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Hepatocellular Carcinoma: Known and Emerging Risk Factors

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Abstract

Hepatocellular carcinoma (HCC) is the most frequent primary liver cancer with a high mortality rate. While chronic hepatitis B virus (HBV) and hepatitis C virus (HCV) infections represent the leading risk factors worldwide, the spreading of metabolic disorders, such as diabetes, obesity and non-alcoholic fatty liver disease (NAFLD) justifies the increasing attention on their oncogenic mechanisms. This review discusses about the main pathogenic mechanisms implicated in occurrence of HCC in presence of viral and metabolic diseases. Additionally, it points to the importance of clinical surveillance for those patients considered at risk of HCC and highlights the strategical role of serum markers, such as alfa-fetoprotein (*a*FP) and Protein Induced by Vitamin K Absence or Antagonist II (PIVKA-II), which, in association to a strictly instrumental follow-up, contribute to the early detection of hepatic nodules with a better prognosis for affected patients.

Keywords

Hepatocellular Carcinoma (HCC), Hepatitis B Virus (HBV), Hepatitis C Virus (HCV), Non-Alcoholic Fatty Liver Disease (NAFLD), Non-Alcoholic Steatohepatitis (NASH), Insulin Resistance (IR), Type 2 Diabetes Mellitus (T2DM), Intestinal Microbiota, Visceral Obesity, Alfa-Fetoprotein (*a*FP), Protein Induced by Vitamin K Absence or Antagonist II (PIVKA-II)

1. Introduction

Hepatocellular carcinoma (HCC) generally develops from a background of chronic inflammatory liver disease and, despite both the imminent disappearance of Hepatitis C Virus (HCV)-related chronic disease and the maintenance of a low or absent replication load in Hepatitis B Virus (HBV) chronic infections,

thanks to new effective antiviral therapies, it represents one of the predominant causes of malignancy-related death worldwide [1]. The leading incidence of HCC in metabolic disorders motivates the clinical approach to the emergent pre-cancerogenic role of non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH) and justifies the leading scientific interest for the metabolic factors that contribute to their pathogenesis. Moreover, the spreading of metabolic disorders, in addition to known viral risk factors, has required a strictly clinical observation in those patients considered at risk in order to achieve an early diagnosis of HCC.

2. Epidemiology

HCC is considered the fifth most common cancer in men and the ninth in the women with a male:female ratio of 2.4 [2]. Moreover, it is the second leading cause of cancer-related death worldwide, preceding lung and stomach cancers [3]. Often, it is diagnosed during the fourth and fifth decades of life with a two times higher incidence among dark-skinned people compared to light-skinned males [4].

HCC distribution is variable worldwide and it has been observed a higher incidence in endemic HBV infection areas, such as sub-Saharan Africa and Eastern Asia (over 20 cases/100.000 population). Conversely intermediate incidence occurs in Mediterranean countries, such as Italy, Greece and Spain, with a rate of 10 - 20 cases/100.000 population, while North and South America have a relatively low incidence < 5 per 100.000 individuals [3] [5].

A multitude of etiological risk factors could contribute to the cancerogenesis of HCC. Firstly, hepatotropic viruses, such as HBV, HCV and Hepatitis D Virus (HDV), are strongly associated with the development of HCC, with an increased risk by two to six folds in the HBV/HCV and/or HBV/HDV coinfections. Similarly, alcohol abuse and consumption of aflatoxin B1-contaminated foods are additional risk factors, in association with the emergent role of NAFLD and metabolic syndrome in the incidence of HCC [6] [7] (**Figure 1**).

3. HBV

Despite the emergent role of metabolic syndrome as further risk factor of HCC, HBV infection remains the leading cause for primary hepatic neoplasia with a prevalence of 3.61% worldwide [8]. As demonstrated by several meta-analysis, the risk of developing HCC is significantly higher (15 - 20 times) in HBV-infected populations compared to uninfected populations [9]. In endemic areas, such as Eastern and African countries, the prevalent HBV transmission is vertical and perinatal and more than 90% of infected patients become chronic HBV carriers. On the contrary, in low prevalence geographic areas, such as Western countries, the HBV infection is acquired during adulthood through sexual or parental routes and it resolves spontaneously in more than 90% of cases.

