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REVIEW ARTICLE

Calprotectin as Diagnostic Marker for Hepatic Encephalopathy and Spontaneous Bacterial Peritonitis in Cirrhosis

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SUMMARY

Background: **Calprotectin is a well-established marker for intestinal inflammation, mainly in inflammatory bowel disease, and represents one of the most studied biomarkers in stool samples.**
Methods: **Apart from its important diagnostic role in inflammatory bowel disease, there are few studies showing that calprotectin can also be used as a diagnostic tool in patients suffering from hepatic encephalopathy (HE) and spontaneous bacterial peritonitis (SBP) in cirrhosis.**
Results: **Since calprotectin concentration in the human stool or in ascites is elevated at an early stage of inflammation, it might serve as an early screening tool for patients suffering from cirrhosis who are at risk to develop these conditions. As detection and monitoring of HE and SBP may be unclear and resource-intensive, identification of valid new markers of disease activity is necessary. In this review, we summarize the current knowledge of calprotectin as a diagnostic biomarker in cirrhosis, indicating that it is a highly promising diagnostic surrogate marker to screen for the presence of HE and SBP.**
Conclusions: **To screen cirrhotic patients for SBP, calprotectin should be assessed in ascitic fluid while it should be measured in feces when screening for HE. However, the value of calprotectin in managing individual patients must be considered in the specific clinical context.**
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INTRODUCTION

The calcium-binding heterodimer calprotectin is an abundant cytosolic antibacterial and antimycotic protein which can be found in neutrophil granulocytes and monocytes [1].
The inhibitory effect of calprotectin on the growth of microbial pathogens is caused by a tight binding of transition metals e.g., manganese and zinc, resulting in reduced uptake and utilization by invading microbial agents [2].
In case of an intestinal inflammation, fecal calprotectin concentrations (FCCs) show a positive correlation with

inflammation activity because it is released during cell activation and cell death [3]. Since calprotectin can differentiate between inflammatory intestinal and non-inflammatory disorders, it can be used as a diagnostic marker for disease activity, mainly in inflammatory bowel disease [1].

The diagnostic value of FCCs, as a marker for mucosal inflammation and as a screening tool for identification of patients who need to undergo endoscopy, has been well examined in numerous studies in both adults and pediatric populations [1-11]. For example, in patients suffering from Crohn´s disease of the small bowel, a positive correlation between disease activity (as measured by leukocyte-scintigraphy) and elevation of FCCs could be shown [4]. Moreover, calprotectin is resistant to colonic degradation and can easily be measured in stool by performing an enzyme-linked immunosorbent assay using polyclonal antibody with high binding affinity [15-17]. Therefore, regarding its high stability and sensitivity, fecal calprotectin represents one of the most studied biomarkers in stool samples. However, studies analyzing the diagnostic value of calprotectin and FCCs in cirrhosis are extremely sparse [12-23].

Various alterations of the GI tract of cirrhotic patients have been demonstrated including modifications of its mucosal barrier [24-26]. Remarkably, cirrhotic patients in particular are susceptible to bacterial infections due to an increased intestinal permeability as the result of a disruption of the gut barrier allowing migration of bacteria and bacterial products to translocate from the intestinal lumen into extraintestinal organs. These conditions - an altered gut flora and bacterial translocation - are known to play an important role in the pathogenesis of certain complications of cirrhosis which are induced by infections, e.g., of the lung or urinary tract [21]. Furthermore, the gastrointestinal tract of cirrhotic patients shows various alterations of its mucosal barrier including infiltrates of neutrophils [26]. Since the occurrence of infections represents a main precipitating factor for the onset and course of hepatic encephalopathy (HE). Calprotectin concentration (e.g., in feces) might be a promising diagnostic parameter to diagnose the onset and severity of these conditions. It is important to recognize these complications and their early stages as soon as possible because adequate and subsequent treatment of HE and SBP reduces morbidity and mortality. The cornerstone of SBP diagnosis is the ascitic fluid analysis regarding polymorphonuclear leukocytes (PMN) cell count [27]. Since accumulation of granulocytes in ascites represents the source of calprotectin, ascitic calprotectin seems to be a potential surrogate marker for SBP. As detection and monitoring of HE and SBP may be unclear and resource-intensive, identification of valid new markers of disease activity is necessary. Since calprotectin concentration in the stool is elevated at an early stage of inflammation, it might serve as an early screening marker for patients suffering from cirrhosis who are at risk for developing HE and SBP. Furthermore, qualities of calprotectin, such as protein stability up to 7 days at room temperature, make this test very attractive for daily routine [1].

PubMed, Embase, and Scopus databases were searched to identify relevant studies published through May 2019. A comprehensive literature search was developed by an experienced medical reference librarian (M. B.). The subsequent literature search was conducted by two independent investigators (JR. A., L. H.). To find relevant publications, MEDLINE, Embase, and Scopus databases were searched through May 1, 2019. The MeSH and keyword search terms included: "calprotectin and cirrhosis," "calprotectin and ascites," and "calprotectin and hepatic encephalopathy". All identified records were screened based on their title and abstract, and the eligible articles were selected to be evaluated at the level of full text. Only English and German language articles were included. In addition, the bibliography of eligible articles was reviewed to identify more relevant studies [12-23].

