Fecal Calprotectin: A Novel Screening Marker for Diagnosis of Gut Flora Associated Complications in Cirrhotic Patients

Sherif Monier Mohamed (MD) [(1)], Nevine Ibrahim Musa (MD) [(1),] Ashraf Albreedy (MD) [(2),] Sally Mohamed Saber (MD)[(3)]

Internal Medicine and Gastroenterology Department [(1)], Tropical Medicine Department [(2)] - Clinical pathology department [(3)] - Faculty of Medicine - Ain Shams University- Cairo –Egypt
Sherifmonier@yahoo.com

Abstract: Background and Aims: The gut flora and bacterial translocation play an important role in the pathogenesis of certain complications of cirrhosis as hepatic encephalopathy (HE), spontaneous bacterial peritonitis (SBP) and Hepatorenal syndrome(HRS). Diagnosis of these complications continues to be a major clinical problem. Calprotectin is a cytosolic protein with immunmodulatory, antimicrobial and antiproliferative action that is predominantly found in neutrophils, monocytes and macrophages as well as in T and B lymphocytes. The measurement of fecal calprotectin (FC) level is a sensitive and non-invasive marker that determines an active inflammation in the gastrointestinal system. The aim of this study was to study the diagnostic value of FC as a predictor for certain complications in cirrhotic patients as HE, SBP and HRS. **Patients and Methods:** This study included 100 subjects.They were divided into five groups: 20 healthy subjects as a control, 20 patients with liver cirrhosis without complications, 20 patients with SBP, 20 patients with HE and 20 patients with HRS. All studied groups were subjected to full history taking, complete clinical examination and routine laboratory investigations and microbiological analysis of ascetic fluid. Fecal calprotectin concentration was measured (by ELISA). **Results:** Fecal calprotectin concentrations were higher in cirrhotics (76±15 mg/kg) compared with controls (31.2±9 mg/kg) (P<0.001). Comparison between HE(364±83 mg/kg), SBP (273±42 mg/kg)and HRS(450±5 mg/kg) groups as regard FC showed statistically highly significant difference (P<0.001). Comparison between cirrhotic(76±15 mg/kg),low grade HE(239±19 mg/kg) and High grade HE (489±23mg/kg)groups as regard FC showed statistically highly significant difference (P<0.001). Fecal calprotectin had sensitivity of 90% and specificity of 60% in prediction of HE with PPV and NPV of 67% and 94% respectively at cut off value of 280mg/kg with AUC of 0.72, on the other hand FC had sensitivity of 95% and specificity of 43% with PPV and NPV of 50% and 96% respectively at cut off value 190mg/kg with AUC of 0.47in prediction of SBP group. As regard HRS group, FC had sensitivity of 90% and specificity of 88% with PPV and NPV of 90% and 97% respectively at cut off value of 340mg/kg with AUC of 0.94. **Conclusions and Recommendations:** FC was significantly elevated in cirrhotic patients with significant correlation emerged between elevated FC and complications as HE, SBP and HRS. And we recommend the use FC as a promising, simple, non-invasive and rapid screening test to make a diagnosis of these complications.
[Sherif Monier Mohamed, Nevine Ibrahim Musa, Ashraf Albreedy, Sally Mohamed Saber. **Fecal Calprotectin: A Novel Screening Marker for Diagnosis of Gut Flora Associated Complications in Cirrhotic Patients.** *Life Sci J* 2014;11(11):709-717] (ISSN: 1097-8135). http://www.lifesciencesite.com. 131

Keywords: Fecal calprotectin, liver cirrhosis, hepatic encephalopathy, spontaneous bacterial peritonitis and hepatorenal syndrome.

1. Introduction

Patients with liver cirrhosis have an increased risk of infections mainly the 'spontaneous' infection of ascites or spontaneous bacterial peritonitis (SBP) which is present in approximately 15% of patients with cirrhosis and ascites [1] due to bacterial translocation (BT) [2]. Over 70% of the cases are caused by intestinal bacteria and can be prevented by selective bacterial decontamination with non-absorbed or absorbed antibiotics, which confirms that the gut is the main source of microorganisms [3].The gut microflora not only takes part in the pathogenesis of overt infective episodes and of the clinical consequences of sepsis, but it also contributes to the pro-inflammatory state of cirrhosis even in the absence of overt infection [4]. Although accurate mechanisms of BT are unknown, small intestinal bacterial overgrowth (SIBO), gut dysmotility, increased intestinal permeability and impaired defense mechanisms are regarded as major risk factors for BT [5]. Small intestinal bacterial overgrowth is common in liver cirrhosis due to various causes and is an independent risk factor for systemic endotoxemia, implicated in various pathophysiological sequelae of cirrhotic patients and in the pathogenesis of certain complications of cirrhosis as HE,SBP and HRS[6].

Endotoxin levels are usually elevated in patients with decompensated liver disease and more so in patients with HRS. This is believed to be due to increased BT and portosystemic shunting.[7].Inflammatory response to infection as estimated by levels of cytokines in plasma or ascetic fluid is increased in cirrhotic patients leading to circulatory dysfunction, splanchnic

vasodilatation and concomitant renal impairment and increased mortality.[8-9]

Including all stages, the prevalence of HE in cirrhosis is presumably high and can be diagnosed in up to 80% of all cirrhotic patients.[10-11].The gut flora and BT also play a role in the pathogenesis of hepatic encephalopathy (HE). A study by **Gupta etal** [12] demonstrated that bacterial overgrowth is a responsible factor for minimal HE in cirrhotic patients.