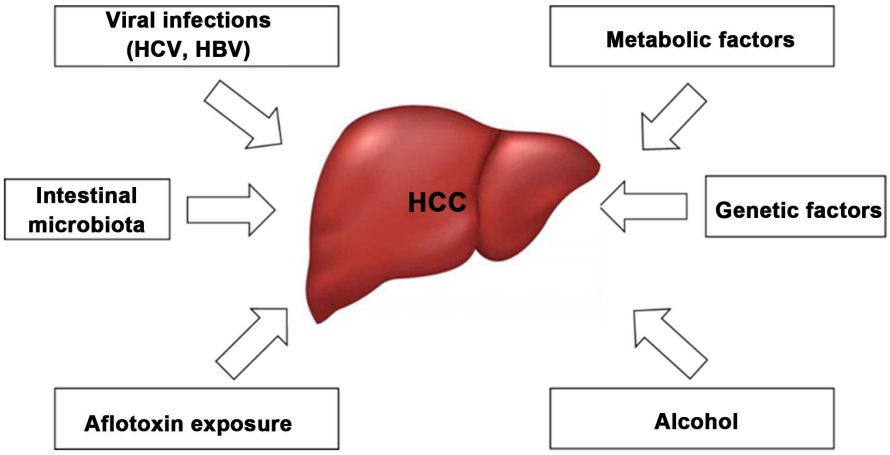

Figure 1. Risk factors for HCC.

It has been reported that annual risk of HCC is 0.5% and 0.8% for asymptomatic HBsAg carriers and for patients with chronic HBV disease, respectively; the effective annual risk increases by 100 times for HBV cirrhotic people [5]. Although 70% - 90% of HCC occurs in cirrhotic HBV carriers, HBV chronic infection is a well-known cause of HCC also in absence of cirrhosis (**Figure 2**). The risk of developing hepatocellular carcinoma in patients with persistent HBV infection is greater among males and correlates with the duration of infection, high levels of HBV viral load and infection with genotype "C" of HBV [10]. Co-factors such as family history of HCC, exposure to mycotoxins (aflatoxin), alcohol abuse or tobacco dependence, HCV and/or HDV co-infections, greatly increase the risk of HCC [11] [12].

HBV is an enveloped virus belonging to the *Hepadnaviridae* family. Its prevalent hepatotropic behaviour is linked to its capacity of recognizing highly heparin sulfate proteoglycans (HSPGs) on the surface of hepatocytes and, through sodium taurocholate cotransporting polypeptide (NTCP or SLC10A1), HBV colonises infected liver cells [13]. Subsequently, HBV partially double-stranded DNA (rcDNA) becomes a completely circular DNA (ccDNA) into the hepatocyte nucleus and it mimics a template for all HBV proteins [14].

It seems that HBV X protein (HBVx) plays a critical role in the development of HCC activating Wnt/β-catenin signalling and promoting tumor initiation through different antiapoptotic mechanisms [15]. First of them, HBVx inhibits p53-mediated apoptosis and it increases the expression of telomerase reverse transcriptase and telomerase activity, thus prolonging the lifespan of hepatocytes and inducing malignant transformation [16]. Additionally, HBVx truncation contributes to the integration of HBV into the host genome and the c-terminal region produced by its truncation is implicated in the cancerogenesis. As matter of facts, this genomic region induces hepatocytes proliferation, oxidative stress through the production of ROS and it increases the invasiveness and the metastasis HCC-related through the activation of MMP10 by C-Jun signalling [17] [18] [19] [20].

