ≥250 cells/mm^3 [9 points if present; 0 if absent] + MR-pro-ADM ≥2.5 nmol/L [4.5 points if present; 0 if absent] + Child–Pugh score [total points]). The 'SBP score' had greater diagnostic accuracy than did the logistic regression model, which included an absolute PMN count ≥250 cells/mm^3 and the Child–Pugh score (difference of AUROCs = 0.073; 95% CI, 0.008–0.137; $p = .03$) (Table 4 and Figure 1A). The 'SBP score' had a median value of 14.5 (IQR, 14.5–22.0) in patients with BC-SBP vs. 9.0 (IQR, 8.0–12.5) in patients without BC-SBP ($p < .0001$). According to the ROC analysis, the optimal cut-off for the 'SBP score' for diagnosing SBP was >13 (AUROC = 0.854; 95% CI, 0.799–0.899; $p < .0001$). Using this cut-off, the sensitivity and specificity of the 'SBP score' were 83% (95% CI, 59–96) and 79% (95% CI, 73–85), respectively. According to the Bayesian analysis, the PPVs and NPVs of the 'SBP score' were systematically greater than 80% when the pre-test probabilities of BC-SBP were greater or less than 50%, respectively (Figure 1B). We then performed a probit regression dose–response analysis using the 'SBP score' and BC-SBP status as the dose and response variables, respectively (Figures 1C and 4). The associations between the 'SBP score' and the series of probabilities are reported in Table 5, Figures 1C and 4. An 'SBP score' ≤13.1 (11.4–14.7) was associated with a probability of BC-SBP ≤10% ($p = .0001$).

TABLE 3 Independent predictors of bacteriologically confirmed spontaneous bacterial peritonitis diagnosis based on multivariable logistic regression analysis in the derivation study.

	Coef.	SE	OR (95% CI)	*P*-value	R^2[a]	AUROC (95% CI)[b]
Logistic regression model: PMN						
Model performance	—	—	—	—	.14	0.636 (0.570–0.698)
Absolute PMN count ≥250 cells/mm^3	2.679	0.640	14.57 (4.16–51.09)	<.0001	—	—
Constant	−2.679	0.276	—	<.0001	—	—
Logistic regression model: MR-pro-ADM						
Model performance	—	—	—	—	.15	0.726 (0.664–0.783)
MR-pro-ADM, ascites ≥2.5 nmol/L	1.966	0.534	7.14 (2.51–20.32)	.0002	—	—
Constant	−3.367	0.455	—	<.0001	—	—
Logistic regression model: (PMN, Child–Pugh score)[c]						
Model performance	—	—	—	—	.25	0.786 (0.724–0.839)
Absolute PMN count ≥250 cells/mm^3	2.744	0.710	15.54 (3.86–62.56)	.0001	—	—
Child–Pugh score	0.481	0.154	1.62 (1.20–2.19)	.002	—	—
Constant	−7.188	1.558	—	<.0001	—	—
Logistic regression model: (MR-pro-ADM, Child–Pugh score)[c]						
Model performance	—	—	—	—	.27	0.832 (0.775–0.879)
MR-pro-ADM, ascites ≥2.5 nmol/L	1.902	0.566	6.70 (2.21–20.31)	.0008	—	—
Child–Pugh score	0.555	0.163	1.74 (1.27–2.40)	.0006	—	—
Constant	−8.589	1.758	—	<.0001	—	—
Logistic regression model: (PMN, MR-pro-ADM)						
Model performance	—	—	—	—	.28	0.825 (0.769–0.873)
Absolute PMN count ≥250 cells/mm^3	2.631	0.721	13.88 (3.38–57.00)	.0003	—	—
MR-pro-ADM, ascites ≥2.5 nmol/L	2.082	0.611	8.02 (2.42–26.57)	.0007	—	—
Constant	−3.876	0.547	—	<.0001	—	—
Logistic regression model: (PMN, MR-pro-ADM, Child–Pugh score) 'SBP score'[c]						
Model performance	—	—	—	—	.36	0.859 (0.804–0.903)
Absolute PMN count ≥250 cells/mm^3	2.666	0.771	14.38 (3.17–65.21)	.0005	—	—
MR-pro-ADM, ascites ≥2.5 nmol/L	1.981	0.648	7.25 (2.04–25.79)	.002	—	—
Child–Pugh score	0.475	0.173	1.61 (1.15–2.26)	.006	—	—
Constant	−8.299	1.858	—	<.0001	—	—

