HEPATOLOGY
Official Journal of the American Association for the Study of Liver Diseases

Serum B-Type Natriuretic Peptide in the Initial Workup of Patients With New Onset Ascites: A Diagnostic Accuracy Study

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Heart failure (HF) is, after cirrhosis, the second-most common cause of ascites. Serum B-type natriuretic peptide (BNP) plays an important role in the diagnosis of HF. Therefore, we hypothesized that BNP would be useful in the differential diagnosis of ascites. Consecutive patients with new onset ascites were prospectively enrolled in this cross-sectional study. All patients had measurements of serum-ascites albumin gradient (SAAG), total protein concentration in ascitic fluid, serum, and ascites BNP. We enrolled 218 consecutive patients with ascites resulting from HF (n = 44), cirrhosis (n = 162), peritoneal disease (n = 10), and constrictive pericarditis (n = 2). Compared to SAAG and/or total protein concentration in ascites, the test that best discriminated HF-related ascites from other causes of ascites was serum BNP. A cutoff of >364 pg/mL (sensitivity 98%, specificity 99%, and diagnostic accuracy 99%) had the highest positive likelihood ratio (168.1); that is, it was the best to rule in HF-related ascites. Conversely, a cutoff ≤182 pg/mL had the lowest negative likelihood ratio (0.0) and was the best to rule out HF-related ascites. These findings were confirmed in a 60-patient validation cohort. *Conclusions*: Serum BNP is more accurate than ascites analyses in the diagnosis of HF-related ascites. The workup of patients with new onset ascites could be streamlined by obtaining serum BNP as an initial test and could forego the need for diagnostic paracentesis, particularly in cases where the cause of ascites is uncertain and/or could be the result of HF. (HEPATOLOGY 2014;59:1043-1051)

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Ascites secondary to heart failure (HF) is, after cirrhosis, the second-most common cause of ascites.[1] The pathophysiology of ascites in both HF and cirrhosis is hepatic sinusoidal hypertension, and therefore the serum-ascites albumin gradient (SAAG) is greater than ≥1.1 g/dL in both conditions.[2] Because the hepatic sinusoids are normal (leaky, i.e., without significant collagen deposition in the space of Disse) in HF and are abnormal in cirrhosis (less leaky as a result of capillarization of sinusoids),[3] ascites total protein content is higher in HF-related ascites than in cirrhotic ascites and has been used to help in the differential diagnosis between these two entities, with a ascites protein level of >2.5 mg/dL suggesting the presence of ascites related to HF. However, a significant number of cases are still misclassified.[2,4] Even the

Abbreviations: ASE, American Society of Echocardiography; BNP, B-type natriuretic peptide; CLD, chronic liver disease; HF, heart failure; HVPG, hepatic venous pressure gradient; INR, international normalized ratio; IQR, interquartile range; LR, likelihood ratio; NPV, negative predictive value; NT-proBNP, N-terminal proBNP; PH, portal hypertension; PPV, positive predictive value; SAAG, serum-ascites albumin gradient; STARD, Standards for reporting Studies of Diagnostic Accuracy; US, ultrasound.

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Received April 19, 2013; accepted July 16, 2013.

This work was supported by the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP; Sao Paulo Research Foundation; grant no.: 2011/09484-5).

standard of care represented by medical history, physical examination, and echocardiography may overlook the diagnosis of HF, because clinical manifestations may be quite similar to other conditions.[5,6]

B-type natriuretic peptide (BNP) is a biologically active 32-amino-acid peptide resulting from the cleavage of the pro-BNP, which is secreted by heart ventricle myocytes in response to volume expansion and pressure overload.[7,8] BNP testing facilitates the diagnosis of HF in patients with dyspnea beyond clinical, radiographic, and echocardiographic data with a sensitivity of 90%, specificity of 76%, and a negative predictive value (NPV) of 96%.[9-12]

Patients with cirrhosis may have a combination of findings, such as increased cardiac output, attenuated systolic contraction and diastolic relaxation, electrophysiological repolarization abnormalities, and reduced response to beta-1 adrenergic stimulation.[13] Thus, BNP levels may also increase in serum of patients with cirrhosis, correlating with severity of liver disease.[14,15]

Nevertheless, because BNP is increased in several body fluids of patients with heart dysfunction[16-18] and is considered the initial test in the workup of patients with suspected HF,[19] we hypothesized that testing for this marker would be a useful diagnostic tool in the diagnosis of HF-related ascites.

The aim of the study was to compare the diagnostic accuracy of serum BNP and ascites BNP to standard used tests, mainly SAAG ± ascites total protein, in the differential diagnosis among the three main causes of ascites (HF, cirrhosis, and peritoneal disease) in patients with new onset ascites.

Patients and Methods

Study Design and Population. From June 2010 to November 2011, all patients with new onset ascites that were admitted to the University of São Paulo School of Medicine (São Paulo, SP, Brazil) were assessed for eligibility. Inclusion criteria were clinically detectable ascites, age over 18 years, and consent to participate. The only exclusion criterion was a creatinine level >2.5 mg/dL (possible unreliability of BNP testing per manufacturer). All patients underwent a stringent protocol that included clinical history, physical examination, rest echocardiography, and collection of blood and ascitic fluid samples for analysis. The study was conducted according to the principles of the Declaration of Helsinki; the protocol was approved by the institutional ethics board review and registered at www.clinicaltrials.gov (NCT01150916). A written informed consent was obtained from patients previous to enrollment.

Adjudicated Final Diagnosis and Diagnostic Criteria. Two independent staff cardiologists and two staff hepatologists interviewed and examined all patients before diagnostic tests (echocardiography, ultrasound [US], and endoscopy) were performed. They were blinded to the results of ascitic fluid biochemistry and BNP, which was not measured for clinical purposes. To adjudicate the correct final diagnosis of each patient, all necessary clinical records, laboratory tests, and imaging findings, including echocardiography (performed in all patients), were reviewed until an agreement was reached. The consensus among the experts was considered the gold standard for diagnosis. The whole cohort had a mean follow-up of 13.1 ± 10.9 (median, 9.3) months, and patient follow-up helped confirm the final diagnosis in all but 8 patients (see below).

