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Impact of Serum and Ascitic Fluid Procalcitonin on Diagnosis and Outcome of Patients with Spontaneous Bacterial Peritonitis

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ABSTRACT

Background: Cirrhosis is the main cause of ascites, where it indicates poor prognosis with approximately 50% mortality at 2 years. The risk is increased with other complications including spontaneous bacterial peritonitis (SBP). Procalcitonin (PCT) is a significant marker for sepsis because its level is usually high in septic conditions but remains low in nonspecific inflammatory diseases and viral infections.

Objective: To assess the impact of serum and ascitic fluid procalcitonin on diagnosis and optimization of antibiotic therapy in SBP in cirrhotic patients.

Patients and Methods: 55 patients with liver cirrhosis and ascites, 25 of them suffered from SBP and the other 30 patients free from bacterial infection were enrolled in the study. All patients were subjected to full history, examination and assessment for serum and ascitic fluid procalcitonin.

Results: The median age was 60.4±10.2 years in the SBP and 61.3±10.6 years in non SBP patients. There was no statistically significant difference between non SBP and SBP groups regarding their age, sex and diabetes meelitus (DM) frequency. Ascitic fluid culture was done to the SBP group where 3 cases were sensitive to ciprofloxacin, one case sensitive to amikacin and 1 case sensitive to meropenem and one case sensitive to vancomycin. A statistically significant higher median ascitic fluid and serum PCT was found among SBP than non SBP group. with every increase one unit increases risk of SBP by 1.01 (Odds ratio= 1.01, 95% CI: 1.0 -1.01). The overall percent predicted was 87.3%.

Conclusion: PCT was significantly high in the serum and ascitic fluid of SBP patients with high specificity, sensitivity, positive and negative predictive value. Ascitic fluid analysis with polymorphonuclear leukocyte (PMN) count remains the standard key to diagnose SBP.

Keywords: Ascitic fluid, Spontaneous bacterial peritonitis, Procalcitonin.

INTRODUCTION

Cirrhosis is the main cause of ascites. Ascites development indicates poor prognosis with approximately 50% mortality at 2 years with increased risk of other complications of hepatic disease including spontaneous bacterial peritonitis (SBP), intractable ascites, and hepatorenal syndrome (HRS) [(1, 2)].

Bacterial infection is one of the most common and serious complications in decompensated cirrhotic patients (DCPs) [(3)]. Bacterial infections are considered the important cause of high mortality and morbidity in these cases. SBP is the most common form of infection in DCPs which accounts for 40%–70% of patients. The early diagnosis of infections improves the prognosis of patients. However, it is so difficult to diagnose SBP early in DCPs because the clinical picture and ascitic biochemical characteristics are usually inconsistent. Last guidelines show that positive culture for a pathogen in ascitic fluid is the gold standard for SBP diagnosis. However, ascitic fluid cultures are negative in about 60% of cases with clinical picture suggestive of SBP and increased ascitic fluid polymorph [(4)].

Procalcitonin (PCT) is a 116 amino acids peptide which is the precursor of calcitonin hormone that is secreted by parafollicular cells (C cells) of the thyroid gland, neuroendocrine cells of the lung, liver and intestine [(5)].

PCT is considered as a significant marker for sepsis because its level is usually high in septic conditions but remains low in nonspecific inflammatory diseases and viral infections, so serum PCT has a diagnostic role in SBP and bacterial infections in advanced hepatic disease. Recent studies proved that the level of PCT can reflect hepatocytes damage and that hepatic disease severity can affect the diagnostic value of PCT in bacterial infection [(6)].

PCT is considered as an early biomarker for diagnosis of bacterial infections in advanced hepatic diseases, and is considered as a good indicator when compared to C-reactive protein (CRP), tumor necrosis factor (TNF) alpha or interleukins 6 and 2 [(7)]. As serum PCT levels increase earlier and normalize faster than CRP, it has benefit of earlier diagnosis of disease, as well as better follow up of the progression of the disease. Rapid identification of infection has a major effect on course, management, and outcome of critically ill intensive care unit (ICU) cases [(8)].

The aim of the present study was to assess the impact of serum and ascitic fluid procalcitonin on diagnosis and optimization of antibiotic therapy in SBP in cirrhotic patients.

