Review article

doi:10.5633/amm.2026.0219

Non-alcoholic fatty liver disease-related hepatocellular carcinoma

Gordana Petrović[1,2], Daniela Benedeto Stojanov[1,2], Biljana Radovanović Dinić[1,2], Vesna Brzački[1,2], Andrija Rančić[1], Milica Bjelaković[1], Marko Stojanović[1], Marko Stamenković[1]

[1]University Clinical Centar Niš, Gastroenterology and Hepatology Clinic, Niš, Serbia

[2]University of Niš, Faculty of Medicine, Niš, Serbia

Contact: Gordana Petrović

48 dr Zoran Đinđić Bulevard, 18000 Niš, Serbia

E-mail: gpetrovicnis@gmail.com

Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease, affecting more than 30% of the global population. It is characterized by excessive accumulation of triglycerides in hepatocytes in the absence of other secondary causes of steatosis. The disease encompasses a broad histological spectrum and is histologically classified into simple steatosis and non-alcoholic steatohepatitis (NASH). Within the NAFLD spectrum, only patients with non-alcoholic steatohepatitis progress to cirrhosis and hepatocellular carcinoma (HCC). Currently, HCC is the sixth most common cancer worldwide and the second leading cause of cancer-related mortality. Projections indicate that the prevalence of HCC in certain regions of the world will increase by up to 122% by 2030. This exponential rise in prevalence is associated with the global pandemics of obesity and diabetes, which represent the two most significant risk factors for the development and progression of NAFLD.The mechanisms underlying HCC development in NAFLD have not yet been fully elucidated; however, metabolic dysregulation in the presence of visceral obesity, hepatocellular lipid accumulation, systemic inflammation, insulin resistance, and dysbiosis lead to oxidative stress, the release of reactive oxygen species in the steatotic liver,

and mitochondrial dysfunction. These processes result in hepatocyte apoptosis, promoting fibrogenesis. Advanced fibrosis in the steatotic liver represents the most important risk factor for the development of HCC; nevertheless, in approximately 20% of patients with NAFLD, HCC develops in the absence of fibrosis.

Key words: non-alcoholic fatty liver disease, diabetes, obesity, hepatocellular carcinoma

Pregledni rad

doi:10.5633/amm.2026.0219

Hepatocelularni karcinom povezan sa nealkoholnom masnom bolešću jetre

Gordana Petrović[1,2], Daniela Benedeto Stojanov[1,2], Biljana Radovanović Dinić[1,2], Vesna Brzački[1,2], Andrija Rančić[1], Milica Bjelaković[1], Marko Stojanović[1], Marko Stamenković[1]

[1]Univerzitetski Klinički centar Niš, Klinika za gastroenterohepatologiju, Niš, Srbija

[2]Univerzitet u Nišu, Medicinski fakultet, Niš, Srbija

Kontakt: Gordana Petrović

Bulevar dr Zorana Đinđića 48, Niš, Srbija

E-mail: gpetrovicnis@gmail.com

Nealkoholna masna bolest jetre (NAFLD) je najčešća hronična bolest jetre, koja zahvata više od 30% populacije. Karakteriše se ekcesivnom akumulacijom triglicerida u hepatocitima, u odsustvu drugih sekundarnih uzroka steatoze. Bolest je širokog histološkog spektra i histološki se klasifikuje na običnu steatozu i nealkoholni steatohepatitis (NASH). Unutar NAFLD spektra samo pacijenti sa nealkoholnim steatohepatitisom razvijaju cirozu i hepatocelularni karcinom (HCC). Danas je HCC šesti najčešći karcinom širom sveta i drugi najčešći razlog mortaliteta povezanog sa karcinomima. Projekcije su da će prevalenca HCC u pojedinim delovima sveta do 2030. godine porasti za 122%. Ovo eksponencijalno povećanje prevalence je povezano sa pandemijom gojaznosti i dijabetesa, dva najznačajnija rizična faktora razvoja i progresije NAFLD. Nisu u potpunosti razjašnjeni mehanizmi razvoja HCC u NAFLD, ali metabolička disregulacija u prisustvu visceralne gojaznosti, akumulacija hepatocelularne masti, sistemska inflamacija, insulinska rezistencija, dizbioza vode oksidativnom stresu, oslobađanju slobodnih kiseoničnih radikala u steatotičnoj jetri, mitohondijalnoj disfunkciji, uzrokujući apoptozu hepatocita i promovišući proces fibroze. Uznapredovala fibroza u steatotičnoj jetri je najznačajniji faktor rizika razvoja HCC, mada se kod 20 % pacijenata sa NAFLD HCC razvija u odsustvu fibroze.

Ključne rečI: nealkoholna masna bolest jetre, dijabetes, gojaznost, hepatocelularni karcinom

Introduction

Non-alcoholic fatty liver disease (NAFLD) represents a growing global health problem and encompasses a disease spectrum that includes non-alcoholic fatty liver (NAFL), non-alcoholic steatohepatitis (NASH), associated cirrhosis, and hepatocellular carcinoma (HCC). The disorder is characterized by excessive accumulation of triglycerides within hepatocytes, manifesting in certain patients as hepatic steatosis in the absence of a history of chronic alcohol consumption (140–350 g/week for women and 210–420 g/week for men) and other secondary causes of hepatic steatosis, such as hepatitis C, hepatotoxic drugs, toxins, Wilson's disease, starvation, or secondary lipodystrophy. NAFLD is currently the most common chronic liver disease, affecting approximately one quarter of the global population, with projections suggesting that its incidence may rise to 56% in the coming decades (1,2).

The prevalence of NAFLD increases progressively in parallel with the rising prevalence of obesity and type 2 diabetes mellitus (T2DM), which represent the principal risk factors for disease development. More than 50% of patients with T2DM and over 90% of individuals with class III obesity are affected by NAFLD. Furthermore, 1.5% to 6% of the general population develops non-alcoholic steatohepatitis, with 10% to 15% of these patients exhibiting progression to fibrosis, which may further advance to liver cirrhosis and hepatocellular carcinoma. NAFLD displays a broad clinical spectrum; despite this, a substantial proportion of affected individuals present with asymptomatic hepatic steatosis and asymptomatic fibrosis. Insufficient awareness of NAFLD-associated HCC, together with these factors, contributes to delayed diagnosis and poor five-year survival outcomes (3,4).

The progressive increase in the incidence of these complications parallels the rising prevalence of NAFLD. NAFLD is currently the second most common indication for liver transplantation; however, it is anticipated to become the leading cause of liver transplantation, HCC development, and liver-related mortality in the coming years (5).

Recently, a new nomenclature has been proposed to define these entities as metabolic dysfunction–associated steatotic liver disease (MASLD) and metabolic dysfunction–associated steatohepatitis (MASH). The presence of hepatic steatosis, confirmed by imaging modalities or liver biopsy, together with at least one cardiometabolic risk factor (obesity, diabetes mellitus, or

metabolic dysregulation) in the absence of other causes of hepatic steatosis, establishes the diagnosis of MASLD (6).

MASLD represents the hepatic manifestation of metabolic syndrome and is a multifactorial disease resulting from the interaction of cardiometabolic and environmental factors in genetically predisposed individuals. Its incidence is rapidly increasing worldwide, and it constitutes a significant risk factor for the development of hepatocellular carcinoma. MASH, first described as a distinct entity in 1980, is characterized by steatosis, inflammation, hepatocellular injury, and apoptosis (7,8).