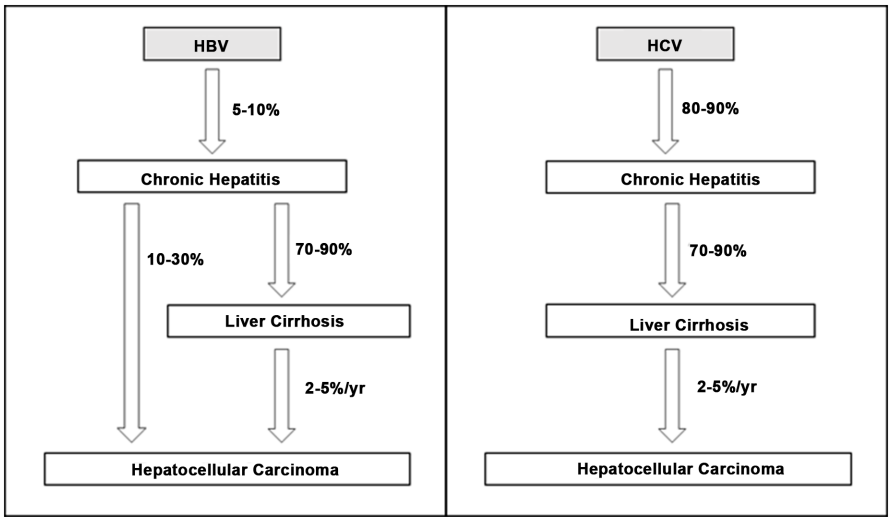

Figure 2. Progression of chronic HBV and HCV infection to HCC.

Small amounts of ccDNA persist in patients with occult HBV infection (e.g. patients HBsAg-negative/anti-HBsAg negative/anti-HBcAg positive) and even in 3% - 5% of patients who have cleared HBV infection with seroconversion to anti-HBsAg, This may represent a risk of HBV reactivation with reappearance of HBsAg in subjects with severe immunosuppression, requiring antiviral prophylaxis during immunosuppressive therapies for malignant, inflammatory and autoimmune diseases [21] [22] [23] [24].

Because of this peculiar ability to integrate into the host genome, control of HBV replication by nucleos(t)ide analogues (NAs: entecavir and tenofovir disoproxil), reduces but doesn't eliminate the risk of HCC in patients with persistent HBsAg positivity [25] [26] [27]. Therefore, despite of serum HBV-DNA negativization, a strictly surveillance is recommended in order to achieve an early stage HCC diagnosis.

4. HCV

Globally, HCV prevalence has been estimated at 2.5%, affecting almost 177 million of people worldwide and 10% - 20% of HCC diagnosed are HCV infection-related [28]. While HBV infection only chronicizes in 5% - 10% of cases, the chronicity of HCV infection occurs in about 85% - 90% of patients [29] (**Figure 2**). In contrast to the Eastern and African countries, where HBV represents the prevalent risk factor for HCC, HCV is considered the leading cause of HCC in developed countries and nowadays the first indication for liver transplantation for HCC in US [30] [31].

HCV, a single-strand RNA virus belonging to the *Flaviviridae* family, enters human hepatocytes by using cell surface receptors (CD81 and SRB-1) and/or tight junction proteins (Occludin-1 and Claudin) [32] [33] [34]. During replication, HCV genome encodes a polyprotein of about 3000 amino acids which, following a proteolysis, produces the different structural (Envelop E1, E2 and Core)

and non-structural proteins (P7, NS2, NS3, NS4A, NS4B, NS5A and NS5B) [35]. HCV-induced chronic damage of infected hepatocytes causes the release of pro-inflammatory and fibrotic mediators such as reactive oxygen species and cell death signals [36]. The activation of hepatic stellar cells, predominantly mediated through intracellular inflammasome activation and nuclear receptor family (farsenoid-X-receptor, peroxisome proliferator-activated receptors and others), promotes hepatic inflammation and, consequently, cirrhosis, but also promotes malignant transformation of hepatic infected cells [7] [37] [38]. Several studies demonstrate that core proteins can alter the MAPk pathway, inducing cell proliferation. Moreover, NS5A inhibits p53 protein and, contrasting its antitumoral effects, causes neoplastic degeneration [39]. Furthermore, Platelet-derived growth factor (PDGF) is the most potent mitogenic signal, inducing expression of beta PDGF receptor in stellate cellstogether with other cell surface receptors of growth signaling such as integrins and contributing to fibrogenesis [40]. On the other side, HCV replication stimulates immune defensive response in the host, through the mediation of tumor necrosis factor (TNF-α) and interferons, results in fibrosis and end stage liver disease. The severity of fibrosis is strictly linked to a higher probability of developing HCC, although different studies highlight the diagnosis of HCC even in low-grade or absence of fibrosis ([41]). Older age at the time of infection, male gender, coinfection with HBV or human immunodeficiency virus (HIV), and metabolic disorders such as obesity, insulin-resistance, diabetes and metabolic syndrome are conditions that significantly increase the risk of developing HCC [42] [43].