SUBJECTS AND METHODS

This study was a prospective analytical study, a sample size of 55 cases (which involved 25 diseased subjects with confirmed SBP) reaches 93% power to determine improvement in sensitivity from 0.5 to 0.82 via a two sided binomial test and 99% power to determine improvement in specificity from 0.5 to 0.86 via a two-sided binomial test. The target significance

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level was 0.05. Actual level of significance was reached via the sensitivity test was 0.0433 and reached via the specificity test was 0.0428. Disease prevalence was 0.456.

Therefore, the study included 25 cases with confirmed SBP and 30 patients without SBP. Those with confirmed SBP were followed up.

Patients

This study involved 55 decompensated HCV-related cirrhotic patients aged between 18-80 years, both sexes with first episode of SBP. Patients with renal impairment (serum creatinine level >2 mg/mL), hepato-celluar carcinoma (HCC), ongoing infections other than SBP, previous episode of SBP, secondary bacterial peritonitis or ongoing organ failure were excluded.

Patients were classified into: Group 1: 25 cases with ascitic neutrophils >250/mm^3 and/or positive ascitic fluid culture (SBP group), and **Group 2:** 30 patients with ascitic neutrophil count <250/mm^3 and ascitic fluid culture was negative (sterile group).

Patients were subjected to the following:

Complete history with stress on: Period of hepatic decompensation, smoking, symptoms like (fever, chills, abdominal pain, dyspepsia, nausea, vomiting, dyspnea), associated hematemesis and melena, known to be infected with HCV by pervious investigations, DM, chronic kidney disease (CKD), and autoimmune diseases.

Laboratory Assessment: Complete blood count (CBC), international normalized ratio (INR), serum creatinine, liver function tests, serum potassium and sodium, alpha feto-protein, CRP and serum procalcitonin were done for all patients. Urine analysis was done to exclude infection. Under complete aseptic maneuver paracentesis of 20 ml was performed and used for:

- Ascitic fluid analysis for neutrophil count, glucose, protein and lactate dehydrogenase (LDH).
- Ascitic fluid culture.
- Ascitic fluid procalcitonin.

Procalcitonin (PCT):

Human PCT ELISA (Enzyme-Linked Immunosorbent Assay) kit is an in vitro ELISA used for quantitative measurement of human PCT in serum. The plate had been pre-coated by PCT antibody, PCT had been added and bound with antibodies present on wells and biotinylated human PCT Antibody had been bound and added to PCT, then biotinylated PCT antibody had been bound with streptavidin-HRP after its addition. During the washing step after the incubation unbound, streptavidin-HRP was cleared away. After addition of substrate solution, color developed in proportion to PCT amount. Reaction had been terminated after addition of stop solution and immersion had been detected in 500 nm (Bioassay Technology Laboratory ® Human Procalcitonin ELISA Kit, USA).

Patients received antibiotics (cefotaxime 2 gm/8 hrs) initially and was modified according to culture and sensitivity results. Patients were followed by ascitic fluid analysis for detection of PMN count every 48 hours during the period of admission.

Ethical consent:

An approval of the study was obtained from Mansoura University Academic and Ethical Committee. Every patient signed an informed written consent for acceptance of participation in the study. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Statistical analysis

Data were entered and analyzed using IBM-SPSS software (version 25). Qualitative data were expressed as percentage and frequency. Quantitative data were tested initially for normality by Shapiro-Wilk test with data being normally distributed if $p > 0.05$.

Quantitative data were expressed as mean ± standard deviation (SD) if distributed normally, or median and interquartile range (IQR) if not. The diagnostic performance of a test, or the accuracy of a test to differentiate infected patients from non-infected patients was estimated by Receiver Operating Characteristic (ROC) curve analysis. If p value ≤ 0.05, results were considered as statistically significant for any of the used tests.

RESULTS

Patient sociodemographic and laboratory characteristics:

Sociodemographic data and medical history are shown in table 1.

Table (1): Sociodemographic and medical history of the studied cases

	Total number =55	%
Age/years		
38-58	16	29.1
59-78	39	70.9
Sex		
Male	41	74.5
Female	14	25.5
Presence of DM	20	36.4

There was no statistically significant difference between non SBP and SBP groups regarding their age, sex and DM frequency (Table 2).