Abbreviations: 95% CI, 95% confidence interval; AUROC, area under the receiver-operating characteristic curve; Coef., coefficient; MR-pro-ADM, mid-regional pro-adrenomedullin; OR, odds ratio; PMN, polymorphonuclear leukocyte; SE, standard error.

[a]Overall logistic regression model fit, Nagelkerke R^2.

[b]Logistic regression model discrimination, ROC analysis.

[c]Total bilirubin, albumin, and prothrombin activity were not included in the multivariable model, as these parameters are included in the calculation of the Child–Pugh score.

3.6 | Validation study

3.6.1 | Description of the studied population in the validation study

Between 1 February 2021 and 30 June 2021, a total of 95 patients were analysed. Ten patients were excluded due to missing data ($n = 6$), urinary tract infection ($n = 2$), peritoneal tuberculosis ($n = 1$) or septic shock ($n = 1$), resulting in 85 patient visits included in the final data set. Of these 85 visits, 58 were categorized as SBP, SBP after antibiotic initiation or 'Cirrhosis without SBP'. The remaining 27 patients were classified as multifocal hepatocellular carcinoma or portal vein thrombosis,

TABLE 4 Pairwise comparison of the diagnostic accuracy of logistic regression models for diagnosing bacteriologically confirmed spontaneous bacterial peritonitis.

	Difference between AUROCs	SE	*z* statistic	*p*-value
Logistic regression model: PMN, MR-pro-ADM, Child–Pugh score (The SBP score), versus				
L RM: MR-pro-ADM, Child–Pugh score	0.034 (−0.012–0.079)	0.023	1.461	.14
LRM: PMN, Child–Pugh score	0.073 (0.008–0.137)	0.033	2.205	.03
LRM: MR-pro-ADM	0.119 (0.024–0.214)	0.049	2.449	.01
LRM: PMN	0.208 (0.097–0.319)	0.057	3.680	.0002
Logistic regression model: MR-pro-ADM, Child–Pugh score, versus				
LRM: PMN, Child–Pugh score	0.039 (−0.050–0.127)	0.045	0.863	.39
LRM: MR-pro-ADM	0.086 (0.019–0.152)	0.034	2.530	.01
LRM: PMN	0.174 (0.036–0.313)	0.071	2.467	.01
Logistic regression model: PMN, Child–Pugh score, versus				
LRM:MR-pro-ADM	0.047 (−0.090–0.183)	0.0695	0.670	.50
LRM:PMN	0.135 (0.046–0.225)	0.0456	2.966	.003
Logistic regression model: MR-pro-ADM, versus				
LRM:PMN	0.089 (−0.078–0.255)	0.0848	1.046	.30

Abbreviations: AUROC, area under the receiver-operating characteristic curve; LRM, logistic regression model; MR-pro-ADM, mid-regional pro-adrenomedullin; PMN, polymorphonuclear leukocyte; SE, standard error.

peritoneal carcinomatosis, portal vein thrombosis without underlying cirrhosis or miscellaneous conditions (Tables S1 and S2).

3.6.2 | Diagnostic accuracy of mid-regional pro-adrenomedullin and the 'SBP score' for diagnosing SBP in the validation study

Concentrations of MR-pro-ADM were significantly greater in ascitic fluids from patients with SBP (4.6 nmol/L; IQR, 6.2–10.9), SBP after antibiotic initiation (3.6 nmol/L; IQR, 6.1–6.4), or multifocal hepatocellular carcinoma and/or portal vein thrombosis (3.4 nmol/L; IQR, 4.8–5.1) than in ascitic fluid from patients without SBP (1.0 nmol/L; IQR, 1.6–2.4) or exhibiting peritoneal carcinosis (1.2 nmol/L; IQR, 2.3–5.1), portal vein thrombosis without underlying cirrhosis (1.7 nmol/L; IQR, 2.5–2.7), or miscellaneous conditions (0.4 nmol/L; IQR, 1.3–1.9) ($p < .0001$).