The diagnosis of cirrhosis was established with a liver biopsy or was based on a compatible clinical history, specifically a history of chronic liver disease (CLD), physical exam, and/or laboratory abnormalities and, importantly, the presence of signs of cirrhosis and/or portal hypertension (PH) on imaging studies (nodular liver, splenomegaly, and/or collaterals). HF was diagnosed according to current diagnostic guidelines.[19,20] Patients had to fulfill both Framingham and Boston criteria[21,22] and have a compatible rest echocardiography (systolic right- or left-ventricle dysfunction with ejection fraction below 50%).

SAAG, Protein Concentration in Ascites, and BNP. All samples were sent to the laboratory immediately after venipuncture and paracentesis. SAAG and protein in ascites were assessed using standard methods. BNP measurements were carried out according to manufacturer's instructions (ADVIA Centaur BNP Siemens Inc, San Diego, CA). This test is a fully automated two-site sandwich immunoassay, based on chemiluminescent technology, and standardized with synthetic purified protein preparation of human BNP (amino acids 77-108), within the range of <2.0-5,000 pg/mL. Inter- and intraassay coefficients of variation at

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DOI 10.1002/hep.26643

Potential conflict of interest: Nothing to report.

different concentrations were, respectively, 2.5% and 2.1% at 48.5 pg/mL, 1.5% and 2.0% at 458 pg/mL, and 0.5% and 2.0% at 1,452 pg/mL.

According to the manufacturer, the BNP assay is reliable in the presence of increased values of several biochemical parameters that can be elevated in patients with decompensated cirrhosis. No interference in measurements has been reported with urea values up to 200 mg/dL, creatinine up to 2.5 mg/dL, unconjugated bilirubin up to 25 mg/dL, conjugated bilirubin up to 25 mg/dL, triglycerides up to 800 mg/dL, and cholesterol up to 1,000 mg/dL. For whole-blood testing, a 4-mL sample was collected in an ethylenediaminetetraacetic acid-containing tube. For ascitic fluid testing, a 10-mL sample was collected at the same time and before infusion of albumin or volume overload. Laboratory staff was unaware of both the clinical diagnosis and routine laboratory results. Kits were purchased from the manufacturers, who had no role in the study design, analysis of data, or writing of the manuscript for this article.

Standard Echocardiography. This was performed at rest in all patients, according to the recommendations of the American Society of Echocardiography (ASE).[23] The following parameters were assessed: left-atrial diameter; systolic left-ventricle diameter; diastolic left-ventricle diameter; left ventricular posterior wall; right ventricular diameter; and interventricular septum. Ventricular ejection fraction was estimated from the Simpson's biplane method.[23] We used tricuspid regurgitation velocity to estimate pulmonary artery systolic pressures and assessed tricuspid regurgitation velocity in the parasternal long- and short-axis and apical four-chamber views. Diastolic function was assessed according to the ASE guideline.[24]

All parameters were recorded in three cycles, and the mean of the measurements was taken for analysis. The examiner was blinded for the clinical diagnosis of the patient.

Validation Cohort. A second set of patients with new onset ascites was recruited for the validation cohort. These patients were prospectively enrolled between January and March 2012 from three secondary and tertiary hospitals in Brazil (Federal University of Espirito Santo [Vitória, Espírito Santo, Brazil], Heart Institute [São Paulo University School of Medicine], and Central Institute of São Paulo University School of Medicine), according to the same protocol as well as inclusion and exclusion criteria used for the training cohort. Similar clinical and laboratory baseline data were available for analysis.

Statistical Analysis. Continuous variables were presented as medians, with the interquartile range (IQR), and categorical variables as numbers and percentages. Sensitivity, specificity, diagnostic accuracy, positive predictive values (PPVs), and negative predictive values (NPVs) were calculated with cutoffs defined by choosing the highest likelihood ratio (LR; to rule in cardiac ascites) and the lowest negative LR (to rule out cardiac ascites). For the validation set, a sample size of at least 50 patients was calculated, assuming a sensitivity of 93% and a 95% confidence interval of width $\pm$ 7%. All hypothesis testing was two-tailed, and P values of less than 0.05 were considered statistically significant. Statistical analyses were performed with SPSS statistical software (version 19.0; IBM, Armonk, NY).

Results

Training Cohort. In the study period, 281 consecutive patients with new onset ascites were admitted and considered for participation in the study. Sixty-three patients were excluded, leaving 218 for analysis (Fig. 1). As noted in Fig. 1, in 8 patients, the experts could not adjudicate the cause of ascites; these were patients with evidence of both CLD and heart disease, but a hepatic venous pressure gradient (HVPG) measurement was not performed to confirm the source of ascites.

Of the 218 patients included in the study, the cause of ascites was HF in 44, cirrhosis in 162, peritoneal disease in 10, and constrictive pericarditis in 2. Notably, in 9 of the 44 patients with HF, the diagnosis was not entirely certain because of the presence of a low serum albumin suggestive of the presence of cirrhosis; however, 6-month follow-up of these patients continued to reveal no evidence of cirrhosis, and hypoalbuminemia was attributed to cardiac cachexia.

Two patients with ascites secondary to constrictive pericarditis are described separately because they did not fit in any of our three main categories.

Of 44 patients with HF, etiology was ischemic (n = 11), Chagas disease (n = 10), valvular (n = 9), idiopathic (n = 7), hypertensive (n = 5), alcoholic (n = 1), and peripartum (n = 1). Eleven patients were found to have class IV functional capacity, 21 class III, and 12 class II, according to the New York Heart Association.[25] No patient with HF had esophageal varices or indirect signs of PH on US.