Table (2): Sociodemographic and medical history of the studied groups

	Non SBP	SBP	Test of significance
	n=30	n=25	
Age/years	N (%)	N (%)	
38-58	7(23.3)	9(36.0)	χ^2=1.06
59-78	23(76.7)	16(64.0)	p=0.303
Mean age ± SD	61.3±10.6	60.4±10.2	
Sex			
Male	20(66.7)	21(84.0)	χ^2=2.16
Female	10(33.3)	4(16.0)	p=0.142
DM	12(40.0)	8(32.0)	χ^2=0.377
			p=0.539

The aboratory findings of the studied cases are shown in table 3.

Table (3): Laboratory findings of the studied cases

	Total number =55 mean±SD
Serum bilirubin (mg/dl)	2.3±0.31
WBCS (mcL)	7±1.1
AST(U/L)	50.0±8.61
ALT(U/L)	40±6.71
INR	1.5±0.21
Serum Albumin (gm/dl)	2.70±0.41
ESR (mm/hr)	30±7.21
CRP (U/L)	48±11.72
Serum sodium (mEq/L)	130.0±7.76

Culture results:

Figure 1 shows the results of asitic fluid culture done to the SBP group, where 3 cases were sensitive to ciprofloxacin, one case sensitive to amikacin and 1 case sensitive to meropenem and one case sensitive to vancomycin.

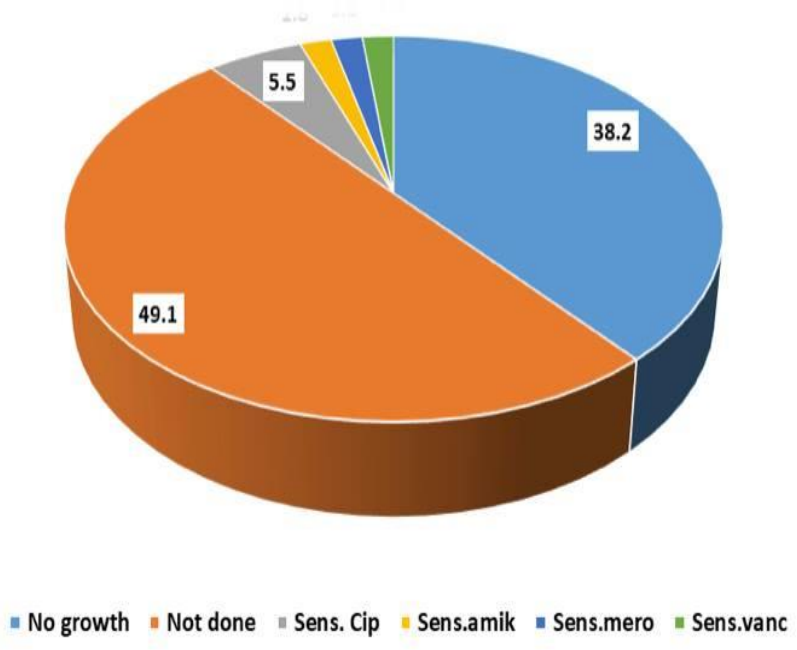

Figure (1): Ascitic fluid culture among studied cases

Serum and ascitic fluid PCT:
Figures 2 and 3 demonstrate a statistically significant higher median ascitic fluid PCT and serum PCT among SBP than non SBP group.

