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Prognostic Value of Ascitic Neutrophil Gelatinase-Associated Lipocalin in Decompensated Liver Cirrhosis with Spontaneous Bacterial Peritonitis Patients

Rashed Mohamed Hassan[1], Ghada Abd Elghafar Salem[1], Bassem Mohamed ALsayed Refaat Mohamed[1], Amal Ahmed Zidan[2], Ahmed M. El-Gebaly[1]

[1]Tropical Medicine, Faculty of Medicine – Zagazig University

[2]Clinical Pathology, Faculty of Medicine, Zagazig University

Corresponding author: Bassem Mohamed ALsayed Refaat Mohamed, **Phone:** 01117587994,

Email: baseem.zamalek2002@gmail.com

ABSTRACT

Background: In cirrhotic individuals, spontaneous bacterial peritonitis (SBP) is a relatively frequent complication. NGAL, or lipocalin linked with neutrophil gelatinase, is a trustworthy indicator of inflammation.

Objective: The aim of this work was to evaluate the prognostic value of ascitic neutrophil gelatinase associated lipocalin in decompensated liver cirrhosis with **SBP** patients.

Patients and Methods: The study comprised 72 patients with cirrhotic liver and ascites, they were categorized into two groups SBP group and non SBP group, each group had 36 patients. Each patient had a thorough history-taking process, physical examination, pelvic-abdominal ultrasonography, laboratory tests, and chemical and bacterial analysis of ascitic fluid samples and specific assay of NGAL levels using ELISA technique.

Results: We found that the optimum cutoff value of NGAL was ≥230.05 ng/ml with (AUC) 0.989, 89.5% positive predictive value, 94.4% specificity, 88.9%, and -94.4% negative predictive value 94.1% and overall accuracy 91.7%. The optimum cutoff of ascitic fluid NGAL in prediction of mortality is ≥401 ng/ml with (AUC) 1, 94.4% overall accuracy, 85.7% of positive predictive value, 100% of negative predictive value, 100% of sensitivity.

Conclusion: Ascitic fluid NGAL can be used not only for SBP diagnosis but also for prediction of SBP patients' short-term prognosis.

Keywords: Neutrophil gelatinase associated lipocalin (NGAL**)**, Ascites, Spontaneous bacterial peritonitis (SBP), Cirrhosis.

INTRODUCTION

In cirrhotic individuals with ascites, spontaneous bacterial peritonitis (SBP) is a common and devastating consequence with very high mortality rate up to 70% [(1,2)]. Diagnosis of SBP up till now is based on ascitic fluid (AF) polymorph neutrophil leucocyte (PMNL) count ≥250/mm3 or positive AF culture which consumes a lot of time for detection of the causative pathogen and unfortunately, it is negative in about 60% of patients **[(3)].** Neutrophil gelatinase-associated lipocalin (NGAL), a member of the lipocalin family, is a low molecular weight secretory protein that was first discovered in active neutrophils that in cirrhotic patients with acute renal injury, urine NGAL can indicate kidney function loss **[(4)]**. Although NGAL and acute kidney damage (AKI) have been the subject of numerous research [(5,6,7)] relation between ascitic NGAL and SBP, have received very less attention. According to earlier investigations, ascitic NGAL may be helpful for monitoring bacterial peritonitis in developing non-malignant ascites **[(8)].** According to a recent study, when it comes to decompensated cirrhosis-related SBP, ascitic NGAL may not only be a biomarker for SBP monitoring but also a predictor of worse outcomes **[(4)]**.

We aimed to evaluate the prognostic Patients with decompensated liver cirrhosis and spontaneous bacterial peritonitis should be tested for ascitic neutrophil gelatinase associated lipocalin.

PATIENTS AND METHODS

This was a case-control study that was carried out in Tropical Medicine Department, Zagazig University Hospitals, during the period from July 2022 to December 2022. Approval was obtained from the institutional Review Board (IRB). A written consent obtained from all patients. The study comprised 72 patients with cirrhotic liver with ascites. 72 individuals with liver cirrhosis and ascites were divided into two groups; group I contained 36 individuals with SBP, while group II contained 36 individuals who did not have SBP.