Calprotectin in cirrhosis

When correlating elevated FCCs with the severity of cirrhosis, the published studies show conflicting results (Table 1, 12 - 23). In a pilot study, Homann et al. first described that high plasma calprotectin levels may characterize a group of cirrhotic patients prone to recurring bacterial infections [12]. However, the same authors found no association between increased plasma calprotectin concentrations and the severity of liver disease [14]. Furthermore, Homann et al. investigated the prognostic value of FCCs in 84 patients suffering from decompensated alcoholic cirrhosis. Interestingly, they were able to show a significant correlation between elevated plasma and ascitic calprotectin levels and overall survival of these patients since high calprotectin concentrations were significantly associated with poor survival [12].

Yagmur et al. also identified higher FCCs in 53 patients with mainly alcoholic cirrhosis compared to healthy controls. In contrast to the results of Homann et al., higher FCCs showed a significant correlation with the severity of cirrhosis (classified using Child Pugh-score). Of note, Yagmur et al. described the highest of all FCCs in two cirrhotic patients with SBP [15]. In studies performed by Gundling et al. and Alempijević et al. there were also higher FCCs in cirrhotic patients compared to healthy controls [17,18].

Burri et al. evaluated the value of fecal calprotectin in 55 patients with cirrhosis concerning its diagnostic accuracy to detect mucosal lesions in the upper intestinal tract including peptic ulcers and signs of portal hypertension compared to well-established endoscopic diagnostics. They could show only limited value of fecal calprotectin measurement since there was no positive correlation between the occurrence of mucosal lesions and the severity of cirrhosis (as assessed by Child Pugh-score) and elevated FCCs [16]. The authors pointed out that there are numerous factors influencing the diagnostic value of fecal calprotectin, including all processes

inducing pharmacological mucosal alterations of the small bowel, for example regular intake of non-steroidal anti-inflammatory drugs (NSAIDs) or active gastrointestinal bleeding [16]. This methodic difficulty has to be taken into consideration when interpreting elevated FCCs and may serve as one possible explanation for the partial discrepant results in the studies mentioned above. Regarding factors influencing the diagnostic value of fecal calprotectin, Poullis et al. showed, that - besides NSAIDs - the intake of proton pump inhibitors (PPIs) leads to significantly elevated FCCs. This effect appears to be independent of the dyspepsia for which the PPIs are prescribed [28].

Other factors which can affect the value of FCC interpretation are inflammatory bowel disease, colorectal cancer, and peptic lesions in the upper intestinal tract [1, 10,11,29-31]. Table 2 summarizes these major factors which can influence the value of FCC interpretation. Taking these factors into consideration in interpreting FCCs may often be a difficult process in daily clinical work, because different complications of cirrhosis frequently occur simultaneously.

Infectious complications in cirrhosis

Patients suffering from cirrhosis are at high risk of infectious diseases of every type, which do not only influence overall prognosis and survival, but also trigger multiple complications of chronic liver disease [32-35]. The great importance and relevance of immediate detection and treatment of these infections was clearly made by Arvaniti et al. They were able to show a four-fold higher mortality in case of bacterial infection in cirrhotic patients compared to cirrhotic patients without any bacterial infection [32].

A key point in the development of manifest infections seems to be an increased bacterial translocation, which occurs in almost all cases of cirrhosis and which can induce numerous frequent complications, e.g., ascites or hepatorenal syndrome [32].

Bacterial translocation itself is defined as migration of bacteria or bacterial fission products from intestinal lumen to extra-intestinal organs via mesenterial lymph nodes [33-35]. In the intestinal tracts of cirrhotic patients many immunological, structural, and microbiological changes have been identified, which enable increased bacterial translocation of infectious pathogens. This “leaky gut” seems to be the Achilles heel of patients suffering from cirrhosis and fecal calprotectin can be used as surrogate marker for its detection [34]. Increased concentrations of fecal calprotectin may indicate a disturbed intestinal barrier function in cirrhotic patients which could be of relevance for the diagnosis of associated complications [24].

Furthermore, qualities of calprotectin such as protein stability up to seven days at room temperature make this test very attractive for daily routine [1].

Hepatic encephalopathy and spontaneous bacterial peritonitis

Hepatic encephalopathy (HE), consisting of a complex of psycho-motoric symptoms, is one of the most serious complications of cirrhosis, which is often triggered or exacerbated by infectious processes [36-38]. Therefore, many therapeutical or preventive approaches in manifest or minimal HE focus on a reduction of bacteria in the intestinal lumen, for example the use of Rifaximine, Metroindazole or non-absorbable amino-glycosides [36-37].