Concentrations of MR-pro-ADM in the ascitic fluid were significantly higher in patients with SBP, SBP after antibiotic initiation, or multifocal hepatocellular carcinoma and/or portal vein thrombosis compared to those without SBP, peritoneal carcinomatosis, portal vein thrombosis without underlying cirrhosis or miscellaneous conditions ($p < .0001$) (Figure 2A and Table S2). The MR-pro-ADM level did not significantly differ between ascitic fluids from patients with SBP and those with SBP within 7 days after antibiotic therapy initiation. Conversely, the 'SBP score' was significantly greater in patients with SBP (23; IQR, 22.5–24.5) than in those with SBP after antibiotic initiation (15.5; IQR, 13.5–16.0), multifocal hepatocellular carcinoma and/or portal vein thrombosis (13.5; IQR, 13.25–14.75), or 'Cirrhosis without SBP' (8 [IQR, 8–10]) ($p < .0001$) (Figure 2B). According to the ROC analysis, the 'SBP score' had an AUROC of 0.993 (95% CI, 0.929–1.000) for diagnosing SBP, which was significantly greater than that of the MR-pro-ADM ≥2.5 nmol/L criterion (AUROC, 0.958; 95% CI, 0.876–0.993) for diagnosing SBP ($p = .0008$) (Figure 3; Table S3). The optimal 'SBP score' cut-off for diagnosing SBP was >17.5, which exhibited a sensitivity of 92%, a specificity of 100%, a PPV of 100%, and an NPV of 98%. In dose–response analysis, an 'SBP score' ≤14.8 (10.3–16.9) was associated with a probability of BC-SBP ≤10% ($p < .0001$) (Figures 1C and 4).

4 | DISCUSSION

The results of this derivation-validation cross-sectional study demonstrated that the combination of the ascitic MR-pro-ADM concentration, absolute PMN count and Child–Pugh score significantly improved the diagnosis of SBP in patients with cirrhosis who underwent abdominal paracentesis. These results prompt re-evaluating the use of an absolute PMN count ≥250 cells/mm^3 as the sole measure for diagnosing SBP in patients with cirrhosis.[7] In addition, our study showed that ascitic procalcitonin was weakly predictive of SBP, in line with the findings of two previously published studies.[32,33]

In the original 1977 study, absolute PMN values largely overlapped between cirrhotic patients with ($n = 11$) or

