Utility of gray-scale histogram analysis in the assessment of treatment response in patients with infected cirrhotic ascites

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Abstract

Objective : **To evaluate the utility of B-mode gray-scale histogram analysis in the management of patients with infected cirrhotic ascites**

Methods : **A total of 97 patients (mean(SD) age : 66.8(14.2) years, 50.5% were males) diagnosed with cirrhotic ascites were included in this non-interventional study. Paracentesis for ascitic fluid analysis [culture tests, white blood cell count, albumin and protein levels, serum ascites albumin gradient (SAAG)] and gray-scale histogram analysis for ascites/subcutaneous echogenicity ratio (ASER) were performed at baseline in each patient and on Day 2 and Day 5 of treatment in patients with infected ascites. Receiver operating characteristics (ROC) curve was plotted to determine performance of ASER in identification of antibiotic resistance with calculation of area under curve (AUC) and ideal cut-off value of % change in ASER to detect antibiotic resistance.**

Results : **Treatment was associated with a significant decrease in median (min-max) ASER [from 0.005(0.0002-0.02) at baseline to 0.003(0.0001-0.01) on Day 2 and 0.0005(0.0001-0.009) on Day 5] and ascitic fluid polymorphonuclear leukocyte (PMNL) count [from 600(300-2200) at baseline to 350(50-1250) on Day 2 and 100(50-1100) on Day 5] ($p<0.001$ for each). ROC analysis revealed that less than 38% reduction in ASER [AUC: 0.923, 95% CI (0.797-0.982), $p<0.001$] was a potential marker of antibiotic resistance with a sensitivity of 90.9% and a specificity of 95.0%.**

Conclusions : **In conclusion, our findings emphasize potential utility of gray-scale histogram based quantitative analysis of ascitic fluid echogenicity as an adjunct non-invasive method in the assessment of treatment response and early recognition of treatment failure in patients with infected ascites.** (Acta gastroenterol. belg., **2018,** 81, **509-516**).

Keywords : Cirrhotic ascites ; infected ascites ; spontaneous bacterial peritonitis ; antibiotic resistance ; gray-scale histogram analysis.

Introduction

Compensated cirrhosis is associated with a low annual mortality rate (approximately 1%) however, a drastic increase in mortality rates occurs with decompensation through development of ascites (up to 20% per year) (1-3).

Ascites is the leading complication of cirrhosis that becomes evident in half of patients over 10 years of follow up (4,5). The development of ascites per se is an important landmark and a poor prognostic factor in the clinical course of cirrhosis, while it also increases the risk of spontaneous bacterial peritonitis (SBP) and hepatorenal syndrome (6-9).

Infections, SBP in particular, are considered amongst the most common events precipitating the development of acute decompensation in cirrhosis (3,10). Hence appropriate diagnosis and prompt management of SBP is of critical importance in the management of cirrhotic ascites given the associated risk of mortality, hepatic encephalopathy and hepatorenal syndrome (8,9,11).

Abdominal paracentesis is considered a mandatory approach in all patients with cirrhosis requiring hospital admission to diagnose infected ascites as well as to assess treatment response (8,9,12,13). However, in accordance with invasiveness of the technique, complications such as abdominal hematoma, hemoperitoneum or bowel perforation are possible, while the difficulty of obtaining samples in some patients necessitates the repeating of procedure and thus increases the risk of iatrogenic peritonitis (8,13-15).

Ultrasound is currently used in screening for morphologic evidence of cirrhosis and portal hypertension and detecting small amounts of ascites undetectable by physical examination as well as to guide paracentesis (11,15,16). However, it has a limited role in the management of ascites (14,17,18).

While the echogenicity assessment in the US is based on qualitative terms that encompass a large spectrum of conditions, gray-scale histogram analysis provides quantitative data on the echogenicity of the lesion or fluid enabling differential diagnosis of various pathologies (14-16,18-22).