HE is a frequent complication in cirrhosis with a high prevalence up to 80% of all cirrhotic patients including all stages [36-38]. Since this condition is associated with poor quality of life and a high risk of traffic violations and accidents resulting in a poorer prognosis compared to cirrhotic patients without HE, diagnostic screening must be performed routinely [38]. The valid diagnosis, especially in early stages, represents a major clinical problem. In the early stages of the condition, HE can be difficult to diagnose since patients may present with only mild cognitive impairment. As a result, HE seems to be an often overlooked condition since the possibilities to diagnose this condition in everyday practice are limited to only a few feasible methods. Therefore, there is a need for new and objective diagnostic markers for detection of HE, especially in early stages [39,40]. Several diagnostic systems have been used to diagnose the severity of HE clinically (e.g., West-Haven criteria) and technically using objective techniques such as critical flicker frequency (CFF) [39,40].

Infections are common among patients with cirrhosis and include e.g., spontaneous bacterial peritonitis, urinary tract infections, pneumonia, skin and soft tissue infections. Intestinal bacterial overgrowth is also common in cirrhosis resulting in hyperammonemia, which leads to hepatic encephalopathy. The presence of infections may serve as a trigger for hepatic encephalopathy which is due to bacterial translocation from the intestinal lumen that occurs as a consequence of intestinal bacterial overgrowth, increased permeability, and decreased motility. Small intestinal bacterial overgrowth is more often detected in cirrhosis than in healthy persons and is associated with some features of cirrhosis. In a recent meta-analysis of all studies performed on this topic, SIBO in cirrhosis was associated with ascites ($p < 0.001$), minimal hepatic encephalopathy ($p = 0.001$), bacterial translocation ($p = 0.026$), spontaneous bacterial peritonitis ($p = 0.008$), and prolonged orocecal transit time ($p < 0.001$) and was not associated with hypocoagulation [41].

Since increased concentrations of fecal calprotectin may indicate a disturbed intestinal barrier function in cirrhotic patients, this could be of relevance for the diagnosis of HE.

Another serious complication triggered by bacterial translocation in patients suffering from cirrhosis is spontaneous bacterial peritonitis (SBP). SBP defines the development of a monomicrobial infection of ascites in

Table 1. Summary of the studies regarding FCCs and cirrhosis.

Author	n	Results	Conclusion	Year
Yagmur et al. [15]	53 cirrhotic patients of different entities 18 healthy controls	significantly higher FCCs in cirrhotic patients significant association between FCCs and stage of liver disease (Child Pugh-score) trend towards higher FCCs in alcoholic cirrhosis compared to other entities no significant correlation of FCCs with systemic inflammatory parameters	elevated FCCs in cirrhotic patients as potential sign of intestinal inflammation	2006
Gundling et al. [17]	61 cirrhotic patients of different entities 42 healthy controls	significantly higher FCCs in cirrhotic patients significant correlation between FCCs and severity of liver disease (Child Pugh- and MELD-score) significant correlation between FCCs and HE grading (West-Haven criteria and CFF) and SBP higher FCCs in cirrhotic patients with additional extra-intestinal inflammation no influence of laboratory parameters of systemic inflammation on FCCs in cirrhotic patients	FCCs as screening tool for HE and SBP assessment of FCCs may facilitate grading of HE severity	2011
Alempijević et al. [18]	60 cirrhotic patients of different entities 37 healthy controls	significantly higher FCCs in cirrhotic patients no significant differences in FCCs between different stages of liver disease (Child Pugh- and MELD-score) significant correlation between FCCs and HE grading via West-Haven criteria no significant correlation between FCCs and HE grading via number connection test and serum concentration of ammonium ion	significantly higher FCCs in cirrhotic patients, especially in HE according to West-Haven criteria	2014
Burri et al. [16]	55 cirrhotic patients of different entities	higher FCCs in patients with peptic lesions in the upper GI tract than in patients without no correlation between peptic lesions and portal hypertension or Child Pugh-score	FCCs do not reliably identify mucosal lesions in the upper GI tract in cirrhotic patients only moderate diagnostic value of FCCs to detect mucosal lesions in cirrhotic patients	2011

Table 2. Factors, which can influence the value of FCC interpretation.

inflammatory bowel disease [1-11,21]
active gastrointestinal bleeding [20]
PPI-intake [20]
NSAID-intake and NSAID-enteropathy [16]
peptic lesions in the upper intestinal tract [22]
colorectal cancer [21-23]

the absence of a contagious source of infection. SBP is a frequent and serious complication of cirrhotic patients with ascites [42-45]. When first described, its mortality exceeded 90%, but in-hospital mortality has been reduced to approximately 20% with early diagnosis and prompt treatment [42-45].

An acute decompensation of cirrhosis might result in organ failure and short-term mortality (acute on chronic liver failure, ACLF). ACLF is characterized by a complex and multifactorial form of systemic inflammatory response which ends up in immunoparalysis predisposing the cirrhotic patient to secondary infectious events such as extraintestinal infections including pneumonia [46].

