The role of fecal calprotectin in assessment of hepatic encephalopathy in patients with liver cirrhosis

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Abstract

Introduction : **Calprotectin is a cytoplasmatic protein of neutrophilic granulocytes and it is an established marker for the assessment of localized intestinal inflammation.**

Aim : **To explore correlation between values of fecal calprotectin and degree of liver cirrhosis and hepatic encephalopathy.**

Methods : We included 60 patients with liver cirrhosis and 37 healthy patients as controls. Patients revealing other causes of abnormal calprotectin results (gastrointestinal bleeding or inflammatory bowel disease) were excluded. The degree of liver insufficiency was assessed according to the Child-Pugh classification and Model of End Stage Liver Disease (MELD), and degree of hepatic encephalopathy by West-Haven criteria, serum concentration of ammonium ion and the number connection test.

Results : The **mean value of fecal calprotectin in patients with liver cirrhosis was 189.1 ± 168.0 µg/g, and 35.0 ± 26.0 µg/g in the control group, respectively. We have confirmed significantly higher fecal calprotectin in patients with cirrhosis ($p < 0.001$). There were no significant differences in values of fecal calprotectin between the patients with different stages of liver cirrhosis according to Child-Pugh classification and MELD score ($p > 0.05$). We observed statistically significant difference comparing fecal calprotectin by West-Haven criteria of hepatic encephalopathy ($p < 0.001$), while there were no correlation with the number connection test and serum concentration of ammonium ion ($p > 0.05$).**

Conclusion : **We confirmed significantly higher values of fecal calprotectin in patients with liver cirrhosis, especially in hepatic encephalopathy according to West-Haven criteria.** (Acta gastroenterol. belg., **2014**, 77, **302-305**).

Key words : fecal calprotectin, liver cirrhosis, hepatic encephalopathy, West-Haven criteria.

Introduction

Over the past years, fecal calprotectin, has been investigated as biological marker of intestinal inflammation, especially in inflammatory bowel disease and less in liver cirrhosis.

The intestinal mucosa is continuously exposed to enormous amounts of microbes and their toxic products, among them are chemotactic substances such as endotoxins. This is probably the explanation for the concentration of calprotectin in normal intestinal environments being many times higher than that in blood. Magne Fagerhol and collaborators first described calprotectin in 1980 (1).

Calprotectin is a calcium and zinc-binding protein, representing more than 60% of the cytosolic proteins in neutrophils. The presence of calprotectin in feces quantitatively relates to neutrophil migration into the gastrointestinal tract (2). Therefore, it is considered as a valid marker of intestinal inflammation because it is released during cell activation and death (3). As the gastrointestinal tract of cirrhotic patients shows various alterations of its mucosal barrier including infiltrates of neutrophils, calprotectin might be a promising diagnostic parameter to diagnose the onset and course of hepatic encephalopathy and also spontaneous bacterial peritonitis (4).

The gut flora and bacterial translocation play an important role in the pathogenesis of hepatic encephalopathy. A recent study demonstrated that bacterial overgrowth is a responsible factor for minimal hepatic encephalopathy in cirrhotic patients (4). For example, bacterial overgrowth is common ranging between 30 until 64% and seems to represent one of the main factors to trigger bacterial translocation (5).

Except patients with inflammatory bowel disease and liver cirrhosis, increased levels of fecal calprotectin have also been described in colorectal neoplasia (6), microscopic colitis (7), bacterial diarrhea (8), after the use of non-steroidal anti-inflammatory drugs (9), in peptic ulcer (10), and gastric cancer (11). It is well known fact that abnormal levels of fecal calprotectin could be find in gastrointestinal bleeding.

The aim of our study was to explore correlation between concentration of fecal calprotectin and degree of liver insufficiency and hepatic encephalopathy.

Methods

We conducted a cross-sectional study of 60 patients with liver cirrhosis examined and treated between March and August 2013 at the Clinic for Gastroenterology and Hepatology, Clinical Center of Serbia, Belgrade. Healthy control group comprised of 37 age and gender-matched subjects. Diagnosis of liver cirrhosis was based on clinical clues from the patient's medical history, physical examination, laboratory tests, abdominal ultrasonography, transient elastography and whenever possible, on liver

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Table 1. — **West-Haven criteria for the diagnosis of Hepatic encephalopathy**

Stage	Distinguishing Features
0	No abnormality detected
1	Trivial lack of awareness Euphoria or anxiety Shortened attention span Impaired performance of addition
2	Lethargy or apathy Minimal disorientation for time or place Subtle personality change Inappropriate behavior Impaired performance of subtraction
3	Somnolence to semistupor, but responsive to verbal stimuli Confusion Gross disorientation
4	Coma (unresponsive to verbal or noxious stimuli)

histology. The degree of liver insufficiency was assessed among the Child-Pugh classification and Model of End Stage Liver Disease (MELD).

