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Biomarkers of bacterial translocation in advanced chronic liver disease: the key to individualizing prognosis

Avik Majumdar, Emmanuel Tsochatzis

Royal Free Hospital, London, UK

The management of patients with liver cirrhosis has evolved significantly in the past decade. No longer considered as having an invariably poor outcome, the need for better risk stratification and prognostic models for this patient group has become increasingly apparent [1-3]. The definition and use of the term "cirrhosis" *per se* is also under scrutiny, with the recent Baveno VI consensus guidelines suggesting that the term "advanced chronic liver disease" (ACLD) should be used interchangeably [4]. This highlights the ongoing paradigm shift where advanced fibrosis and cirrhosis are perceived as two often clinically indistinguishable points on the same spectrum, with both attributing the risk of developing complications of chronic liver disease.

Portal hypertension has long been recognized as the main pathophysiological process governing the prognosis of patients with ACLD. However, the development of clinical signs of portal hypertension is an event already too late in the clinical trajectory of patients. This is illustrated by the substantial increase in the 1-year mortality of patients from 3.4% to 20% for stage 2 and stage 3 ACLD respectively, with the distinction between these stages being the development of ascites [5,6]. Earlier identification of those at risk of disease progression is therefore urgently needed.

The importance of bacterial translocation as an underlying mechanism for the clinical consequences of ACLD is being increasingly recognized. The migration of bacteria or bacterial products from the intestinal lumen to mesenteric lymph nodes or other non-intestinal sites is a controlled physiological process in healthy individuals. However, this process becomes progressively deregulated in the presence of advancing chronic liver disease and culminates in pathological bacterial translocation (PBT), which in turn results in inflammation, immune activation and eventual clinical sequelae such as spontaneous bacteremia and spontaneous bacterial peritonitis [7]. Once infection develops in patients with ACLD, their 1-year mortality increases four-fold [8,9]. Furthermore, the altered inflammatory response to bacterially derived products has been implicated in the development of hepatic encephalopathy, hepatorenal syndrome and advancing portal hypertension [7,10].

Sheila Sherlock Liver Unit and UCL Institute for Liver and Digestive Health, Royal Free Hospital, London, UK

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Correspondence to: Dr Avik Majumdar, Royal Free Hospital, Pond St, London NW3 2QG, United Kingdom, Tel.: +44 7941638842, e-mail: avik.majumdar@nhs.net

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However, no reliable measure of PBT currently exists in the cirrhotic population, nor is a quantifiable threshold defined where bacterial translocation becomes pathologic [7,11,12]. Peripheral blood levels of bacterial DNA and endotoxin levels have traditionally been used as markers of PBT and have been correlated with severity of liver disease, as measured by Child-Pugh score. However, the correlation with the degree of portal hypertension has been less robust. This suggests that an alternative mechanism associated with immune activation may underlie the pathogenic process of translocation of bacterial products, as opposed to viable bacteria [13-15]. A better understanding of the complex interactions between luminal bacteria, the innate mucosal defence system and downstream immune and inflammatory responses are required.

In this issue of *Annals of Gastroenterology*, Kaltsa *et al* investigate the role of human beta defensin-1 (hBD-1), an intestinal mucosal antimicrobial peptide, as a surrogate marker of pathological bacterial translocation using the pattern-recognition receptor, soluble CD14 (sCD14), as a reference [16]. sCD14 is expressed primarily by monocytes and binds bacterial lipopolysaccharide (LPS) in the presence of LPS binding protein (LBP). Elevated concentrations of sCD14 have been previously demonstrated in patients with cirrhosis, but have not been widely validated as a marker of PBT [17,18].

In this study, the systemic expression of hBD-1 expression was investigated via peripheral vein sampling with a subset of patients undergoing hepatic vein sampling. Real-time PCR of the *DEFB1* gene (which encodes hBD-1) and other antimicrobial peptide genes from terminal ileal and colonic biopsies were obtained. Three cohorts of patients were compared; cirrhosis, non-cirrhotic chronic viral hepatitis and healthy controls who were age- and gender-matched. The cirrhosis group comprised 51 patients of various etiologies and was defined by the presence of clinical decompensation, or in the four patients who had compensated disease, either a consistent liver biopsy or a liver stiffness measurement of greater than 12kPa. In contrast, where biopsies were available for the chronic viral hepatitis group, patients had minimal liver fibrosis (Ishak Stage 0-1).

Kaltsa *et al* found higher plasma concentrations of hBD-1 in cirrhotic patients compared to both other groups, a difference that was sustained when cirrhotic viral hepatitis patients were compared to non-cirrhotic viral hepatitis patients. Levels of sCD14 significantly correlated with hBD-1 in the hepatic venous blood of cirrhotic patients resulting in a Spearman r of 0.6 (P=0.0045) but not in the peripheral blood (P=0.0528); however this could be due to a type II error. Interestingly, LBP correlated well with sCD14 in peripheral blood (r=0.5,

P=0.0021), however this was in an undefined subset of cirrhotic patients. Conversely, there was no increased mucosal expression of antimicrobial peptide genes, including *DEFB1*, in the cirrhotic group compared with healthy controls. This could be due to a number of confounding issues such as variable intestinal sites of expression of hBD-1 or extraintestinal expression, as has been described in monocytes and platelets.

Although the study by Kaltsa *et al* has a number of limitations, including the non-robust definitions of study cohorts, the lack of consistency of tests performed amongst all recruited patients and the heterogeneous group of cirrhotic patients with an under-representation of non-alcoholic fatty liver disease, the authors should be commended in aiming to identify a much needed surrogate of PBT. The pursuit of an endogenous antimicrobial peptide expressed in plasma is a valid concept. However, the strength of association of hBD-1 expression with PBT is hard to justify without bacterial DNA levels. The absence of a defined reference standard for PBT in patients with ACLD is a major challenge, however, measuring bacterial DNA appears to be the best available at present. Future research could include correlating hBD-1 levels with hepatic venous pressure gradient, inflammatory cytokines and hard clinical endpoints including decompensation and mortality.

The potential applications for a reproducible and reliable peripheral biomarker of PBT in ACLD are immense. Similar to the advent of non-invasive markers for pre-cirrhotic fibrosis, the early identification of those who are at risk of developing complications of advanced chronic liver disease will allow better risk-stratification and earlier treatment, which ideally will translate to better clinical outcomes. Finding these elusive biomarkers of PBT may just be the key to unlocking a new era in the management of patients with chronic liver disease.

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