Table 3. Summary of the studies regarding calprotectin levels (in ascites, feces and serum) and cirrhosis.

Author	n	Results	Conclusion	Year
Lutz et al. [22]	120 ascites samples of 100 cirrhotic patients of different entities 8 patients with malignant peritoneal effusion as disease control	significantly lower calprotectin levels in samples without infection compared to SBP samples and malignant effusions higher calprotectin levels in Child Pugh stage B versus C and in alcoholic versus viral cirrhosis in non-infected ascites ratio of ascites calprotectin to total protein as a better marker for SBP than calprotectin alone	ratio of ascites calprotectin to total protein as a promising new diagnostic and prognostic marker in patients with liver cirrhosis and SBP	2015
Burri et al. [23]	130 ascites samples of 71 cirrhotic patients of different entities	positive correlation between polymorphonuclear (PMN) cell count and ascitic calprotectin levels	Ascitic calprotectin levels reliably predicts PMN cell count > 250/µL and therefore may prove useful in diagnosis of SBP	2013
Homann et al. [12]	180 cirrhotic patients of different entities 30 healthy controls	significantly higher plasma and ascites calprotectin levels in malignant disease compared to non-malignant disease association between high plasma and ascites calprotectin levels and increased mortality in decompensated cirrhosis lower plasma calprotectin levels in viral liver disease compared to non-viral liver disease and healthy controls plasma calprotectin level as highly significant marker of poor survival in alcohol-induced cirrhosis, no prognostic value in non-alcohol-induced cirrhosis	prognostic importance of calprotectin in alcohol-induced cirrhosis is specific for alcohol-induced liver disease low calprotectin levels are indicated in viral liver disease association between high ascites calprotectin levels and malignant ascites	2003
Homann et al. [13,14]	84 alcohol-induced cirrhotic patients 16 healthy controls	positive correlation between high plasma calprotectin levels and poor survival prognostic value of plasma calprotectin levels is independent of severity of liver disease (eight clinical and biochemical variables) higher prognostic value of plasma calprotectin levels than albumin, prothrombin complex, bilirubin, and ascites positive correlation between increased plasma calprotectin levels and recurrent infection no difference in plasma calprotectin levels when comparing healthy controls with patients with compensated cirrhosis and those with decompensated cirrhosis	plasma calprotectin as new prognostic marker of survival in alcoholic cirrhosis, which seems independent of severity of liver disease high plasma calprotectin levels may characterize a group of cirrhotic patients with recurring bacterial infections	1995 and 1996
Weil et al. [21]	236 ascites samples of 119 cirrhotic patients of different entities	significantly lower calprotectin levels in samples without infection compared to SBP samples no correlation of calprotectin levels with Child Pugh and Model for End-Stage Liver disease scores	dosage of ascites calprotectin is a rapid and reliable marker in patients with liver cirrhosis and SBP	2019
Fernandes et al. [19]	ascites samples of 88 cirrhotic patients of different entities	positive correlation between polymorphonuclear (PMN) cell count and ascitic calprotectin levels in samples without infection compared to SBP samples	Ascitic calprotectin levels reliably predict PMN cell count > 250/µL and therefore may prove useful in diagnosis of SBP	2016
Abdel-Razik et al. [20]	Ascites samples if 79 cirrhotic patients of different entities, mainly hepatitis B and C	Both serum procalcitonin and ascitic calprotectin were significantly higher in SBP patients than in non-SBP patients. In addition, at a cutoff value of 445 ng/mL, ascitic calprotectin had 95.4% sensitivity and 85.2% specificity for detecting SBP positive correlation with ascitic fluid proteins and PMN count.	Ascitic calprotectin levels reliably predict PMN cell count > 250/µL and therefore may prove useful in diagnosis of SBP	2016

Similar to HE, patients with SBP are frequently asymptomatic which implicates the danger of overlooking these complications in daily routine. Due to the increased mortality caused by SBP, it is a complication of cirrhosis, which also has to be diagnosed and sufficiently treated immediately. Up to date, diagnostic paracentesis and analysis of ascitic fluid is considered the diagnostic gold standard. Normally, diagnosis of SBP can be made when a polymorphonuclear (PMN) leukocyte cell count of more than 250/µL in ascites is determined by performing a differential cell count using light microscopy and counting chambers [42-45]. However, the diagnosis is time consuming and often delayed. Therefore, an accurate and convenient method of rapid diagnosis of SBP is needed. Even though diagnostic paracentesis is regarded as a safe procedure, there are undoubtedly complications inherent with the test. These include bleeding, visceral perforation, local infection and persistent leaks [39]. However, diagnostic alternatives are sparse. The use of diagnostic tools such as leukocyte esterase reagent strips, pH-testing or analysis of procalcitonin and lactoferrin of ascitic fluid is doubtful [47].