The recent introduction of directly acting antivirals (DAAs) allows achieving a sustained virologic response (SVR) in more than 90% of treated patients, whatever the stage of liver fibrosis. Moreover, the excellent safety profile of DAAs has allowed treatment to a larger number of HCV patients with more advanced liver cirrhosis and a higher risk of HCC [44]. HCV eradication reduces risk of hepatic decompensation and both liver-related and overall mortality, in these subjects [45]. Similarly, the risk of new occurrence and/or recurrence of HCC in patients with advanced liver disfunction or cirrhosis is also significantly reduced but not completely eliminate, despite of HCV infection clearance [46] [47] [48] [49] [50]. Thus, regular surveillance is mandatory amon these patients, even after clinical stabilization and improvement of the fibrosis.

5. Alcohol

Alcoholic abuse is considered the oldest risk factor for HCC, particularly in the case of heavy consumption (50 - 70 gr/die) [5]. Furthermore, in the Western developed countries it is considered the leading cause of mortality among people aged between 15 - 49 year [51] [52].

Alcohol is metabolised in acetaldehyde by both alcohol dehydrogenase (at low alcohol concentrations) and CYP2E1 (at higher concentrations), thus it is further metabolised by aldehyde dehydrogenase to acetate. Ethanol's metabolites

are responsible for the chronic hepatocytes injury; particularly, excessive production of reactive oxygen species (ROS) causes protein, lipid, and DNA oxidation, additionally to a direct cell injury by DNA damage, lipid peroxidation and tumour necrosis factor (TNF) pathway signalling via nuclear factor kappa B [53]. Further mechanisms include iron overload, decreased vitamin A (retinoid acid) levels and downregulation of S-adenosyn-methionine [54].

Although alcohol abuse increases the risk of HCC through the development of fibrosis, there is no evidence that highlights its direct cancerogenic potential. Conversely, different meta-analysis show the synergic actions of alcohol and HBV and HCV infection in the process of fibrosis and progression to cirrhosis with a significant higher rate among those patients with a higher consumption [55].

6. Aflatoxin Exposure

Aflatoxins are secondary metabolites of *Aspergillus flavus* and *Aspergillus parasiticus*, which are fungus that grow under climatic factors, such as high moisture and temperature, typically of Asia and sub-Saharan Africa. Furthermore, they are common contaminants of food, especially in maize, groundnuts, wheat, soy and rice [56].

There are several types of aflatoxin and, according to the International Agency for research on cancer, Aflatoxin B1 (AFB1) is the most toxic and carcinogenic of the family [57]. As matter of fact, while AFB1 is absorbed in the enterocytes of the small intestinal epithelium, liver injury is related to the conversion into aflatoxin-8,9-exo-epoxid thru hepatic cytochrome P450. This metabolite is highly reactive with the p53 tumor suppressor gene and it generates mutation at codon 249 that has been observed in more than 50% of HCC cases aflatoxin exposure-related [58] [59]. Subsequently, aflatoxin-8,9-exo-epoxid binds hepatocyte DNA forming procancerogenic adducts that cause further mutations in nucleotides [58].

It has been observed that areas with high prevalence of HCC-aflatoxin related coincide with areas with endemic HBV infection, suggesting a synergic mutagenic role of HBV and AFB1 and justifying the highest risk among those people exposed to both [60] [61] [62].

7. Metabolic Risk Factors for HCC

7.1. NAFLD/NASH

NAFLD is the most common hepatic disorder in developed countries, with a prevalence ranging from 6% to 35% [63]; moreover, it is considered the emerging leading cause of chronic liver disease, with an estimated incidence in Western countries (20% - 30%) higher than in Asia (5% - 18%) [64]. Additionally, the prevalence of NAFLD is increasing worldwide, depending on the current trends in dietary irresponsibility and preponderance of a sedentary lifestyle and showing a significant linear rise with diabetes and metabolic syndrome [65]. Cur-

rently, NAFLD represents the second common indication to be listed in liver transplantation and it has been projected that, within the next 10 - 20 years, it will become the major cause of liver-related morbidity and mortality, as well as a leading indication for liver transplantation [66].

NAFLD is considered a heterogeneous clinical entity that, starting off the simple hepatic deposition of triglycerides (TG) and steatosis, might evolve to NASH, liver fibrosis, cirrhosis, and eventually hepatocellular carcinoma [65]. Furthermore, it has been observed that progression to cirrhosis or HCC is slower in the presence of NAFLD/NASH than in other chronic liver diseases, occurring in 2.5% of patients [67]. Additionally, an increased risk of cirrhosis and HCC has been reported in patients with NAFLD and metabolic syndrome (MS)-associated clinical disorders, such as insulin-resistance (IR)/type 2 diabetes mellitus (T2DM), visceral obesity, dyslipidaemia and arterial hypertension [68] [69] [70] [71] [72] (**Figure 3**).