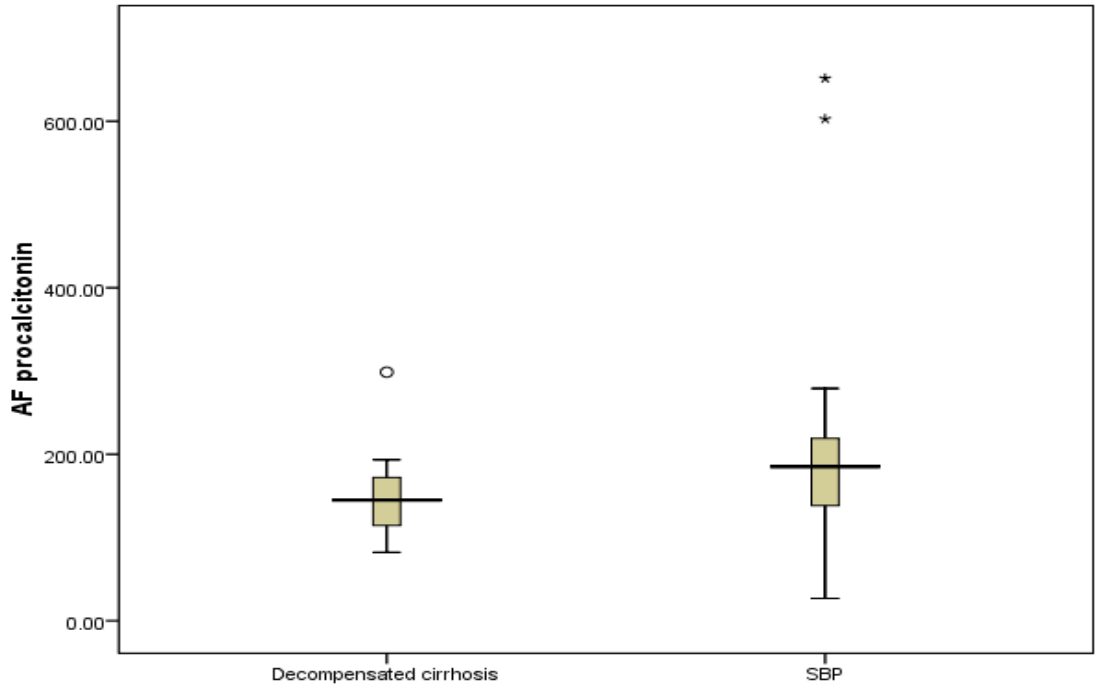

Figure (2): Box and Whisker plot of ascitic fluid PCT between non SBP and SBP group

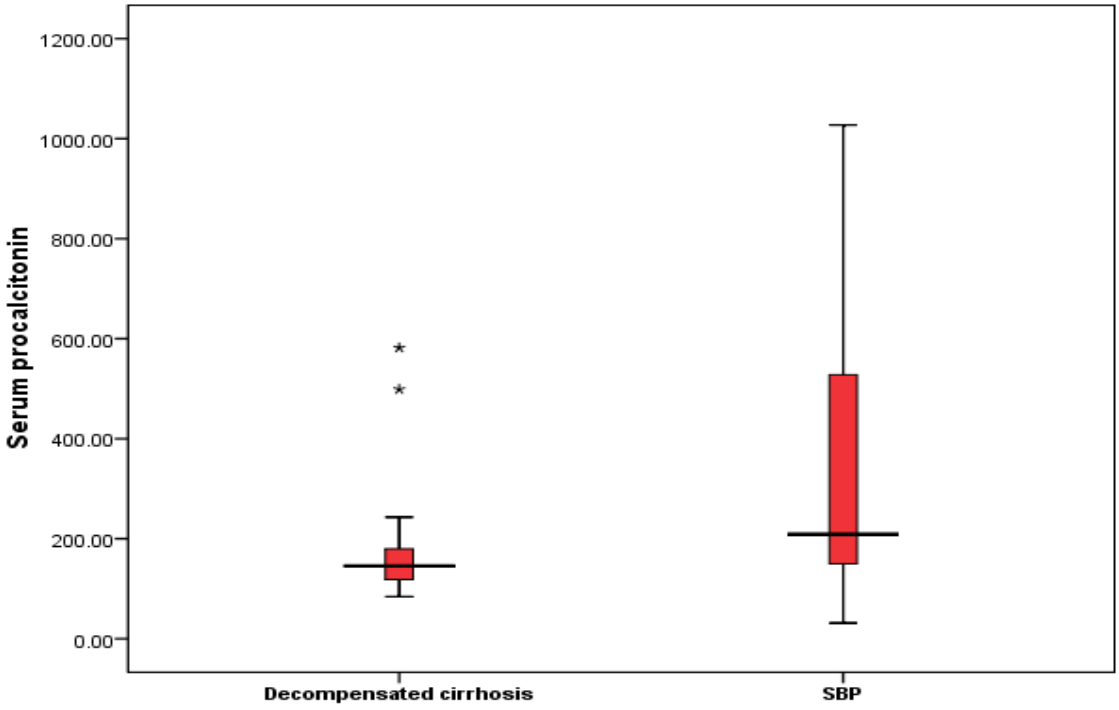

Figure (3): Box and Whisker plot of serum PCT between non SBP and SBP group

Table (4) illustrates that area under curve for ascitic fluid PCT and serum PCT was good (with the best detected cut off point from the curve is 150.16 yielding accuracy of 66.04 and 67.31, respectively in differentiating SBP group from non SBP group. Table (5) illustrates that serum PCT was statistically significant predictor of SBP among studied cases with every increase one unit increases risk of SBP by 1.01. The overall percent predicted was 87.3%.