Numerous risk factors are associated with the development of MAFLD, including insulin resistance, elevated body mass index, atherogenic dyslipidemia, diabetes mellitus, systolic hypertension, and chronic kidney disease, all of which contribute to metabolic dysregulation, disease onset, and progression. Currently, approximately two-thirds of the global population is overweight, and one-third is obese. In younger individuals, MAFLD is more prevalent among men; however, with advancing age, the incidence increases overall, and the sex-related distribution of the disease shifts (9).

Ethnicity and genetic background also play important roles in the development of MAFLD. Polymorphisms in the patatin-like phospholipase domain containing 3 (PNPLA3) gene are a well-recognized predisposing factor explaining the higher prevalence of the disease among Hispanic populations compared with African Americans, who, despite a high prevalence of diabetes mellitus and obesity, carry a protective gene variant (S453I allele) that reduces susceptibility to hepatic steatosis. Several other genetic variants, including TM6SF2, MBOAT7, APOC3, NCAN, GCKR, LYPLAL1, and PPP1R3B, have been shown to predispose individuals to the development and progression of MAFLD (10).

Despite substantial advances in the understanding of pathophysiological mechanisms—particularly the central roles of insulin resistance and adipose tissue dysfunction—there remains significant heterogeneity among patients with respect to clinical presentation, disease progression, and response to therapy.

Epidemiology and Clinical Characteristics of NAFLD-Associated Hepatocellular Carcinoma

According to statistics from 2020, hepatocellular carcinoma (HCC) represents the sixth most frequently diagnosed malignancy worldwide and ranks third among causes of cancer-related mortality. The most common etiological factors contributing to the development of HCC include chronic hepatitis B and hepatitis C virus infections, excessive alcohol consumption, aflatoxin exposure, and schistosomiasis. The annual incidence of HCC ranges from 1% to 4% among patients with cirrhosis of any etiology, while the five-year survival rate remains as low as 18%. HCC occurs more frequently in men, with the highest incidence observed between the ages of 60 and 70 years. Since the year 2000, the global incidence of HCC related to viral hepatitis has declined, largely due to the implementation of hepatitis B vaccination programs and the availability of highly effective antiviral therapies. Non-alcoholic fatty liver disease (NAFLD) is associated with an increased risk of hepatocellular carcinoma, particularly among patients with cirrhosis or advanced fibrosis; however, 20–30% of NAFLD-associated HCC cases occur in the absence of advanced fibrosis (11,12).

Steatohepatitic hepatocellular carcinoma (SH-HCC) was first described in 2010 as a histological variant of HCC characterized by morphological features resembling non-neoplastic steatohepatitis and a strong clinical association with metabolic syndrome (13,14). Early observations demonstrated that patients with SH-HCC had a significantly higher burden of metabolic syndrome risk factors (2.44 vs. 1.48, $p = 0.01$) and a greater proportion of individuals with at least three components of metabolic syndrome (50% vs. 22.5%, $p = 0.02$) compared with patients with conventional HCC (14).

The rising incidence of NAFLD-associated HCC in Western countries has been attributed to increasing rates of childhood obesity, early onset of NASH, and higher alcohol consumption during early adolescence. The annual incidence of non-cirrhotic HCC among patients with NAFLD ranges from 0.1% to 1.3 per 1,000 individuals, while the incidence of HCC in NASH-related cirrhosis ranges from 0.5% to 2.6%. Younossi and colleagues, using registry data and Medicare-linked files to monitor HCC in the United States, reported that during a six-year follow-up period involving 4,929 patients with HCC and 14,937 controls, 14.1% of HCC cases were associated with NAFLD, with an annual increase in newly diagnosed cases of 9% between 2004 and 2009. Patients

with NAFLD-associated HCC tend to be older and have a higher prevalence of metabolic and cardiovascular comorbidities, as well as diabetes mellitus, compared with patients with HCC arising from other chronic liver diseases. These factors contribute to a poorer prognosis, reduced survival, and a diminished response to immunotherapy (15,16).

A retrospective study conducted in Japan involving more than 6,500 patients with ultrasound-confirmed NAFLD reported cumulative incidence rates of NAFLD-associated HCC of 0.02% at 4 years, 0.19% at 8 years, and 0.51% at 12 years of follow-up. Advanced fibrosis was identified histologically in 184 patients, who demonstrated a significantly higher cumulative incidence of HCC (hazard ratio [HR] 25.03; 95% confidence interval [CI] 9.02–69.52). The annual incidence of newly diagnosed HCC in this cohort was 0.043% (17).

In a study involving more than 10 million Medicare beneficiaries, the prevalence of NAFLD was estimated at 5.7%, with cirrhosis identified in 30% of affected patients. The calculated cumulative risks of progression from NAFLD to compensated cirrhosis and decompensated cirrhosis were 39% and 45%, respectively, while the cumulative risk of developing HCC reached 76.2% over eight years of retrospective follow-up. Independent predictors of disease progression included cardiovascular disease, renal impairment, dyslipidemia, and diabetes mellitus (3).

Available evidence suggests that NAFLD-associated HCC constitutes the predominant category of non-cirrhotic HCC, with 58.3% to 77.2% of NAFLD-related HCC cases developing in a non-cirrhotic liver, in contrast to HCC arising from alcoholic liver disease or chronic hepatitis C (12).

NAFLD-associated HCC exhibits morphological features reminiscent of non-neoplastic steatohepatitis, including macrovesicular steatosis, inflammation, ballooning of malignant hepatocytes, fibrosis, and Mallory–Denk bodies (18). For a diagnosis of NAFLD-related HCC, at least 5% to 50% of the tumor area must demonstrate steatohepatitic features. Phenotypically, NASH-related HCC differs from HCC of other etiologies. Histologically, NASH-HCC tends to be less aggressive, follows a more indolent course, exhibits a more pronounced inflammatory response, consists of well-differentiated tumors of larger diameter, shows a lower frequency of satellite nodules, and is associated with a reduced likelihood of extrahepatic metastases and less frequent intravascular invasion. At the time of diagnosis, patients with NAFLD-associated HCC generally

exhibit less severe underlying liver dysfunction compared with patients with HCC related to hepatitis C. In the cirrhotic stage, steatosis and necroinflammatory activity may disappear, resulting in a condition known as “burned-out NASH,” with cirrhosis histologically classified as cryptogenic, thereby contributing to delayed recognition of the increased risk of HCC (19,20).

Since the beginning of the millennium, the progressive increase in NAFLD prevalence has led to an eightfold rise in the proportion of HCC cases attributable to NAFLD. Compared with other chronic liver diseases, HCC in NAFLD develops five times more frequently in the pre-cirrhotic stage; however, from another perspective, patients with NAFLD without cirrhosis have a 100-fold lower risk of developing HCC (21,22).

A meta-analysis of 19 studies including approximately 170,000 patients with NASH demonstrated a prevalence of HCC of 38% in the absence of cirrhosis, compared with 14% in other liver diseases (23).

In 2002, Bugianesi and colleagues followed 641 patients with HCC, among whom 6.9% developed HCC in the setting of cryptogenic cirrhosis. These patients were compared with individuals who developed HCC on the background of hepatitis C virus–related cirrhosis, hepatitis B virus–related cirrhosis, and alcoholic cirrhosis. Comparative analysis revealed that obesity, diabetes mellitus, dyslipidemia, and insulin resistance—metabolic disturbances commonly associated with NASH—were significantly associated with the presence of cryptogenic cirrhosis (20).

Risk Factors for the Development of NASH and Hepatocellular Carcinoma

Given the global trend of increasing NAFLD incidence, the identification of high-risk subgroups among patients with NAFLD for the development of HCC is of paramount importance.