Calprotectin is a cytosolic protein that belongs to the S-100 protein group that increases under conditions such as inflammation, infection, and malignancy. Fecal calprotectin may increase under many conditions such as inflammatory bowel diseases (IBDs). Calprotectin can also be measured in plasma, synovial fluid, cerebrospinal fluid, oral fluids and urine. Calprotectin is a calcium and zinc binding protein. It decreases the local zinc intensity by binding to zinc. In this way, it deprives microorganisms of zinc and additionally inhibits many zinc-dependent enzymes.[13] Calprotectin is a cytosolic protein with immunmodulatory, antimicrobial and antiproliferative action that is predominatly found in neutrophils, monocytes and macrophages as well as in T and B lymphocytes. The measurement of FC levels is a sensitive and non-invasive marker that determines an active inflammation in the gastrointestinal system.[14]

Aim of the work

The aim of this study was to assess the diagnostic value of FC as a predictor for GIT inflammation induced complications of cirrhosis as HE, SBP and HRS.

2. Patients and methods

This case control study was conducted on 100 subjects who were admitted to Ain Shams University Hospital after written consent. They were divided into five groups: 20 healthy non cirrhotic subjects as a control, 20 patients with liver cirrhosis without complications, 20 patients with liver cirrhosis complicated by SBP, 20 patients with liver cirrhosis complicated by HE and 20 patients with liver cirrhosis complicated by HRS

The diagnosis of liver cirrhosis was based on clinical, laboratory and radiological findings [15-16].The disease severity was based on Child-Pugh Classification [17].

- Assessment of hepatic encephalopathy was calculated according to West-Haven criteria for hepatic encephalopathy [18]. which classify HE into 5 stages from 0 to 4 according to Consciousness (0= Normal,1= Mild lack of awareness, 2= Lethargic, 3= Somnolent and4= Coma), Intellect and behavior (0=Normal, 1=Shortened attention span; impaired addition or subtraction,2= Disoriented;,3= Gross disorientation and 4=coma) and Neurological findings (0= Normal,1= Mild asterixis.2= Obvious asterixis,3= rigidity, clonus. Hyper-reflexia and 4= Decerebrate posturing). In this study, HE was either divided into low grade HE (stage 1) or high grade HE (> stage 1) while stage 0 excluded HE.

- The diagnosis of SBP in patients with ascites is established by definition as ascitic fluid neutrophil count of more than 250 cells/µL determined via cytological (manual microscopic cell count) with or without positive ascetic fluid culture as the concentration of bacteria in ascitic fluid of patients with SBP is usually very low (1 microorganism/ml or less).[19]

- The diagnosis of HRS was selected according to diagnostic criteria of HRS. [20-24]

1-Major diagnostic criteria: Cirrhosis with ascites. serum creatinine > 1.5 mg/dL. no improvement in serum creatinine (decrease to a level of <1.5 mg/dL) after at least 2 days with diuretic withdrawal and volume expansion with albumin. The recommended dose of albumin is 1 g/kg of body weight per day up to a maximum of 100 g/day. Absence of shock. No current or recent treatment with nephrotoxic drugs,and absence of parenchymal kidney disease as indicated by proteinuria >500 mg/day, microhematuria (<50 RBC/high power field) and/or abnormal renal ultrasonography.

2-The minor diagnostic criteria are: Urine volume < 500 mL/24 h. Urine sodium <10 mEq/L. Urine osmolality greater than plasma osmolality. Urine red blood cells < 50 per high power field and serum sodium <130 mEq/L.

Exclusion criteria: Include all causes of abnormal FC including inflammatory bowel disease(IBD), coeliac disease, colorectal carcinoma, active GI bleeding, certain drugs [e.g. non-steroidal anti-inflammatory drugs(NSAIDs), anticoagulants, antibiotic therapy, proton pump inhibitors(PPI)], food allergy, ongoing alcohol abuse and all patients who reported about diarrhea were also excluded from this study as GI infections may cause elevated FC.

Methodology: All studied groups were subjected to:

1- Full history taking and complete clinical examination
2- Routine laboratory investigations including complete blood count (CBC), liver function tests(LFT) including serum bilirubin(total and direct), serum albumin, prothrombin time, serum aspartate transaminase (AST),serum alanine transaminase (ALT), and alkaline phosphatase, kidney function tests(KFT) including serum urea, creatinine and blood urea nitrogen, electrolytes including serum sodium, potassium and urinary sodium excretion, viral markers including HBsAg and HCVAb,blood sugar, urine and stool analysis, and alpha fetoprotein (AFP).

3- Ascetic fluid sample for all patients with liver cirrhosis and ascites was obtained under complete aseptic conditions. Approximately 30 ml of ascetic fluid was aspirated and divided into two syringe one for cell count type and differential, chemistry including protein, albumin, glucose, lactate dehydrogenase (LDH) and the other for bedside inoculation in two blood culture bottles (Oxoid gas capture system aerobic and anaerobic) followed by subculture of bottles with positive signal on conventional plates.[19] Microbial identification was done using routine biochemical reactions & culture morphology. Antimicrobial susceptibility testing was done for each pathogen according to CLSI recommendation 2014[25]. Additionally rapid diagnosis of spontaneous bacterial peritonitis was done using of reagent strips that detect leukocyte esterase which was developed for use in urine analysis (Combur strips, USA) with a threshold of >50 PMN cells/mm3.[26]
4- Measurement of fecal calprotectin concentration: Samples obtained for FC measurement were collected in screw- caped disposable plastic containers and stored in aliquots at -20 ^{0}C until analysis. Fecal calprotectin concentration was measured using a commercial enzyme linked immunoassay (ELISA) method (Calprest, Dynex Elisa Eurospital, Trieste, Italy). The FC concentration results were expressed in mg of FC per kilogram of wet feces.
Normal ranges: according to the manufacturer's instructions, the median value in healthy adults is about 25 mg/kg while samples giving values above 50 mg/kg are regarded as positive.
5- Abdominal ultrasound (US).