All 162 patients with cirrhosis had clinically significant PH (esophageal varices at endoscopy: n = 119; venous collaterals identified by imaging methods: n = 43). Etiology was hepatitis C (n = 56), alcoholic (n = 48), hepatitis B (n = 6), nonalcoholic steatohepatitis (n = 7), autoimmune hepatitis (n = 5), biliary disease (n = 2), and others (n = 38). None of the patients

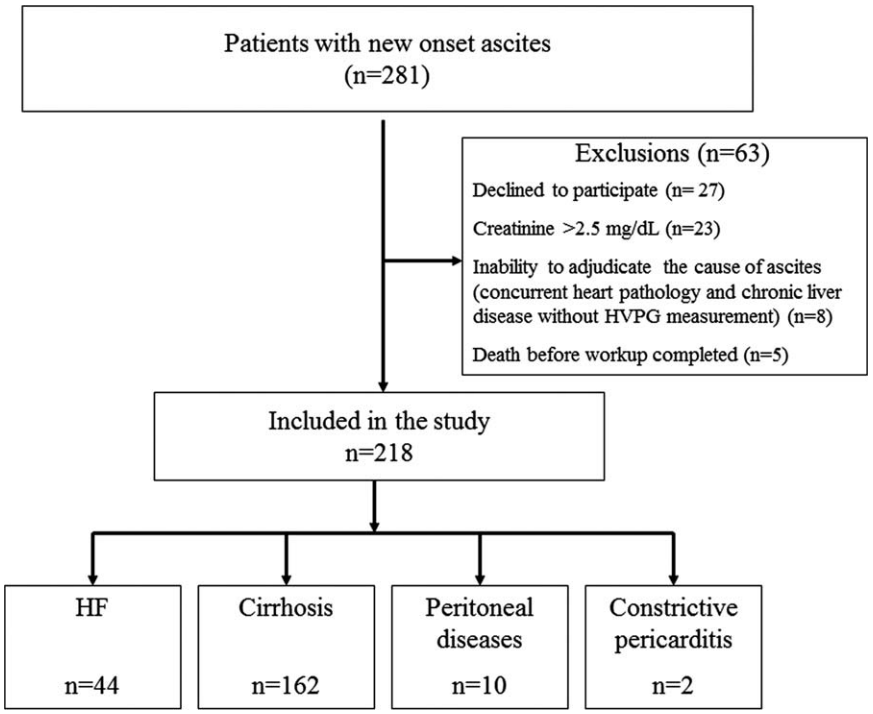

Fig. 1. Enrollment of patients.

had any evidence of heart disease or HF, and 113 were classified Child-Pugh B and 49 Child-Pugh C.

Peritoneal disease was diagnosed in 10 patients (peritoneal tuberculosis: n = 3; carcinomatosis, n = 6; pancreatic ascites: n = 1). No patient in this group was found to have cirrhosis or HF.

Characteristics of patients and laboratory tests at the time of inclusion in the study are shown in Table 1. As expected, arterial hypertension, dyslipidemia, and echocardiographic abnormalities were more common in the HF group, whereas albumin was lower and bilirubin was higher in the group with cirrhosis. Groups were comparable regarding age and glomerular filtration rates, factors that could influence results of BNP measurements. Table 2 shows echocardiography findings in the study groups.

Diagnostic Accuracy of SAAG, Ascites Protein Concentration, Serum BNP, and Ascites BNP in the Training Cohort. Figure 2 shows the individual results of SAAG, ascites total protein, serum BNP, and ascites BNP in the study groups.

A SAAG $\geq$1.1 was present in all 162 patients with cirrhosis, whereas an ascites protein concentration $\leq$2.5 mg/dL was present in 142 of 162 (87.7%) of patients with cirrhosis. Of the 20 patients with an ascites protein concentration >2.5 mg/dL, 18 were Child-Pugh B and only 2 belonged to Child-Pugh C classification. In fact, patients with an ascites protein level >2.5 had a significantly better liver synthetic function than those with an ascites protein level <2.5, as evidenced by a higher serum albumin level (3.5 $\pm$ 0.5 versus 3.0 $\pm$ 0.6 g/dL; P = 0.0003), a lower serum bilirubin level (1.4 $\pm$ 1.5 versus 3.0 $\pm$ 3.6 mg/dL; P = 0.001), and a lower international normalized ratio (INR; 1.3 $\pm$ 0.3 versus 1.4 $\pm$ 0.4; P = 0.0023). In the group of patients with HF, a SAAG level <1.1 was present in 9 of 44 (20%) patients, and this subset of patients characteristically had lower serum albumin values, when compared with patients with HF and a SAAG level >1.1 (2.8 $\pm$ 0.8 versus 3.5 $\pm$ 0.5; P = 0.0031). In the group of patients with peritoneal disease, a SAAG level <1.1 was present in 9 of 10 (90%) of patients and ascites protein concentration >2.5 mg/dL was present in 8 of 10 (80%). The 2 patients with constrictive pericarditis had a SAAG level of 1.2 and 1.1, ascites protein level of 4.3 and 5.8 mg/dL, serum BNP level of 167 and 31 pg/mL, and ascites BNP level of 54 and 38 pg/mL, respectively.

Table 3 shows the frequency of patients in different groups, according to the cutoff of SAAG, protein in ascites, serum BNP, and BNP in ascites. The sensitivity, specificity, diagnostic accuracy, NPV and PPV, and the LR^+ and LR^- are shown in Table 4.