Demographic characteristics (age, sex, and ethnicity), metabolic syndrome, obesity, diabetes mellitus, alcohol consumption, and the severity and activity of liver disease—particularly the presence of inflammation and fibrosis—significantly contribute to the risk of HCC development (12).

Obesity has well-established implications in the pathogenesis of multiple malignancies, including colorectal, endometrial, renal, esophageal, gastric, pancreatic, and gallbladder cancers, and, in conjunction with insulin resistance (IR), represents a major risk factor for HCC. Individuals with a body mass index (BMI) greater than 40 kg/m² exhibit a 50–60% higher risk of malignancy compared with normal-weight individuals (24,25). Obesity (BMI >30 kg/m²) doubles the risk of HCC, while individuals with a BMI exceeding 35 kg/m² have a fourfold increased risk of developing HCC (3).

Obesity induces insulin resistance in adipose tissue, promotes enhanced lipolysis, inflammatory cell infiltration, and alterations in adipocytokine secretion. Specific adipocytokines link metabolic syndrome, T2DM, and NAFLD, and imbalance in their expression plays a central role in disease progression toward NASH and cirrhosis (26).

Assessment of visceral obesity is particularly important in estimating HCC risk, with waist circumference serving as a reliable anthropometric measure of visceral adiposity, given its strong association with increased all-cause mortality.

In a prospective study by Williams et al. in 2011, a markedly higher prevalence of NAFLD and NASH was observed among patients with diabetes compared with the general study population (74% vs. 46% and 22% vs. 12%, respectively). Insulin resistance was identified as an independent risk factor for the development of NASH across the entire cohort, and patients with advanced fibrosis demonstrated a higher degree of insulin resistance compared with those with moderate fibrosis (27).

Prospective studies have consistently shown that T2DM is an independent risk factor for NAFLD progression, fibrosis development, HCC occurrence, and overall mortality (28). Diabetes mellitus doubles the risk of HCC and increases HCC-related mortality by approximately 1.5-fold. When combined with metabolic syndrome, T2DM confers a fivefold increased risk of developing HCC (3).

Younossi et al., in a prospective study of patients with histologically confirmed NAFLD, demonstrated that individuals with diabetes had a higher prevalence of cirrhosis (25% vs. 10%, $p = 0.04$), greater overall mortality (56.8% vs. 27.3%, $p = 0.001$), and increased liver-related mortality (18.2% vs. 2.3%, $p = 0.02$) compared with patients without diabetes (29).

Patients with HCC arising in the setting of cryptogenic cirrhosis exhibit a high prevalence of obesity and diabetes compared with those with HCC secondary to alcoholic or viral cirrhosis, suggesting that NAFLD represents the underlying cause of cryptogenic cirrhosis complicated by HCC in this patient population (20).

A systematic review and meta-analysis of 29 studies by Guo et al., aimed at identifying the risk factors for HCC development in patients with NAFLD, demonstrated a strong association between diabetes mellitus and HCC, a weaker association with hypertension and obesity, and no significant association with dyslipidemia. The oncogenic effects of diabetes and obesity are mediated through the induction and promotion of inflammatory cascades, increased production of pro-inflammatory cytokines and reactive oxygen species, genomic instability, and hepatocyte apoptosis. Collectively, these processes lead to continuous cycles of hepatocellular injury and regeneration, promoting replication-associated mutations, ultimately facilitating hepatocarcinogenesis. Additional risk factors identified for HCC development in NAFLD included older age, male sex, alcohol consumption, smoking, elevated liver enzymes, reduced platelet count and serum albumin levels, and PNPLA3 gene polymorphisms (30).

Consistent with these findings, a meta-analysis of eight cohort studies by Chen et al. concluded that diabetes mellitus and overweight/obesity are strong risk factors for HCC development in individuals with NAFLD, whereas hypertension and dyslipidemia do not exert a significant influence (31).

Among all identified factors, the most potent risk factor for NAFLD-associated HCC is the presence of advanced fibrosis, as assessed by histology, non-invasive scoring systems, and elastography (32).

Pathogenesis of Hepatocellular Carcinoma in NAFLD

Metabolic dysfunction–associated fatty liver disease (MAFLD) represents the phenotypic manifestation of metabolic dysregulation and may exhibit heterogeneous clinical presentations

depending on the interplay between genetic predisposition and environmental factors. The mechanisms underlying HCC development in the steatotic liver have not yet been fully elucidated. Currently, the "multiple parallel hits" hypothesis is widely accepted to explain the progression from NAFLD to non-alcoholic steatohepatitis (NASH) and fibrosis (33). Over the past decade, adipose tissue and the gastrointestinal tract have been recognized as key drivers of inflammation and fibrosis in NAFLD. Insulin resistance constitutes the central pathogenic mechanism underlying hepatic steatosis, while hepatic insulin resistance and lipotoxicity represent additional hallmarks of NAFLD (33).

Disease progression is believed to be driven by a combination of factors, including genetic variants, oxidative stress, abnormal lipid metabolism, altered immune responses, mitochondrial and endoplasmic reticulum dysfunction, and dysregulation of the gut microbiota.

The most important triggers of chronic inflammation leading to hepatocyte injury and subsequent HCC development are insulin resistance, accumulation of lipids and toxic lipid metabolites, and infiltration of pro-inflammatory cells with cytokine release.

Insulin resistance promotes peripheral lipolysis, resulting in increased delivery and hepatic uptake of free fatty acids. Chronic hyperinsulinemia stimulates lipogenesis through the regulation of lipogenic transcription factors, such as peroxisome proliferator–activated receptor gamma (PPAR-γ) and sterol regulatory element–binding protein-1c (SREBP-1c) in the liver (34,35). Hepatic lipid accumulation results from increased lipogenesis, enhanced portal influx of fatty acids, or impaired β-oxidation of fatty acids. Insulin resistance also induces adipocyte dysfunction, leading to increased secretion of adipocytokines—peptides derived from visceral adipose tissue—including tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6), and leptin. Imbalance in the secretion of these cytokines represents an additional mechanistic link between obesity and NAFLD. Pro-inflammatory cytokines promote the migration of inflammatory cells into the liver and activate hepatic stellate cells, resulting in increased extracellular matrix protein production.These processes induce structural and morphological alterations in hepatocyte mitochondria and stimulate the synthesis and release of hepatotoxic reactive oxygen species (ROS) (9,26).

Excessive ROS production within a lipid-rich hepatic environment leads to lipid peroxidation, further impairment of mitochondrial respiratory chains, and enhanced release of pro-inflammatory cytokines, including tumor necrosis factor alpha (TNF-α), transforming growth factor beta (TGF-β), interleukin-6 (IL-6), leptin, and adiponectin. IL-6 represents a key mediator of ROS-induced liver injury. Elevated ROS levels cause mitochondrial and DNA damage in hepatocytes, activate caspases 3 and 9, and induce hepatocyte apoptosis. In parallel, IL-6 stimulates cellular proliferation and activates anti-apoptotic pathways through signal transducer and activator of transcription 3 (STAT3) signaling, a well-established oncogenic transcription factor. TNF-α contributes to disease progression and hepatocarcinogenesis through the activation of the JAK2/STAT signaling pathway (36,37).