Statistical Methodology:

Analysis of data was done using SPSS (statistical program for social science version 17) as follows: Description of quantitative variables as mean, SD and range, description of qualitative variables as number and percentage. Chi-square test was used to compare qualitative variables between groups. Unpaired t-test was used to compare quantitative variables, in parametric data (SD<50% mean),Mann Whitney Willcoxon test was used instead of unpaired t-test in non parametric data (SD>50%mean),ANOVA test (one way analysis of variance) was used to compare more than two groups as regard quantitative variable,Spearman correlation co-efficient test was used to rank variables versus each other positively or inversely.ROC Curve (receiver operator characteristic curve) was used to find out the best cut off value, and validity of certain variable. sensitivity = true +ve /true +ve + false –ve = ability of the test to detect +ve cases, specificity = true -ve/true-ve+ false +ve = ability of the test to exclude negative cases, positive predictive value (PPV) = true+ve /true+ve +false +ve = % of true +ve cases to all positive, and negative predictive value (NPV)= true-ve /true-ve + false –ve = % of the true –ve to all negative cases. P value >0.05 insignificant, P<0.05 significant and P<0.01 highly significant [27]

3. Results

Comparison between the studied groups as regard age and gender showed statistically no significant difference. **(Table 1)**

Comparison between the studied groups as regards various laboratory data showed statistically highly significant difference using one way ANOVA test. **(Table 2)**

Comparison between the studied groups as regard FC showed statistically highly significant difference using one way ANOVA test (P<0.001)with lowest value in the control group (31.2±9 mg/kg) and highest value in the HRS group (450±5 mg/kg). **(Table 3&figure 1)**

Comparison between control (31.2±9 mg/kg) and cirrhotic (76±15 mg/kg) groups as regard FC showed statistically highly significant difference **(Table 4)**

Comparison between control, cirrhotic and SBP groups as regard FC showed statistically highly significant difference using one way ANOVA test (P<0.001)with lowest value in the control group (31.2±9 mg/kg) followed by cirrhotic(76±15 mg/kg) with highest value in the SBP group(273±42 mg/kg). **(Table 5)**

Comparison between control, cirrhotic and HRS groups as regard FC showed statistically highly significant difference using one way ANOVA test (P<0.001) with lowest value in the control group(31.2±9 mg/kg) followed by cirrhotic(76±15 mg/kg) with highest value in the HRS group (450±5 mg/kg). **(Table 6)**

Comparison between control, cirrhotic and HE groups as regard FC showed statistically highly significant difference using one way ANOVA test(P<0.001) with lowest value in the control group (31.2±9 mg/kg) followed by cirrhotic(76±15 mg/kg)with highest value in the HE group(364±83 mg/kg). **(Table 7)**

Comparison between cirrhotic,low grade HE and High grade HE groups as regard FC showed statistically highly significant difference using one way ANOVA test (P<0.001) with lowest value in the cirrhotic group (76±15 mg/kg)followed by low grade HE (239±19mg/kg) with highest value in the high grade HE group(489±23mg/kg). **(Table 8)**

Comparison between HE, SBP and HRS groups as regard FC showed statistically highly significant difference using one way ANOVA test

with lowest value in the SBP group (273±42 mg/kg) followed by HE group (364±83)and highest value in the HRS group (450±5 mg/kg). **(Table 9)**

Table 10 showed that FC had sensitivity of 90% and specificity of 60% in prediction of HE with PPV and NPV of 67% and 94% respectively at cut off value of 280mg/kg with AUC of 0.72, on the other hand FC had sensitivity of 95% and specificity of 43% with PPV and NPV of 50% and 96% respectively at cut off value 190mg/kg with AUC of 0.47in prediction of SBP group. As regard HRS group, FC had sensitivity of 90% and specificity of 88% with PPV and NPV of 90% and 97% respectively at cut off value of 340mg/kg with AUC of 0.94.**(Figure2-4)**

Table 11 and 12 showed culture results of ascetic fluid in SBP patients, it was divided into culture positive neutrocytic ascites of monomicrobial infection 9/20 (45%) and culture negative neutrocytic ascitis 11/20 (55 %) with no statistical significant difference p>0.05. However, leucocyte reagent strips showed 100% sensitivity compared to neutrophil count. Culture results showed growth of Ecoli 5/9(55.6%) followed by klebsiella pneumonia 3/9(33.3%), both isolates showed extended spectrum beta lactamase production (ESBL) which was only sensitive to carbapenems (imipenem& meropenem) and one Enterococci isolate 1/9(11.1%) which was sensitive to penicillin and ampicillin.