Given the likelihood of gray-scale histogram analysis to provide more objective and detailed quantitative information about the echogenicity of the ascitic fluid (14,15,18), the present study was designed to investigate utility of ascites/subcutaneous echogenicity ratio (ASER) determined via gray-scale histogram analysis in patients with cirrhotic ascites in terms of identification and management of infected ascites.

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Methods

Study population

Patients of both gender aged ≥18 years admitted to Antalya Training and Research Hospital Gastroenterology Clinic and diagnosed with cirrhotic ascites associated with portal hypertension (serum ascites albumin gradient (SAAG) >1.1) between June 2015 and January 2016 were included in this prospective study. For all the patients included in the study, the cause of ascites was related to cirrhosis (uncomplicated or infected), whereas patients with other types of the ascites such as echogenic ascites due to peritoneal hemorrhage and peritoneal carcinomatosis, ascites with SAAG <1.1 (exudate), chylous ascites and lymphocyte-dominant ascites in smear analysis were excluded. Patients with a concurrent secondary infection and/or malignancy were also excluded from the study population.

Written informed consent was obtained from each subject following a detailed explanation of the objectives and protocol of the study which was conducted in accordance with the ethical principles stated in the "Declaration of Helsinki" and approved by the Clinical Research and Ethics Committee of Antalya Training and Research Hospital (Date of Approval : 19/01/2017 ; Reference number/Protocol No: 2/9)

Study procedures and parameters

All patients were examined for infected ascites. Those with ascitic fluid polymorphonuclear leukocyte (PMNL) count >250/mm^3 were given antibiotic treatment and re-evaluated at 2 and 5 days for treatment response.

Data on patient demographics (age, gender), etiology and severity of cirrhosis (Child-Pugh score) were recorded at study enrolment. Serum analysis for albumin, C-reactive protein (CRP) and alpha-fetoprotein (AFP), ascites fluid analysis for culture tests, PMNL count, albumin and protein levels] and ultrasound examination of the abdomen were performed and SAAG was calculate at baseline in each patient, as well as on Day 2 and Day 5 of treatment in patients with infected ascites.

No response (<%25 reduction in ascitic fluid PMNL count in 2 days) to antibiotic treatment considered as developed antibiotic resistance. Ascites/subcutaneous echogenicity ratio (ASER) was calculated by gray-scale histogram analysis upon US images.

Paracentesis and culture techniques

Bedside diagnostic paracentesis was performed using a sterile method with a 23-G needle attached to a 20-cc syringe. Ascitic fluid was centrifuged in the laboratory for 3 min and analyzed for total protein and total and differential leukocyte counts. A smear was prepared and stained with Giemsa. Peritoneal fluid collected from the patients was cultured using two methods. In the first method, 20 mL of peritoneal fluid was inoculated in aerobic blood culture bottles. These bottles were then placed in an automated BacT/Alert 3D culture system. In the second method, the remaining sample was cultured using conventional culture methods (i.e., inoculation using blood agar, MacConkey agar, and thioglycollate broth). The conventional agar and broth media were incubated at 35°C for up to 3 days before being discarded as negative. (18).

Diagnosis of infected and sterile ascites and antibiotic resistance

The presence of ascitic fluid infection was determined on the basis of PMNL counts and culture positivity in ascitic fluid. Patients with PMNL >250/mm^3 in ascitic fluid were considered to have infected ascites, while those with PMNL count <250/mm^3 in ascitic fluid and with a negative culture were considered to have sterile ascites. Antibiotic resistance was defined via paracentesis as the presence of <25% decrease in pretreatment ascitic fluid PMNL count after 2-day treatment (8,9,12,13).

