REVIEW

Markers of bacterial translocation in end-stage liver disease

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Abstract

Bacterial translocation (BT) refers to the passage of viable bacteria or bacterial products from the intestinal lumen, through the intestinal epithelium, into the systemic circulation and extraintestinal locations. The three principal mechanisms that are thought to be involved in BT include bacterial overgrowth, disruption of the gut mucosal barrier and an impaired host defence. BT is commonly observed in liver cirrhosis and has been shown to play an important role in the pathogenesis of the complications of end stage liver disease, including infections as well as hepatic encephalopathy and hepatorenal syndrome. Due to the importance of BT in the natural history of cirrhosis, there is intense interest for the discovery of biomarkers of BT. To date, several such candidates have been proposed, which include bacterial DNA, soluble CD14, lipopolysaccharides endotoxin, lipopolysaccharide-binding protein, calprotectin and procalcitonin. Studies on the association of these markers with BT have demonstrated not only promising data but, oftentimes, contradictory results. As a consequence, currently, there is no optimal marker that may be used in clinical practice as a surrogate for the presence of BT.

Key words: End stage liver disease; Cirrhosis; Soluble CD14; Bacterial DNA; Lipopolysaccharides endotoxin; Procalcitonin; Bacterial translocation; Calprotectin; Biomarkers; Lipopolysaccharide-binding protein

Core tip: The exact mechanism behind bacterial translocation in patients with cirrhosis has not been fully elucidated. The discovery of reliable biomarkers for this phenomenon would be of significant clinical importance, as bacterial translocation is closely associated with the development of severe complications. Various molecules have been identified as candidates for serving as markers of bacterial translocation in this patient population. This mini-review attempts to summarize the most recent available data regarding the potential use of such markers as clinical and prognostic tools in the management of end-stage liver disease.

INTRODUCTION

Bacterial infections are frequent complications of cirrhosis and have been associated with significantly increased mortality rate[1]. Spontaneous bacterial peritonitis (SBP), urinary tract infections, pneumonia and sepsis are the most common infections, with gram-negative and gram-positive bacteria being equally detected as the causative organisms. In particular, cirrhotic patients with gastrointestinal bleeding have higher risk for developing bacterial infections during hospitalization and, thus, antibiotic prophylaxis is recommended in this scenario[2,3].

Bacterial translocation (BT) refers to the entry of viable bacteria or their products into the regional lymph nodes, the systemic circulation, and possibly extraintestinal organs. The origin of such microorganisms is the enteric flora and translocation occurs *via* a defective mucosal barrier[4]. BT is considered the key step in the pathogenesis of SBP and bacteremia in cirrhotic patients, as well as a critical factor that triggers host immune responses and secretion of inflammatory mediators, which, ultimately, mediate the hemodynamic changes that are present in portal hypertension and cirrhosis[5]. The main three mechanisms involved in BT include bacterial overgrowth, physical disruption of the gut mucosal barrier and an impaired host defence (Figure 1)[6]. In the present article, we will briefly review the pathogenesis of BT and analyze the literature regarding surrogate markers for this condition.

Pathogenesis of BT

Small intestinal bacterial overgrowth (SIBO) is multi-factorial and may be the result of defective gastric acid secretion and compromised small intestinal motility, as well as dysregulated mucosal and systemic immunity. The currently accepted criterion for the diagnosis of SIBO is the presence of $> 10^5$ colony-forming units/mL of coliform bacteria in aspirates from the proximal jejunum. Alternatively, breath tests have been used as sensitive and simpler tools for diagnosis of bacterial overgrowth, by measuring an increase in breath hydrogen or methane concentration, produced from intestinal bacterial fermentation after glucose or lactulose ingestion[7]. Experimental data has shown that cirrhotic rats with SIBO had a significantly higher rate of BT and slower intestinal transit than those without SIBO[8]. In clinical studies, the prevalence of SIBO in cirrhotic patients was found to be significantly higher than that in non-cirrhotic controls, and that it is related to the severity of liver disease[9]. Furthermore, the incidence of SIBO was higher in patients with a previous history of SBP than in SBP-naïve patients[10].