The test that best discriminated HF-related ascites from other causes of ascites was serum BNP levels. Serum BNP at a cutoff of 364 pg/mL had a sensitivity of 98%, specificity of 99%, diagnostic accuracy of 99%, NPV of 99%, and PPV 98%. In fact, only 1 patient with cirrhosis and none of the patients with a peritoneal process as cause for ascites had a BNP level

Table 1. Baseline Characteristics of the Patients and Laboratory Tests

Parameter	HF n = 44	Cirrhosis n = 162	Peritoneal Diseases n = 10
Age, years	55 (49-62)	57 (49-63)	42 (37-66)
Medical history, no. (%)			
Diabetes mellitus	15 (34.1)	52 (33.3)	2 (20.0)
Arterial hypertension	25 (56.8)	37 (26.8)	3 (30.0)
Heavy alcohol intake	7 (15.9)	45 (26.1)	4 (40.0)
Current smoker	14 (31.8)	64 (41.1)	3 (30.0)
Dyslipidemia	12 (27.3)	4 (2.6)	1 (10.0)
Clinical findings			
Heart rate, beats/min	72 (64-84)	80 (68-84)	92 (80-100)
Mean arterial pressure, mmHg	80 (70-85)	86 (80-93)	96 (83-100)
Biochemistry			
Aspartate aminotransferase, IU/L	30 (20-40)	49 (36-76)	26 (18-50)
Alanine aminotransferase, IU/L	28 (20-33)	32 (22-46)	27 (10-39)
Alkaline phosphatase, IU/L	145 (113-193)	129 (96-193)	122 (104-140)
Bilirubin, mg/dL	1.4 (0.9-1.8)	1.7 (1.1-3.1)	0.5 (0.4-0.8)
Serum protein, mg/dL	6.8 (6.4-7.3)	6.9 (6.3-7.4)	6.6 (5.8-7.9)
Albumin, g/dL	3.4 (3.0-3.8)	3.0 (2.6-3.5)	3.1 (2.2-4.1)
INR	1.4 (1.2-1.5)	1.3 (1.2-1.6)	1.0 (1.0-1.2)
Platelets, $\times 10^3$ mm^3	208 (153-250)	109 (72-174)	255 (189-415)
Cholesterol, mg/dL	122 (100-155)	127 (101-151)	181 (125-199)
Creatinine, mg/dL	1.45 (1.09-1.93)	0.97 (0.82-1.33)	0.78 (0.52-0.98)
Glomerular filtration rate, mL/min/1.73 m^2	63 (47-91)	76 (58-107)	89 (54-108)

Continuous variables are expressed as median (IQRs).

>364 pg/mL, and this patient with cirrhosis had a borderline elevation of serum BNP at 384 pg/mL. A cutoff of >364 pg/mL had the highest LR^+ (168.1), that is, it was the best to rule in HF-related ascites. Conversely, a cutoff <182 pg/mL had the lowest LR^- (0.0) and was the best to rule out HF-related ascites. Neither ascites BNP levels nor a combination of SAAG level >1.1 and ascites total protein level >2.5 mg/dL were as effective as serum BNP in establishing the diagnosis of ascites resulting from HF. However, ascites BNP level >229 ng/mL had a higher LR^+ (121.18) than the combination of high SAAG and high ascites protein that is currently used to suggest a diagnosis of cardiac ascites (LR^+, 9.6).

Table 2. Echocardiography Findings of the Patients

Parameter	HF n = 44	Cirrhosis n = 162	Peritoneal Diseases n = 10
Rest echocardiography			
Ejection fraction, %	29 (20-45)	67 (64-70)	66 (62-69)
Left-atrial diameter, mm	50 (48-55)	40 (36-43)	39 (34-43)
Systolic left-ventricle diameter, mm	54 (41-60)	29 (26-32)	26 (22-30)
Diastolic left-ventricle diameter, mm	63 (54-70)	46 (42-50)	41 (37-46)
Right ventricular diameter, mm	34 (22-43)	22 (20-25)	20 (18-27)
Diastolic dysfunction, %			
Grade 0	1 (5.6)	65 (44.8)	4 (50.0)
Grade 1 and 2	5 (27.8)	80 (55.2)	4 (50.0)
Grade 3 and 4	12 (66.7)	0 (0.0)	0 (0.0)
Pulmonary artery systolic pressure, mmHg	49 (37-60)	30 (27-34)	31 (31-51)

Continuous variables are expressed as median (IQRs).

Complications. Minor complications of paracentesis were observed in 8 (3.7%) of the 218 patients (ascitic fluid leakage: n = 7; abdominal wall hematoma: n = 1). No hospitalization was required for treatment. No death related to the procedure occurred.

Validation Cohort. Sixty consecutive patients with new onset ascites constituted the validation cohort. Of these, HF was the cause of ascites in 15 and cirrhosis the cause in 45. None of the patients with cirrhosis had a BNP >364, and none of the patients with HF had a BNP <182 (Table 3). This yields a sensitivity and specificity of 100% (Table 5).

These results confirm the findings of the training cohort, in that serum BNP is the best test that discriminates HF as a cause of ascites from other causes of ascites and also confirms that the cutoff of 362 pg/mL is the best to rule in ascites secondary to HF and that <182 pg/mL rules out HF-related ascites.

Discussion

Ascites secondary to HF is the second-most common cause of ascites in the Western world. Making the differential diagnosis among the three-most common causes of ascites (i.e., HF, cirrhosis, and peritoneal processes) is important because the diagnostic workup and management will be different depending on the probable diagnosis.