Ultrasound histogram analysis

All radiological examinations were performed by the same experienced radiologist and correlated by another radiologist who was blinded to the study protocol. US images were obtained from patients in the supine position via US device (Hi-Vision Prerius ; Hitachi medical systems, Tokyo, Japan) with a 7.5 MHz linear-array transducer. All US images were analyzed using gray-scale US and gray-scale histogram methods. Gray-scale US images were analyzed with Image J software (National Institutes of Health, USA). Echogenicity values were measured as 0-256 (0 : black, 256 : white) using histogram analysis in a sampling area (diameter, 5-10 mm). The quantitative measurements of ascitic fluid were made at the subcutaneous and deepest midpoint level. Reference subcutaneous tissue measurements were obtained using US from the hypoechoic fatty tissue

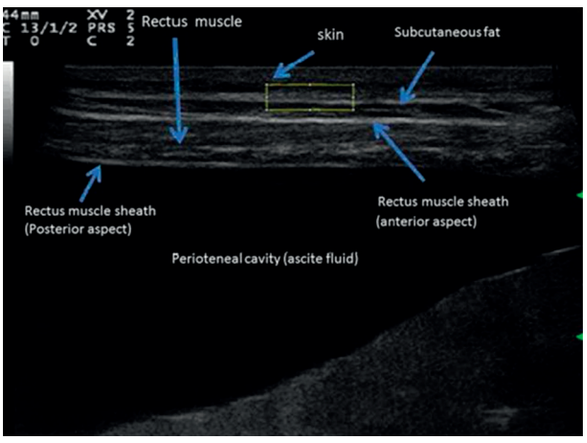


Fig 1. — Ultrasound anatomy of the subcutaneous tissue and contiguous structures measured with gray-scale histogram.

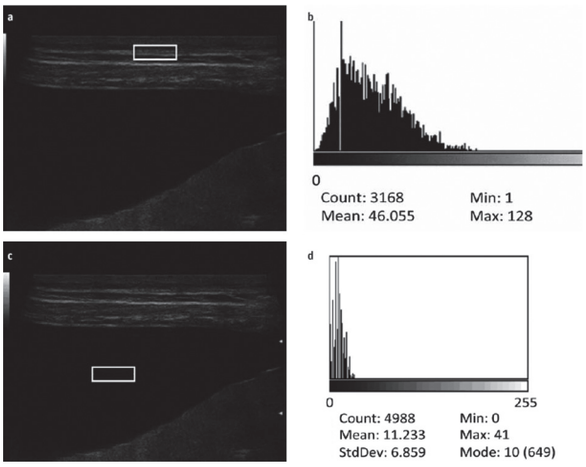


Fig 2. — Measurement performed from the subcutaneous fatty tissue (a), gray-scale histogram values of the subcutaneous fatty tissue (b), measurement performed from ascitic fluid (c), gray-scale histogram values of ascitic fluid (d).

between the hyperechoic skin layer and hyperechoic anterior aspect of the rectus abdominis muscle sheath to minimize the probable disparity of the gray-scale echogenicity values between patients (Fig. 1).

Measurements were repeated three times, and an average of three measurements was estimated. ASER was determined using the Image J software in a single-blinded fashion by the same radiologist and were correlated by a second radiologist. (Fig. 2).

Statistical analysis

Statistical analysis was made using IBM SPSS Statistics for Windows, Version 22.0 (IBM Corp., Armonk, NY). Fisher's exact test and Pearson chi-square analysis performed for categorical variables. The normality assumptions of the analysis of the two-group measurement differences were controlled by the Shapiro-Wilk test. Mann-Whitney U test was used for analysis of non-normally distributed numerical data. Friedman's test was performed to test any significant differences in ASER and PMNL on different time points. Friedman's test was performed to test any significant differences in ASER and PMNL on different time points and Bonferroni-Dunn test was used for post-hoc analysis. Correlations of ASER and ascitic fluid PMNL count with study parameters were analyzed in patients with infected ascites. Spearman correlation test was performed to test relationships in ordinal or quantitative variable with non-normal distribution corrected by multiple testing and $p<0.006$ were considered significant.