Table (1) Comparison between the studied groups as regard age and gender

Variables	control (n=20)	cirrhosis (n=20)	HE (n=20)	SBP (n=20)	HRS (n=20)	*P* value
Age (mean±SD)	49.5±9	52.6±8	50.5±4	50.3±2	51.6±1.7	>0.05
Gender						
Male	8(40%)	15(75%)	10(50%)	11(55%)	14(70%)	>0.05
Female	12(60%)	5(25%)	10(50%)	9(45%)	6(30%)	

Table (2) Comparison between the studied groups as regards various laboratory data

Variables	control (n=20)	cirrhosis (n=20)	HE (n=20)	SBP (n=20)	HRS (n=20)	*P* value
HB (gm/dl.)	12.6±1.	9.9±2	9,1±0.8	10±0.8	8.6±1	**<0.001**
Platelets(mm3)	352±38	100±20	75±20	79.9±26	68±9	**<0.001**
WBCs(mm3)	5.5±1	4±1.3	4.6±1.6	9.5±4	4.3±2	**<0.001**
PT (Sec.)	12.1±2	15.2±1.6	18.5±2	18.6±2	20±3	**<0.001**
INR	1±0.04	1.4±0.6	1.8±0.4	1.9±0.4	1.9±0.3	**<0.001**
Bilirubin (mg/dl.)	0.85±0.2	1.5±0.3	2.9±0.3	1.8±0.3	4.6±2	**<0.001**
ALT (IU/L)	22.6±4	49±22	80±39	73±37	108±90	**<0.001**
AST (IU/L)	25±15	53±20	61±23	57±23	88±29	**<0.001**
Albumin (gm/dl.)	3.9±1.2	3.5±1.1	2.2±0.3	2.1±0.4	1.9±0.3	**<0.001**
Na(mmol/L)	135±1	137±1.1	126±2	126.7±5	119±6	**<0.001**
K(mmol/L)	4.1±0.4	4±0.3	3.6±0.4	3.4±0.5	4.7±1	**<0.001**
RBS(mg/dl)	114±7.7	148±41	135±75	124±40	108±14	**<0.001**
Cr(mg/dl)	0.83±0.2	0.86±0.2	1.15± 0.6	0.97±0.40	3.2±0.5	**<0.001**
BUN(mg/dl)	15.1±3	21.5±8	27±11	26±10	81±12	**<0.001**

Table (3) comparison between studied groups as regard fecal calprotectin

Variables	control (n=20)	cirrhosis (n=20)	HE (n=20)	SBP (n=20)	HRS (n=20)	*P* value
Calprotectin (mg/kg)	31.2±9	76±15	364±83	273±42	450±5	**<0.001**

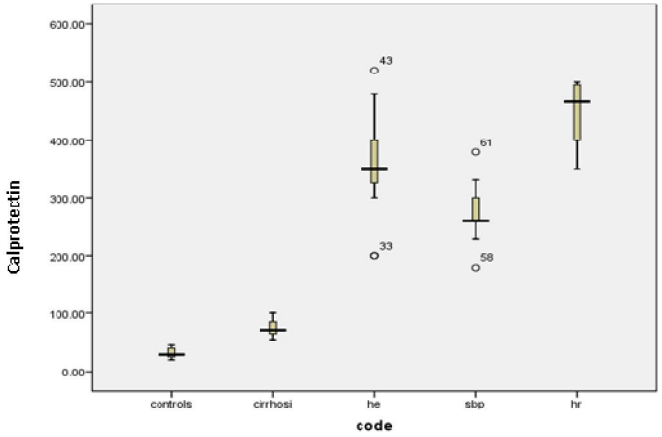

Figure (1) comparison between studied groups as regard fecal calprotectin

Table (4) comparison between control and cirrhotic groups as regard fecal calprotectin

Variables	control (n=20)	cirrhosis (n=20)	*P* value
Calprotectin (mg/kg)	31.2±9	76±15	**<0.001**

Table (5) comparison between control, cirrhotic and SBP groups as regard fecal calprotectin

Variables	control (n=20)	cirrhosis (n=20)	SBP (n=20)	*P* value
Calprotectin (mg/kg)	31.2±9	76±15	273±42	**<0.001**

Table (6) comparison between control, cirrhotic and HRS groups as regard fecal calprotectin

Variables	control (n=20)	cirrhosis (n=20)	HRS (n=20)	*P* value
Calprotectin (mg/kg)	31.2±9	76±15	450±5	**<0.001**

Table (7) comparison between control, cirrhotic and HE groups as regard fecal calprotectin

Variables	control (n=20)	cirrhosis (n=20)	HE (n=20)	P value
Calprotectin (mg/kg)	31.2±9	76±15	364±83	**<0.001**

Table (8) comparison between cirrhotic, low grade HE and high grade HE groups as regard fecal calprotectin.