Alterations in cellular redox status impair the protein-folding capacity of the endoplasmic reticulum and trigger the unfolded protein response (UPR). In NAFLD, UPR promotes activation of c-Jun N-terminal kinase (JNK) and nuclear factor kappa B (NF-κB), which are actively involved in hepatocyte inflammation and apoptosis. Oxidative stress–mediated activation of NF-κB results in increased production of pro-inflammatory cytokines, further driving neutrophil chemotaxis, hepatocyte apoptosis, and stellate cell activation.[81] Chronic inflammation in the context of hepatic steatosis, together with sustained NF-κB activation, plays a pivotal role in carcinogenesis and HCC development.

Persistent hyperinsulinemia in the setting of insulin resistance, along with increased secretion of insulin-like growth factor 1 (IGF-1), further contributes to hepatocarcinogenesis. Binding of insulin and IGF-1 to their respective receptors activates the phosphoinositide 3-kinase (PI3K) and mitogen-activated protein kinase (MAPK) signaling pathways, which play central roles in tumorigenesis. Activation of MAPK pathways induces transcription of proto-oncogenes such as *c-fos* and *c-jun*, promoting cellular proliferation and subsequent activation of the Wnt/β-catenin pathway, both of which are critically involved in fibrosis and tumor development (36,37).

Patients with NAFLD-associated HCC generally exhibit a poor prognosis. Therefore, accurate risk stratification of patients with hepatic steatosis and an increased risk of HCC—based on clinical parameters, disease activity and stage, and genetic information—is essential for early detection and improved outcomes (11).

The Role of the Microbiota in the Pathogenesis of NASH-Related Hepatocellular Carcinoma

An increasing body of evidence supports the role of intestinal barrier dysfunction in initiating hepatic inflammation, promoting NAFLD progression, and driving fibrogenesis. Alterations in gut permeability facilitate the translocation of bacteria and bacterial membrane components—such as lipopolysaccharides (LPS)—via the portal circulation to the liver, where they activate immune responses and induce hepatocellular injury.

Kupffer cells, through their Toll-like receptors (TLRs), recognize LPS and other pathogen-associated molecular components. Through the expression of numerous cytokines, including TNF-α, IL-1β, IL-6, IL-12, and IL-18, they mobilize the inflammatory cells. This cytokine release promotes the recruitment and activation of inflammatory cells such as neutrophils, T lymphocytes, and monocytes, thereby amplifying hepatic inflammation (38).

Ponziani et al. investigated alterations in gut microbiota composition, intestinal permeability, inflammatory markers, and circulating mononuclear cells in patients with NAFLD-related cirrhosis and HCC, patients with cirrhosis without HCC, and healthy controls (39). They demonstrated an increased abundance of *Bacteroides* and *Ruminococcaceae* in patients with HCC, accompanied by a reduction in *Bifidobacteria*. Intestinal permeability was comparable between the two cirrhotic groups; however, fecal calprotectin levels were significantly higher in patients with cirrhosis and HCC. Moreover, the HCC group exhibited elevated plasma levels of IL-8, IL-13, and chemokines including C–C motif ligand (CCL) 3, CCL4, and CCL5. These findings underscore the importance of gut microbiota dysbiosis and systemic inflammation in hepatocarcinogenesis (39).

A key gut bacterial metabolite implicated in obesity-associated HCC is deoxycholic acid (DCA), which induces DNA damage and plays a critical role in hepatocarcinogenesis. Experimental mouse models have demonstrated that increased enterohepatic circulation of DCA is associated with enhanced secretion of inflammatory cytokines (such as IL-8) and tumor-promoting factors in the liver, thereby facilitating HCC development.

Dietary fiber fermentation by intestinal bacteria produces short-chain fatty acids (SCFAs), primarily acetate, butyrate, and propionate. Among these, butyrate exhibits the most potent anti-

inflammatory properties through the activation of regulatory T cells. Diets rich in fats and carbohydrates promote gut dysbiosis, characterized by reduced colonization with beneficial bacteria (*Bifidobacterium*, *Lactobacillus*, *Bacteroides*) and predominance of *Prevotella* and *Firmicutes*. Dysbiosis leads to decreased SCFA production, increased inflammation, dyslipidemia, and obesity.Reduced butyrate levels are associated with increased intestinal permeability, enhanced translocation of bacterial endotoxins, and hepatic steatosis. In addition, SCFAs regulate host metabolism by activating free fatty acid receptors that stimulate the secretion of hormones such as glucagon-like peptide-1 (GLP-1) and peptide YY, which modulate glucose homeostasis, appetite, and energy metabolism. Beneficial effects of sodium butyrate on increased intestinal GLP-1 expression and release, along with upregulation of hepatic GLP-1 receptors and attenuation of NAFLD progression, have been demonstrated in animal models (40,41,42).

As early as 2001, Wigg et al. reported a higher prevalence of small intestinal bacterial overgrowth (SIBO) and elevated TNF-α levels in patients with NASH compared with controls, supporting the role of SIBO and endotoxemia in NASH pathogenesis (43). TNF-α and IL-8 play key roles in the induction and progression of NAFLD to NASH and cirrhosis, as evidenced by their elevated serum concentrations in affected patients.

Activation of TLR-4 and TLR-9 receptors secondary to gut dysbiosis stimulates Kupffer cells to secrete IL-1β, promoting lipid accumulation and hepatocyte apoptosis, thereby driving steatosis and inflammation. Hepatic stellate cells produce fibrogenic mediators that contribute to fibrosis.

Gut dysbiosis also alters bile acid metabolism, with a predominance of deoxycholic acid, which exerts its effects via hepatic stellate cells and the production of inflammatory and tumor-promoting factors, further facilitating the development of hepatocellular carcinoma (44).

PNPLA3 I148M and NAFLD-Associated Hepatocellular Carcinoma

The strongest genetic variant predisposing to MASLD and its progression to HCC is the rs738409 C>G polymorphism of the *PNPLA3* gene, corresponding to the I148M variant. This polymorphism involves a cytosine-to-guanine nucleotide substitution, resulting in an amino acid

change at position 148, with methionine replacing isoleucine. The *PNPLA3* gene encodes a protein belonging to the patatin-like phospholipase family, whose progenitor, patatin, exhibits non-specific lipid acyl hydrolase activity, catalyzing the conversion of lysophosphatidic acid to phosphatidic acid (45).

It is postulated that the I148M variant of *PNPLA3* reduces its enzymatic activity toward glycerol lipids, leading to subsequent hepatic fat accumulation. Valenti et al. were the first to demonstrate, in two independent cohorts of patients with histologically confirmed NAFLD, that homozygosity for the I148M allele confers a 3.3-fold increased risk of developing NASH and fibrosis (46). The association between the *PNPLA3* I148M polymorphism and fibrosis severity has been further confirmed in a meta-analysis, which showed that GG homozygotes exhibit a 3.24-fold higher necroinflammatory score and a 3.2-fold increased risk of fibrosis compared with CC homozygotes (47).

A study by Liu et al. confirmed a strong association between the *PNPLA3* rs738409 C>G polymorphism and HCC in patients with NAFLD ($p < 0.0001$). The strength of this association was proportional to the number of G alleles present and was independent of established risk factors such as age, sex, obesity, diabetes mellitus, and the presence of cirrhosis. Carriers of the GG genotype exhibited up to a fivefold increased risk of developing HCC and were, on average, up to four years younger at diagnosis than carriers of the CC genotype (48).