Liver accumulation of TG represents the direct metabolic consequence of insulin-resistance (IR) and hyperinsulinemia; in fact, IR increases lipoprotein lipase (LpL) activity of adipocytes, with lipolysis and free fat acids (FFA) mobilization through liver, where they are reassembled in TG. Furthermore, hyperinsulinemia reduces microsomal triglyceride transfer protein (MTP) activity, involved in Apo-B secretion, resulting in lower VLDL synthesis and, finally, in a reduced release of TG from the liver [73].

Accumulation of TG in hepatocytes may induce activation of mitochondrial lipoxygenase that increases production of ROS. The alteration of the oxidative state results in micro-inflammation and liver fibrosis [74] [75] [76]. ROS oxygen species induce Kupffer cells (KCs) to produce pro-inflammatory cytokines such as TNFα, TGF-β and IL-8, which are involved in the activation and migration of hepatic stellate cells (HSCs) [77] [78] [79]. HSCs produce lots off collagen fibres resulting in fibrotic state. Under physiological condition, liver injury is rapidly repaired and the fibrotic material (collagen type I, III and IV) is degraded to allow parenchymal tissue regeneration [80]. On the contrary, when the damage persists, the repair mechanisms are continuously activated by depositing, especially in the periportal and perisinusoidal zones, of high amounts of extracellular matrix, predominantly composed by proteoglycans, hyaluronic acid, collagen

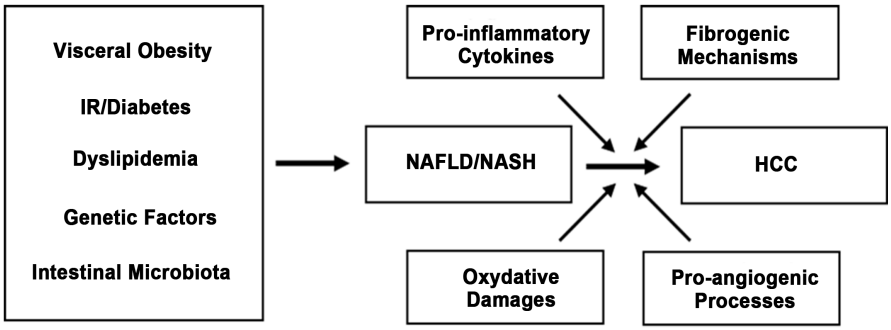

Figure 3. Metabolic Syndrome, NAFLD/NASH and risk of progression to HCC.

type I and III. This extracellular matrix organizes to form fibrous septa which connect portal areas to one another and often to central areas. Meanwhile, due to the lack of a specific orientation, hepatocytes regenerate by forming nodular structures that do not repeat the typical liver structure [77] [78].

Although HCC usually develops in patients who have progressed to NASH and liver cirrhosis, there are evidences that it may occur in patients with NAFLD in absence of advanced liver fibrosis, making liver steatosis itself a risk factor for HCC. Sanyal *et al.* reported that the majority of HCC associated with NAFLD/NASH occur in patients who haven't International Classification of Disease criteria for cirrhosis [81]. Moreover, the risk of developing HCC without cirrhosis is higher in patients with liver steatosis and metabolic syndrome criteria, particularly IR/T2DM and visceral obesity [82].

7.2. Visceral Obesity

Epidemiological reports demonstrate that overweight and obesity, two rapidly increasing conditions in developed countries and defined by a body mass index (BMI) higher than 25, significantly correlate with an elevated cancer risk [83] [84]. More than 90,000 cancer-related deaths per year in the US are linked to excessive body weight [85].