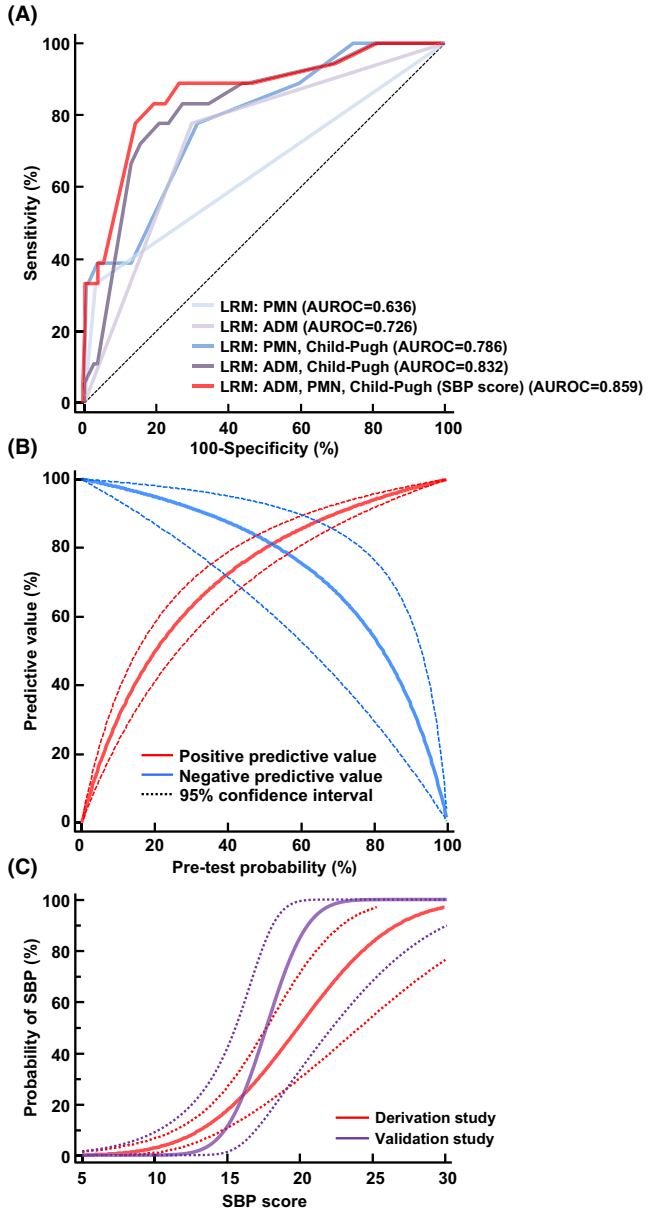

FIGURE 1 (A) Receiver-operating characteristic (ROC) curves for the diagnostic accuracy of the spontaneous bacterial peritonitis (SBP) score (red line) for diagnosing bacteriologically confirmed spontaneous bacterial peritonitis (BC-SBP); logistic regression models based on an absolute polymorphonuclear (PMN) neutrophils count ≥250 cells/mm^3 (light blue line); an MR-pro-ADM concentration of ≥2.5 nmol/L (light purple line); a PMN count of ≥250 cells/mm^3 and a Child–Pugh score (dark blue line); an MR-pro-ADM concentration of ≥2.5 nmol/L and a Child–Pugh score (dark purple line) for the diagnosis of SBP in the derivation study; (B) Probit sigmoid dose–response curve showing the SBP score as the dose variable and BC-SBP status as the response variable. The red and purple lines show the probability and corresponding dose, respectively, in the derivation and validation studies. The dashed curves represent the 95% confidence intervals (CIs) for the respective doses. The dose and 95% confidence interval corresponding to a particular probability are taken from a horizontal line at that probability level. (C) Bayesian estimation of the positive and negative predictive values of an 'SBP score' >13 for BC-SBP diagnosis for varying expected prevalence values of BC-SBP in the derivation study. Red line: Positive predictive value; blue line: Negative predictive value; dashed line: 95% confidence interval.

without ($n = 57$) ascitic fluid infection according to bacteriological analysis-based diagnosis.[7] The median absolute PMN counts in patients with and without SBP were 1470 and 32 cells/mm,^{3} respectively.[7] Thus, the authors suggested that 'a granulocyte count greater than 250 cells per mL was a relatively sensitive and specific indication of bacterial peritonitis, either spontaneous or secondary to other intra-abdominal infections' but failed to assess the diagnostic accuracy of this measure or report an effect size for the relationship between PMN count and SBP risk.[7] However, due to the lack of systematic research, most current guidelines and clinicians continue to use this diagnostic criterion.

ADM is a potent, 52-residue vasodilator peptide that belongs to the calcitonin gene-related peptide (CGRP) family and was first described in 1993 as a hypotensive peptide isolated from human pheochromocytoma.[34,35] ADM is synthesized from a larger 185-residue precursor, pre-pro-adrenomedullin, which is successively cleaved into four peptides: the pro-ADM N-terminal 20 peptide, MR-pro-ADM, the mature ADM molecule and the C-terminal fragment (adrenotensin).[36] Adrenomedullin is ubiquitously expressed in virtually all human tissues, with the highest concentrations detected in the adrenal medulla, cardiac atria and lungs.[37,38] It is produced by various cell types, including endothelial cells, monocytes, macrophages, and neutrophils. The quantification of ADM is hampered by its short half-life (~22 min) and high affinity for human complement factor H, making its analysis technically challenging.[39,40] Conversely, MR-pro-ADM is more stable than the other tested agents and can be easily measured through a sandwich immuno-luminometric assay using two polyclonal antibodies directed against residues 45–92 of pro-ADM.[41]