Hepatic encephalopathy was observed according to West-Haven criteria (Table 1) (12), serum concentration of ammonium ion and the number connection test. Because of the last one parameter, we included only patients without (Stage 0), or with first (Stage 1) or second (Stage 2) degree of hepatic encephalopathy through West-Haven criteria. Because patients with higher degree of encephalopathy have gastrointestinal bleeding, spontaneus bacterial peritonitis and other complication of end stage liver disaese which influence the calprotectin levels, this group of patients was excluded. Only patients with same treatment of encephalopathy (nutitional intervenction, peoral lactulose and antibiotics,parenteran branched-chained amino acids) were included.

The values of serum ammonium ion between 9 and 35 μmol/L were considerd as normal. Serum amonia level is usually elevated in patients with hepatic encephalopathy, yet the utility of this test is contreversial. Although this findings may aid in correctly diagnosing patients with cirrhosis who present with altered mental status, serial ammonia measurements are inferior to clinical assessment in improvement or deterioration in a patient under therapy for hepatic encephalopathy. The number connection test is a test of visuo-spatial orientation and psychomotor speed. The subject is shown a sheet of paper with 25 numbered circles which are randomly spread over the paper. The task is to connect the circles from 1-25 as quick as possible. Test result is the time needed by the subject including error correction time (Table 2) (13,14).

The *BÜHLMANN Quantum Blue® Calprotectin Assay* was used as an immunoassay designed for the quantitative determination of calprotectin in stool samples in combination with the *BÜHLMANN Quantum Blue® Reader*. Calprotectin values < 50 μg/g were considered as normal and not indicative of inflammation in the gastrointestinal tract (15).

Table 2. — **Normal values of number connection test**

Grades of hepatic encephalopathy	Completion time (s)
Grade 0	15 – 31
Grade 1	31 – 50
Grade 2	51 – 80
Grade 3	81 – 120
Grade 4	Can not be completed

All patients underwent gastroscopy and colonoscopy. Patients revealing other causes of abnormal fecal calprotectin results (gastrointestinal bleeding, inflammatory bowel disease, bacterial diarrhea, use of non-steroidal anti-inflammatory drugs, peptic ulcer and gastric or colon cancer confirmed by endoscopy) were excluded.

One-sample Kolmogorov-Smirnov and Shapiro-Wilk tests were performed to determine whether the data showed normal distribution. Mann Whitney test was used for categorical characteristics. Kruskal-Wallis test was applied to assess the influence of the investigated parameters if there is more than two observed groups. Post-hoc multiple comparison procedures with Bonferonni correction were performed to determine individual differences between the groups. Spearman's correlation procedure was performed to evaluate the relationship between different variables. The Statistical Package for Social Sciences version 17 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis, at the 0.05 level of significance.

Results

The patient group comprised 48 (80%) males and 12 (20%) females. The average age of the patients was 55.2 ± 15.1 years and mean BMI 28.6 ± 5.8. Alcoholic cirrhosis (70%) was the most common etiology (42/60). We detected no significant correlation of fecal calprotectin with age and BMI (Table 3), neither significant difference among the sex ($p > 0.05$).

Increased fecal calprotectin values in study group were detected in 44 patients (73.3%). The mean value of fecal calprotectin in patients with liver cirrhosis was 189.1 ± 168.0 μg/g (median 121.5 μg/g), while it was 35.0 ± 26.0 μg/g (median 31.0 μg/g) in the control group. Comparing these groups, we have confirmed significantly higher fecal calprotectin in patients with cirrhosis ($p < 0.001$).

The liver insufficiency degree, determined by generally accepted Child-Pugh classification, was divided in three stages : A 20 (33.3%), B 28 (46.7%) and C 12 (20%) patients. There were no significant differences in values of fecal calprotectin between the patients with different stages of liver cirrhosis ($p > 0.05$) (Table 4). Also there was no correlation with MELD score (Table 3), which average value in the study group was 14.7 ± 5.5 ($p > 0.05$).

Table 3. — **Corelation with fecal calprotectin**

	r_s[†]	*P* value
Age (years)	-0.055	0.774
BMI[‡]	0.071	0.708
MELD[§]	-0.007	0.972
Ammonium ion (μmol/L)	0.089	0.640
The number conection test (s)	0.119	0.531

[†] r_s : Spearman's rank correlation coefficient ; [‡] : Body mass index ; [§] : Model of end stage liver disease.