Patients carrying the mutant G allele tend to develop more aggressive, poorly differentiated tumors and experience worse clinical outcomes. The impact of the *PNPLA3* rs738409 variant on HCC risk and prognosis is particularly pronounced in homozygous carriers. Homozygosity for the *PNPLA3* I148M variant has been associated with shorter median survival, and this effect appears to be consistent across different etiologies of liver disease. In the study by Valenti et al., *PNPLA3* I148M homozygosity emerged as the only independent negative prognostic factor for survival in patients with metabolic- and alcohol-related liver disease in Cox regression analysis (hazard ratio [HR] 1.87; 95% confidence interval [CI] 1.12–2.78) (49).

A longitudinal prospective study by Trifò et al. identified a potential synergistic interaction between the *PNPLA3* rs738409 polymorphism and environmental and metabolic factors—such as obesity and alcohol consumption—in modulating the risk of HCC (50).

Incorporation of *PNPLA3* into validated predictive models may substantially improve risk stratification for HCC development. Furthermore, combining *PNPLA3* with other genetic variants associated with progressive liver disease has facilitated the development of polygenic risk scores (PRS) (51).

Screening of Patients with NAFLD: Whom and When?

Patients with NAFLD-associated hepatocellular carcinoma (HCC) generally have a poor prognosis. Therefore, risk stratification of patients with hepatic steatosis and an increased risk of HCC—based on clinical characteristics, disease activity and stage, and genetic information—is crucial (11).

In high-risk populations, such as patients with chronic hepatitis B infection and those with cirrhosis of any etiology, HCC surveillance is recommended in order to increase the proportion of tumors detected at an early stage. Among patients with decompensated NASH-related cirrhosis, the incidence of HCC is higher than in those with compensated cirrhosis. Additional risk factors for HCC in patients with NASH-related cirrhosis include male sex, the presence of diabetes mellitus, obesity, dyslipidemia, alcohol consumption, and, in the United States Hispanic ethnicity. Patients with stage F3 fibrosis are considered to be at intermediate risk for HCC development. In contrast, patients with NAFLD and lower stages of fibrosis (F1 and F2), as determined by a combination of the FIB-4 index and transient elastography, have a low incidence of HCC, and routine screening is not recommended in this group (52).

Identifying patients at high risk remains a major challenge. Screening for HCC in patients with NAFLD without cirrhosis is costly and not cost-effective. Given the projected global incidence of more than one million HCC cases by 2025, it is estimated that excluding patients with non-cirrhotic NAFLD from surveillance programs could result in missing approximately 60,000 new HCC cases. The primary risk stratification tool for HCC development in NAFLD is the assessment of cirrhosis or advanced fibrosis using non-invasive techniques. To reduce the likelihood of misclassification, current recommendations favor the combination of at least two non-invasive fibrosis assessment methods from different categories (i.e., serum-based tests and imaging-based techniques). Concordant findings indicating advanced fibrosis or cirrhosis support the

initiation of HCC surveillance.The FIB-4 index can be easily calculated using online calculators based on age, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and platelet count. Patients with a FIB-4 score greater than 2.67, irrespective of cirrhosis status, have an increased risk of developing HCC and should be considered for screening (52). Additionally, mathematical models named NAFLD fibrosis score, incorporating six variables—age, body mass index (BMI), AST/ALT ratio, fasting glucose, platelet count, and serum albumin—can identify patients with advanced fibrosis; values greater than 0.675 suggest advanced fibrosis, while values ≤1.455 exclude it (3). Transient elastography (FibroScan) is an inexpensive, painless bedside method with acceptable accuracy and reproducibility for the diagnosis of liver fibrosis. However, its diagnostic performance may be limited by obesity, the presence of ascites, and narrow intercostal spaces.

Current guidelines of the American, European and Japanese associations for the study of liver diseases recommend HCC surveillance in patients with advanced fibrosis, particularly those older than 65 years, individuals with diabetes mellitus and retinopathy, and patients with persistently elevated transaminases. The cost-effectiveness of HCC surveillance is greatest in patients with NAFLD and Child–Pugh class A cirrhosis. In contrast, the benefit of surveillance in patients with more advanced liver dysfunction is questionable, given the competing risks of liver-related and cardiovascular mortality (53).

As a primary surveillance strategy, both AASLD and EASL recommend abdominal ultrasonography every six months, with or without measurement of alpha-fetoprotein (AFP). The advantages of ultrasonography include wide availability, non-invasiveness, low cost, and safety, without exposure to ionizing radiation or contrast agents (54,55).

Patients with NAFLD-related cirrhosis and liver stiffness values exceeding 15 kPa on elastography should undergo semiannual HCC surveillance. However, surveillance in this population is complicated by the reduced diagnostic accuracy of semiannual ultrasonography due to steatosis-related deep ultrasound attenuation. Increased BMI further compromises ultrasound sensitivity and accuracy. Emerging evidence indicates that ultrasonographic visualization may be inadequate to reliably exclude liver lesions in approximately one-fifth of patients. Consequently, the diagnostic accuracy of ultrasound for HCC detection in NAFLD-related cirrhosis is approximately threefold lower than in cirrhosis due to other chronic liver diseases (56).

A study involving 941 patients with cirrhosis demonstrated that ultrasound was inadequate as a screening modality in 20% of cases, with elevated BMI and NASH-related cirrhosis identified as the two main contributing factors. Although repeated ultrasound examinations may improve visualization, patients with persistently poor imaging quality—particularly those with NAFLD—require alternative imaging modalities such as computed tomography (CT) or magnetic resonance imaging (MRI).

Similar to ultrasonography, AFP measurement offers advantages of low cost and wide availability, but it has limited sensitivity for detecting early-stage HCC. A recent meta-analysis demonstrated that AFP significantly improves the sensitivity of ultrasound for early HCC detection in patients with NAFLD-related cirrhosis, with sensitivity increasing from 45% using ultrasound alone to 63% when combined with AFP (57).

These limitations highlight the need for alternative imaging modalities, particularly CT and MRI. However, the use of CT for surveillance is limited by cumulative radiation exposure and the risks associated with contrast agents. The prospective PRIUS study conducted in South Korea demonstrated significantly higher sensitivity and specificity of MRI compared with ultrasound for early HCC detection in high-risk cirrhotic patients (58). Nevertheless, the cost-effectiveness of MRI and limitations in radiological capacity must be carefully considered.

The integration of demographic and clinical variables with blood-based biomarkers has led to the development of biomarker panels aimed at identifying patients at increased risk of NAFLD-associated HCC. One such model, the HCCrisk score, combines age, sex, diabetes status, platelet count, aminotransferase levels, and serum albumin to stratify HCC risk (59).

The GALAD score, proposed by Best et al., is currently undergoing phase III validation following encouraging results demonstrating high accuracy for early-stage HCC detection in patients with NASH, with or without fibrosis. In a case–control study of patients with NAFLD, a GALAD cutoff value of −0.63 yielded a sensitivity of 68% and a specificity of 95% for early-stage HCC detection. The GALAD score incorporates age, sex, serum AFP, AFP-L3 isoform, and des-γ-carboxy prothrombin (60). However, despite its promise, the GALAD score still requires validation in phase III and IV studies before it can be routinely adopted for HCC screening.

Conclusion

The growing contribution of non-alcoholic fatty liver disease to the global prevalence of HCC is largely driven by the pandemics of obesity and diabetes mellitus. The prevalence of cirrhosis among patients with NAFLD-associated HCC is lower than that observed in HCC arising from other etiologies. When cirrhosis is present, it is frequently classified histologically as cryptogenic due to the absence of steatosis and necroinflammatory activity. Owing to its prolonged and indolent course, non-alcoholic steatohepatitis is often overlooked, leading to delayed diagnosis. Consequently, patients typically present at advanced stages of disease, with multiple comorbidities, larger tumor size, late-stage tumor detection, and significantly reduced survival.