Visceral adiposity causes a low grade chronic inflammation with a massive release of pro-inflammatory cytokines. In particular, adipocytes secrete several pro-inflammatory molecules including tumor necrosis factor-alpha (TNFα) and interleukin-6 (IL-6) which are more clearly linked to the progression from NAFLD to NASH and HCC by stimulating pro-oncogenic signal pathways [84]. TNFα activates c-Jun NH_2-terminal kinase (JNK) phosphorylating c-Jun at Ser-63 and Ser-73 by mitogen-activated protein kinases (MAPKs) activity, resulting inapoptosis inhibition and cancerogenesis [86]. Moreover, IL-6 binds to IL-6 receptors (IL-6R) on hepatocytes and liver non-parenchymal cells (biliary epithelial cells and HSCs) to promote binding of the signal-transducing receptor gp130 to the IL-6R complex, thus activating Janus Kinase1 (JAK1). Subsequently, JAK1 activates several intracellular signalling pathways including signal transducer and activator of transcription-3 (STAT3) factor, a known mediator of apoptosis inhibition and cell proliferation [87] [88].

Furthermore, adipose tissue remodelling modifies the hormonal circulation assessment causing adipokines imbalance with increased leptin and decreased adiponectin [87] [89]. It has been showed that leptin is implicated in pro-inflammatory, pro-fibrogenic and pro-angiogenic processes by activating different molecular pathways, such as JAK2/STATs, posphatidylinositide-3-kinases (PI-3Ks)/protein kinase B (Akt) and MAPKs/extracellular regulated kinase 1 and 2 (ERK 1/2). Conversely, adiponectin antagonizes leptin effects with ant-inflammatory, anti-fibrotic, anti-angiogenic and anti-proliferative activities by reducing STAT3 and Akt phosphorylation and by upregulating the expression of suppressor of cytokine signalling (SOCS3) [90] [91].

7.3. IR/T2DM

Almost 70% of obese patients affected by T2DM develops NAFLD and almost 30% of them progress to NASH, highlighting that T2DM increases risk for cirrhosis and HCC [92] [93]. Large population-based cohort studies showed a 1.86 to 4-fold increase in risk of HCC among patients with diabetes [94].

As already mentioned, IR causes the release of free fatty acids (FFA) towards hepatocytes inducing oxidative stress through overproduction of ROS, cellular inflammation and cancerogenesis. It has been reported that trans-4-hydroxy-2-nonenal, a product of lipid peroxidation, causes mutation of p53 tumor suppressor gene which is associated with more than half of human cancers, including HCC [94].

Hyperinsulinemia IR-induced increases bioavailable insulin-like growth factor-1 (IGF-1) released by hepatocytes because of lower plasmatic concentration of IGF binding proteins. Levels of insulin-like growth factor-2 (IGF-2) are also increased in type 2 diabetic patients; however, IGF-2 is highly expressed in fetal liver and its physiologic function in adult liver remains obscure [95]. The IGF-1 receptor (IGF-1R) is a tyrosine kinase cell-surface receptor that is activated by both IGF-1 and IGF-2, and by insulin with a lower affinity. IGF-1R signalling pathway causes phosphorylation of insulin receptor substrate 1 (IRS-1), a key protein involved in cellular proliferation through phosphatase and tensin homolog (PTEN)/PI-3Ks/Akt and MAPKs cascades.

8. Microbiota

Several observations demonstrate that intestinal microbiota (IM) contributes to NAFLD and its status may promote progression to NASH, particularly in obese patients [96] [97].

IM is mainly composed ofat least 500 - 1000 different species and trillions of aerobes and anaerobes microorganisms and, although more than 50 bacterial phyla have been detected, it is dominated by the Bacteroidetes and the Firmicutes [98]. The gut bacteria are able to synthesize vitamins and all essential and nonessential amino acids, contributing to biotransformation of bile [99]. Additionally, they provide the biochemical substrates for the metabolism of host-derivated mucines and nondigestible carbohydrates (large polysaccharides, such as starches, cellulose, hemicellulose, pectins, and gums; some oligosaccharides escaped from digestion; unabsorbed sugars and alcohols from the diet) [100]. These metabolic functions results in the recovery of energy for the host and a supply of energy for the microbiota growth and proliferation. Function and compositionof the IM are influenced by several factors such as age, pH of the intestinal enviroment, high fat diet and probiotics [101].