According to West-Haven criteria of hepatic encephalopathy there were 18 (30%) patients without abnormality detected (Stage 0), with average values of fecal calprotectin 66.5 ± 78.4 μg/g, while others with signs of encephalopathy in Stage 1 (26 patients, 43.3%) and Stage 2 (16 patients, 26.7%) had average values of 156.0 ± 143.0 μg/g and 380.7 ± 107.4 μg/g, respectively. There was statistically significant difference comparing fecal calprotectin by West-Haven criteria (p = 0.001). Post-hoc comparisons showed statistically significant differences between stages 0 and 2 ($p = 0.001$), and between 1 and 2 ($p = 0.003$), while there was no difference between 0 and 1 stages ($p > 0.05$).

Increased ammonium ion in study group were detected in 44 patients (73.3%), with average value of 54.4 ± 27.6 μmol/L. No significant differences was observed between levels of ammonium ion among Child-Pugh stages ($p > 0.05$) (Table 4), neither significant correlation with fecal calprotectin ($p > 0.05$) (Table 3).

Observing the third parameter in assessment of hepatic encephalopathy, the number connection test, we reported significant differences according to Child-Pugh classification ($p = 0.048$). Post-hoc comparisons showed statistically significant differences between stages A and C ($p = 0.020$), while there was no difference between A and B, and B and C stages ($p > 0.05$) (Table 4). However, we detected no relationship between fecal calprotectin and the number connection test ($p > 0.05$) (Table 3).

Discussion

While there is extensive literature about the diagnostic significance of fecal calprotectin in patients with inflammatory bowel disease, non-steroidal anti-inflammatory enteropathy and patients with irritable bowel disease, studies about the value of calprotectin in patients with cirrhosis are extremely sparse (16,17).

An altered gut flora and bacterial translocation are known to play an important role in the pathogenesis of certain complications of cirrhosis such as HE and SBP (18). Furthermore, qualities of calprotectin such as protein stability up to 7 days at room temperature make this test very attractive for daily routine (16).

Fecal calprotectin in cirrhosis was firstly investigated by Yagmur et al. who found significantly elevated values in patients with advanced disease and a trend towards higher levels of fecal calprotectin in subjects with alcoholic cirrhosis (19). Significantly increased levels of fecal calprotectin in patients with cirrhosis confirmed Gundling F. *et al*. in their study (4).

Patients in our control group were between 22 and 60 years, and mean values of fecal calprotectin responds to letarture date. In the study of Joshi S. *et al*. it was 34 μg/g in the healthy patients between 10 and 59 years (20).

Body weight does not play a big role in levels of fecal calprotectin. Comparing values of fecal calprotectin before and after weight loss in obese and overweight subjects didn't show significant difference (21).

We observed no correlation in calprotectin values and degre of liver function impairment, so further investigations are needed to give definite conclusions.

To the best of our knowledge, analysis of cirrhosis-associated complications, including hepatic encephalopathy and correlation to fecal calprotectin have been performed only in study of Gundling F. *et al*, where thay demonstrated high significant difference, with significantly elevated values of fecal calprotectin dependent on the severity of encephalopathy (4).

Patients with higher grade of encephalopathy (Stage 3 and Stage 4) were not included in the study due to possible influence of complications of end stage liver disease to calprotectin levels.

Among 12 patients (20%) with Child Pugh stage C of liver insufficiency in our reaserch, there was no spontaneus bacterial peritonitis diagnosed.

In our study there was no significant difference in values of fecal calprotectin between patients without hepatic encephalopathy and stage 1 according to West-Haven criteria. For assessing minimal hepatic encephalopathy, which would correspond to stage 1 of West-Haven criteria, it could be used critical flicker frequencies.

However we confirmed that fecal calprotectin is more sensitive marker of hepatic encephalopathy than serum ammonium ion and the number connection test.

Table 4. — **Fecal calprotectin, ammonium ion and connect the number test according to Child Pugh score**

	Child Pugh score (mean ± SD)			*P*
	A	B	C	
Fecal calprotectin (μg/g)	194.6 ± 180.5	171.8 ± 162.1	220.3 ± 186.4	0.842
Ammonium ion (μmol/L)	45.0 ± 29.3	56.4 ± 28.2	65.5 ± 21.7	0.295
The number connection test (s)	49.9 ± 10.4	59.9 ± 23.9	103.3 ± 78.6	0.048

Conclusion

We confirmed that concentration of fecal calprotectin is elevated in patients with cirrhosis, and there are significant correlation between its values and grade 0, 1 and 2 of hepatic encephalopathy according to West-Haven criteria.

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