The high proportion of HCC developing in the pre-cirrhotic stage of NAFLD, combined with underdiagnosis of advanced fibrosis—the principal risk factor for HCC—and insufficient awareness of the importance of regular surveillance in patients with NAFLD-related cirrhosis, represent major contributors to the failure of effective HCC screening in this population. Efforts should therefore be focused on the prevention of NAFLD and NASH, proactive identification of advanced fibrosis among obese individuals and patients with diabetes mellitus, and increasing global awareness of the clinical significance of NAFLD.

Incorporating patients with NAFLD and multiple risk factors into structured HCC surveillance programs may substantially enhance HCC prevention strategies. There is an urgent need for improved non-invasive biomarkers to enable accurate risk stratification and precise identification of individuals at high risk for HCC, including the development and validation of clinical and polygenic risk scores, as well as integrated models combining these approaches.

References

1. *Angulo P. Nonalcoholic fatty liver disease. N. Engl. J. Med. 2002;346:1221–1231. doi: 10.1056/NEJMra011775.*
2. *Younossi ZM, Rinella ME, Sanyal AJ, Harrison SA, Brunt EM, Goodman Z, et al. From NAFLD to MAFLD: implications of a premature change in terminology. Hepatology 2021; 73:1194–1198. doi: 10.1002/hep.31420.*
3. Younossi ZM, Henry L. Epidemiology of non-alcoholic fatty liver disease and hepatocellular carcinoma. JHEP Rep. 2021 May 11;3(4):100305. doi: 10.1016/j.jhepr.2021.100305. PMID: 34189448; PMCID: PMC8215299.
4. Allen AM, Therneau TM, Ahmed OT, Gidener T, Mara KC, Larson JJ, Canning RE, Benson JT, Kamath PS. Clinical course of non-alcoholic fatty liver disease and the implications for clinical trial design. J Hepatol. 2022 Nov;77(5):1237-1245. doi: 10.1016/j.jhep.2022.07.004. Epub 2022 Jul 16. PMID: 35843374; PMCID: PMC9974107.
5. Younossi Z.M., Stepanova M., Ong J., Trimble G., AlQahtani S., Younossi I., Ahmed A., Racila A., Henry L. Nonalcoholic Steatohepatitis Is the Most Rapidly Increasing Indication for Liver Transplantation in the United States. Clin. Gastroenterol. Hepatol. 2020;19:580–589.e5. doi: 10.1016/j.cgh.2020.05.064
6. Rinella ME, Lazarus JV, Ratziu V, Francque SM et al. NAFLD Nomenclature consensus group. A multisociety Delphi consensus statement on new fatty liver disease nomenclature. Hepatology. 2023 Dec 1;78(6):1966-1986. doi: 10.1097/HEP.0000000000000520. Epub 2023 Jun 24. PMID: 37363821; PMCID: PMC10653297.
7. Kim CH, Younossi ZM. Nonalcoholic fatty liver disease: a manifestation of the metabolic syndrome. Cleve Clin J Med. 2008 Oct;75(10):721-8. doi: 10.3949/ccjm.75.10.721. PMID: 18939388.
8. Rinella ME, Sookoian S. From NAFLD to MASLD: updated naming and diagnosis criteria for fatty liver disease. J Lipid Res. 2024 Jan;65(1):100485. doi: 10.1016/j.jlr.2023.100485. Epub 2023 Dec 14. PMID: 38103785; PMCID: PMC10824973.
9. Bae SDW, George J, Qiao L. From MAFLD to hepatocellular carcinoma and everything in between. Chin Med J (Engl). 2022 Feb 21;135(5):547-556. doi: 10.1097/CM9.0000000000002089. PMID: 35191421; PMCID: PMC8920461.
10. Yu J. Obesity, Fatty Liver and Liver Cancer. Singapore: Springer; 2018. 157.
11. Michelotti A, de Scordilli M, Palmero L, Guardascione M, Masala M, Roncato R, Foltran L, Ongaro E, Puglisi F. NAFLD-Related Hepatocarcinoma: The Malignant Side of Metabolic Syndrome. Cells. 2021 Aug 9;10(8):2034. doi: 10.3390/cells10082034. PMID: 34440803; PMCID: PMC8391372.
12. Takahashi Y, Dungubat E, Kusano H, Fukusato T. Pathology and Pathogenesis of Metabolic Dysfunction-Associated Steatotic Liver Disease-Associated Hepatic Tumors. Biomedicines. 2023 Oct 12;11(10):2761. doi: 10.3390/biomedicines11102761. PMID: 37893134; PMCID: PMC10604511.
13. Olofson, A.M.Gonzalo D.H. Chang M. Liu X. Steatohepatitic variant of hepatocellular carcinoma: A focused review. Gastroenterol. Res. 2018; 11: 391–396.
14. Salomao, M.; Remotti, H.; Vaughan, R.; Siegel, A.B.; Lefkowitch, J.H.; Moreira, R.K. The steatohepatitic variant of hepatocellular carcinoma and its association with underlying steatohepatitis. Hum. Pathol. 2012, 43, 737–746.
15. *Huang D.Q., El-Serag H.B., Loomba R. Global epidemiology of NAFLD-related HCC: Trends, predictions, risk factors and prevention. Nat. Rev. Gastroenterol. Hepatol. 2021;18:223–238. doi: 10.1038/s41575-020-00381-6.* [DOI] [PMC free article] [PubMed] [Google Scholar]
16. Younossi, Z.M.; Otgonsuren, M.; Henry, L.; Venkatesan, C.; Mishra, A.; Erario, M.; Hunt, S. Association of nonalcoholic fatty liver disease (NAFLD) with hepatocellular carcinoma (HCC) in the United States from 2004 to 2009. Hepatology **2015**, 62, 1723–1730.
17. Kawamura, Y.; Arase, Y.; Ikeda, K.; Seko, Y.; Imai, N.; Hosaka, T.; Kobayashi, M.; Saitoh, S.; Sezaki, H.; Akuta, N.; et al. Large-scale long-term follow-up study of Japanese patients with non-alcoholic fatty liver disease for the onset of hepatocellular carcinoma. Am. J. Gastroneterol. 2012, 107, 253–261