ADM exhibits immunosuppressive and antimicrobial activities and acts as a complement system activator.[40,42–46] Notably, epithelial cells and macrophages express ADM as the first line of defence against various pathogens, such as *Escherichia coli* and *Staphylococcus aureus*, by inducing intramembranous pores in the bacterial cell membrane.[43,44,47] Ultrastructural analyses of *Escherichia coli* treated with ADM revealed marked cell wall disruption within 30 min.[43] Moreover, the antimicrobial activity of C-terminal ADM fragments was shown to be up to 250-fold greater than that of the full-length molecule.[43] Interestingly, the most active ADM fragments are present within MR-pro-ADM.[43] Taken together, these

TABLE 5 Probit regression analysis of the 'SBP score' for diagnosing bacteriologically confirmed spontaneous bacterial peritonitis or spontaneous bacterial peritonitis in the derivation and validation studies, respectively.

Outcome probability	SBP score (95% CI), Derivation study[a]	SBP score (95% CI), Validation study[b]
1.0%	7.6 (3.6–9.7)	12.5 (3.9–14.8)
2.0%	9 (5.8–10.9)	13.1 (5.7–15.3)
2.5%	9.5 (6.5–11.3)	13.3 (6.3–15.4)
5.0%	11.2 (8.9–12.7)	14 (8.2–16.1)
10.0%	13.1 (11.4–14.7)	14.8 (10.3–16.9)
20.0%	15.4 (13.9–17.6)	15.8 (12.5–18.3)
25.0%	16.3 (14.7–18.8)	16.1 (13.3–18.9)
50.0%	19.9 (17.7–24.1)	17.6 (15.5–22.1)
75.0%	23.4 (20.4–29.6)	19.1 (17–26.2)
80.0%	24.3 (21.1–31)	19.5 (17.3–27.2)
90.0%	26.6 (22.8–34.6)	20.5 (18.1–30)
95.0%	28.5 (24.3–37.6)	21.3 (18.6–32.4)
97.5%	30.2 (25.5–40.3)	22 (19.1–34.5)
98.0%	30.7 (25.9–41.1)	22.2 (19.3–35.1)
99.0%	32.1 (26.9–43.3)	22.8 (19.7–36.9)

Note: SBP: bacteriologically confirmed spontaneous bacterial peritonitis.

[a]Overall Model Fit, significance level, $p < .0001$; Nagelkerke $R^2 = .35$.

[b]Overall Model Fit, significance level, $p < .0001$; Nagelkerke $R^2 = .91$.

data highlight the functional role of ADM as an innate immune mediator in the peritoneal fluid, particularly in patients with cirrhosis with an increased bacterial load in the ascitic fluid.[48,49]

Our study demonstrated that patients with severe portal hypertension linked to multifocal hepatocellular carcinoma or portal vein thrombosis have high MR-pro-ADM levels, potentially reflecting inflammatory changes in the peritoneum. However, these patients had lower SBP scores compared to those with ascitic fluid infections. This result underscores the importance of a multimodal approach to diagnose ascitic fluid infection in patients with cirrhosis, particularly by combining MR-pro-ADM, the absolute PMN count and the Child–Pugh score within the SBP score, which exhibits high diagnostic accuracy for SBP.

