NAFLD in 2019

What's new in NAFLD pathogenesis, biomarkers and treatment?

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Advances have been made in the field of nonalcoholic fatty liver disease in 2019. One paper highlights the role of gut microbiota in hepatocellular carcinoma (HCC) pathogenesis, another presents a non-invasive algorithm for detecting advanced liver fibrosis and another suggests a potential novel approach to treating nonalcoholic steatohepatitis and suppressing HCC development.

Nonalcoholic fatty liver disease (NAFLD) was first described by Dr. Jürgen Ludwig 40 years ago. NAFLD encompasses a spectrum of liver-lipid associated liver conditions that begins with hepatic steatosis and progresses over time to steatohepatitis (nonalcoholic steatohepatitis or NASH), NASH with fibrosis and cirrhosis. With the development of advanced fibrosis or cirrhosis, there is a marked increase in risk of development of hepatocellular carcinoma (HCC)[1]. The past decade has seen a phenomenal year by year increase in NAFLD-related research, with 2019 proving no exception. We have chosen to highlight three 2019 papers related to the following: understanding the role of gut microbiota in the pathogenesis of hepatocellular carcinoma (HCC); new biomarkers for nonalcoholic steatohepatitis (NASH) and advanced liver fibrosis; and a new, and perhaps unexpected, therapeutic approach to treating NASH and ameliorating risk of HCC.

Early in 2019, Ponziani and colleagues reported a link between the gut microbiota and HCC in patients with NAFLD[2]. Whether changes in gut microbiota (dysbiosis) causally influence pathogenesis of NAFLD remains uncertain, but Ponziani and colleagues addressed a related question, investigating whether dysbiosis and related factors are associated with HCC. They recruited 21 patients with

NAFLD-related cirrhosis and HCC, 20 patients with NAFLD-related cirrhosis without HCC, and 20 healthy controls. Intestinal permeability was assessed by quantifying circulating zonulin-1 (ZO1) and lipopolysaccharide (LPS) concentrations. Intestinal inflammation was evaluated by measuring faecal calprotectin, an intracellular protein of myeloid lineage cells. Systemic inflammation was assessed by measuring a panel of 27 cytokines, chemokines and growth factors.

If intestinal permeability is increased, bacteria or proinflammatory bacterial components (such as LPS) can gain access to the liver via the portal circulation. LPS-mediated activation of toll-like receptor-4 (TLR-4) has previously been shown to be capable of initiation and promotion of tumorigenesis in rodents[3], suggesting that LPS-mediated TLR-4 activation may promote HCC development. In their study, Ponziani and colleagues showed that patients with cirrhosis and HCC had higher levels of faecal calprotectin than those without HCC, whereas intestinal permeability (although higher in patients with cirrhosis than in healthy controls) was similar in patients with or without HCC. Plasma levels of interleukin (IL)-8, IL-13, chemokine (C-C motif) ligand (CCL)-3, CCL4 and CCL5 were higher in patients with cirrhosis and HCC than in those with cirrhosis without HCC and associated with an activated status of circulating monocytes. The faecal microbiota of the two groups of patients with cirrhosis had an increased abundance of Enterobacteriaceae and *Streptococcus* and a reduction in *Akkermansia* compared with healthy controls *Bacteroides* and Ruminococcaceae were higher in the HCC group than in patients with cirrhosis without HCC and *Bifidobacterium* was reduced. *Akkermansia* and *Bifidobacterium* inversely correlated with faecal calprotectin concentration, which was in turn associated with humoral and cellular inflammatory markers. These thought-provoking data suggest for the first time that the gut microbiota profile is associated with systemic inflammation in patients with NAFLD-related cirrhosis, and that markers of systemic inflammation are further increased in patients with cirrhosis who also have HCC.

Advanced liver fibrosis (Kleiner stage F3 on histology) is a key risk factor for end-stage liver disease and HCC development, so clinicians need easy-to-use, inexpensive tests with good diagnostic performance for diagnosing and excluding advanced fibrosis[4]. Daniels and colleagues investigated the diagnostic performance of serum concentration of PRO-C3 (a neo-epitope specific marker of type III collagen formation) for identifying advanced liver fibrosis in patients with NAFLD. They measured serum PRO-C3 levels using enzyme-linked immunosorbent assay in two large independent cohorts that had extensive clinical phenotyping and liver biopsy data. The derivation cohort consisted of 150 adults with biopsy-proven NAFLD and the validation cohort consisted of 281 patients with biopsy-proven NAFLD. The researchers developed a PRO-C3-based fibrosis algorithm — the ADAPT score — that included age, presence of diabetes, PRO-C3 level, and platelet count. Serum level of PRO-C3 increased with stage of liver fibrosis and was independently associated with