Inclusion criteria:

All patients in this study diagnosed as cirrhotic patient based on clinical and laboratory tests,(clinical presence of ascities, hepatic encephalopathy,jaundice or hematemesis and melena)(laborartory CBC platelet <150000 , liver function test albumin<3.5 ,INR >1.1 and coagulation profile) with ascites caused by chronic liver illness and ultrasound criteria, after that they were divided into two groups, the first one with SBP and the other one without SBP as a control group.

Exclusion criteria:

Cirrhotic patient with hepatocellular carcinoma, peritonitis due to any cause other than SBP, patients with portal hypertension and ascites from any cause other than cirrhosis, patients with liver transplantation or any organ transplantation and patients with renal diseases.

To identify spontaneous bacterial peritonitis, all cases underwent a detailed history taking, thorough physical examination, laboratory tests (abdominal paracentesis and SBP was diagnosed by PMNL count

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≥250/mm3, or/and with positive fluid cultures), ascitic fluid sample chemical and bacteriological analysis, and single organism culture isolation, with specific assay of Lipocalin linked with Neutrophil Gelatinase in ascetic fluid by (Human Lipocalin linked with Neutrophil Gelatinase Kit, Sun Red bio company, China), using double-antibody sandwich ELISA technique.

Child-Pugh classification determines the degree of ascites and encephalopathy, the serum concentrations of bilirubin and albumin, and the prothrombin time with points to each item. A total Child-Pugh score of 5 to 6 is considered Child-Pugh class A, A score of 7 to 9 is class B, and a score of 10 to 15 is class C. MELD score includes serum bilirubin, serum creatinine, INR, and etiology of the liver disease (cholestatic or alcohol-associated versus other etiologies) with normal score ranges from 6 to 40.

Ethics approval:

The protocol for this study was approved by both the Institutional Review Board [IRB] and the local ethics committee at Zagazig University's Faculty of Medicine. The study was conducted according to the sound clinical practice guidelines and according to the declaration of Helsinki, the World Medical Association's code of ethics for studies that involve humans.

Statistical Analysis

Data analysis was done using SPSS (Statistical Package for the Social Sciences) version 28. The means, standard deviations, median, and range of quantitative variables were utilized, depending on the type of data, to describe it. They were described using the categorical variables' absolute frequencies, and they were compared using the chi square test. To compare ordinal data between two groups, a chi square trend test was performed. Shapiro-Wilk test was used to check the assumptions used in parametric tests. The quantitative variables' means, standard deviations, medians, and interquartile ranges were utilized to describe them depending on the type of data.

Quantitative data from two groups were compared using the Kruskal-Wallis test for irregularly distributed data and the one-way ANOVA test for normally distributed data. Quantitative data from two groups were compared using the Mann Whitney test (for data that are not normally distributed) and the independent sample t test (for data that are normally distributed). For data that were not regularly distributed, Spearman rank correlation coefficients were utilized to determine the strength and direction of the association between two continuous variables. The best cutoff value for a particular quantitative measure was determined using the ROC curve to diagnose a particular medical condition. Using binary logistic regression, it was possible to pinpoint independent risk variables linked to certain health issues. To quantify the associated independent components for the dependent factor, linear regression analysis was done. P 0.05 was used as the statistical significance level. If p0.001, a highly significant difference was detected.

RESULTS

Table (1) Comparison between the studied groups regarding ascitic fluid NGAL level

Parameter	Group I	Group II	Z	P
	Median (IQR)	Median (IQR)		
NGAL (ng/mL)	826.55 (413.25 – 992.95)	146.35 (122.43 – 188.5)	-7.135	<0.001**

Z Mann Whitney test IQR interquartile range **p≤0.001 is statistically highly significant.

Table (1) exhibits that there is ascitic fluid NGAL, which was much greater in the case group, showed a statistically significant difference between the examined groups.