Variables	cirrhosis (n=20)	Low grade HE (n=10)	High grade HE (n=10)	P value
Calprotectin (mg/kg)	76±15	239±19	489±23	**<0.001**

Table (9) comparison between HE, SBP and HRS groups as regard fecal calprotectin

Variables	HE (n=20)	SBP (n=20)	HRS (n=20)	*P* value
Calprotectin (mg/kg)	364±83	273±42	450±5	**<0.001**

Table (10) Validity of calprotectin in prediction of HE, SBP and HRS

Variables	Best cut	AUC	Sensitivity	Specificity	PPV	NPV
HE	280 mg/kg	0.72	90%	60%	67%	94%
SBP	190 mg/kg	0.47	95%	43%	50%	96%
HRS	340 mg/kg	0.94	90%	88%	90%	97%

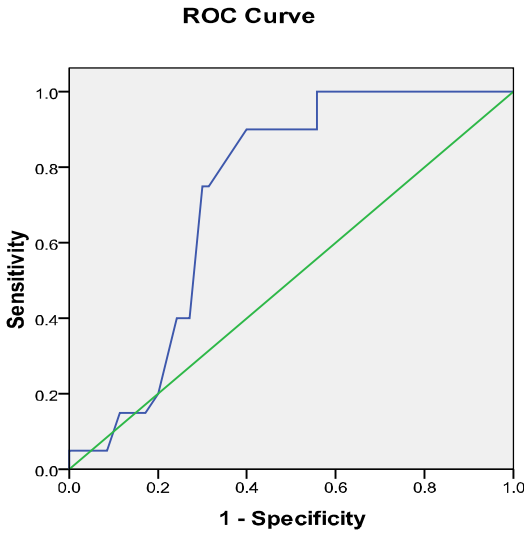

Figure (2): Roc curve for prediction of HE by using fecal calprotectin

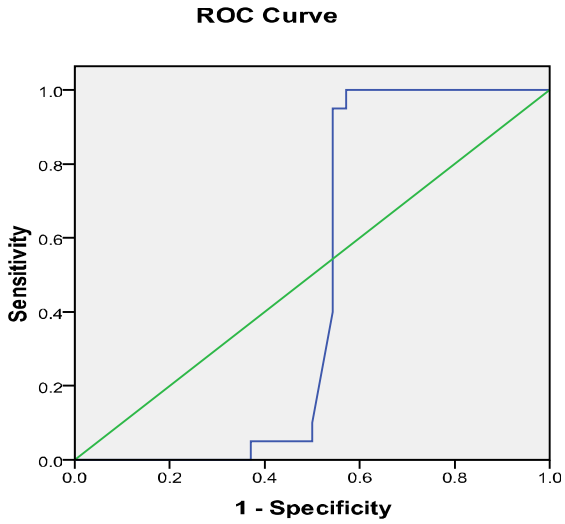

Figure (3): Roc curve for prediction of SBP by using fecal calprotectin

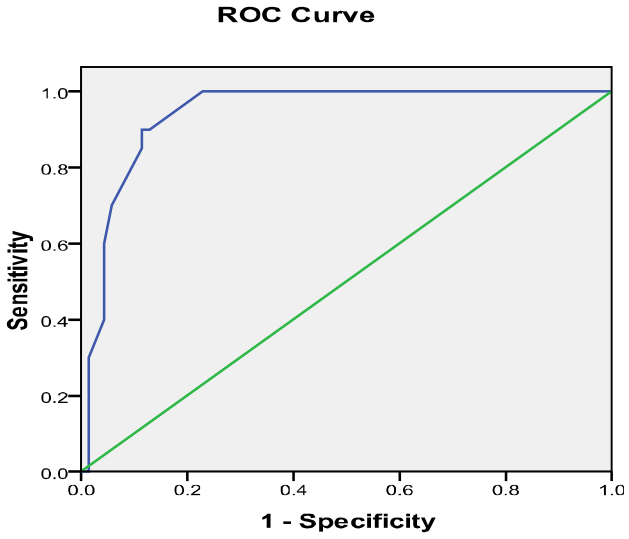

Figure (4): Roc curve for prediction of HRS by using fecal calprotectin

Table (11): Culture results of neutrocytic ascites in SBP patients. (n=20)

Variables	positive		Negative		*P* value
	no	%	no	%	
	9	45	11	55	P >0.05

Table (12): Isolated organisms in culture positive ascetic fluid in SBP patients (n=9)

E coli		Klebsiella pneumonia		Enterococci	
no	%	no	%	no	%
5	55.6	3	33.3	1	11.1

4. Discussion

In the present study, the median FC were significantly higher in cirrhotic patients (76±15 mg/kg) compared with control group (31.2±9 mg/kg) despite of a careful exclusion of other causes of abnormal FC ie IBD, coeliac disease, colorectal carcinoma, active GI bleeding, certain drugs (e.g. NSAIDs, anticoagulants, antibiotic therapy and PPI), food allergy, alcohol abuse and diarrhea. This may be explained by Small Intestinal bacterial overgrowth in patients with liver cirrhosis and intestinal barrier dysfunction in liver cirrhosis leading to infiltrates of the intestinal mucosa with neutrophils, transmucosal passage of bacteria, the essential step for bacterial translocation, which has been identified as a key event in the pathogenesis of life-threatening infections in cirrhosis.[28, 29] this result agree with **Gundling *et al***. [30] who reported that the median FC were significantly higher in cirrhotic patients (65.8 mg/kg)compared with control group(17.5 mg/kg) [P < 0.001] and concluded that, elevated FC in cirrhotic patients as opposed to the control group may be caused by a regional (primary) intestinal inflammation which is not secondary the result of a systemic inflammatory reaction. Therefore, FC is even increased in cirrhotic patients without inflammation of GI tract. This result also agree with **Yagmur *et al*** [31] who found significantly elevated FC in patients with advanced disease and a trend towards higher levels of FC in subjects with alcoholic cirrhosis. However, **Montalto *et al.*** [32] and **Gundling *et al*.**[30] found no significant correlation with a certain etiology of cirrhosis and level of FC.