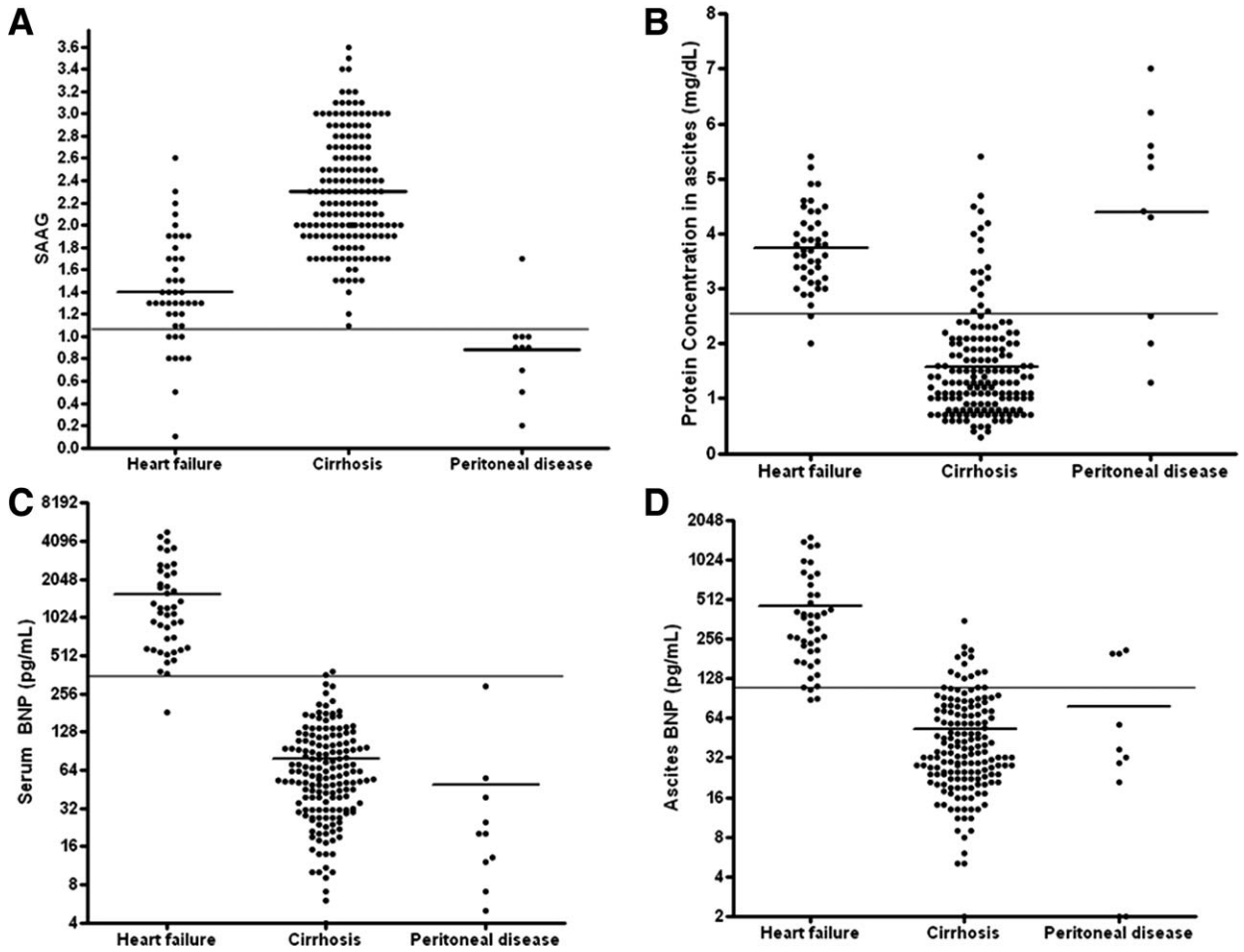

Fig. 2. SAAG (A), protein concentration in ascites fluid (B), serum BNP (C), and BNP in ascitic fluid (D), according to the cause of ascites in the training cohort. Note that the scale for serum and ascites BNP is a semilogarithmic scale.

The diagnosis of HF is initially based on clinical findings (history, physical exam, and routine tests), but is often difficult to establish with certainty. Although current guidelines for HF[20,21] indicate that the diagnosis should be made with a combination of typical signs and symptoms, this approach is limited by low accuracy rates (25%-50%),[7,26,27] when compared to echocardiography results. BNP testing is largely used in the primary practice setting, avoiding unnecessary investigations of HF. A systematic review[28] examined the results of 20 studies of BNP testing for the diagnosis of clinically defined HF and showed a consistently high sensitivity, but a variable specificity, for the diagnosis of HF, concluding that an elevated BNP does not confirm the diagnosis of clinically defined HF, but normal levels rule out the diagnosis.

Table 3. Frequency of Patients in Different Groups in the Training and Validation Cohorts*

	Training Cohort			Validation Cohort	
Parameter	**HF n = 44**	**Cirrhosis n = 162**	**Peritoneal Disease n = 10**	**HF n = 15**	**Cirrhosis n = 45**
SAAG ≥1.1/protein ≥2.5 mg/dL† (%)	35 (79.5)	20 (12.3)	0	8 (53.3)	6 (13.3)
SAAG ≥1.1/protein <2.5 mg/dL† (%)	0	142 (87.7)	1 (10)	2 (13.3)	39 (86.7)
SAAG <1.1/protein <2.5 mg/dL† (%)	0	0	1 (10)	0	0
SAAG <1.1/protein ≥2.5 mg/dL† (%)	9 (20.5)	0	8 (80)	5 (33.3)	0
Ascites BNP >229 pg/mL‡ (%)	31 (70.5)	1 (0.6)	0	13 (86.7)	1 (2.2)
Serum BNP >364 pg/mL‡ (%)	43 (97.7)	1 (0.6)	0	15 (100)	0
Serum BNP ≤182 pg/mL‡ (%)	1 (2.3)	153 (94.4)	9 (90)	0	41 (91.1)

*According to the cutoff of SAAG, protein in ascites, serum BNP, and BNP in ascites.
†Cutoffs selected based on current practice.
‡Cutoff is a point estimate based on the LR.