Our study has several strengths. First, the SBP scoring model successfully challenged the current diagnostic method for SBP, with an AUROC of 0.854. Second, we used data from a standardized prospectively maintained database and electronic health records, thus reducing the risk of bias. We also employed strict criteria for BC-SBP diagnosis, including review by senior microbiologists and subsequent pathogen identification by mass spectrometry or 16S rRNA gene sequencing. Third, MR-pro-ADM is a rapid-assay biomarker that can be measured using sensitive, automated KRYPTOR™ assays with an incubation time of 29 min. Nevertheless, our study has several limitations. First, this was a single-centre study, and the limited size of the validation cohort, comprising only 85 patients, highlights the need for further validation of the SBP score in an independent cohort. Second, despite clearly defined risks, patient selection requirements, and outcome criteria with objective and validated methods, the study is retrospective. Retrospective studies are valuable for evaluating new potential biomarkers and serve as a preliminary step in hypothesis generation.[50] In this study, the retrospective design allowed the inclusion of diverse patient subgroups and facilitated the identification of biomarker limitations that may not be evident in prospective studies with pre-defined inclusion criteria. To further enhance methodological rigour, we mitigated selection bias by implementing strict standards for patient identification through expert chart review. Moreover, misclassification bias was minimized by employing a robust and standardized bacteriological approach for diagnosing BC-SBP. Third, in the present study, we focused on two biomarkers in ascitic fluid, namely procalcitonin and MR-pro-ADM, recognizing that other biomarkers, including C-reactive protein and interleukin-6, have been evaluated in previous studies with inconsistent results.[51,52] Notably, the biomarkers assessed here are innate immune markers belonging to the CALC peptide family, which have been extensively studied in the context of septic conditions.[13,15–19]

The integration of the SBP score into routine clinical practice may offer significant potential to improve the diagnosis and management of SBP in patients with cirrhosis.

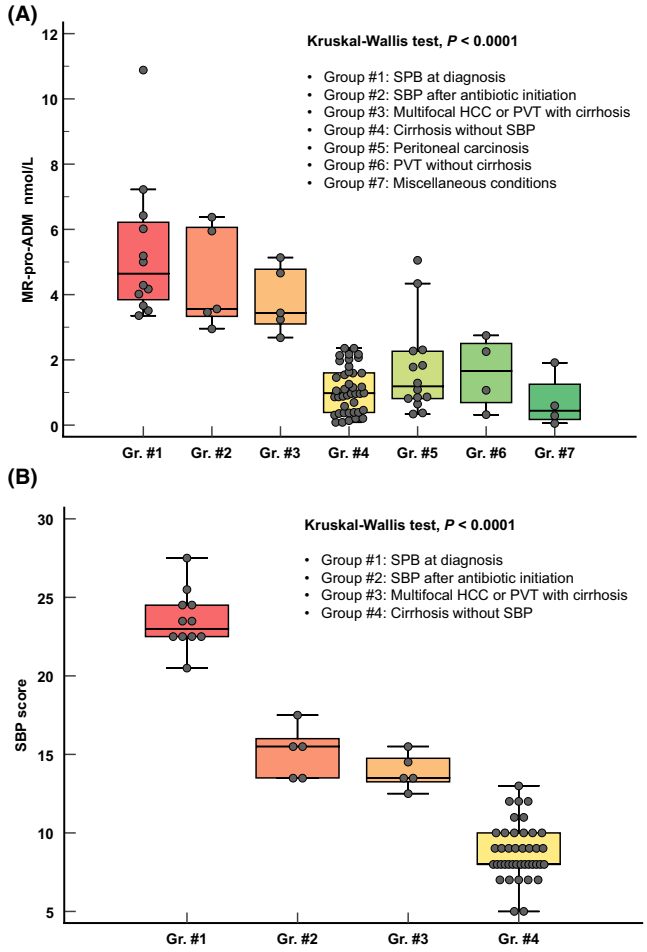

FIGURE 2 (A) Distribution of MR-pro-ADM in the ascitic fluid of patients included in the validation study. The seven patient subgroups were identified as follows: Group #1: Spontaneous bacterial peritonitis (SBP) at diagnosis, Group #2: SBP after antibiotic initiation, Group #3: Multifocal hepatocellular carcinoma (HCC) with severe hypertension or portal vein thrombosis (PVT) with cirrhosis, Group #4: 'Cirrhosis without SBP', Group #5: Peritoneal carcinosis, Group #6: PVT without cirrhosis and Group #7: Miscellaneous conditions. (B) Distribution of the SBP score in cirrhotic patients included in the validation study (Groups #1–#4).