Hepatic encephalopathy (HE) describes a spectrum of potentially reversible neuropsychiatric abnormalities seen in patients with liver dysfunction after exclusion of unrelated neurologic and/or metabolic abnormalities. It may be clinically apparent in as many as one third of cirrhotic patients and, if rigorously tested, up to two thirds have some degree of mild or subclinical HE.[33] In our study, a significant association emerged between elevated FC and HE with mean value of (364+83 mg/kg) and when we use FC in prediction of HE, it had a sensitivity of 90% and specificity of 60% with PPV and NPV of 67% and 94% respectively at cut off value of 280mg/kg with AUC of 0.72.

Comparison between cirrhotic,low grade HE and High grade HE groups as regard FC showed statistically highly significant difference using one way ANOVA test (P<0.001) with lowest value in the cirrhotic group (76±15 mg/kg)followed by low grade HE (239±19mg/kg) with highest value in the high grade HE group(489±23mg/kg).

Gundling and colleagues [30] found a significant association emerged between elevated FC and HE grading as measured by West–Haven criteria ($P < 0.001$). Differentiating gradings of 0 and 1 from 2 and 3 resulted in a sensitivity of 94% and a specificity of 95% using an optimal cut point of 164 mg/kg ($P < 0.001$). And conclude that, FC may serve as a screening tool to identify cirrhotic patients with HE. Furthermore, assessment of FC may facilitate grading of HE-severity which may be sometimes subjective when using only clinical criteria.

The high level of FC in patients with HE may be explained by SIBO. **Gupta and colleagues** [12] studied the role of bacterial overgrowth of the small intestine among patients with minimal HE, 55.9% of patients with cirrhosis had minimal HE. Among these patients with minimal HE, (38.6%) had SIBO, while (8.9%) of patients without Minimal HE had SIBO ($p < 0.001$). The prevalence of SIBO was higher in patients with Child- Pugh classes B and C (69.2%) compared to those in class A (30.8%).

Spontaneous bacterial peritonitis is a frequent and severe complication in patients with cirrhosis and ascites that occurs in the absence of an evident intra-abdominal and surgically treatable source of infection, such as the perforation or inflammation of intra abdominal organs. [34-35]. Although the precise mechanism(s) underlying the development of SBP have not been fully clarified, bacterial translocation is believed to be the most important causative factor. In addition, several conditions frequently noted in cirrhotic patients, including alterations in gut flora, increased intestinal permeability and a compromised immune system have been reported to be involved in disease-related bacterial translocation and the subsequent onset of SBP [26].

As regards diagnosis of SBP, the ascitic fluid neutrophil count of more than 500 cells/µL is the single best predictor of spontaneous bacterial peritonitis, with a sensitivity of 86% and specificity of 98%. Lowering the ascitic fluid neutrophil count to more than 250 cells/µL results in an increased sensitivity of 93% but a lower specificity of 94%. (For simplicity, a threshold of 250 cells/µL) was used in this study with or without positive ascetic fluid culture) according to **lata *et al*** [19]. In this study ascetic fluid culture succeeded in diagnosis of 9/20 (45%) of SBP cases (monomicrobial infection by Ecoli,klebsiella and Enterococci) compared to neutrophils count, while 11/20 (55%) was CNNA with no significant difference. This was in agreement with **Enomoto *et al*** [26] who recommended to inoculate the ascitic fluid into blood culture bottles at the patient's bedside in order to increase the sensitivity of the bacterial culture approximately 80%, namely, between 72% and 90% of cases assessed using the culture-bottle method. However, **lata *et al*** [19] reported that 50% of patients with SBP were CNNA. It may be due to different stages of infection either early or late stages with bacterial colony count below the

detection limit despite use of bedside inoculation culture that improve the sensitivity. Nonetheless, these patients should be treated just as aggressively as those with positive culture results.

An exciting new development in the rapid diagnosis of spontaneous bacterial peritonitis is the proposed use of reagent strips that detect leukocyte esterase, which can be read at the bedside. In a pilot study done by **Enomoto *et al*** [26] that compared the reagent strips with the manual laboratory polymorphonuclear leukocyte count, the strips achieved a 100% sensitivity in diagnosis of spontaneous bacterial peritonitis this was in agreement with our results.This diagnostic method holds promise in replacing manual cell counting, which is time-consuming and is often unavailable in many laboratories "after hours". Use of these reagent strips may result in a significant reduction of the time from paracentesis to presumptive diagnosis and antibiotic treatment of spontaneous bacterial peritonitis.

In our study, culture results showed growth of enterobacterecae resistant strain,this was in accordance with **Dănulescu and his colleague**[36],they recommended that Empirical therapy of nosocomial SBP with third-generation cephalosporin was often inefficient due to the high prevalence of multiresistant (MR) bacteria.

In our study we compared FC between control and cirrhotic subjects with and without SBP. Median FC was higher when SBP was present. This difference was highly significant ($P <$ 0.01). A significant association emerged between elevated FC and SBP with mean value of (273±42mg/kg) and when we use FC in prediction of SBP, it had a sensitivity of 95% and specificity of 43% with PPV and NPV of 50% and 96% respectively at cut off value of 190mg/kg with AUC of 0.47. Therefore, FC may serve as a screening tool to identify cirrhotic patients with SBP. **Gundling and colleagues** [30] compared cirrhotic subjects with and without ascites, they found that the median FC was higher when ascites was present (79.3 Vs 65.8 mg/kg respectively). However, this difference was not significant ($P >$ 0.05). A sensitivity of 42 % and a specificity of 74% could by observed at an optimal cut off point of 123 mg/kg. In cirrhotic patients with SBP, median FC was higher than in patients without (275.4 Vs 48.5mg/kg respectively). Explorative data analysis of this association was significant [$P <$ 0.01]. At an optimal cut off point of 140 mg/kg the sensitivity and specificity were found to be 71% and 79% ($P < 0.01$). These results agree with **Yagmur *et al*** [31] who described in their study that the highest of all FC concentrations were present in cirrhotic patients with SBP.