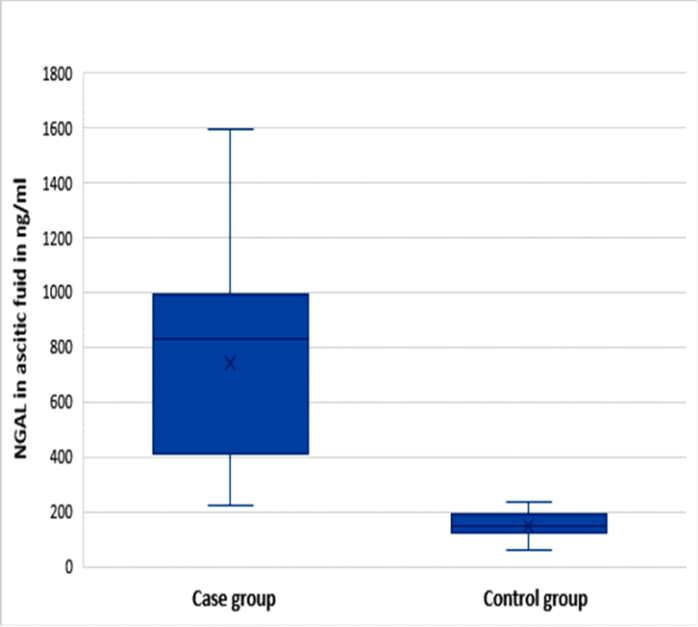

Figure (1) Boxplot showing comparison between groups regarding ascitic fluid NGAL

Table (2) Multivariate regression analysis of predictors of mortality among studied patients:

	B	p	AOR	95% C.I.	
				Lower	Upper
NGAL in ascitic fluid (ng/ml)	2.894	0.003*	18.074	.000	3.34 45

AOR adjusted odds ratio CI confidence interval

Table (2) exhibits that among factors significantly associated with mortality among studied patients, only ascitic fluid NGAL independently increased risk by 18.074 folds.

Table (3) Performance of AF NGAL in prediction of spontaneous bacterial peritonitis:

Cutoff	AUC	Sensitivity	Specificity	PPV	NPV	Accuracy	P
≥230.05	**0.989**	**94.4%**	**88.9%**	**89.5%**	**94.1%**	**91.7%**	**<0.001****

**p≤0.001 is statistically highly significant AUC area under curve PPV positive predictive value NPV negative predictive value

Table (3) exhibits that the best cutoff of ascitic fluid NGAL in diagnosis of spontaneous bacterial peritonitis is ≥230.05 ng/ml with area under curve 0.989, sensitivity 94.4%, specificity 88.9%, positive predictive value 94.1%, negative predictive value 94.1% and overall accuracy 91.7% (p<0.001).

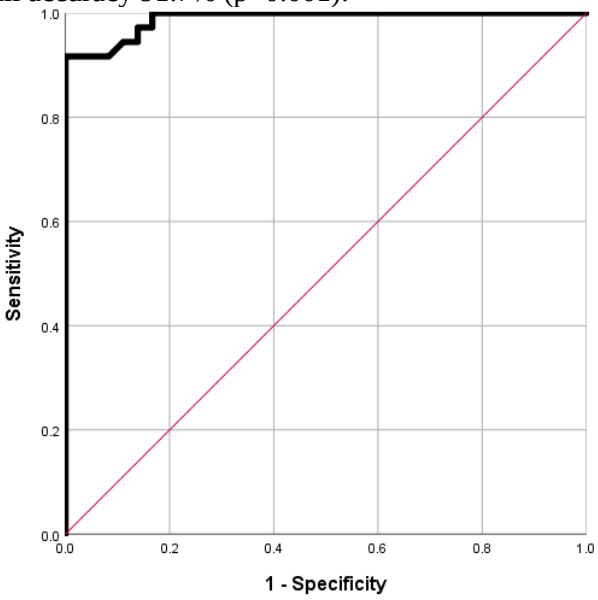

Figure (2) ROC curve showing performance of AF NGAL in prediction of spontaneous bacterial peritonitis

Table (4) Linear regression analysis of factors significantly related to ascitic fluid NGAL among patients with SBP:

	Unstandardized Coefficients		Standardized Coefficients	t	p	95.0% Confidence Interval	
	β	Std. Error	Beta			Lower	Upper
(Constant)	1226.309	312.497		3.924	<0.001**	588.967	1863.652
AF TLC	0.107	0.015	0.676	7.277	<0.001**	.077	.137
3 months mortality risk	6.968	2.532	0.259	2.752	0.01*	1.803	12.132
Spleen diameter	-54.996	20.207	-0.246	-2.722	0.011*	-96.208	-13.784

**p≤0.001 is statistically highly significant *p<0.05 is statistically significant

Table (4) exhibits that among factors significantly correlated to ascitic fluid NGAL, only AF TLC (unstandardized β=0.107), 3 months mortality risk (unstandardized β=6.698) and spleen diameter (unstandardized β=-54.996) significantly independently associated with NGAL.

Table (5) Performance of AF NGAL, MELD score in prediction of mortality among studied patients:

	Cutoff	AUC	Sensitivity	Specificity	PPV	NPV	Accuracy	p
AF NGAL	≥401	1	100%	91.7%	85.7%	100%	94.4%	<0.001**
MELD	≥16.5	0.659	62.5%	62.5%	76.9%	45.5%	62.5%	0.029*

**p≤0.001 is statistically highly significant AUC area under curve PPV positive predictive value NPV negative predictive value

Table (5) exhibits that the best cutoff of ascitic fluid NGAL sensitivity, specificity, positive predictive value, negative predictive value, and overall accuracy are measured by the area under curve 1 (AUC1) (p 0.001) are all 100%. In the prediction of death, the value is 401 ng/ml.

The optimal MELD threshold for predicting death is AUC of 0.659, sensitivity, specificity, positive predictive value, and positive predictive value for 16.5, negative predictive value, and overall accuracy of 62.5% (p0.05).

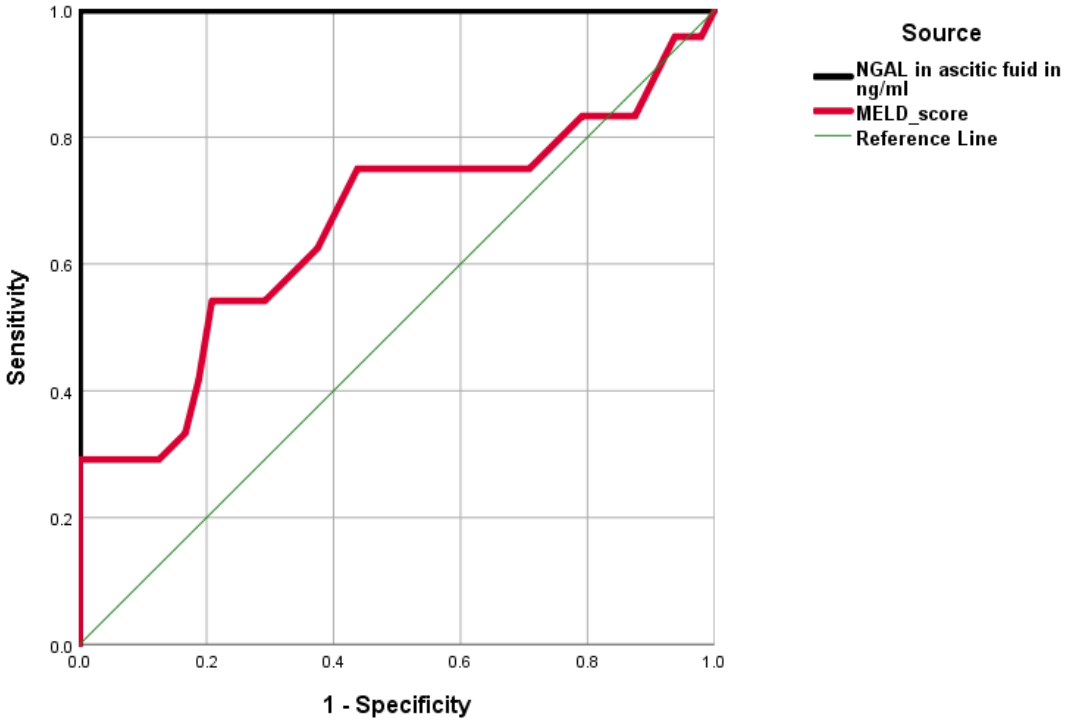

Figure (3) ROC curve showing performance of AF NGAL, MELD score in prediction of mortality among studied patients