Table 4. Diagnostic Performance of Tests for HF-Related Ascites in the Training Cohort

Parameter	Sensitivity	Specificity	Accuracy	NPV	PPV	LR$^-$	LR$^+$
SAAG $\geq$1.1/protein $\geq$2.5 mg/dL*	0.630	0.935	0.855	0.877	0.773	0.396	9.633
SAAG $\geq$1.1/protein $<$2.5 mg/dL*	0.007	0.317	0.101	0.123	0.023	3.128	0.010
SAAG $<$1.1/protein $<$2.5 mg/dL*	0.000	0.786	0.783	0.994	0.000	1.272	0.000
SAAG $<$1.1/protein $\geq$2.5 mg/dL*	0.529	0.816	0.792	0.951	0.205	0.577	2.874
Ascites BNP $>$229 pg/mL†	0.705	0.994	0.935	0.929	0.969	0.297	121.18
Serum BNP $>$364 pg/mL†	0.977	0.994	0.991	0.994	0.977	0.023	168.09
Serum BNP $\leq$182 pg/mL†	1.000	0.936	0.949	1.000	0.800	0.000	15.63

*Cutoffs selected based on current practice.
†Cutoff is a point estimate based on the LR.

This concept has been adopted by the UK National Institute for Health and Clinical Excellence clinical practice guideline that recommends that patients without previous myocardial infarction should undergo measurement of serum BNP as the first step, with subsequent echocardiography, and specialist evaluation is indicated if these levels are elevated.[29]

Ascites findings are very useful to make a differential diagnosis. As mentioned previously, a high ascites protein level in the setting of an elevated SAAG favors a diagnosis of HF. In cirrhosis, although SAAG is also elevated, ascites protein is low, whereas in ascites as a result of local peritoneal processes, the SAAG is low (because there is no sinusoidal hypertension), but the ascites protein is elevated. However, there is a significant number of cases in which results are borderline, and we have to resort to measurements of HVPG to make the final diagnosis. In cirrhotic ascites the HVPG is high, in cardiac ascites the wedged hepatic venous pressure is increased, but the gradient is normal (because of elevated systemic pressures), and in ascites secondary to peritoneal processes both the wedge and the gradient are normal.[30]

Given the diagnostic accuracy of BNP in the diagnosis of HF, its widespread use, and the fact that results can be obtained in 18 minutes,[8-13] the present study sought to determine whether serum and/or ascites BNP is more accurate than the SAAG, the ascites protein, or the SAAG/ascites protein combination in establishing the cause of ascites in patients with new onset ascites.

Our study shows that serum BNP accurately identified HF as the cause of ascites with a higher sensitivity, specificity, and diagnostic accuracy, when compared to the standard methods, SAAG, and/or ascites total protein. These results are strengthened by similar findings in the validation cohort. We could also establish cutoff levels that would reliably rule in the diagnosis of HF-related ascites (serum BNP $>$364 pg/mL) and rule out this diagnosis (serum BNP $\leq$182 pg/mL). Notably, none of the patients in the training cohort fell in the "gray zone" (serum BNP between 183 and 363 pg/mL) and only 4 in the validation cohort did so, but they were all patients with cirrhotic ascites, all with levels below 363 pg/mL (216, 224, 285, and 334, respectively).

Regarding the standard tests, we could confirm that SAAG $\geq$1.1 is present in nearly all patients with cirrhosis, but low SAAG values were present in 20% of patients with HF, particularly in the presence of hypoalbuminemia.

We also confirmed that most patients with HF have an ascites protein concentration $>$2.5 g/dL.[2] However, we also found that 12% of patients with cirrhosis had a high ascites protein, indicating that sinusoids are normal ("leaky"). Because this could indicate that capillarization was not extensive (and therefore cirrhosis was "milder"), we looked at these patients with

Table 5. Diagnostic Performance of Tests for HF-Related Ascites in the Validation Cohort

Parameter	Sensitivity	Specificity	Accuracy	NPV	PPV	LR$^-$	LR$^+$
SAAG $\geq$1.1/protein $\geq$2.5 mg/dL†	0.533	0.867	0.783	0.848	0.571	0.538	4.000
SAAG $\geq$1.1/protein $<$2.5 mg/dL†	0.133	0.133	0.133	0.316	0.049	6.500	0.154
SAAG $<$1.1/protein $<$2.5 mg/dL†	0.000	1.000	0.750	0.750	N/A	1.000	N/A
SAAG $<$1.1/protein $\geq$2.5 mg/dL†	0.333	1.000	0.833	0.818	1.000	0.670	N/A
Ascites BNP $>$229 pg/mL‡	0.867	0.978	0.950	0.957	0.929	0.136	39.000
Serum BNP $>$364 pg/mL‡	1.000	1.000	1.000	1.000	1.000	0.000	N/A
Serum BNP $\leq$182 pg/mL‡	0.000	0.089	0.067	0.211	0.000	0.000	11.250

Abbreviation: N/A, not applicable because specificity is 1.000.
†Cutoffs selected based on current practice.
‡Cutoff is a point estimate based on the LR.

cirrhosis and ascites protein >2.5 g/dL and found that they in fact had a milder liver disease, as evidenced by higher serum albumin, lower serum bilirubin, and lower INR.

When combining SAAG and total ascites protein, we still found that the diagnostic accuracy was not optimal (85%), with 63% sensitivity and 93% specificity.

Ascites BNP, although not as accurate as serum BNP, had a greater diagnostic accuracy than standard tests. In ascites resulting from PH, collagen deposition in the Disse space leads to varying degrees in loss of sinusoidal endothelial cell fenestration and formation of basement membrane. The obliteration of this opening between the sinusoidal lumen and the Disse space is expected to affect the filtration of particles by the liver.[31-33] One could speculate that the explanation for the higher performance of ascites BNP testing may be related to the lower molecular weight of this peptide (4 kDa), when compared with human serum albumin (67 kDa), used in SAAG calculation, which could lead to better diffusion of the former.

Interestingly, both patients with ascites secondary to constrictive pericarditis had SAAG ≥1.1 and very high ascites protein levels (4.3 and 5.8 mg/dL). Notably, both patients had a low serum BNP (31 and 167 pg/mL, respectively), confirming that BNP becomes elevated when there is heart enlargement. In this context, it is important to note that all patients included in the study had new onset ascites and none had received intravascular volume replacement before performing the diagnostic tests. It is conceivable that a patient with cirrhosis who is undergoing vigorous volume replacement (as would occur in an intensive care unit) could have elevated serum BNP.