Both complications of elevated bacterial translocation - HE as well as SBP - have in common, that it is hard to set reliable and clear diagnosis in daily clinical work, especially in an early stage of disease. Moreover, many of the required diagnostic tools are not available in broad clinical care, for example CFF as an important objective diagnostic procedure for HE. Therefore, a simple, non-invasive, rapid, and cheap screening test to make a presumptive diagnosis of HE and SBP in cirrhotic patients would be really helpful in the clinical management of these complications of liver cirrhosis.

Calprotectin in diagnosis of HE and SBP

Knowing this background, the use of calprotectin (assessed in feces, serum or ascites) as diagnostic marker in patients suffering from cirrhosis is of great clinical interest, especially in detection of HE and SBP, which represent complications triggered by inflammatory processes. In the exclusive setting of HE and SBP, a significant correlation between elevated FCCs was shown by Gundling et al. and Alempijevic et al. when common factors influencing calprotectin values (like active gastrointestinal bleeding, chronic inflammatory bowel disease or colorectal carcinoma) were carefully ruled out [17,18].

One paper which was published from the author's group was able to show that there is not only a positive correlation of elevated FCCs with the severity of liver disease as assessed by Child Pugh- and MELD-score and with SBP, but also with the stage of HE as classified by both West-Haven criteria as well as CFF. When comparing FCCs in cirrhotic patients with healthy controls, there were significantly higher values in cirrhotic patients ($p < 0.001$). Among cirrhotic patients, FCCs were elevated dependent on the severity of liver disease as assessed by Child Pugh- and MELD-score. Furthermore, there was a significant correlation between elevated FCCs and HE grading as measured by West-Haven criteria and CFF. In this study, FCCs were higher in cirrhotic patients with additional extra-intestinal inflammation, while there was no influence of laboratory parameters of systemic inflammation on FCCs in the cirrhotic subgroup. Therefore, FCCs may serve as a screening tool for HE and SBP and assessment of FCCs may facilitate grading of HE-severity [17].

Our findings in this study were confirmed by the studies of Alempijević et al. when they examined the role of fecal calprotectin in the assessment of HE in patients with cirrhosis. In this study they also showed that there are higher FCCs in cirrhotic subjects compared to healthy controls, especially in HE according to West-Haven criteria [18].

Several studies have been performed showing a significant correlation between ascitic fluid calprotectin and PMN cell count. Remarkably, this correlation was shown in cirrhotic populations with different underlying etiologies of chronic liver disease. It could be demonstrated that FCCs in active-drinking alcoholics are not significantly different compared with the healthy controls, while FCCs do not significantly differ according to the alcohol drinking status [48]. Therefore, ascitic calprotectin represents an independent marker of SBP independent from the cause of cirrhosis.

Burri et al. were able to show that measurement of calprotectin levels in ascitic fluid correlates with the polymorphonuclear cell count and reliably predicts levels > 250/µL (Table 3) [23]. Confirming these findings, Lutz et al. showed that ascitic calprotectin levels were significantly lower in cirrhotic patients without SBP compared to those suffering from SBP or malignant effusions. In addition, ascitic calprotectin levels are higher in Child Pugh B compared to C and in alcoholic compared to viral cirrhosis [22]. Furthermore, they pointed out that the ratio of ascitic calprotectin levels to total protein levels is a better marker for SBP than calprotectin alone [38-41].

Fernandes et al. analyzed the accuracy of a point-of-care test (POCT) for ascitic calprotectin in diagnosing patients with SBP by using a quantitative POCT developed by Bühlmann [19]. Calprotectin levels correlated with PMN cell count and other inflammatory markers and were significantly higher in patients with SBP. An optimal cutoff of calprotectin above 1.57 µg/mL presented high sensitivity (87.8%), specificity (97.9%), and positive (97.3%) and negative (90.2%) predictive values for diagnosing SBP. Using calprotectin selectively in patients with a serum albumin-ascites gradient above 11 g/L further increased the sensitivity and negative predictive values of the test.

Abdel-Razik et al. performed a prospective study in cirrhotic patients with ascites to evaluate the impact of serum procalcitonin and calprotectin in ascitic fluid [20]. In their study, the majority of patients (n = 79) were suffering from cirrhosis due to chronic hepatitis B and C. Both serum procalcitonin and ascitic calprotectin

were significantly higher in SBP patients than in non-SBP patients. In addition, at a cutoff value of 445 ng/mL, ascitic calprotectin had 95.4% sensitivity and 85.2% specificity for detecting SBP while there was a positive correlation with ascitic fluid proteins and PMN count. Calprotectin was measured by an ELISA using Immundiagnostik AG ELISA kit (MRP 8/14; Immundiagnostik AG, Bensheim, Germany).