18. Qin, J.; Higashi, T.; Nakagawa, S.; Fujiwara, N.; Yamashita, Y.; Beppu, T.; Baba, H.; Kobayashi, M.; Kumada, H.; Gunasekaran, G.; et al. Steatohepatitic variant of hepatocellular carcinoma is associated with both alcoholic steatohepatitis and nonalcoholic steatohepatitis: A study of 2 cohorts with molecular insights. *Am. J. Surg. Pathol.* 2020, *44*, 1406–1412.
19. Takahashi Y, Fukusato T. Histopathology of nonalcoholic fatty liver disease/nonalcoholic steatohepatitis. World J Gastroenterol. 2014 Nov 14;20(42):15539-48. doi: 10.3748/wjg.v20.i42.15539. PMID: 25400438; PMCID: PMC4229519.
20. Bugianesi E, Leone N, Vanni E, Marchesini G, Brunello F, Carucci P, MussoA, De Paolis P,Capussotti L, Salizzoni M, Rizzetto M. Expanding the natural history of nonalcoholic steatohepatitis: from cryptogenic cirrhosis to hepatocellular carcinoma. Gastroenterology 2002; 123:134 –140
21. Tan DJH, Ng CH, Lin SY, Pan XH, Tay P, Lim WH, Teng M, Syn N, Lim G, Yong JN, Quek J, Xiao J, Dan YY, Siddiqui MS, Sanyal AJ, Muthiah MD, Loomba R, Huang DQ. Clinical characteristics, surveillance, treatment allocation, and outcomes of non-alcoholic fatty liver disease-related hepatocellular carcinoma: a systematic review and meta-analysis. Lancet Oncol. 2022 Apr;23(4):521-530. doi: 10.1016/S1470-2045(22)00078-X. Epub 2022 Mar 4. PMID: 35255263; PMCID: PMC9718369.
22. Kanwal F, Kramer JR, Mapakshi S, Natarajan Y, Chayanupatkul M, Richardson PA, Li L, Desiderio R, Thrift AP, Asch SM, Chu J, El-Serag HB. Risk of Hepatocellular Cancer in Patients With Non-Alcoholic Fatty Liver Disease. Gastroenterology. 2018 Dec;155(6):1828-1837.e2. doi: 10.1053/j.gastro.2018.08.024. Epub 2018 Aug 23. PMID: 30144434; PMCID: PMC6279617.
23. *Stine J.G., Wentworth B.J., Zimmet A., Rinella M.E., Loomba R., Caldwell S.H., Argo C. Systematic review with meta-analysis: Risk of hepatocellular carcinoma in non-alcoholic steatohepatitis without cirrhosis compared to other liver diseases. Aliment Pharmacol. Ther. 2018;48:696–703. doi: 10.1111/apt.14937.* [DOI] [PMC free article] [PubMed] [Google Scholar]
24. Lu K, Song XL, Han SL, Wang CH, Zhong N, Qi LF. Potential study perspectives on mechanisms and correlations between adiposity and malignancy. Asian Pac J Cancer Prev. 2014;15(2):1057-60. doi: 10.7314/apjcp.2014.15.2.1057. PMID: 24568450.
25. Bechmann LP, Hannivoort RA, Gerken G, Hotamisligil GS, Trauner M, Canbay A. The interaction of hepatic lipid and glucose metabolism in liver diseases. J Hepatol 2012; 56: 952-964
26. Mirza MS. Obesity, Visceral Fat, and NAFLD: Querying the Role of Adipokines in the Progression of Nonalcoholic Fatty Liver Disease. ISRN Gastroenterology 2011;2011:592404.
27. Williams CD, Stengel J, Asike MI, Torres DM, Shaw J, Contreras M, Landt CL, Harrison SA. Prevalence of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis among a largely middle-aged population utilizing ultrasound and liver biopsy: a prospective study. Gastroenterology 2011; 140:124-131
28. Leite NC, Villela-Nogueira CA, Cardoso CRL, Salles GF. Non-alcoholic fatty liver disease and diabetes: From physiopathological interplay to diagnosis and treatment. World J Gastroenterol. 2014;20(26):8377-8392.
29. Younossi ZM, Gramlich T, Matteoni CA, Boparai N, McCullough AJ. Nonalcoholic fatty liver disease in patients with type 2 diabetes. Clin Gastroenterol Hepatol 2004; 2: 262-265.
30. Guo WP, Zhang HY, Liu LX. Risk factors of hepatocellular carcinoma in non-alcoholic fatty liver disease: a systematic review and meta-analysis. Eur Rev Med Pharmacol Sci. 2023 Dec;27(24):11890-11903. doi: 10.26355/eurrev_202312_34788. PMID: 38164853.
31. Chen J, Song S, Li X, Bian D, Wu X. Association of metabolic traits with occurrence of nonalcoholic fatty liver disease-related hepatocellular carcinoma: A systematic review and meta-analysis of longitudinal cohort studies. Saudi J Gastroenterol 2022; 28: 92-100.
32. Pons M, Rivera-Esteban J, Manzano R, Bañares J, Bermúdez M, Vargas V, Salcedo-Allende MT, Castells L, Augustin S, Mínguez B, Pericàs JM. Non-Invasive Tests of Liver Fibrosis Help in Predicting the Development of Hepatocellular Carcinoma among Patients with NAFLD. J Clin Med. 2022 Apr 27;11(9):2466. doi: 10.3390/jcm11092466. PMID: 35566592; PMCID: PMC9103029.

33. Tilg H, Adolph TE, Moschen AR. Multiple Parallel Hits Hypothesis in Nonalcoholic Fatty Liver Disease: Revisited After a Decade. Hepatology. 2021 Feb;73(2):833-842. doi: 10.1002/hep.31518. Epub 2021 Feb 6. PMID: 32780879; PMCID: PMC7898624.
34. Anty R, Lemoine M. Liver fibrogenesis and metabolic factors. Clin Res Hepatol Gastroenterol. 2011 Jun;35 (1): 10-20.
35. Stefan N, Kantartzis K, Haring HU. Causes and metabolic consequences of fatty liver. Endocr Rev.2008;29:939–60.
36. Kutlu O, Kaleli HN, Ozer E. Molecular pathogenesis of nonalcoholic steatohepatitis-(NASH-) related hepatocellular carcinoma. Can J Gastroenterol Hepatol 2018;2018:8543763. doi: 10.1155/2018/8543763
37. Michelotti A, de Scordilli M, Palmero L, Guardascione M, Masala M, Roncato R, Foltran L, Ongaro E, Puglisi F. NAFLD-Related Hepatocarcinoma: The Malignant Side of Metabolic Syndrome. *Cells*. 2021; 10(8):2034. https://doi.org/10.3390/cells10082034
38. Chu H, Williams B, Schnabl B. Gut microbiota, fatty liver disease, and hepatocellular carcinoma. Liver Res. 2018 Mar;2(1):43-51. doi: 10.1016/j.livres.2017.11.005. Epub 2018 Feb 21. PMID: 30416839; PMCID: PMC6223644.
39. Ponziani FR, Bhoori S, Castelli C, Putignani L, Rivoltini L, Del Chierico F, Sanguinetti M, Morelli D, Paroni Sterbini F, Petito V, Reddel S, Calvani R, Camisaschi C, Picca A, Tuccitto A, Gasbarrini A, Pompili M, Mazzaferro V. Hepatocellular Carcinoma Is Associated With Gut Microbiota Profile and Inflammation in Nonalcoholic Fatty Liver Disease. Hepatology. 2019 Jan;69(1):107-120. doi: 10.1002/hep.30036. Epub 2018 Jul 10. PMID: 29665135.
40. Beisner J, Filipe Rosa L, Kaden-Volynets V, Stolzer I, Günther C, Bischoff SC. Prebiotic Inulin and Sodium Butyrate Attenuate Obesity-Induced Intestinal Barrier Dysfunction by Induction of Antimicrobial Peptides. Front Immunol. 2021 Jun 11;12:678360. doi: 10.3389/fimmu.2021.678360. PMID: 34177920; PMCID: PMC8226265.
41. Zhou D, Pan Q, Xin FZ, Zhang RN, He CX, Chen GY, Liu C, Chen YW, Fan JG. Sodium butyrate attenuates high-fat diet-induced steatohepatitis in mice by improving gut microbiota and gastrointestinal barrier. World J Gastroenterol. 2017 Jan 7;23(1):60-75. doi: 10.3748/wjg.v23.i1.60. PMID: 28104981; PMCID: PMC5221287.
42. Kaji I, Karaki S, Kuwahara A. Short-chain fatty acid receptor and its contribution to glucagon-like peptide-1 release. Digestion. 2014;89(1):31-6. doi: 10.1159/000356211. Epub 2014 Jan 20. PMID: 24458110.
43. Wigg AJ, Roberts-Thomson IC, Dymock RB, McCarthy PJ, Grose RH, Cummins AG. The role of small intestinal bacterial overgrowth, intestinal permeability, endotoxaemia, and tumour necrosis factor alpha in the pathogenesis of non-alcoholic steatohepatitis. Gut. (2001) 48:206–11. doi: 10.1136/gut.48.2.206
44. Chu H, Williams B, Schnabl B. Gut microbiota, fatty liver disease, and hepatocellular carcinoma. Liver Res. 2018 Mar;2(1):43-51. doi: 10.1016/j.livres.2017.11.005. Epub 2018 Feb 21. PMID: 30416839; PMCID: PMC6223644.
45. Romeo S, Kozlitina J, Xing C, Pertsemlidis A, Cox D, Pennacchio AL, Boerwinkle E, Cohen CJ & Hobbs HH. Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. Nat Genet. 2008; 40(12): 1461-5.
46. Valenti L, Al-Serri A, Daly AK, et al. Homozygosity for the PNPLA3 / adiponutrin I148M polymorphism influences liver fibrosis in patients with nonalcoholic fatty liver disease. Hepatology 2010; 51: 1209-17.
47. Sookoian S, Pirola CJ. Meta-analysis of the influence of I148M variant of patatin-like phospholipase domain containing 3 gene (PNPLA3) on the susceptibility and histological severity of nonalcoholic fatty liver disease. Hepatology 2011; 53: 1883-1894.
48. Liu YL, Patman GL, Leathart JB, Piguet AC, Burt AD, Dufour JF, Day CP, Daly AK, Reeves HL, Anstee QM. Carriage of the PNPLA3 rs738409 C >G polymorphism confers an increased risk of non-alcoholic fatty liver disease associated hepatocellular carcinoma. J Hepatol. 2014;61(1):75-81.
49. Valenti L, Motta BM, Soardo G, Iavarone M, Donati B, Sangiovanni A, Carnelutti A, Dongiovanni P, Rametta R, Bertelli C, Facchetti F, Colombo M, Fargion S, Fracanzani AL. PNPLA3 I148M polymorphism, clinical presentation, and survival in patients with hepatocellular carcinoma. PLoS One. 2013 Oct 14;8(10):e75982. doi: 10.1371/journal.pone.0075982. PMID: 24155878; PMCID: PMC3796509.
50. Thrift AP, Kanwal F, Lim H, Duong H, Liu Y, Singal AG, Khaderi S, Asrani SK, Amos CI, El-Serag HB. PNPLA3, Obesity, and Heavy Alcohol Use in Cirrhosis Patients May Exert a