Receiver operating characteristics (ROC) analysis was done to compare reduction in ASER with reduction in ascitic fluid PMNL count as the golden standard to diagnose development of antibiotic resistance. ROC curve was plotted to determine performance of ASER in identification of antibiotic resistance in patients with infected ascites with calculation of area under curve (AUC) values to estimate overall accuracy of ASER, while ideal cut-off value of % change in ASER to detect treatment failure was calculated via ROC analysis. ROC curve was plotted to determine performance of ASER with calculation of AUC values to estimate overall accuracy of ASER, while ideal cut-off value of % change in ASER to detect treatment failure was calculated via ROC analysis. P values <0.05 were considered statistically significant.

Results

Baseline characteristics (n=97)

Mean(SD) age of 97 consecutive patients included in the study was 66.8(14.2) years ; and 50.5% were males. HBV infection (33.0%) as followed by HCV infection (22.7%) and alcoholic hepatitis (24.7%) were the most common etiologies underlying cirrhosis. Infected ascites was identified in 42(43.3%) patients and cefotaxime was administered as empirical antibiotic treatment in these patients. Culture positivity was detected in 16(38.1%) patients with infected ascites and Escherichia coli (*E. coli*) was the most commonly isolated pathogen (62.5) (Table 1).

Comparison of sterile versus infected ascites groups

No significant difference was noted between patients with sterile vs. infected ascites in terms of demographic

Table 1. — **Baseline characteristics in the overall study population (n=97)**

Age (year), mean(SD)	66.8(14.2)
Gender, n(%)	
Female	48(49.5)
Male	49(50.5)
Cirrhosis etiology, n(%)	
HBV infection	32(33.0)
HCV infection	22(22.7)
Alcoholic	24(24.7)
Cryptogenic	16(16.5)
Autoimmune	3(3.1)
Type of ascites, n(%)	
Sterile	55(56.7)
Infected	42(43.3)
Spontaneous bacterial peritonitis	16(16.5)
Culture negative neutrocytic ascites	26(26.8)
Culture findings (n=42), n(%)	
Culture positive	16(38.1)
Escherichia Coli	10(62.5)
Staphylococcus aureus	2(12.5)
Klebsiella pneumonia	2(12.5)
Pneumococci	2(12.5)

HBV : Hepatitis B Virus ; HCV : Hepatitis C virus.

Table 2. — **Patient demographics, serum and ascitic fluid parameters sterile versus infected ascites groups**

		Sterile ascites (n=55)	Infected ascites (n=42)	p value[1]
Gender, n(%)	Female	30(54.5)	18(42.9)	0.254[2]
	Male	25(45.5)	24(57.1)	
		Median (min-max)	**Median (min-max)**	
Age (year)		73(44-84)	64.95(36-88)	0.268
Serum analysis				
CRP (mg/L)		45(3-175)	38(4-140)	0.983
Albumin (g/dL)		3.3(2.1-5.0)	3.2(2.2-4.2)	0.430
AFP (ng/mL)		4(1-120)	9.0(2-2200)	**0.037**
Ascitic fluid analysis				
PMNL count (cells/mm^3)		100(50-250)	600(300-2200)	**0.001**
Total protein (gr/dL)		2600(200-2845)	2600(200-3100)	0.562
Albumin (gr/dL)		1.1(0.2-2.7)	1.2(0.3-2.3)	0.209
ASER		0.0007(0.0001-0.002)	0.005(0.0002-0.02)	**0.001**
Child-Pugh score		8(6-12)	9(6-13)	**0.001**
SAAG		2(0.7-3.5)	2(1.3-2.9)	0.086
Culture, n(%)	Negative	55(100.0)	26(61.9)	**0.001**[2]
	Positive	0(0.0)	16(38.1)	

AFP : Alpha-feto protein ; ASER : Ascites/subcutaneous echogenicity ratio ; CRP : C-reactive protein ; SAAG : Serum ascites albumin gradient ; PMNL : Polymorphonuclear leukocyte ; Values in bold indicate statistical significance (p<0.05) ; [1]Mann Whitney U test ; [2]χ^2 test.

characteristics, SAAG, serum levels for CRP and albumin, ascitic fluid protein and albumin content (Table 2).