DISCUSSION

Bacterial infection in decompensated liver cirrhosis is horribly to blame for 35% of cirrhosis-related deaths. Particularly SBP, which represents a frequent type of infection (approximately 28%) in cirrhotic patients **Fukui *et al.*** [(9)], Until recently, the gold standard for the identification of the condition has been ascitic fluid with a PMN concentration of 250 cells/mm^3 of SBP, but sadly, many individuals with high risk factors cannot be identified by PMN. As a result, a measure that can help with both SBP screening and patient prognosis is becoming more and more necessary [(4)].

NGAL, a lipocalin family member glycoprotein with a 25-kDa molecular weight and was first discovered in active neutrophils, can also be partially synthesized by renal tubular cells. NGAL is a versatile protein that is rapidly released after an injury, such as acute renal tubular damage inflammation, ischemia, metabolic disorders, and also implicated in the infection-specific innate immune response [(10)].

There are comparatively few investigations on NGAL, particularly the relation between ascitic NGAL and SBP, Even though several research have focused on the relationship between NGAL and acute kidney injury (AKI) [(11-13)]. Ascitic NGAL has been demonstrated to be useful tool to keep an eye on bacterial peritonitis in developing nonmalignant ascites in prior studies [(8)]. According to a recent study, in decompensated cirrhosis-related SBP, Ascitic NGAL may be used to predict worse outcomes in addition to acting as a biomarker for SBP monitoring [(4)]. In a second study utilizing a different detection technique, of the 146 individuals with liver cirrhosis, 29 SBP patients had plasma NGAL levels that were obviously high [(14)].

In this study we found that Statistically extremely significant differences between the two groups revealed that SBP patients had significantly greater ascitic NGAL levels than non-SBP patients with median (IQR) (826.55(413.25 – 992.95) vs 146.35(122.43 – 188.5) ng/mL; P <.001) in agreement with **Cullaro *et al.*** [(14)] who between the two groups that was significantly different with median (IQR) [221.3 (145.9–392.9) vs 139.2 (73.9–237.2) ng/mL; P <.001] and in agreement with **Liu *et al.*** [(4)] who discovered that between the study groups, there was a statistically significant difference in terms of ascitic NGAL level with median (IQR) [111(83.9-178) vs 48 (35.4-63) ng/mL; P <.001] and in agreement with **Biomy *et al.*** [(15)] who found that SBP Ascitic NGAL levels were noticeably greater in the patients than non SBP with statistically highly significant difference between the both groups [(171.55+89.13) vs (45.95+18.95) ng/mL; P <.001].

We found that ascitic fluid NGAL was the only factor that independently increased risk by 18.074 folds among mortality significantly associated factors in studied patients in agreement with **Liu *et al.*** [(4)] who found that 11 survivors of the SBP group showed dynamic changes in ascitic NGAL levels, which sharply decreased after taking Cephalosporins of the third generation for seven days [149 (124-308.9) vs 54.3 (29-106) ng/mL; P =.001]. Contrarily, despite the use of vigorous antibiotic therapy, five non survivors of the SBP group had significantly increased levels of ascitic NGAL [111 (88.75-520.1) vs 228 (178.5-717) ng/mL; P =.043] due to the emergence of HCC.

We discovered that NGAL's ideal cutoff value used to diagnose aortic dissection in SBP was ≥230.05 ng/ml with area under curve **(**AUC**)** 0.989, sensitivity 94.4%, overall accuracy 91.7%, 89.5% positive predictive value, 94.1% negative predictive value, and 88.9% specificity (p<0.001).