Structural and functional alterations of the gut mucosa that lead to increased intestinal permeability to bacteria and their products have been described in cirrhosis[11]. Bile secretions may also play a role in the prevention of BT by inhibiting bacterial overgrowth, exerting a trophic effect on intestinal mucosa and neutralizing endotoxin[12].

Increased intestinal permeability has been linked to the progression of liver disease and the complications of cirrhosis[13]. However, increased intestinal permeability cannot fully account for the pathophysiology of BT; moreover, it is not clear whether these structural changes are the cause or the result of BT[5].

The intestinal immune system is comprised of Peyer's patches, the mesenteric lymph nodes (MLNs) and a large number of cells distributed throughout the lamina propria and epithelium of the intestine[14,15]. Patients with cirrhosis exhibit systemic immune altera-tions that may promote the development of infections and BT. Advanced cirrhosis is associated with decrease in the cellular and humoral components of immune response, decreased activity of the reticuloendothelial system, decreased phagocytic capacity of Kupffer cells, as well as restricted recruitment of leucocytes in response to inflammatory stimuli due to portal hypertension-associated splanchnic hyperaemia[16-18]. The inflammatory response induced by BT, with the synthesis of cytokines, particularly tumor necrosis factor-alpha (TNF-α), interleukins and nitric oxide (NO) also increases intestinal barrier permeability, which, in turn, favours BT, thus creating a feedback in which BT promotes its own causative mechanisms[5].

Bacterial translocation is also associated with systemic complications and deterioration of the hyper-dynamic circulation in cirrhosis (Figure 1). Human studies have shown that cirrhotic patients with increased levels of lipopolysaccharide binding protein (LBP), a marker of BT, are found to have a significant immune and haemodynamic derangement, which is ameliorated by norfloxacin administration, by causing selective intestinal decontamination and inhibiting BT[19]. Additionally, the presence of bacterial DNA (bDNA), another marker of BT, in patients with cirrhosis and ascites, has been correlated with aggravation of peripheral vasodilation and with worsening of intrahepatic endothelial dysfunction[20]. Kidney tissue in cirrhosis shows increased expression of the toll-like receptor 4 (TLR4), nuclear factor κB (NF-κB), and TNF-α molecules, which makes the renal system further susceptible to the effects of cirrhosis during BT, highlighting the fact that BT contributes to the development of hepatorenal syndrome (HRS)[21]. Moreover, it has been reported that the non-absorbable antibiotic rifaximin improves systemic hemodynamics and renal function in patients with alcohol-related cirrhosis and ascites, by suppressing intestinal bacterial overgrowth and preventing BT[22]. It has been also suggested that cirrhotic patients may have compromised ability to upregulate sufficient dilatory forces [*i.e.*, endothelial nitric oxide synthase (NOS), inducible NOS, and heme oxygenase-1] to counterbalance the constrictive effect of endothelin-1 upon a secondary insult of endotoxemia. This is indicated by the presence of lipopolysaccharides (LPS) endotoxin during BT, and the net effect of this phenomenon is the establishment of increased intrahepatic resistance[23]. Endotoxemia may also be a trigger factor for variceal bleeding,
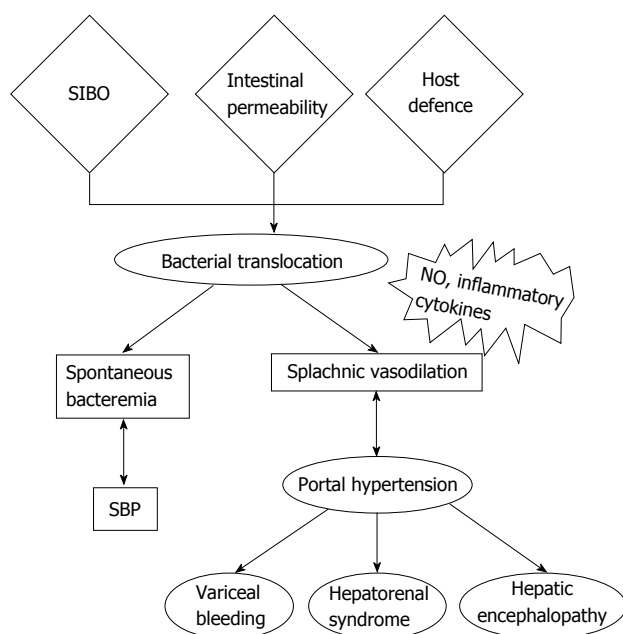
Figure 1 The main three mechanisms involved in bacterial translocation include intestinal bacterial overgrowth, increased intestinal permeability to bacteria and an impaired host defence. BT is considered the key step in the pathogenesis of bacteremia and SBP in cirrhotic patients, as well as a critical factor that triggers host immune responses and secretion of inflammatory mediators from macrophages, hepatocytes and other cells which, ultimately, mediate the hemodynamic changes that are present in portal hypertension and cirrhosis. BT contributes to the development of hepatorenal syndrome, and it may also be a trigger factor for variceal bleeding. Infection and the resulting systemic inflammatory response are considered important factors contributing to worsening hepatic encephalopathy in cirrhotic patients. SIBO: Small intestinal bacterial overgrowth; SBP: Spontaneous bacterial peritonitis; NO: Nitric oxide; BT: Bacterial translocation.