Table (4): Validity of serum and ascitic fluid PCT in predicting SBP among studied cases

	AUC (95% CI)	P Value	Cut off point	Sensitivity	Specificity	PPV	NPV	Accuracy
Ascitic fluid PCT	0.702 (0.545-0.859)	0.015*	150.16	75	58.6	60.0	73.9	66.04
Serum PCT	0.700 (0.550-0.851)	0.015*	145.72	83.3	53.6	60.6	78.9	67.31

AUC: Area Under curve, PPV: Positive predictive value, NPV: Negative predictive value

Table (5): Binary logistic regression for prediction of SBP

Predictors	B	P-value	Odds ratio (95% CI)
Ascitic fluid PCT	0.008	0.093	1.01(0.99-1.02)
Serum PCT	0.004	0.026*	1.01(1.0-1.01)
Bilirubin	0.199	0.317	1.22(0.826-1.80)
WBCS	0.338	0.009	1.40(1.09-1.81)
AST	-0.008	0.382	0.992(0.974-1.0)
INR	0.005	0.726	1.01(0.976-1.04
Overall % predicted=87.3% Modelχ^2=30.94 P<0.001			

DISCUSSION

This study is a prospective case-control that involved 55 patients with liver cirrhosis and ascites, 25 of them with SBP and the other 30 patients were free from bacterial infection and considered as a control group. In our study we found that there was no statistically significant difference between non SBP and SBP groups regarding their age, sex and DM frequency, 70.9% of the studied cases aged from 59 to 78 years (median age is 60.4±10.2 in SBP group and 61.3±10.6 in non SBP group), 74.5% were males (21 cases with confirmed SBP while 20 cases with sterile ascites) and 36.4% had DM (12 diabetic patients in SBP group and 8 diabetic patients in non SBP group). **Abdel-razik *et al.*** [9] found that median age in non SBP cases was 57.5±10.7 while median age in SBP cases was 58.4±10.2. Moreover 38 cases with SBP were males and 21 cases with sterile ascites were males in this study and this is in agreement with our study. Higher median age in these studies in addition to our study can be explained may be due to the fact that all these studies were done in decompensated cirrhosis and fibrosis usually takes long time to be happened after the primary disease.

In our study, we found positive culture in 6 cases, 3 cases were sensitive to ciprofloxacin (organism was Escherichia coli), one case sensitive to amikacin (organism was Streptococcus pneumonia) and 1 case sensitive to meropenem (organism was Escherichia coli) and one case sensitive to vancomycin (organism is Staph aureus). In a study by **Zeni *et al.*** [10], positive ascitic fluid cultures were detected in 16 patients; microorganisms isolated were Escherichia coli in 9 patients, Streptococcus pneumoniae in 4 patients, Listeria monocytogenes in two patients and Bacteroides fragilis in one patient. Other SBP patients had negative bacteriological culture of ascitic fluid. Our expalanation for this difference is methodology and techniques of cultures in addition to the community acquired infections.

We demonstrated a statistically significant higher bilirubin, WBCS, AST and INR among SBP than non SBP group. This is in contrast to **Zeni *et al.*** [10] who showed low AST among SBP than sterile ascites. **Abdel-razik *et al.*** [9] showed that there was no statistically significant difference in AST and serum bilirubin. This difference may be due to the cause of cirrhosis. In our study all patients were post-HCV, but in **Zeni *et al.*** [10] study, 55 patients were alcoholics and 2 patients were post hepatitic, which may influence liver enzymes or bilirubin level. In **Abdel-razik *et al.*** [9] study, 56 patients were post HCV, 13 patients were post HBV, 6 patients were due to autoimmune hepatitis, 4 patients were due to nonalcoholic steatohepatitis (NASH).