Hepatorenal syndrome (HRS) is a unique form of acute kidney injury seen in patients with acute liver failure or chronic liver disease in absence of any other identifiable cause of renal failure. It is primarily a diagnosis of exclusion [37]. The development of bacterial infections, particularly SBP, is the most important risk factor for HRS [38-49]. HRS develops in approximately 30% of patients who develop SBP and treatment of SBP with albumin infusion together with antibiotics reduces the risk of developing HRS and improves survival [40-42]

To the best of our knowledge, no systematic multivariate analysis of cirrhosis-associated HRS and their relation to FC have been performed so far. In our study we compared FC between control and HRS subjects'.The mean value of FC was higher in HRS group. This difference was highly significant ($P < 0.01$). A significant association emerged between elevated FC and HRS with mean value of (450±5mg/kg) and when we use FC in prediction of HRS, it had a sensitivity of 90% and specificity of 88% with PPV and NPV of 90% and 97% respectively at cut off value of 340mg/kg with AUC of 0.94. Therefore, FC may serve as a screening tool to identify cirrhotic patients with HRS.

Comparison between studied groups as regard FC revealed that HRS group (450±5 mg/kg)had the highest level, followed by HE group(364±83 mg/kg),SBP group(273±42 mg/kg),liver cirrhosis group(76±15 mg/kg) and lowest level was in the control group(31.2±9 mg/kg).

Conclusions and Recommendations: FC was significantly elevated in cirrhotic patients and a significant correlation emerged between elevated FC and complications as HE, SBP and HRS. We recommend the use of FC as a simple, non-invasive and rapid screening test to make a diagnosis of these complications. However, further studies are needed to investigate FC prospectively in cirrhotic patients with SBP, HE and HRS before and after medical treatment to find out whether FC might be a useful parameter for monitoring the further course.

References

1. **Moore KP, Aithal GP.** Guidelines on the management of ascites in cirrhosis. *Gut* 2006; 55: 1–12.
2. **Almeida J, Galhenage S, Yu J, etal.** Gut flora and bacterial translocation in chronic liver disease. World J Gastroenterol 2006; 12(10):1493-502.
3. **Rasaratnam B, Connelly N, Chin-Dusting J.** nitric oxide and the hyperdynamic circulation in cirrhosis: is there a role for selective intestinal decontamination? Clin Sci (Lond) 2004; 107: 425-434.
4. **Gerova VA, Nakov VN, Stoynov SG, etal**. Prevalence of small intestinal bacterial overgrowth in patients with liver cirrhosis. Journal of GHR 2013; August 21 2(8): 740-743.
5. **Rasaratnam B, Connelly N, Chin-Dusting J.** Nitric oxide and the hyperdynamic circulation in cirrhosis: is there a role for selective intestinal decontamination? Clin Sci (Lond) 2004; 107: 425-434.
6. **Lakshmi CP, Ghoshal Uday C, Sunil K, etal.** Frequency and factors associated with small intestinal

bacterial overgrowth in patients with cirrhosis of the liver and extra hepatic portal venous obstruction. Dig Dis Sci 2010; 55: 1142-1148.