Data on the usefulness of natriuretic peptides testing in distinguishing ascites resulting from cirrhosis from ascites caused by HF are scanty in the literature. This is the first prospective study that included consecutive patients with ascites and a wide spectrum of disease, fulfilling the Standards for reporting Studies of Diagnostic Accuracy (STARD)[34] initiative requirements. According to STARD, a valid diagnostic study must comply with a list of 25 requirements that includes assembling an appropriate number and spectrum of patients with well-defined clinical diagnosis of the disease entities, application of both the diagnostic (BNP testing) and reference standard (SAAG and protein in ascites) to all of them, blind interpretation of the results from the clinical diagnosis, and validation in a second set of patients. Importantly, the gold standard was a clinical diagnosis established by adjudication by experts and based on well-established clinical parameters.

A previous retrospective small study of banked serum and ascites samples in 58 patients with cirrhosis and 18 with HF showed that, using the cutoff of 1,000 pg/mL, serum N-terminal proBNP (NT-proBNP) had a sensitivity of 100%, specificity of 97%, and PPV of 90%, and that ascites NT-proBNP had a sensitivity of 93.3% and specificity of 97.5% in predicting HF as the cause for ascites.[35] However, the study was retrospective and patients were not consecutive, thereby with the potential of introducing bias.

We chose to measure BNP rather than NT-proBNP, which may be more utilized in some centers, because the latter is more dependent on sex, age, and renal dysfunction[36] and because it is a more expensive test. We excluded patients with serum creatinine levels >2.5 mg/dL because the manufacturer stated that BNP determination is not reliable at these levels. However, recent data show that BNP continues to be the strongest predictor of the presence of HF when holding all other predictors equal, including renal function.[37]

Apart from the typical HF setting, a number of factors, unmet by the patients of the current study, have been associated with higher natriuretic peptide levels. Conditions such as advanced age, sepsis, hyperthyroidism, acute coronary syndromes, acute respiratory distress syndrome, and pulmonary embolism can be associated with a rise in BNP blood levels, even in the absence of concomitant HF.[38] Thus, the findings of this study may not be applicable to all patients with high BNP. However, these clinical scenarios are easily acknowledged and are not associated with ascites, helping to clarify what might otherwise seem to be a discordant result.

In this study, we have shown that, in patients with new onset ascites, serum BNP is more accurate in establishing the diagnosis of HF-related ascites than ascites analysis. The workup of patients with new onset ascites could be streamlined by obtaining serum BNP as an initial test and could potentially forego the need for diagnostic paracentesis, particularly in cases where the cause of ascites is uncertain or when HF-related ascites is suspected. Even if the diagnosis of HF-related ascites is established by serum BNP, a paracentesis would still be indicated if a superimposed infection is suspected or for therapeutic purposes (relief of patient discomfort).

Acknowledgment: The authors thank Rafael Ximenes, M.D., for referring patients for the study.