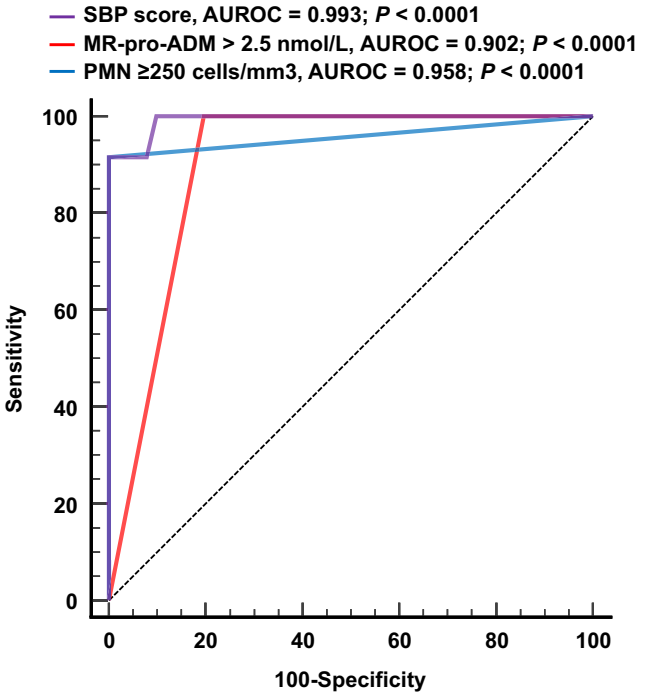

FIGURE 3 Receiver-operating characteristic curves for the diagnostic accuracy of the spontaneous bacterial peritonitis (SBP) score (purple line), mid-regional pro-adrenomedullin (MR-pro-ADM) ≥2.5 nmol/L (red line) and absolute PM neutrophils count ≥250 cells/mm^3 (blue line) for the diagnosis of SBP in the validation study. For each item, the area under the ROC curve is reported with the associated *p*-value.

By incorporating markers of liver dysfunction, such as components of the Child–Pugh score, alongside MR-pro-ADM, which reflects innate immune function, the score provides a nuanced assessment of the underlying pathophysiology. This approach enhances diagnostic sensitivity, particularly in patients with early-stage infection and PMN cell counts below 250/mm,^{3} who might otherwise be overlooked. The inclusion of hepatic dysfunction parameters aligns with emerging evidence, highlighting the role of Child–Pugh components as independent risk factors for SBP.[53] From a clinical management perspective, early and accurate diagnosis facilitated by the SBP score could enable timely initiation of appropriate therapy, improving patient prognosis and potentially reducing hospital length of stay. Conversely, its strong negative predictive value minimizes the risk of overdiagnosis and unnecessary treatment in patients with conditions such as hemorrhagic ascites contaminated by PMNs. This contributes to more effective antibiotic stewardship by limiting exposure to antibiotics in cases where SBP is unlikely, thereby mitigating risks associated with antimicrobial resistance and adverse drug effects. Future prospective studies should assess the impact of implementing the SBP score on clinical outcomes, including mortality, and investigate its potential integration with automated laboratory systems to enhance diagnostic efficiency.

In conclusion, our findings urge the reappraisal of the use of the absolute PMN count as the sole measure for diagnosing SBP in patients with cirrhosis. MR-pro-ADM represents a promising, rapid assay sepsis-related biomarker that enables personalized clinical decision-making in patients with cirrhosis with suspected SBP. Future prospective studies should assess the utility of the SBP score in the diagnostic algorithm for SBP among patients with cirrhosis.