advanced fibrosis. The area under the receiver operating characteristic curve (AUROC) for the identification of patients with advanced fibrosis for the ADAPT score was 0.86 (95% CI 0.79–0.91) in the derivation cohort and 0.87 in the validation cohort (95% CI 0.83–0.91), which was superior to the AUROCs of other commonly used non-invasive scores for advanced fibrosis (the NAFLD fibrosis score, the fibrosis [FIB]-4 score and the AST to platelet ratio index [APRI])[5]. The diagnostic performance of another peptide related to type III collagen was also tested in 2019[6]. In 204 children and adolescents with biopsy-proven NAFLD, the AUROC of plasma N-terminal propeptide of type III procollagen (PIIINP) levels was 0.92 (95% CI 0.87–0.97) for diagnosis of F≥2 fibrosis and 0.993 (95% CI 0.98–1.0) for diagnosis of F3 fibrosis (none of these children had cirrhosis). Measurements of biomarkers of collagen formation in blood therefore seem to be reliable proxies for the presence of liver fibrosis in both adults and children[6].

The mechanisms underlying immune cell recruitment in NASH and the consequences of immune cell recruitment for the development of HCC are unclear. A growing body of evidence suggests that platelets are involved in inflammatory processes in liver disease. For example, activated platelets may contribute to cytotoxic T lymphocyte (CTL)-mediated liver damage in a model of viral hepatitis[7]. Furthermore, antiplatelet therapy inhibits immune-mediated hepatocarcinogenesis in a mouse model of viral hepatitis[8], suggesting that platelets might be key players in the pathogenesis of HCC in humans[9]. In 2019, Malehmir and colleagues showed that antiplatelet therapy suppressed HCC development in a murine hIL4rα/GP1bα transgenic mouse model of NASH treated with aspirin (a cyclooxygenase-1 inhibitor) and ticagrelor (an ADP-P2Y12 receptor inhibitor),[10], mostly by suppressing hepatic inflammation. Furthermore, these investigators showed that treatment with anti-GP1bα antibodies abrogated NASH. Although these findings were obtained solely in mice, Malehmir and colleagues also tested whether antiplatelet therapy modified liver volume and liver fat in patients being considered for cardiac catheterization: 12 patients were treated with antiplatelet therapy for 6 months and 10 patients not treated with antiplatelet therapy acted as controls. The sample size was far too small to adjust for differences between groups in baseline measurements or confounders, but the results suggested that antiplatelet therapy was associated with a decrease in both liver fat and liver volume. Taken together, these results suggest that blocking platelet activation might be a useful therapeutic strategy to ameliorate NASH and thereby to decrease risk of HCC development.

We selected three 2019 papers that have relevance to NAFLD (**Figure 1).** NAFLD prevalence has increased markedly in recent years and advanced fibrosis is a key risk factor for development of HCC. The ADAPT algorithm showed excellent diagnostic performance for detecting advanced fibrosis, and

might prove useful in clinical practice. The other two highlighted papers illustrate novel mechanisms involving dysbiosis and platelet activation that might contribute to the pathogenesis of HCC associated with NAFLD. Whether inhibiting platelet adhesion, aggregation or activation benefits NASH and decreases risk of HCC should be quite easy to test.

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Competing interests

The authors declare no competing interests.

Key advances

- The altered gut microbiota profile is associated with systemic inflammation in patients with nonalcoholic fatty liver disease (NAFLD)-related cirrhosis who have HCC[2]
- Measuring blood markers related to type III collagen might be useful for staging liver fibrosis in both adults and children with NAFLD[5],[6]
- Platelet count, activation and aggregation are increased in nonalcoholic steatohepatitis (NASH) and anti-platelet therapies should be tested[10]

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Figure 1 New findings in NAFLD in 2019. An unhealthy calorie-dense, fibre-poor diet results in changes in the intestinal microbiota (dysbiosis). Dysbiosis and decreased colonic carbohydrate available for fermentation by gut microbiota can lead to alterations in short chain fatty acids (SCFAs) which in turn lead to poor colonic epithelial integrity and increased permeability. Dysbiosis can increase production of ethanol production, SCFAs, incretins and metabolites such as trimethylamine, cresol and indole. All of these products, gut bacteria and bacterial products such as lipopolysaccaride (LPS) can then gain access to the liver via the portal circulation, and various proinflammatory pathways are activated via binding to toll-like receptor-4 (TLR-4) to promote development of hepatocellular carcinoma (HCC)[2]. Platelet activation results in release of platelet-derived growth factors, cytokines and chemokines that promote stellate cell and Kupffer cell activation and angiogenesis, increasing risk of HCC[9,10]. Stellate cells produce collagenous matrix and type III collagen-related molecules can be detected in the blood and used to stage liver fibrosis in both adults and children with NAFLD[5,6].