References

1. Runyon BA. Management of adult patients with ascites due to cirrhosis: an update. HEPATOLOGY 2009;49:2087-2107.
2. Runyon BA, Montano AA, Akriviadis EA, Antillon MR, Irving MA, McHutchison JG. The serum-ascites albumin gradient is superior to the exudate-transudate concept in the differential diagnosis of ascites. Ann Intern Med 1992;117:215-220.
3. Henriksen JH, Horn T, Christoffersen P. The blood-lymph barrier in the liver. A review based on morphological and functional concepts of normal and cirrhotic liver. Liver 1984;4:221-232.
4. Christou L, Economou M, Economou G, Kolettis M, Tsianos EV. Characteristics of ascitic fluid in cardiac ascites. Scand J Gastroenterol 2007;42:1102-1105.
5. Stevenson LW, Perloff JK. The limited reliability of physical signs for estimating hemodynamics in chronic heart failure. JAMA 1989;261: 884-888.
6. Remes J, Miettinen H, Reunanen A, Pyorala K. Validity of clinical diagnosis of heart failure in primary health care. Eur Heart J 1991;12: 315-321.
7. de Lemos JA, McGuirre DK, Drazner MH. B-type natriuretic peptide in cardiovascular disease. Lancet 2003;362:316-322.
8. Suttner SW, Boldt J. Natriuretic peptide system: physiology and clinical utility. Curr Opin Crit Care 2004;10:336-341.
9. Maisel A, Krishnaswamy P, Nowak RM, McCord J, Hollander J, Duc P, et al. Rapid Measurement of B-type natriuretic peptide in the emergency diagnosis of heart failure. N Engl J Med 2002;347:161-167.
10. Maisel AS, McCord J, Nowak RM, Hollander JE, Wu AH, Duc P, et al. Bedside B-type natriuretic peptide in the emergency diagnosis of heart failure with reduced or preserved ejection fraction. J Am Coll Cardiol 2003;41:2010-2017.
11. Maisel A, Mueller C, Adams K, Jr., Anker SD, Aspromonte N, Cleland JGF, et al. State of the art: using natriuretic peptide levels in clinical practice. Eur J Heart Fail 2008;10:824-839.
12. Mueller C, Scholer A, Laule-Kilian K, Martina B, Schindler C, Buser P, et al. Use of B-type natriuretic peptide in the evaluation and management of acute dyspnea. New Engl J Med 2004;350:647-654.
13. Zardi EM, Abbate A, Zardi DM, Dobrina A, Margiotta D, Van Tassell BW, et al. Cirrhotic cardiomyopathy. J Am Coll Cardiol 2010;56:539-549.
14. Yildiz R, Yildirim B, Karincaoglu M, Harputluoglu M, Hilmioglu F. Brain natriuretic peptide and severity of disease in non-alcoholic cirrhotic patients. J Gastroenterol Hepatol 2005;20:1115-1120.
15. Henriksen JH, Gøtze JP, Fuglsang S, Christensen E, Bendtsen F, Møller S. Increased circulating pro-brain natriuretic peptide (proBNP) and brain natriuretic peptide (BNP) in patients with cirrhosis: relation to cardiovascular dysfunction and severity of disease. Gut 2003;52: 1511-1517.
16. Porcel JM, Martínez-Alonso M, Cao G, Bielsa S, Sopena A, Esquerda A. Biomarkers of heart failure in pleural fluid. Chest 2009;136:671-677.
17. Kolditz M, Halank M, Schiemanck CS, Schmeisser A, Höffken G. High diagnostic accuracy of NT-proBNP for cardiac origin of pleural effusions. Eur Respir J 2006;28:144-150.
18. Tomcsányi J, Nagy E, Somlói M, Moldvay J, Bezzegh A, Bózsik B, et al. NT-brain natriuretic peptide levels in pleural fluid distinguish between pleural transudates and exudates. Eur J Heart Fail 2004;6:753-756.
19. Jessup M, Abraham W, Casey DE, Feldman A, Francis GS, Ganiats TG, et al. 2009 focused update: ACCF/AHA guidelines for the diagnosis and management of heart failure in adults: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines: developed in collaboration with the International Society for Heart and Lung Transplantation. Circulation 2009; 119:1977-2016.
20. Dickstein K, Cohen-Solal A, Filippatos G, McMurray JJ, Ponikowski P, Poole-Wilson PA, et al. ESC guidelines for the diagnosis and treatment of acute and chronic heart failure. Eur Heart J 2008;29:2388-2442.
21. McKee PA, Castelli WP, McNamara PM, Kannel WB. The natural history of congestive heart failure. The Framingham Study. N Engl J Med 1971;285:1441-1446.
22. Carlson KJ, Lee DC, Goroll AH, Leahy M, Johnson RA. An analysis of physicians' reasons for prescribing long-term digitalis therapy in outpatients. J Chronic Dis 1985;38:733-739.
23. Lang RM, Bierig M, Devereux RB, Flachskampf FA, Foster E, Pellikka PA, et al. Recommendations for chamber quantification: a report from the American Society of Echocardiography's Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Cardiology. J Am Soc Echocardiogr 2005;18:1440-1463.
24. Nagueh SF, Appleton CP, Gillebert TC, Marino PN, Oh JK, Smiseth OA, et al. Recommendations for the evaluation of left ventricular diastolic function by echocardiography. J Am Soc Echocardiogr 2009;22: 107-212.
25. The Criteria Committee of the New York Heart Association. Nomenclature and Criteria for Diagnosis of Diseases of the Heart and Great Vessels. 9th ed. Boston, MA: Little, Brown & Co; 1994:253-256.
26. Wheeldon NM, MacDonald TM, Flucker CJ, McKendrick AD, McDevitt DG, Struthers AD. Echocardiography in chronic heart failure in the community. Q J Med 1993;86:17-23.
27. Davis RC, Hobbs FD, Kenkre JE, Roalfe AK, Hare R, Lancashire RJ, et al. Prevalence of heart failure in high risk groups: community based epidemiological study. BMJ 2002;325:1156.
28. Mant J, Doust J, Roalfe A, Barton P, Cowie MR, Glasziou P, et al. Systematic review and individual patients data meta-analysis of diagnosis of heart failure, with modeling of implications of different diagnostic strategies in primary care. Health Technol Assess 2009;13:1-207.
29. Mant J, Al-Mohammad A, Swain S, Laramee P. Management of chronic heart failure in adults: synopsis of the National Institute for Health and Clinical Excellence. Ann Intern Med 2011;155:252-259.
30. Garcia-Tsao G. Ascites. In: Boyer TD, Manns MP, Sanyal AJ, eds. Zakim and Boyer's Hepatology. A Textbook of Liver Disease. 6th ed. Philadelphia, PA: Elsevier Saunders; 2012:283-295.
31. Rider H, Meyer zum Büschenfelde KH, Ramadori G. Functional spectrum of sinusoidal endothelial liver cells: filtration, endocytosis, synthetic capacities, and intercellular communication. J Hepatol 1992;15: 237-250.
32. Wisse E, De Zanger RB, Charels K, Van Der Smissen P, McCuskey RS. The liver sieve: considerations concerning the structure and function of endothelial fenestrae, the sinusoidal wall and the space of Disse. HEPATOLOGY 1985;5:683-692.
33. Fraser R, Dobbs BR, Rogers GW. Lipoproteins and the liver sieve: the role of the fenestrated sinusoidal endothelium in lipoprotein metabolism, atherosclerosis, and cirrhosis. HEPATOLOGY 1995;21:863-874.
34. Bossuyt PM, Reitsma JB, Bruns DE, Gatsonis CA, Glasziou PP, Irwig LM, et al. The STARD statement for reporting studies of diagnostic accuracy: explanation and elaboration. Ann Intern Med 2003;138:W1-W12.
35. Sheer TA, Joo E, Runyon BA. Usefulness of serum N-terminal-ProBNP in distinguishing ascites due to cirrhosis from ascites due to heart failure. J Clin Gastroenterol 2010;44:e23-e26.
36. Manzano-Fernández S, Januzzi JL, Boronat-García M, Pastor P, Albaladejo-Otón MD, Garrido IP, et al. Impact of kidney dysfunction on plasma and urinary N-terminal pro-B-type natriuretic peptide in patients with acute heart failure. Congest Heart Fail 2010;16:214-220.
37. McCullough PA, Duc P, Omland T, McCord J, Nowak RM, Hollander JE, et al. B-type natriuretic peptide and renal function in the diagnosis of heart failure: an analysis from the Breathing Not Properly Multinational Study. Am J Kidney Dis 2003;41:571-579.
38. Daniels LB, Maisel AS. Natriuretic peptides. J Am Coll Cardiol 2007; 50:2357-2368.