7. **Triger DR**. Endotoxemia in liver disease-time for re-appraisal? J Hepatol 1991; 12: 136-8.
8. **Sheron N, Bird G, Goka J, *et al*.** Elevated plasma interleukin-6 and increased severity and mortality in alcoholic hepatitis. Clin Exp immunol 1991; 84: 449-53.
9. **Gines P.** Diagnosis and treatment of hepatorenal syndrome. Bailliere's clinical Gastroenterology 2000; 14 (6): 945-57.
10. **McPhail MJ, Bajaj JS, Thomas HC, *et al*.** Pathogenesis and diagnosis of hepatic encephalopathy. Expert Rev Gastroenterol Hepatol 2010; 4: 365–78.
11. **Ferenci P, Lockwood A,Mullen K, *et al*.** Hepatic encephalopathy – definition, nomenclature, diagnosis, and quantification: final report of the working party at the 11th World Congress of Gastroenterology, Vienna, 1998. Hepatology 2002; 35: 716–721
12. **Gupta A, Dhiman RK, Kumari S, *et al*.** Role of small intestinal bacterial overgrowth and delayed gastrointestinal transit time in cirrhotic patients with minimal hepatic encephalopathy. J Hepatol 2010; 53: 849–55.
13. **Beşer OF, Sancak S, Erkan T, *et al*.** Can Fecal Calprotectin Level Be Used as a Markers of Inflammation in the Diagnosis and Follow-Up of Cow's Milk Protein Allergy? Allergy Asthma Immunol Res. 2014 January; 6(1):33-38.
14. **D' Inca R, Dal Pont E, Di Leo V, *et al*.** Can calprotectin predict relapse risk in inflammatory bowel disease? Am JGastroenterol 2008; 103: 2007–14.
15. **Schuppan D, Afdhal NH**: Liver cirrhosis. Lancet 2011; 371: 838–51.
16. **Berg T**: Diagnostik bei erhöhten Leberwerten. Der Gastroenterologe 2012; 4: 557- 72.
17. **Hofmann WP, Rädle J, Moench C, *et al***. Prediction of perioperative mortality in patients with advanced liver disease and abdominal surgery by the use of different scoring systems and tests. *Z Gastroenterol* 2011; 46: 1283–9.
18. **Ferenci P, Lockwood A, Mullen K, *et al*.** Hepatic encephalopathy – definition, nomenclature, diagnosis, and quantification: final report of the working party at the 11th World Congress of Gastroenterology, Vienna, 1998. *Hepatology* 2012; 35: 716–721.
19. **Lata J, Stiburek O, Kopacova M.** Spontaneous bacterial peritonitis: a severe complication of liver cirrhosis. World J Gastroenterol2009; Nov 28; 15(44):5505-10.
20. **Ginès P, Schrier RW**. Renal failure in cirrhosis. N Engl J Med 2012; 361:1279.
21. **Ginès P, Guevara M, Arroyo V, Rodés J**. Hepatorenal syndrome. Lancet 2013; 362:1819.
22. **Wadei HM, Mai ML, Ahsan N, Gonwa TA**. Hepatorenal syndrome: pathophysiology and management. Clin J Am Soc Nephrol 2012; 1:1066.
23. **Ginès A, Escorsell A, Ginès P, *et al***. Incidence, predictive factors, and prognosis of the hepatorenal syndrome in cirrhosis with ascites. Gastroenterology2012; 105:229.
24. **Arroyo V, Ginès P, Gerbes AL, *et al***. Definition and diagnostic criteria of refractory ascites and hepatorenal syndrome in cirrhosis. International Ascites Club. Hepatology2013; 23:164.
25. **Clinical & Laboratory Standard institute (CLSI) (2014):** performance standard for antimicrobial susceptibility testing, 24 informational standard M100, S24 document.
26. **Enomoto H, Inoue S, Matsuhisa A**. Diagnosis of peritonitis and an in situ hybridization approach to detect an "unidentified" pathogen. Int J Hepatol 2014; 617 -634.
27. **Miller MC, Knapp RG:** Clinical epidemiology and biostatistics, published by Williams & Wilkins, Maryland: 3rd edition 1992.
28. **Palma P, Mihaljevic N, Hasenberg T, *et al*.** Intestinal barrier dysfunction in developing liver cirrhosis: An in vivo analysis of bacterial translocation Hepatology Research 2007; 37(1):6-12.
29. **Gerova VA, Nakov VN, Stoynov SG.** Prevalence of small intestinal bacterial overgrowth in patients with liver cirrhosis. Journal of Gastroenterology and Hepatology Research 2013; 21 2(8):740-743.
30. **Gundling F, Schmidtler F, Hapfelmeier A, *et al*.**Fecal calprotectin is a useful screening parameter for hepatic encephalopathy and spontaneous bacterial peritonitis in cirrhosis. Liver international. 2011; 31(9):1406-1415.
31. **Yagmur E, Schnyder B, Scholten D, *et al*.** Elevated concentrations of fecal calprotectin in patients with liver cirrhosis. Dtsch Med Wochenschr 2006; 131: 1930–4.
32. **Montalto M, Gallo A, Ferrulli A, *et al*.** Fecal calprotectin concentrations in alcoholic patients: a longitudinal study. Eur J Gastroenterol Hepatol 2011; 23: 76–80.
33. **Li SW, Wang K, Yu YQ, *et al*.** Psychometric hepatic encephalopathy score for diagnosis of minimal hepatic encephalopathy in China. World J Gastroenterology 2013 ; 14; 19(46): 8745-8751.
34. **Wiest R, Krag A, and Gerbes A**. "Spontaneous bacterial peritonitis: recent guidelines and beyond,". Gut 2012; 61(2):.297–310.
35. **Piroth L, Pechinot A, Di Martino V, etal**. Evolving epidemiology and antimicrobialresistance in spontaneous bacterial peritonitis: a two-year observational study. BMC Infectious Diseases2014, 14:287:1-8
36. **Dănulescu RM, Stanciu C, Trifan A.** Assessing the risk of decompensation by ascites and spontaneous bacterial peritonitis in cirrhosis. Rev Med Chir Soc Med Nat Iasi 2014 ;118(2):320-6.
37. **Pandey CK, Karna ST, Singh A.** Hepatorenal syndrome: a-A decade later. Journal of the association of physicians of India 2014; 62:36-42.
38. **Fasolato S, Angeli P, Dallagnese L, *et al***. Renal failure and bacterial infections in patients with cirrhosis: epidemiology and clinical features. Hepatology 2007; 45:223–229.
39. **Terra C, Guevara M, Torre A, *et al***. Renal failure in patients with cirrhosis and sepsis unrelated to spontaneous bacterial peritonitis: value of MELD score. Gastroenterology 2005; 129: 1944–1953.
40. **Kopacova M.** Hepatorenal syndrome**.** World J Gastroenterol 2012; September 28; 18(36): 4978-4984
41. **Strauss E**. The impact of bacterial infections on survival of patients with decompensated cirrhosis. Annals of hepatology 2014; 13(1):7-19.
42. **Shizuma T.** Spontaneous Bacterial Peritonitis and Bacteremia in Patients with Liver Cirrhosis. J Med Microb Diagn 2014; 3:2:1-6.