Weil et al. aimed to evaluate the accuracy of the dosage of calprotectin in ascitic fluid using the Quantum Blue assay for diagnosis of SBP [21]. In this prospective study, 236 ascitic fluid samples from 119 hospitalized cirrhotic patients were assessed in two French centers between May 2016 and May 2017. The Quantum Blue Reader selectively measured the calprotectin antigen within the measurable range from 0.18 to 1.80 µg/mL. SBP had significantly higher median levels of calprotectin than non-SBP while calprotectin levels were positively and significantly correlated with neutrophils in ascites and C-reactive protein but not with the Child-Pugh and Model for End-Stage Liver Disease scores. The authors conclude that the dosage of calprotectin in ascites using the Quantum Blue assay is a rapid and reliable method of ruling out SBP in hospitalized cirrhotic patients.

CONCLUSION

To summarize, measuring calprotectin in patients suffering from cirrhosis is a highly promising diagnostic tool to identify patients at a higher risk for developing HE or SBP. However, calprotectin was analyzed differently in the available published studies. Similar results were demonstrated in different studies for ascitic fluid, showing that calprotectin correlates well with PMN count. While the use of calprotectin in cirrhosis was investigated by analysis of measuring calprotectin in serum, feces, and ascites. Most studies evaluated the value of FCCs and ascitic calprotectin.

In most studies evaluating its role in screening for SBP, calprotectin was assessed in ascitic fluid. Since accumulation of granulocytes in ascites represents the cornerstone of SBP diagnosis and the source of calprotectin, ascitic calprotectin seems to be superior to its assessment in serum or feces. Therefore, ascitic calprotectin levels may also be a promising new diagnostic and prognostic marker in cirrhotic patients with SBP, especially with a readily available bedside testing device, which allows its measurement in settings with limited equipment and/or technical personnel.

In HE, calprotectin serves as a surrogate marker for an altered gut flora and bacterial translocation which are known to play an important role as a trigger factor for HE [19,20]. Since bacterial translocation from the gastrointestinal tract is a frequent source for HE, the assessment of calprotectin from feces seems to be superior to its assessment in serum. Regarding this complication of cirrhosis, calprotectin might be a valid parameter to diagnose the onset and severity of HE.

Of note, qualities of calprotectin such as protein stability up to 7 days at room temperature make this test very attractive for daily routine [1].

Nevertheless, in analogy to other surrogate markers like ammonia or neuronal markers (NSE, S100beta), calprotectin is not a specific marker for these complications and frequent factors influencing its levels have to be carefully taken into consideration. Additionally, cirrhotic patients often undergo conditions which can also cause (e.g., fecal or ascitic) calprotectin elevations independent from HE and SBP such as gastrointestinal bleeding or intake of certain drugs e.g., PPIs or NSAIDs. Therefore, the limitations of this diagnostic procedure and the danger of misinterpretation, e.g., in the case of variceal bleeding, must be carefully considered in specific clinical contexts when calprotectin is used in patients with cirrhosis. Furthermore, future prospective studies evaluating calprotectin before and after successful treatment of HE and SBP are needed.

Author Contribution:

B. M.: development of comprehensive literature search
A. JR, H. L.: subsequent literature search
G. F.: wrote the manuscript
S. W.: corrections of grammar and style of the manuscript

Declaration of Interest:

The authors declare that there is no conflict of interest regarding the publication of this paper.

References:

1. van Rheenen PF, Van de Vijver E, Fidler V. Faecal calprotectin for screening of patients with suspected inflammatory bowel disease: diagnostic meta-analysis. BMJ 2010;341:c3369 (PMID: 20634346).

2. Besold AN, Culbertson EM, Nam L, et al. Antimicrobial action of calprotectin that does not involve metal withholding. Metallomics. 2018; 10:1728-1742 (PMID: 30206620).

3. D'Inca R, Dal Pont E, Di Leo V, et al. Can calprotectin predict relapse risk in inflammatory bowel disease? Am J Gastroenterol 2008;103(8):2007-14 (PMID: 18802997).

4. Hoffmann JC, Preiss JC, Autschbach F, et al. [Clinical practice guideline on diagnosis and treatment of Crohn's disease]. Z Gastroenterol 2008;46(9):1094-146 (PMID: 18810679).

5. Montalto M, Curigliano V, Santoro L, et al. Fecal calprotectin in first-degree relatives of patients with ulcerative colitis. Am J Gastroenterol 2007;102(1):132-6 (PMID: 17100982).

6. Poullis A, Foster R, Mendall MA, Fagerhol MK. Emerging role of calprotectin in gastroenterology. J Gastroenterol Hepatol 2003; 18(7):756-62 (PMID: 12795745).

7. Gisbert JP, McNicholl AG. Questions and answers on the role of faecal calprotectin as a biological marker in inflammatory bowel disease. Dig Liver Dis 2009;41(1):56-66 (PMID: 18602356).

8. Shitrit AB, Braverman D, Stankiewics H, Shitrit D, Peled N, Paz K. Fecal calprotectin as a predictor of abnormal colonic histology. Dis Colon Rectum 2007;50(12):2188-93 (PMID: 17963005).