Infected ascites was associated with significantly higher mean(IQR) levels of serum AFP (9.0(2-2200) vs. 4(1-120), p=0.037) and ASER (0.005(0.0002-0.02) vs. 0.0007(0.0001-0.002), p=0.001) values (Table 2).

Change in ASER and ascitic fluid PMNL count from baseline in patients with infected ascites

A significant decrease was noted in median(min-max) ASER [from 0.005(0.0002-0.02) at baseline to 0.003(0.0001-0.01) on Day 2 and 0.0005(0.0001-0.009) on Day 5] and ascitic fluid PMNL count [from 600(300-2200) at baseline to 350(50-1250) on Day 2 and 100(50-1100) on Day 5] from baseline to Day 2 and Day 5 as well as from Day 2 to Day 5 (p<0.001 for each) (Table 3). Based on Day 2 ascitic fluid PMNL counts, antibiotic resistance was identified in 20(47.6%) of 42 patients with infected ascites.

Table 3. — **Change in ASER and ascitic fluid PMNL count from baseline to Day 2 and Day 5 in patients with infected ascites (n=42)**

	ASER	Ascitic fluid PMNL count
	Median (min-max)	Median (min-max)
Baseline	0.005(0.0002-0.02)	600(300-2200)
Day 2	0.003(0.0001-0.01)[*]	350(50-1250)[*]
Day 5	0.0005(0.0001-0.009)[*,q]	100(50-1100)[*,q]

ASER : Ascites/subcutaneous echogenicity ratio ; PMNL : Polymorphonuclear leukocyte ; [*]p<0.001 compared to baseline levels and [q]p=0.001 compared to Day 2 levels (Friedman's test)

Correlations of ASER and ascitic fluid PMNL levels with study parameters in patients with infected ascites

Significant positive correlations were found between ASER and ascitic fluid PMNL at baseline (r=0.972, p=0.001), on Day 2 (r=0.983, p<0.001) and Day 5 (r=0.932, p<0.001) (Fig. 3, Table 4). ASER was also positively correlated with SAAG (r=0.421, p=0.005) on Day 5 (Table 4).

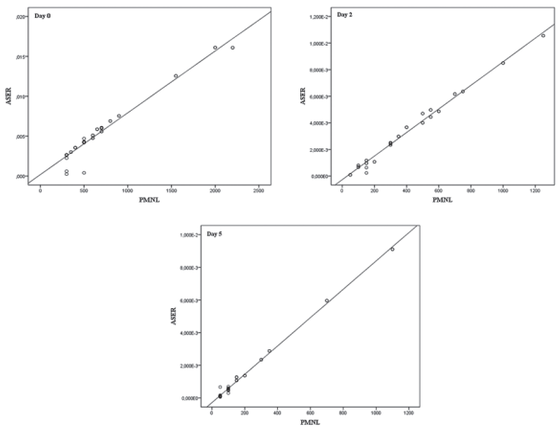


Fig 3. — Day 0, Day 2 and Day 5 correlations between ascites/subcutaneous histogram ratio (ASER) and ascitic fluid PMNL count.

Table 4. — **Correlations of ASER and ascitic fluid PMNL count with study parameters in patients with infected ascites (n=42)**