either by worsening liver function or causing an acute increase in portal hypertension[24]. Infection and the resulting systemic inflammatory response (SIRS) are considered important factors contributing to worsening hepatic encephalopathy (HE) in cirrhotic patients[25]. BT-associated inflammatory response may therefore have a role in the pathogenic mechanisms involved in HE. Consistent with this are the results from studies showing either improvement of minimal encephalopathy in cirrhotic patients receiving rifaximin, or the effectiveness of probiotics for secondary prophylaxis of HE, overall suggesting that BT plays an important role in the pathogenesis of HE[26].

Considering the eminent role of BT in the progression of liver disease and the subsequent complications of cirrhosis, it is not surprising that the elucidation of the underlying mechanisms as well as the discovery of possible markers of BT, that may be easily measured and have prognostic value for the severity of liver disease, has been at the center of attention of many research groups. In this review, we aimed to summarize the most prominent of the proposed markers of BT, such as bDNA, LPS, LBP, sCD14, calprotectin and procalcitonin (Table 1).

Bacterial DNA

The occurrence of bacterial-derived material in extrain-

testinal locations has been long recognized in clinical and experimental cirrhosis. At the time of liver surgery, one third of cirrhotic patients with Child-Pugh stage C demonstrate infected MLNs; this percentage is reduced to the level of non-cirrhotic patients after selective intestinal decontamination[27]. In contrast, most of BT episodes in cirrhotics remain undetected as its diagnosis relies on blood or ascitic fluid cultures, which are more often negative than positive. This may be the result of bacterial opsonization which renders bacteria nonviable in routine cultures. Despite the process of opsonization, bacterial components might remain in biological fluids and could be detected with more sensitive analytical methods. Taken together, these findings emphasize the need for the discovery of novel and reliable markers with high sensitivity and specificity for the diagnosis of BT[28].

The application of bDNA as a marker of BT was initially shown in animal models of experimental cirrhosis. Specifically, in cirrhotic rats the presence of bDNA of a certain bacterial species in blood, ascites or pleuritic fluid was always associated with its simultaneous presence in MLNs. Moreover, the presence of bDNA was associated with marked inflammatory responses[29]. Importantly, these findings occurred independently of the blood culture status (positive or negative).