Biomy *et al.* [15] found that cutoff value of ascitic fluid NGAL was 100.8 ng/dl with (AUC) of 0.974, sensitivity of 97.62% and specificity of 97.67%**. Lippi *et al*. [16]** reported that cutoff value of ascitic fluid NGAL was ≥ 120 ng/ml sensitivity of 96%, specificity of 100%, and (AUC) of 0.89 of 75% **Liu *et al.* [4]** found that cutoff value of ascitic fluid NGAL was 108.95 ng/mL with (AUC) 0.702, sensitivity of 76.9% and a specificity of 45.1% **Cullaro *et al.* [14]** found that cutoff value of ascitic fluid NGAL was 211.0 ng/mL with AUC .79, sensitivity 87.5% and specificity 75.1%.

We found that, ascitic fluid TLC was statistically highly significant independently associated with ascitic fluid NGAL while, 3 months mortality risk and spleen diameter were statistically significant independently associated with it in agreement with **Liu *et al.* [4]** but not in agreement with **Biomy *et al.* [15]** who found that white blood cells, The connection between NGAL in the non-SBP group and all of the ascitic fluid PMNL, Child Pugh, MELD, and uMELD scores were negative.

We found that, the optimum cutoff value of ascitic fluid NGAL in prediction of mortality is ≥401 ng/ml with (AUC) 1, 85.7% of predictions were correct, while only 0.3% were incorrect of 100%, specificity of 100%, and 91.7% and overall accuracy 94.4% (p<0.001) in agreement with **Liu *et al.* [4]** who found that the cutoff value of ascitic fluid NGAL level was 108.95 ng/ml with (AUC) 0.702, sensitivity of 76.9% and a specificity of 45.1% and that study also approved that ascitic fluid all SBP patients' NGAL levels were the most accurate predictors of mortality and NGAL level was statistically AKI and SBP are closely associated with two cirrhosis-related mortality-related consequences and in agreement with **Cullaro *et al.* ([14]** who found that ascitic NGAL concentrations greater than 221.3 ng/ml were sensitive (73.3%) and specific (71.2%) for in-hospital death.

We found that the best cutoff value of MELD score in prediction of mortality is ≥16.5 with (AUC) 0.659, 76.9% for positive predictive value, 45.5% for negative predictive value, 62.5% for specificity and overall accuracy 62.5% (p<0.05) in agreement with **Liu *et al.* [4]** They used Cox regression to demonstrate that the MELD score and ascitic NGAL levels were separate risk variables for decompensated liver cirrhosis with SBP and in agreement with **Cullaro *et al.*[14]** who found that NGAL was more precise predictor of mortality than bilirubin and INR, the other two parts of the MELD score. These results imply that ascitic NGAL is a composite of hepatic insults and a total of the ischemia, inflammation, and infection levels.

This study showed a substantial difference in ascitic NGAL levels between the SBP and non-SBP groups. The infection-related upregulation of neutrophils in the ascitic fluid may not be the only source of this increase; as general decompensation in liver function may also play a golden role. The liver is a well-known human immunological organ, and studies on animals had shown that hepatocyte-derived NGAL is crucial for controlling bacterial infection [17].

According to the results of our investigation, detecting the presence of SBP in patients with decompensated liver cirrhosis who had elevated levels of ascitic NGAL is crucial and optimum cutoff value was ≥230.05 ng/ml with (AUC) 0.989, 89.5% in terms of positive predictive value and negative predictive value, respectively, 94.4% for specificity and overall accuracy 91.7%. We found that optimum cutoff of ascitic fluid NGAL in prediction of mortality is ≥401 ng/ml with (AUC) 1, 94.4% overall accuracy, 85.7% positive predictive value, 100% negative predictive value, 100% sensitivity, 100% specificity. So ascitic fluid NGAL can be used not only for SBP diagnosis but also for prediction of cirrhosis SBP patients' short-term prognosis.

CONCLUSION

Ascitic fluid NGAL can be used not only for SBP diagnosis but also for prediction of SBP patients' short-term prognosis.

RECOMMENDATION

Additional research with a larger sample size and longer follow-up times is required.

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Conflicts of interest: There are no conflicts of interest, according to the authors.

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