9. Schoepfer AM, Trummler M, Seeholzer P, Seibold-Schmid B, Seibold F. Discriminating IBD from IBS: comparison of the test performance of fecal markers, blood leukocytes, CRP, and IBD antibodies. Inflamm Bowel Dis 2008;14(1):32-9 (PMID: 17924558).

10. Tibble J, Teahon K, Thjodleifsson B, et al. A simple method for assessing intestinal inflammation in Crohn's disease. Gut 2000; 47(4):506-13 (PMID: 10986210).

11. Roseth AG, Aadland E, Jahnsen J, Raknerud N. Assessment of disease activity in ulcerative colitis by faecal calprotectin, a novel granulocyte marker protein. Digestion 1997;58(2):176-80 (PMID: 9144308).

12. Homann C, Christensen E, Schlichting P, Philipsen EK, Graudal NA, Garred P. Ascites fluid and plasma calprotectin concentrations in liver disease. Scand J Gastroenterol 2003;38(4):415-20 (PMID: 12739714).

13. Homann C, Garred P, Graudal NA, et al. [Plasma calprotectin. A new prognostic marker of survival in alcoholic liver cirrhosis]. Ugeskr Laeger 1996;158(21):2980-4 (PMID: 8686035).

14. Homann C, Garred P, Graudal N, et al. Plasma calprotectin: a new prognostic marker of survival in alcohol-induced cirrhosis. Hepatology 1995;21(4):979-85 (PMID: 7705809).

15. Yagmur E, Schnyder B, Scholten D, et al. [Elevated concentrations of fecal calprotectin in patients with liver cirrhosis]. Dtsch Med Wochenschr 2006;131(36):1930-4 (PMID: 16967390).

16. Burri E, Manz M, Rieder S, Beglinger C. Limited value of fecal calprotectin in patients with liver cirrhosis. Scand J Gastroenterol 2011;46(12):1527-8 (PMID: 21905979).

17. 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 Int 2011; 31(9):1406-15 (PMID: 22093455).

18. Alempijevic T, Stulic M, Popovic D, Culafic D, Dragasevic S, Milosavljevic T. The role of fecal calprotectin in assessment of hepatic encephalopathy in patients with liver cirrhosis. Acta Gastroenterol Belg 2014;77(3):302-5 (PMID: 25509200).

19. Fernandes SR, Santos P, Fatela N, et al. Ascitic Calprotectin is a Novel and Accurate Marker for Spontaneous Bacterial Peritonitis. J Clin Lab Anal. 2016;30(6):1139-45 (PMID: 27184969).

20. Abdel-Razik A, Mousa N, Elhammady D, et al. Ascitic Fluid Calprotectin and Serum Procalcitonin as Accurate Diagnostic Markers for Spontaneous Bacterial Peritonitis. Gut Liver. 2016 Jul 15;10(4):624-31 (PMID: 26601826).

21. Weil D, Heurgue-Berlot A, Monnet E, et al. Accuracy of calprotectin using the Quantum Blue Reader for the diagnosis of spontaneous bacterial peritonitis in liver cirrhosis. Hepatol Res. 2019; 49(1):72-81 (PMID: 30084186).

22. Lutz P, Pfarr K, Nischalke HD, et al. The ratio of calprotectin to total protein as a diagnostic and prognostic marker for spontaneous bacterial peritonitis in patients with liver cirrhosis and ascites. Clin Chem Lab Med 2015 Nov;53(12):2031-9 (PMID: 26035112).

23. Burri E, Schulte F, Muser J, Meier R, Beglinger C. Measurement of calprotectin in ascitic fluid to identify elevated polymorphonuclear cell count. World J Gastroenterol 2013;19(13):2028-36 (PMID: 23599621).

24. Alexopoulou A, Agiasotelli D, Vasilieva LE, Dourakis SP. Bacterial translocation markers in liver cirrhosis. Ann Gastroenterol. 2017;30(5):486-97 (PMID: 28845103).

25. Fukui H, Wiest R. Changes of Intestinal Functions in Liver Cirrhosis. Inflamm Intest Dis. 2016 Apr;1(1):24-40 (PMID: 29922655).

26. Wiest R, Lawson M, Geuking M. Pathological bacterial translocation in liver cirrhosis. Journal of Hepatology 2014 Jan;60(1): 197-209.

27. Wong F, Bernardi M, Balk R, et al. Sepsis in cirrhosis: report on the 7th meeting of the International Ascites Club. Gut 2005; 54(5):718-25 (PMID: 15831923).

28. Poullis A, Foster R, Mendall MA, Shreeve D, Wiener K. Proton pump inhibitors are associated with elevation of faecal calprotectin and may affect specificity. Eur J Gastroenterol Hepatol 2003;15(5):573-4; author reply 574 (PMID: 12702920).

29. Roseth AG, Fagerhol MK, Aadland E, Schjonsby H. Assessment of the neutrophil dominating protein calprotectin in feces. A methodologic study. Scand J Gastroenterol 1992;27(9):793-8 (PMID: 1411288).