Several studies in humans have now tested the validity of molecular detection of bDNA as a surrogate marker of BT. Using a polymerase chain reaction (PCR) - based method, Such *et al*[28] reported that bDNA in serum and ascitic fluid was present in 32% of cirrhotic patients with culture negative ascites, and that this likely represented episodes of single clone translocation and systemic seeding. *E. coli* was the most frequently identified microorganism, while *S. aureus* was responsible less frequently. The same group showed that bacteria persist in the blood of cirrhotic patients during variable periods of time after the completion of therapeutic paracentesis, therefore suggesting that this phenomenon is related to the existence of repeated episodes of BT from the intestinal lumen. The presence of identical sequences of nucleotides in all bDNA PCR fragments detected in every patient, strongly supports the existence of the repeated episodes of BT being caused by the same bacteria specie[30]. The presence of bDNA in patients with decompensated cirrhosis has also been associated with marked activation of peritoneal macrophages, as evidenced by NO synthesizing ability along with enhanced interferon-γ, TNF-α, interleukin (IL)-2, and IL-12 production[31]. Serum and ascitic fluid TNF-α levels were significantly higher in patients with bDNA compared to those without this marker on admission. Additionally, the relative risk of death, HRS and SBP was higher in patients with bDNA[32]. A sub-group of patients with translocation of Gram-positive microorganisms showed increased proinflammatory cytokines unrelated to endotoxin[33]. Furthermore, the presence of bDNA in patients with cirrhosis during an episode of ascites was shown to be an indicator of poor prognosis. When

Table 1 Biological markers for assessment of bacterial translocation in liver cirrhosis

	Origin	Comments	Ref.
Bacterial DNA	Bacteria	Pros: Long half-life, association with cytokine production, hemodynamic changes and prognosis Cons: Variable rates of detection (maybe depending on methodology used), lower prevalence in outpatients	Bellot *et al*[20], Francés *et al*[31], El-Naggar *et al*[32], González-Navajas *et al*[33], Zapater *et al*[34], Jun *et al*[35], Fagan *et al*[36] Rincón *et al*[39], Sersté *et al*[40], Feng *et al*[41], Fujita *et al*[42], Vlachogiannakos *et al*[43], Mortensen *et al*[44], Mortensen *et al*[45], Appenrodt *et al*[46]
LPS	Gram (-) bacteria	Pros: Correlation with TNF-α, stage of cirrhosis, prognostic value for severity of liver damage Cons: Short half-life, variation of detection rates	Hanck *et al*[52], Lin *et al*[54], Chan *et al*[55] Bellot *et al*[5], Fukui *et al*[53], Kaser *et al*[56], Stadlbauer *et al*[57]
LBP	Acute phase protein triggered by LPS	Pros: Long half-life, correlation with TNF-RI, TNF-α, IL-6, and hyperdynamic circulation Cons: Low detection rates, elevated in systemic infection from Gram (-) bacteria	Albillos *et al*[19], Albillos *et al*[59] Albillos *et al*[19], Albillos *et al*[59]
sCD14	Monocytes, liver	Pros: Prognostic marker of disease progression in HBV/HCV/HIV, NAFLD and alcoholic liver disease, correlation with liver fibrosis, easily measured	Landmann *et al*[61], Tuomisto *et al*[62], Sandler *et al*[63], Balagopal *et al*[64], French *et al*[65], Ogawa *et al*[66], Campos *et al*[67]
Calprotectin	Neutrophils	Pros: Easily measured, fecal levels associated with stage of liver disease, SBP and HE, ratio of ascites calprotectin/total protein may be better Cons: Plasma levels do no distinguish cirrhotic patients from healthy controls, weak association with alcoholic liver disease	Gundling *et al*[77], Lutz *et al*[81] Homann *et al*[75], Homann *et al*[76], Montalto *et al*[78]
Procalcitonin	Neutrophils, liver, thyroid	Pros: Ascitic levels may differentiate between cirrhotic subgroups Cons: No correlation with HE, conflicting results depending on etiology of liver disease	Attar *et al*[97] Spahr *et al*[92], Elefsiniotis *et al*[94], Rahimkhani *et al*[95], Villarreal *et al*[96]
ANCAs (IgA)	Neutrophils	Pros: Associated with ascites and advanced cirrhosis, predicts time to the first infectious complication Cons: Single study	Papp *et al*[99]