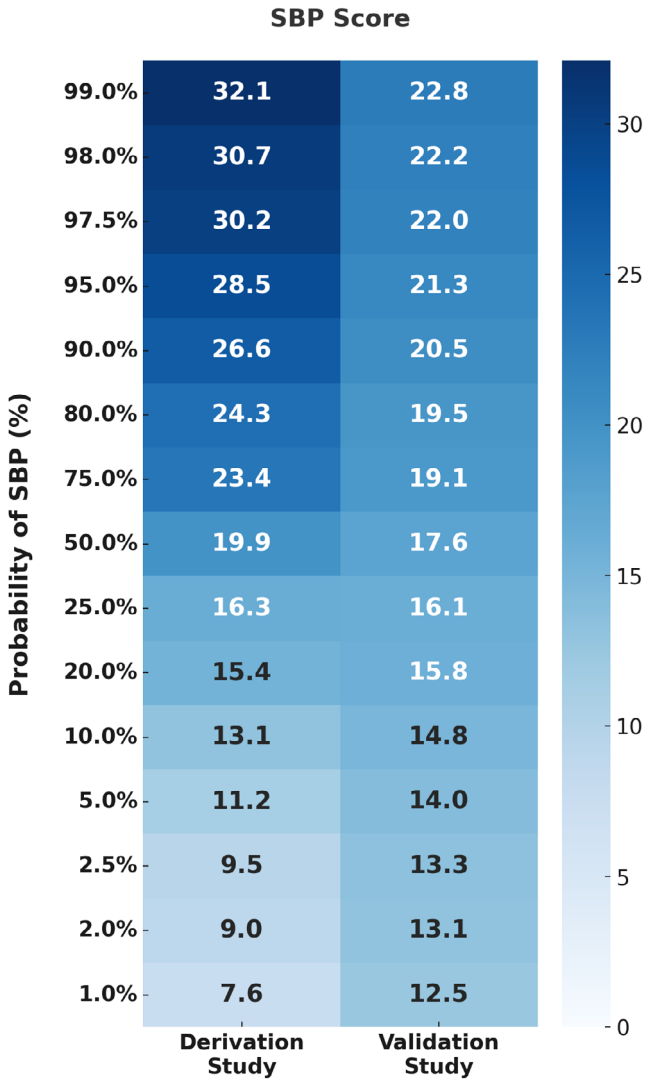

FIGURE 4 Nomogram of spontaneous bacterial peritonitis (SBP) scores across probability of SBP (%) from the derivation and validation studies. The heatmap represents SBP scores obtained from derivation and validation studies across increasing probabilities of SBP (%). Scores are depicted in a gradient of blue colours, with darker shades indicating higher SBP scores. The *Y*-axis denotes the probability of SBP, ranging from 1.0% to 99.0%. The two columns represent data from the derivation study (left) and validation study (right), separated by clear visual boundaries.

AUTHOR CONTRIBUTIONS

A.O.: Conceptualization; Methodology; Software; Validation; Formal analysis; Investigation; Resources; Writing – original draft preparation; Writing – review and editing; Visualization; Supervision; Project administration; Funding acquisition; V.H.: Investigation; Resources; Writing – original draft preparation; M.S.R.: Investigation; Writing – review and editing; A-S.L.: Resources; Writing – review and editing; T.A.: Resources; Writing – review and editing; P.F-T.: Investigation; Resources; Writing – review and editing; J.F.: Investigation; Resources; Writing – review and editing; J.J.: Investigation; Resources; Writing – review and editing; J.B.: Resources; Writing – review and editing; S.S.: Resources; Writing – review and editing; A.L.: Investigation; Methodology; Software; Formal analysis; Writing – review and editing; C.B.: Investigation; Methodology; Software; Formal analysis; Writing – review and editing; P.S.: Investigation; Writing – original draft preparation; Resources; Writing – review and editing; A.L.: Investigation; Writing – original draft preparation; Resources; Writing – review and editing; K.P.: Investigation; Writing – original draft preparation; Resources; Writing – review and editing; H.P.: Investigation; Writing – original draft preparation; Resources; Writing – review and editing; J-L.G.: Investigation; Writing – original draft preparation; Resources; Validation; Writing – review and editing; J-P.B.: Investigation; Formal analysis; Writing – original draft preparation; Resources; Writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors who have taken part in this study declare that they have no conflicts of interest concerning this manuscript to disclose.

DATA AVAILABILITY STATEMENT

Anonymized patient data are available for collaborative research upon reasonable request. Access to the data will be granted following the review and approval of a research proposal, including a detailed statistical analysis plan, and the completion of a data-sharing agreement. Requests for raw data will be evaluated by the Institutional Review Board (IRB) of the University Hospital of Nancy.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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