30. Summerton CB, Longlands MG, Wiener K, Shreeve DR. Faecal calprotectin: a marker of inflammation throughout the intestinal tract. Eur J Gastroenterol Hepatol 2002;14(8):841-5 (PMID: 12172403).

31. Kristinsson J, Roseth A, Fagerhol MK, et al. Fecal calprotectin concentration in patients with colorectal carcinoma. Dis Colon Rectum 1998;41(3):316-21 (PMID: 9514426).

32. Arvaniti V, D'Amico G, Fede G, et al. Infections in patients with cirrhosis increase mortality four-fold and should be used in determining prognosis. Gastroenterology 2010;139(4):1246-56 (PMID: 20558165).

33. Wiest R, Garcia-Tsao G. Bacterial translocation (BT) in cirrhosis. Hepatology 2005;41(3):422-33 (PMID: 15723320).

34. Benten D, Wiest R. Gut microbiome and intestinal barrier failure--the "Achilles heel" in hepatology? J Hepatol 2012;56(6):1221-3 (PMID: 22406521).

35. Garcia-Tsao G, Wiest R. Gut microflora in the pathogenesis of the complications of cirrhosis. Best Pract Res Clin Gastroenterol 2004;18(2):353-72 (PMID: 15123075).

36. Dhiman RK, Saraswat VA, Sharma BK, et al. Minimal hepatic encephalopathy: consensus statement of a working party of the Indian National Association for Study of the Liver. J Gastroenterol Hepatol 2010;25(6):1029-41 (PMID: 20594216).

37. Ferenci P, Lockwood A, Mullen K, Tarter R, Weissenborn K, Blei AT. Hepatic encephalopathy-definition, nomenclature, diagnosis, and quantification: final report of the working party at the 11th World Congresses of Gastroenterology, Vienna, 1998. Hepatology 2002;35(3):716-21 (PMID: 11870389).

38. Vilstrup H, Amodio P, Bajaj J, et al. Hepatic encephalopathy in chronic liver disease: 2014 Practice Guideline by the American Association for the Study of Liver Diseases and the European Association for the Study of the Liver. Hepatology. 2014;60(2):715-35 (PMID: 25042402).

39. Sharma P, Sharma BC, Sarin SK. Critical flicker frequency for diagnosis and assessment of recovery from minimal hepatic encephalopathy in patients with cirrhosis. Hepatobiliary Pancreat Dis Int 2010;9(1):27-32 (PMID: 20133225).

40. Kircheis G, Fleig WE, Gortelmeyer R, Grafe S, Haussinger D. Assessment of low-grade hepatic encephalopathy: a critical analysis. J Hepatol 2007;47(5):642-50 (PMID: 17869373).

41. Maslennikov R, Pavlov C, Ivashkin V. Small intestinal bacterial overgrowth in cirrhosis: systematic review and meta-analysis. Hepatol Int. 2018 Nov;12(6):567-76 (PMID: 30284684).

42. Garcia-Tsao G. Current management of the complications of cirrhosis and portal hypertension: variceal hemorrhage, ascites, and spontaneous bacterial peritonitis. Gastroenterology 2001;120(3): 726-48 (PMID: 11179247).

43. Runyon BA; AASLD Practice Guidelines Committee. Management of adult patients with ascites due to cirrhosis: an update. Hepatology 2009;49(6):2087-107 (PMID: 19475696).

44. European Association for the Study of the Liver. EASL clinical practice guidelines on the management of ascites, spontaneous bacterial peritonitis, and hepatorenal syndrome in cirrhosis. J Hepatol 2010;53(3):397-417 (PMID: 20633946).

45. Koulaouzidis A, Bhat S, Saeed AA. Spontaneous bacterial peritonitis. World J Gastroenterol 2009;15(9):1042-9 (PMID: 19266595).

46. Laleman W, Claria J, Van der Merwe S, Moreau R., Trebicka J. Systemic Inflammation and Acute-on-Chronic Liver Failure: Too Much, Not Enough. Can J Gastroenterol Hepatol. 2018 Aug 1; 2018:1027152 (PMID: 30155448).

47. Nguyen-Khac E, Cadranel JF, Thevenot T, Nousbaum JB. Review article: the utility of reagent strips in the diagnosis of infected ascites in cirrhotic patients. Aliment Pharmacol Ther. 2008; 28(3):282-8 (PMID: 19086234).

48. Montalto M, Gallo A, Ferrulli A, et al. Fecal calprotectin concentrations in alcoholic patients: a longitudinal study. Eur J Gastroenterol Hepatol. 2011;23(1):76-80 (PMID: 21030869).

49. Leblhuber F, Geisler S, Steiner K, Fuchs D, Schütz B. Elevated fecal calprotectin in patients with Alzheimer's dementia indicates leaky gut. J Neural Transm (Vienna). 2015 Sep;122(9):1319-22 (PMID: 25680441).