		ASER			Ascitic fluid PMNL count		
		Baseline	**Day 2**	**Day 5**	**Baseline**	**Day 2**	**Day 5**
PMNL	r	0.972	0.983	0.932	1.000	1.000	1.000
	p	**0.001**	**<0.0001**	**<0.0001**	.	.	.
	N	42	42	42	42	42	42
ASER	r	1.000	1.000	1.000	0.972	0.983	.932
	p	.	.	.	**0.001**	**<0.0001**	**<.0001**
	N	42	42	42	42	42	42
Child Score	r	0.052	-0.206	-0.205	-0.001	-0.252	-0.076
	p	0.745	0.191	0.192	0.997	0.108	0.631
	N	42	42	42	42	42	42
AFP	r	-0.073	0.062	-0.076	-0.054	0.030	-0.256
	p	0.648	0.697	0.630	0.735	0.848	0.101
	N	42	42	42	42	42	42
CRP	r	0.087	0.108	0.248	0.115	0.156	0.163
	p	0.584	0.497	0.114	0.467	0.323	0.303
	N	42	42	42	42	42	42
SAAG	r	-0.003	0.248	0.421	-0.020	0.228	0.416
	p	0.986	0.113	**0.005**	0.901	0.146	**0.006**
	N	42	42	42	42	42	42
Serum albumin	r	0.013	-0.106	-0.029	-0.036	-0.128	-0.122
	p	0.933	0.505	0.857	0.819	0.419	0.442
	N	42	42	42	42	42	42
Ascites albumin	r	-0.160	-0.276	-0.157	-0.206	-.285	-0.266
	p	0.311	0.077	0.322	0.190	0.068	0.088
	N	42	42	42	42	42	42
Ascites total protein	r	0.076	-0.046	-0.394	0.054	-0.123	-0.389
	p	0.632	0.773	0.010	0.732	0.437	0.011
	N	42	42	42	42	42	42

AFP : Alpha-fetoprotein ; ASER : Ascites/subcutaneous echogenicity ratio ; CRP : C-reactive protein ; r : correlation coefficient ; SAAG : Serum ascites albumin gradient ; PMNL : Polymorphonuclear leukocyte ; Values in bold indicate statistical significance (p <0.006; Bonferroni correction) Spearman's correlation analysis.

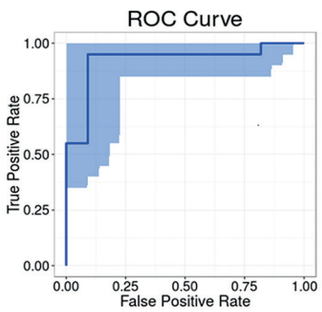
A negative relationship was found between ascites total protein levels and both ASER (r=-0.394) and ascetic fluid PMNL count (r=-0.389) on day 5, but the correlation coefficient could not reach the level of statistical significance after Bonferroni correction.

ROC analysis

ROC analysis revealed that less than 38% reduction in ASER [AUC: 0.923, 95% CI (0.797-0.982), p<0.001] was a potential marker of antibiotic resistance with a sensitivity of 90.9% and a specificity of 95.0% (Fig. 4). Positive predictive value was 95.2 [95% CI (76.2-99.9)] % and negative predictive value was 90.5 [95% CI (69.6-98.8)] %.

Discussion

Our findings in a cohort of patients with cirrhotic ascites revealed that infected ascites was associated with identification of higher ASER in gray-scale histogram analysis when compared to sterile ascites. Consistent



ASER % change*					
AUC	Cut-off value	Sensitivity	95%CI	Specificity	95%CI
0.923. 95% CI (0.797–0.982) (p<0.001)	38.0216	90.91	70.8-98.9	95.00	75.1-99.9
Classification variable : antibotic resistance (<25% reduction in ascitic fluid PMNL count from baseline to Day 2)					

Fig 4. — ROC curve analysis for ascites/subcutaneous histogram ratio (ASER) in identification of antibiotic resistance in patients with infected ascites.

with significant positive correlations between baseline, Day 2 and Day 5 values for ASER and ascitic fluid PMNL count in patients with infected ascites, a significant reduction was noted in ASER from baseline to Day 2 and Day 5 alongside a parallel decline in ascitic fluid PMNL count on the same days. As a result, less than 38% reduction in ASER [AUC: 0.923, 95% CI (0.797-0.982), p<0.001] during follow up of infected ascites was shown to be a potential marker of antibiotic resistance. ASER was also positively correlated with SAAG on Day 5.