LPS: Lipopolysaccharides; HE: Hepatic encephalopathy; SBP: Spontaneous bacterial peritonitis; LBP: Lipopolysaccharide binding protein; ANCA: Anti-neutrophil cytoplasmic antibodies; TNF-α: Tumor necrosis factor-alpha; IL: Interleukin; HCV: Hepatitis C virus; HBV: Hepatitis B virus; HIV: Human immunodeficiency virus; NAFLD: Non-alcoholic fatty liver disease.

considering only patients with MELD score < 15, mortality was significantly higher in those positive for bDNA. In this study, SBP developed independently of the bDNA status at admission[34]. The presence of bDNA was also associated with peripheral vasodilation and deterioration of intrahepatic endothelial function[20]. Another study highlighted the strong correlation between SIBO and the presence of bDNA in the peripheral blood of patients with cirrhosis[35]. Moreover, the high bDNA detection frequency was recently confirmed as it was shown that it was detected in ascitic fluid from 23 of 25 patients with culture-negative, non-neutrocytic ascites. Again, bDNA levels were a poor prognostic factor for a 6-mo clinical outcome. High bDNA burden was also associated with reduced major histocompatibility complex class Ⅱ expression on macrophages isolated from ascites[36-38].

Studies on the importance of bDNA detection in cirrhotics have occasionally created contradictory results. bDNA was identified by gel electrophoresis of a multiplex PCR-based product which amplified selected prokaryotic nucleic acids and was detected in 5/5 culture-positive neutrocytic, 1/6 culture-negative neutrocytic and 8/56 culture-negative non-neutrocytic samples. Three-month mortality was increased in the presence of ascitic bDNA only for patients with a MELD score > 15[39]. Contrary to hospitalized patients, bDNA was rarely detected in ascitic fluid and serum of asymptomatic

outpatients with cirrhosis and non-neutrocytic ascites, after repeated paracentesis[40]. This finding may account for the markedly low prevalence of SBP in cirrhotic outpatients, when compared with their hospitalized counterparts. In another study, bacterial-specific 16S ribosomal RNA was not detected in blood samples from systemic or splanchnic circulation of cirrhotic patients on days 0 and 29 after rifaximin administration. In this study, plasma bDNA concentration did not correlate with systemic hemodynamic parameters[43]. Furthermore, bDNA did not correlate with markers of inflammation, such as C-reactive protein, TNF-α, IL-6 and IL-8. Additionally, it did not accurately predict the presence of SBP[44]. bDNA was also found to be largely unrelated to a panel of markers of inflammation and without association with portal pressure in patients with cirrhosis undergoing transjugular intrahepatic portosystemic shunt insertion[45]. No correlation between detection of bDNA in ascites and SBP was found[46]. In another study, administration of a probiotic mixture improved the hepatic and systemic haemodynamics in cirrhotic patients, but these changes were not related to the detection of bDNA[39].

In all, these contradictory results may be accounted for by differences in the tested populations and, also, in the specific methodologies used for the detection of bDNA[41,42]. These discrepancies obviate the need

for larger studies which will include well-characterized patient subpopulations, including chronic hepatitis, non-alcoholic fatty liver disease (NAFLD), as well as compensated and decompensated cirrhosis. Moreover, standardisation of methodology is required in order to determine the applicability of bDNA usage in clinical practice as a surrogate marker for BT. Finally, testing of larger cohorts of patients may define the significance of detectable bDNA as a prognostic tool for systemic responses and survival in cirrhosis.

LPS-LBP-sCD14

LPS or endotoxin is a lipopolysaccharide, which is part of the outer membrane of Gram-negative bacteria. In circulation, it is recognized by LBP. The LPS-LBP complex binds to membrane CD14 (mCD14) on myeloid cells or to circulating CD14 (soluble CD14, sCD14)[47,48] and promotes a cascade of inflammatory responses *via* myeloid differentiation-2/TLR4 activation of NF-κB[49,50]. Kupffer cells, which are specialized macrophages located in the liver, are activated when exposed to LPS and can produce a spectrum of cytokines and reactive oxygen intermediates, including the proinflammatory cytokine TNF-α. Thus, LPS is a potent stimulator of Kupffer cell TNF-α production, and this pathway has been implicated in the pathogenesis of many types of liver injury. It has also been proposed that through this mechanism hepatic stellate cells are activated towards the production of inflammatory and adhesion molecules, thus inflicting liver damage[51].