Synergistic Increase Hepatocellular Carcinoma Risk. Clin Gastroenterol Hepatol. 2024 Sep;22(9):1858-1866.e4. doi: 10.1016/j.cgh.2024.04.006. Epub 2024 May 9. PMID: 38729396; PMCID: PMC11615715.

51. Kim HS, Xiao X, Byun J, Jun G, DeSantis SM, Chen H, Thrift AP, El-Serag HB, Kanwal F, Amos CI. Synergistic Associations of PNPLA3 I148M Variant, Alcohol Intake, and Obesity With Risk of Cirrhosis, Hepatocellular Carcinoma, and Mortality. JAMA Netw Open. 2022 Oct 3;5(10):e2234221. doi: 10.1001/jamanetworkopen.2022.34221. PMID: 36190732; PMCID: PMC9530967.
52. Kanwal F, Kramer JR, Mapakshi S, Natarajan Y, Chayanupatkul M, Richardson PA, Li L, Desiderio R, Thrift AP, Asch SM, Chu J, El-Serag HB. Risk of Hepatocellular Cancer in Patients With Non-Alcoholic Fatty Liver Disease. Gastroenterology. 2018 Dec;155(6):1828-1837.e2. doi: 10.1053/j.gastro.2018.08.024. Epub 2018 Aug 23. PMID: 30144434; PMCID: PMC6279617.
53. Singal AG, El-Serag HB. Rational HCC screening approaches for patients with NAFLD. J Hepatol. 2022 Jan;76(1):195-201. doi: 10.1016/j.jhep.2021.08.028. Epub 2021 Sep 9. PMID: 34508791; PMCID: PMC8688224.
54. *Marrero JA, Kulik LM, Sirlin CB, Zhu AX, Finn RS, Abecassis MM, Roberts LR, et al. Diagnosis, Staging, and Management of Hepatocellular Carcinoma: 2018 Practice Guidance by the American Association for the Study of Liver Diseases. Hepatology 2018;68:723–750.* [DOI] [PubMed] [Google Scholar]
55. *European Association for the Study of the Liver. Electronic address eee, European Association for the Study of the L. EASL Clinical Practice Guidelines: Management of hepatocellular carcinoma. J Hepatol 2018;69:182–236.*
56. Simmons O, Fetzer DT, Yokoo T, Marrero JA, Yopp A, Kono Y, Parikh ND, Browning T, Singal AG. Predictors of adequate ultrasound quality for hepatocellular carcinoma surveillance in patients with cirrhosis. Aliment Pharmacol Ther. 2017 Jan;45(1):169-177. doi: 10.1111/apt.13841. Epub 2016 Nov 8. PMID: 27862091; PMCID: PMC7207219.
57. Tzartzeva K, Obi J, Rich NE, Parikh ND, Marrero JA, Yopp A, Waljee AK, Singal AG. Surveillance Imaging and Alpha Fetoprotein for Early Detection of Hepatocellular Carcinoma in Patients With Cirrhosis: A Meta-analysis. Gastroenterology. 2018 May;154(6):1706-1718.e1. doi: 10.1053/j.gastro.2018.01.064. Epub 2018 Feb 6. PMID: 29425931; PMCID: PMC5927818.
58. Kim SY, An J, Lim YS, Han S, Lee JY, Byun JH, Won HJ, Lee SJ, Lee HC, Lee YS. MRI With Liver-Specific Contrast for Surveillance of Patients With Cirrhosis at High Risk of Hepatocellular Carcinoma. JAMA Oncol. 2017 Apr 1;3(4):456-463. doi: 10.1001/jamaoncol.2016.3147. PMID: 27657493; PMCID: PMC5470420.
59. Ioannou GN, Green P, Kerr KF, Berry K. Models estimating risk of hepatocellular carcinoma in patients with alcohol or NAFLD-related cirrhosis for risk stratification. J Hepatol. 2019 Sep;71(3):523-533. doi: 10.1016/j.jhep.2019.05.008. Epub 2019 May 28. PMID: 31145929; PMCID: PMC6702126.
60. Best J, Bechmann LP, Sowa JP, Sydor S, Dechêne A, Pflanz K, Bedreli S, Schotten C, Geier A, Berg T, Fischer J, Vogel A, Bantel H, Weinmann A, Schattenberg JM, Huber Y, Wege H, von Felden J, Schulze K, Bettinger D, Thimme R, Sinner F, Schütte K, Weiss KH, Toyoda H, Yasuda S, Kumada T, Berhane S, Wichert M, Heider D, Gerken G, Johnson P, Canbay A. GALAD Score Detects Early Hepatocellular Carcinoma in an International Cohort of Patients With Nonalcoholic Steatohepatitis. Clin Gastroenterol Hepatol. 2020 Mar;18(3):728-735.e4. doi: 10.1016/j.cgh.2019.11.012. Epub 2019 Nov 8. PMID: 31712073.