Our results demonstrate a statistically significant higher median ascitic fluid procalcitonin,

serum procalcitonin among SBP group than non SBP group; (185.07 Versus 145.07 for ascitic fluid procalcitonin) and (208.94 versus 145.54 for serum procalcitonin), cut off point for ascitic and serum procalcitonin were 150.16 and 145.72 yielding accuracy of 66.04 and 67.31 respectively in differentiating SBP from non SBP group. This is in agreement with **Estakhri *et al.*** [11] study, which included 120 cirrhotic patients and 59.16% had confirmed SBP with sensitivity of 90.91% and specificity of 91.5%, so serum PCT is considered a less invasive method when compared to ascitic fluid analysis in SBP diagnosis.

In our study we found that area under curve for ascitic fluid PCT and serum PCT was good (AUC =0.702 and 0.700) with the best detected cut off point from the curve was 150.16 yielding accuracy of 66.04 and 67.31, respectively in differentiating cases with SBP from non SBP group. Also **Zeni *et al.*** [10] showed that the best marker for the diagnosis of SBP was serum levels of PCT. A cut off value of 0.76 ng/ml of serum PCT gives a sensitivity and a specificity of 95% and 98%, respectively. By contrast, serum CRP and PMN count had a low sensitivity of 62 and 57%, respectively and this agrees with our study.

Our study is in agreement with **Abdel-razik *et al.*** [9] who showed that there was a statistically significant increase in serum PCT in SBP versus non SBP cases, serum PCT had a diagnostic specificity 91.8% and sensitivity of 94.3% for SBP detection (AUC=0.941 with positive predictive values [PPV, 95%] and negative predictive values [NPV, 93%]).

In contrast to our results, **Lesińska *et al.*** [12] showed that the serum and ascitic PCT levels did not differentiate between cases with and without SBP with very low sensitivities of 20% and 30%, respectively. The majority of serum PCT levels were below 0.5 ng/ml in these cases with sterile ascites, but serum PCT level was higher than 1 ng/ml in only 3 of 10 patients with SBP.

Lesińska *et al.* [12] explained that significantly elevated serum PCT levels in other studies is due to recruitment of patients with severe SBP, presenting clinical signs of sepsis and hospitalized in emergency units. It is known that highly elevated levels of PCT happen in diseases of infectious etiology accompanied by systemic signs. In decompensated patients, clinical signs of systemic inflammatory response are often lacking even in life-threatening infections. This finding proves that PCT has very large area of overlapping results and low sensitivity for diagnosis of SBP.

Our study results are in agreement with **Barutcu *et al.*** [13] who enrolled 50 patients with cirrhotic ascites who were divided into three groups: Group A: 20 patients with SBP diagnosed laboratoraly. Group B: 20 patients with criteria suggestive of SBP, but shows neutrophil count in ascitic fluid <250. Only patients with positive culture were included. Group C: 10 patients with ascites, but no evidence of SBP as the control and the results showed that ascitic fluid PCT level was significantly higher in both groups A and B than group C.

As regards group A VS group B+ group C: The cut off point was 520 (pg). Its sensitivity was 95%. Its specificity was 53.3%. The AUC was 0.633. The positive predictive value was 57.6%. The negative predictive value was 94.1%. Regarding group B VS group C: The cut off point was 300 (pg). Its sensitivity was 85%. Its specificity was 70%. The AUC was 0.823. The positive predictive value was 85%. The negative predictive value was 70%, so from this study we conclude that ascitic fluid PCT showed a high specificity and sensitivity in SBP diagnosis. PCT is so valuable in SBP diagnosis and prognosis.

Wu *et al.* [14] found that PCT showed a higher AUC in ROC curve for prediction of SBP. Also, their results showed that ascitic fluid WBC was correlated with level of PCT, so levels of serum PCT with ascitic fluid WBC should be used for early SBP diagnosis.

CONCLUSION

From our results, we concluded that PCT was significantly high in serum and ascitic fluid of SBP patients and showed a high specificity, sensitivity, positive and negative predictive value. Ascitic fluid analysis with PMN count remains the standard key to diagnose SBP.

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Author contribution: Authors contributed equally in the study.

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