Due to its aforementioned properties, LPS was one of the first molecules that was proposed as a marker of BT. The working hypothesis is that its presence in the sera of cirrhotic patients directly indicates endotoxemia from Gram negative bacteria. In alcoholic cirrhosis, LPS has been found to correlate with TNF-α levels and with the stage of liver disease (Child-Pugh score); thus, it has been suggested that LPS may be a key player in the progression of alcoholic liver disease[52]. Levels of LPS in plasma of patients with alcoholic cirrhosis were higher than in non-alcoholics, pointing to a critical role for alcohol consumption in the development of endotoxemia and liver damage[53]. Taking into consideration that LPS plasma levels of cirrhotic patients are elevated compared to chronic hepatitis and the reported correlation with the stage of cirrhosis, it is probable that LPS may serve as a prognostic factor of disease severity[54] and short-term survival of cirrhotic patients[55]. It should be noted, however, that not all studies have demonstrated a positive correlation between serum LPS and stage of cirrhosis[53,56]. Therefore, LPS has not been clearly established as a reliable marker of BT[57]. This may be due to the short half-life of LPS or to the interference of various factors with the detection of LPS[5].

LBP is a 65 kDa, acute phase protein that is predominantly produced in the liver by hepatocytes. Bacterial LPS triggers the production of LBP and the peripheral levels of LBP are significantly elevated in the setting of bacterial presence. LBP is known to specifically bind and transfer bacterial LPS, and the LPS-LBP complex binds mCD14 on myeloid cells or to sCD14[58]. Consequently, several studies have tested the validity of the expression of LBP as an indicator of a systemic response to LPS, and, thus, as an indirect marker of BT. Albillos et al[19] demonstrated that BT, expressed by elevated plasma levels of LBP, leads to derangement of patients with decompensated cirrhosis. Cirrhotic patients with high LBP levels had enhanced expression of sCD14 and sTNF-RI as well as elevated circulating levels of TNF-α and IL-6. Furthermore, an intense hyperdynamic circulatory state was described in this population. Interestingly, LPS was detectable only in one third of the patients with high LBP. The latter observation strengthens the suggestion that LPS cannot easily serve as a marker of BT, due to its short half-life, so transient episodes of bacteremia may remain undiagnosed. On the other hand, the subsequent expression of LBP after endotoxemia is detectable for a much longer period. Moreover, a study performed by the same group suggested that patients with high LBP circulating levels have increased susceptibility to infections[59]. Different studies have proposed that increased levels of LBP in cirrhosis are the result of chronic endotoxemia and that also no difference occurs in the expression of LBP between alcoholic and non-alcoholic cirrhosis. Kaser et al[56] reported a strong correlation between the two binding proteins of LPS, LBP and sCD14. Although its longer half-life makes LBP a more attractive marker of BT than LPS, its use has certain drawbacks. First, systemic LBP elevation exists only in response to Gram-negative bacteria, and, second, it may not only be present in BT, but also in systemic infection resulting in SIRS (systemic inflammatory response syndrome)[60].

Recently, the target molecule of the complex LPS-LBP, CD14 was also proposed as a surrogate marker of BT. mCD14 is expressed mainly by macrophages and at a lesser extent by neutrophils and dendritic cells. sCD14 is secreted by hepatocytes and monocytes. The expression of sCD14 is upregulated as a result of the presence of LPS. CD14 acts as a co-receptor along with TLR-4 for the detection of bacterial LPS[61]. LPS induces the CD14/TLR4 complex endocytosis in human monocytes and macrophages and the consequent NF-κB activation. Tuomisto et al[62] showed a significant correlation between the levels of bDNA in the liver and the local expression of CD14 in alcoholic liver disease. These findings emphasize the role of CD14 as a marker of BT. Sandler et al[63] proposed that sCD14 may not only be a marker of BT, but also a prognostic marker of disease progression in HBV and HCV infection. Specifically, sCD14 in patients with severe fibrosis was highly elevated not only in peripheral blood, but also within the hepatic parenchyma, measured by CD14 (+) hepatic cells. Regardless of the etiology of cirrhosis, microbial translocation as identified by the presence of sCD14, is believed to play a key role in the progression of liver disease[56]. Studies concerning cirrhosis in HIV-infected patients support this hypothesis[64]. French et al[65] provided evidence