Our findings support that SBP cases were predominantly caused by gram-negative bacteria such *E. coli* and *K. pneumoniae* (23,24). However, a change in bacterial profile of SBP has been documented in recent years with an increase in the incidence of gram-positive pathogens (23,25-27). This change has been attributed to antibiotic-mediated alteration in the intestinal flora with translocation of gram-positive bacteria and increased resistance to antibiotics commonly used for the treatment of SBP such as quinolones (23,25-27).

Given the identification of antibiotic resistance in nearly half of our patients with infected ascites, our findings emphasize the critical importance of in detail assessment of pathogen profiles as well as antibiotic resistance of pathogens in the management of SBP among cirrhotic patients to control the emergence and development of pathogenic bacteria-resistant strains (23).

Diagnostic paracentesis is considered mandatory in all patients with cirrhosis requiring hospital admission to identify infected ascites and is repeated after two days of antibiotic treatment to monitor treatment response (8,9,12,13). However, paracentesis technique is not exempt from certain drawbacks such as complications due to invasiveness of the method, likelihood of inadequacy of collected sample for proper analysis and the risk of iatrogenic peritonitis in some patients (8,13-15).

In addition influx, efflux and kinetics of neutrophils in ascitic fluid has not yet been elucidated alongside the potential source of error in the PMNL count due to hemorrhage and entry of leukocytes into the ascitic fluid during a traumatic paracentesis (27-30). Thus, vast array of potential analyses have been addressed in studies searching for alternative methods of SBP diagnosis including detection of leukocyte enzymes by strip tests, identification of bacterial DNA by molecular methods, ascetic fluid lactoferrin levels an inflammation or immune-derived mediators (6,30-33).

While the clinical and diagnostic relevance of these methods is not yet conclusive, more extensive data are available on serum procalcitonin levels indicating its significant contribution to the diagnosis of SBP. Findings from a meta-analysis of 181 SBP episodes showed that serum procalcitonin (cut-offs ranged from 0.58 to 0.75 ng/mL) displayed a high accuracy for diagnosis of SBP, with a pooled AUC value of 0.95 (95% CI, 0.82-0.99), a sensitivity of 0.86 (95% CI, 0.73-0.94), and a specificity of 0.80 (95% CI, 0.72-0.87) (32).

In a previous study conducted by our team, ROC analysis revealed serum procalcitonin levels to be a useful marker in differentiating ascites infections in hospitalized cirrhotic patients with a cut off value >0.5 mg/dL and AUC value of 0.860 (95% CI: 0.884-0.999) (14). In the same study, identification of ASER via gray-scale histogram analysis was found to be a better diagnostic marker than serum procalcitonin in diagnosing infected ascites with cut off value of >0.0019 and AUC value of 0.974 (95% CI: 0.884-0.999) for ASER (14). Similarly, in the present study, ASER values were below 0.002 in patients with sterile ascites, confirming the previously shown diagnostic value of ASER.

The present findings revealed significant reduction in ASER from baseline to Day 2 and Day 5 of antibiotic treatment in patients with infected ascites alongside positive correlations between ASER and ascitic fluid PMNL count on each day of measurement. As a result, less than 38% reduction in ASER [AUC: 0.923, 95% CI (0.797-0.982), p<0.001] during follow up of infected ascites was shown to be a potential marker of antibiotic resistance defined otherwise via paracentesis as <25% decrease in pretreatment ascitic fluid PMNL count after 2-day treatment.

Accordingly, our findings emphasize the role of gray-scale histogram analysis of echogenicity as a potential non-invasive marker not only in the differential diagnosis of infected vs. sterile cirrhotic ascites (14) but also in the monitoring of treatment response in infected ascites. This seems notable given that antibiotic resistance was evident in nearly half of patients with infected ascites in our cohort, supporting the change in antibiotic sensitivity in SBP with an increased frequency of bacteria resistant to multiple antibiotics (34,35).

Gray-scale histogram analysis was shown to be an easily applicable noninvasive quantitative sonographic method with high sensitivity and specificity in differentiating exudative from transudative ascites (15), malignancy in solid breast lesions (36) and malignant microcalcifications in thyroid nodules (37).