that in HIV/hepatitis C virus (HCV)-coinfection, levels of sCD14 and IL-6 were mostly elevated in patient with disease progression than in non-progressors. Studies in patients with NAFLD reached similar results, suggesting a positive correlation between serum sCD14, hepatic CD14 expression and liver inflammation[66]. Attention has also been drawn towards polymorphisms of the promoter region of the *CD14* gene, shedding light to the role of CD14/-159TT genotype in the progression of liver injury in alcoholic liver disease[67]. Taking into account the aforementioned studies, it appears that sCD14 may represent a promising marker of BT as it can easily be measured in circulation and correlates with progression of liver disease.

Calprotectin - procalcitonin - anti-neutrophil cytoplasmic antibodies

Calprotectin is a calcium and zinc binding protein with a molecular weight of 36 kDa[68]. It has been estimated that it may account for more than 60% of the soluble cytosolic proteins in human neutrophil granulocytes[69]. Moreover, it was shown that calprotectin measurements in fecal samples not only correlate with the degree of neutrophil migration in the gastrointestinal mucosa[70], but also serve as reliable surrogate marker of intestinal inflammation[71]. As a result, fecal calprotectin has been studied in depth in gastrointestinal disorders, and has assumed an important role in monitoring the activity and response to treatment in patients with inflammatory bowel disease[72,73].

The pathogenesis of BT in patients with cirrhosis has been associated with alterations in gut mucosal immune responses and intestinal permeability. In addition, neutrophil infiltrates are detected in the gastrointestinal mucosa of cirrhotics. Consequently, calprotectin has been investigated as a possible diagnostic marker for the existence and natural history of SBP and HE. Initial studies conducted by a Danish research group, focused on the possible prognostic significance of calprotectin levels in the plasma and ascitic fluid samples from patients with end-stage liver disease. The authors did not find a significant difference between healthy controls and patients with cirrhosis (irrespectively if liver disease was compensated or decompensated), a finding that was confirmed in additional studies[74,75]. On the other hand, they reported that high plasma calprotectin levels were an indicator of poor survival in alcohol-related cirrhosis[74]. The most important finding, however, regarding the role of calprotectin in relation to BT, was that during follow up of the patients higher calprotectin levels were an independent predictor of recurrent bacterial infections[76].

Arguably the most important study regarding the role of fecal calprotectin in the diagnosis of BT complications, is the one conducted by Gundling *et al*[77]. They investigated the relationship between fecal calprotectin levels and the onset and course of SBP and HE. They confirmed that patients with cirrhosis had significantly elevated fecal calprotectin levels

when compared to healthy controls. Moreover, this increase correlated with the severity of liver disease (assessed by Child-Pugh and MELD scores). Even more significantly, higher calprotectin values were associated with advanced stages of HE, the presence of SBP, as well as extraintestinal infections. Finally, calprotectin strongly correlated with serum ammonia levels[77]. The aforementioned observations reinforced the hypothesis that fecal calprotectin may be a reliable surrogate marker for BT and provide important assistance in the diagnosis and clinical management of patients with decompensated liver disease. It should be noted that the finding of a possible selective calprotectin upregulation in alcoholic liver disease that was reported in initial studies, was not confirmed in a longitudinal study of active alcoholics, where alcoholics and controls had similar fecal calprotectin measurements[78].