Our findings indicate potential utility of gray-scale histogram analysis as an adjunct non-invasive method in early recognition of treatment failure and prompt modification of antibiotic treatment in patients with SBP (8), and an easy to use and rapid alternative with high sensitivity and specificity in conditions challenging the implementation of invasive paracentesis. In fact, the major utility of ASER values seems to be in the therapeutic monitoring stage by avoiding an invasive paracentesis-based re-evaluation on Day 2 of treatment for patients with favorable ASER values indicating a good response to antibiotics.

Gray-scale histogram analysis provides quantitative information about the echogenicity of the lesion or fluid by graphing the number of pixels at each color intensity level and illustrates the quantity, reflexivity and disposition of the pixels in the specific area of interest aiding the differential diagnosis of several pathologies

(14-16,18-22). Thus, it provides more comprehensive echogenicity assessment than routine ultrasonography which assess echogenicity only in qualitative terms such as anechoic or hypoechoic characteristics encompassing a large spectrum of conditions with no objective information on the degree of echogenicity (14-16,18).

Since US imaging is included in the routine ascites management protocol, identification of ASER via gray-scale histogram analysis may offer a readily available and easy to use method leading to improved diagnostic accuracy and therapeutic monitoring in patients with cirrhotic ascites at no additional healthcare cost.

Despite no significant difference was noted between infected and sterile ascites groups in terms of ascitic fluid protein levels and SAAG, ASER was also positively correlated with SAAG on day 5 of antibiotic treatment in patients with infected ascites. A negative relationship was found between ascites total protein levels and both ASER and ascetic fluid PMNL count in accordance with the consideration of low concentration of ascitic fluid proteins amongst the risk factors for infection in patients with cirrhosis (10,38-40) ; however the correlation coefficient could not reach the level of statistical significance.

Subclinical low dose endotoxemia due to circulating bacterial DNA has been associated with a systemic inflammatory response and increased risk of bacterial infections even in stable cirrhotic patients (41-44). Hence, the localized nature of early stage infection in cirrhotic ascites along with typical delayed immune response in the cirrhotic patients may have contributed to the identification of similarly high baseline CRP levels in infected and sterile cirrhotic ascites groups in our cohort. This seems to emphasize the overall imbalance between pro-inflammatory and anti-inflammatory signaling pathways in immune cells in cirrhotic patients (45).

Certain limitations to this study should be considered. First, the cross-sectional design made it impossible to establish any cause and effect relationships. Second the employed gray-scale histogram analysis is subject to impact of changes in fluid and electrolyte balance, acoustic impedance, and sonographic pattern on echogenicity, if not performed by an experienced radiology specialist. However, given the entire dependency of US on the performer with marked inter- and intra-observer variability, consideration of echogenicity in quantitative terms in gray-scale histogram analysis seems to overcome this limitation (21,22). Nonetheless, the area to be measured should be chosen with extreme care, multiple measurements should be made from the areas free from ultrasonographic artifacts, and the final score should be based on the average of these measurements (14). In addition, slight differences in time-gain compensation adjustments can alter echogenicity and histogram values, but obtaining measurements from the same horizontal level can overcome this effect. Optimization of the B-mode US parameters seem also important to provide standard image quality for numeric analysis (15).

In conclusion, our findings indicate association of infected ascites with significantly higher ASER, along with a significant decline in pretreatment ASER during 5-day antibiotic treatment in patients with infected ascites in accordance positive correlation between ASER and ascitic fluid PMNL count. Accordingly, our findings emphasize potential utility of gray-scale histogram based quantitative analysis of ascitic fluid echogenicity an adjunct non-invasive method not only in diagnosing infected ascites but also in the assessment of treatment response and early recognition of treatment failure in patients with infected ascites. Future larger scale studies addressing use of gray-scale histogram analysis in a wider disease spectrum are needed to better define the reference values and to justify feasibility and clinical relevance of using this method as a readily available tool in the routine management of patients with ascites.

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