It was recently reported that ascitic calprotectin may be utilized (with the help of a point-of-care assay) to reliably predict an elevated polymorphonuclear count (> 250) in ascitic fluid, allowing for faster diagnosis of SBP[79]. Another study by Alempijević *et al*[80] focusing exclusively on HE confirmed that fecal calprotectin levels were positively correlated with HE grading according to the West-Haven grouping criteria, although it did not show a correlation with serum ammonia levels as Gundling *et al*[77] did. Finally, the ratio of ascites calprotectin to total protein was proposed as a better marker than ascitic fluid calprotectin alone for use in the diagnosis and prognosis of SBP. The authors report satisfactory sensitivity and specificity for this new marker, as well as a statistically significant correlation of higher values with poor 30-d survival[81].

In all, calprotectin remains a promising surrogate marker for BT in cirrhosis. It demonstrates many advantages, especially in its fecal measurement, as it is a non-invasive, quick and relatively easy to perform assay, with proven clinical value in other disease states[82].

Procalcitonin (PCT) is a 116 amino acid propeptide of calcitonin. It has been established as a valuable biomarker for the diagnosis and monitoring of bacterial infections to the point that is being used as a guide for antibiotic use[83]. Concerning advanced liver disease, PCT has been studied for the past 15 years regarding its potential for the diagnosis of SBP in decompensated patients and subsequently for its utility as an indirect marker of BT. As the liver is believed to be a key source of PCT, there were initially concerns that hepatic impairment may result in downregulated serum PCT levels. Although, this was not proven to be the case[84], and early reports were encouraging about the use of PCT in the diagnosis of SBP, its potential role remains currently unclear as several studies provided conflicting results[85-93]. A solution has been proposed with the use of an ultra-sensitive PCT assay[87], and possible explanations for the discrepancies noted between studies include higher baseline levels in patient with alcoholic[94] or specific viral-related[95] causes of cirrhosis and the

presence of other bacterial infections[96]. Furthermore, it was reported that PCT levels in the ascitic fluid, but not in serum may differentiate between different cirrhotic subgroups, reflecting a possible localized role in the interplay between ascites and BT[97]. Interestingly, in contrast to calprotectin, PCT has not been found to correlate with the presence of HE, another possible important component of the BT phenotype in cirrhosis. It should be also noted, that in several of the PCT-related studies the role of CRP was assessed as well and it was usually found to have an inferior potential as a SBP marker when compared to PCT[91,98].

Finally, as part of the potential of established auto-immune and inflammatory markers of BT, a recent Hungarian study is worth mentioning. Therein, it was reported that the prevalence of anti-neutrophil cyto-plasmic antibodies (ANCA) of the IgA subtype was higher in patients with cirrhosis in comparison to patients with non-cirrhotic chronic liver disease or healthy controls. Moreover, the presence of IgA ANCA was associated with the presence of ascites and advanced cirrhosis. Even more significant was the finding that, during follow up, patients with IgA ANCA had not only a higher complication risk but also IgA ANCA positivity correlated with a shorter time to the first infectious complication[99]. As IgA antibodies are linked to the gut immune system, these results may reflect a component in the pathogenesis of BT involving alterations in the local immune system and the intestinal barrier. In spite of the promise shown by these observations, further research is required to elucidate if IgA ANCA may serve as a possible marker of BT.

CONCLUSION

BT plays an important role in the pathogenesis of end stage liver disease complications. Therefore, its early and reliable detection would be significant for accurately identifying a particular subset of patients with potentially adverse prognosis, who may benefit from increased vigilance and aggressive management. Several biological molecules (bacterial DNA, soluble CD14, LPS/endotoxin, lipopolysaccharide-binding protein, calprotectin, pro-calcitonin) have been tested as potential biomarkers for BT, each with its own merits and flaws (Table 1). The literature available on the subject is intriguing and expanding. In all, while no single marker has emerged as optimal for the identification of BT, there is an obvious need for better designed and more focused research. These studies will not only enable us to understand the underlying mechanisms of BT, but may also allow for implementing a timely intervention in patients with liver cirrhosis. This may, in turn, alter the natural history of this ominous disease and improve its currently unfavorable prognosis.

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