Emergent evidences show a strong cross-talk between gut and liver, the so called “gut-liver axis”, and it seems that intestinal bacteria are responsible of the maintenance of a healthy axis; furthermore, almost 70% of liver blood

derivates from intestine, justifying its role of first line defense against gut-derivated antigens. The findings of NAFLD/NASH as a common complication of both jejuno-ileal bypass surgery for obesity andjejunal diverticulosis, as well as Intestinal Bacterial Overgrowth (IBO), supportthe strictly correlation between NAFLD/NASH and gut-liver axis alterations [102]. In fact, IM-related progression of NAFLD to NASH and HCC depends on increased gut permeability andendotoxin translocation towards liver, inducing the transcriptional activation of a wide variety of proinflammatorygenes and cytokines, suchas TNF-a, and stimulating pro-apoptotic cascades [103] [104]. Additionally, levels of bacterialli popolysaccharide (LPS), a component of theouter membrane of Gram-negative bacteria, are highestin the portal and/or systemic circulation in several types ofchronic liver diseases [105]; LPSbehaves as a hepatotoxin, binds the Kupffer cells toll-like receptor 4 (TLR4) and triggers an intracellular inflammatory cascade that, through the activation of nuclear factor kappa (NF-κB), induces the production of proinflammatory cytokines, such as TNF-α, IL-1, and IL-6 [106]. Furthermore, TLR4 expressed on hepatic stellate cells (HSCs), mostly implicated in the extracellular matrix deposition, is responsible for the fibrotic process LPS-induced [107].

9. Polymorphisms

Emergent evidences show that specific gene polymorphisms are determinant factors for susceptibility and progression of NAFLD. Among them, single-nucleotide polymorphism (SNP) in the patatin-like phospholipase domain-containing protein 3 (PNPLA 3) gene seems to play a key role in TAG remodelling [72]. Asreported by Romeo *et al*., rs 738409 C > G SNP in the PNPLA3 gene, also called adiponutrin, is significantly associated with NAFLD and it increases the risk of NAFLD-related HCC in European and Japanese populations [108] [109].

PNPLA3, a 481-residue protein, has been identified in various tissues but mostly expressed in the liver, particularly in hepatocytes and hepatic stellate cells, where it explicates hydrolase activity towards triglycerides and retinyl esters, respectively [110]. The cytosine (C) to guanine (G) transversion in rs738409 causes an isoleucine (I) to methionine (M) exchange at amino acid 148 (I148M) with loss of its hydrolysefunction and consequent retention of fat in hepatocytes and retinol in hepatic stellate cells [111] [112]. Additionally, the I148M mutation also reduces very low density lipoproteins (VLDL) secretion and it partially justifies the fat depots in *PNPLA*3 mutation carriers [113].

Retention of retinoids in hepatic stellate cells results in cell proliferation and differentiation [112] [114] [115]; moreover, the detection of a highest lactate-to-pyruvate ratio in patients with PNPLA3 I148M mutation suggests a shift to anaerobic metabolism probably as a consequence of hepatic hypoxia, mitochondrial dysfunction, and oxidative stress [116]. These pathogenic mechanisms could explain the increased risk of progression to NASH and HCC in patients with Met148 PNPLA3 over expression [117].

Table 1. Sensitivity, specificity and predictive value of PIVKA-II and *a*FP in the diagnosis of HCC (134).

	Sensitivity	Specificity	PPV	NPV
PIVKA-II	60%	88%	80%	73%
*a*FP	67%	68%	63%	72%
PIVKA-II + *a*FP	70%	94%	91%	79%

Two other specific gene polymorphisms, such as TM6SF2 E167K and GCKR rs780094, also seem to be associated with a higher risk of fatty liver and liver fibrosis [72]. These evidences underline the role of steatosis as a strong precancerogenic factor that, in association to genetic environment, could contribute to progression to HCC.

10. Surveillance

Nowadays, almost 50% of HCC are diagnosed at advanced stage with a poor prognosis and a 5-year overall survival rate lower than 10%. On the contrary, in patients who receive an earlier diagnosis, the 5-year survival rate increases to 70% [117]. As suggested by American Association for the Study of Liver (AASLD) and European Association for the Study of Liver (EASL) guidelines for the management of HCC, the implementation of surveillance by a combination of instrumental investigations such as ultrasounds (US), computed tomography (CT) and/or magnetic resonance imaging (MRI), allows to significantly improve the prognosis thanks to the detection of smaller tumors [118] [119]. The availability of serum biomarkers such as alpha-fetoprotein (*a*FP) and Protein Induced by Vitamin K Absence or Antagonist II (PIVKA-II) in combination with imaging also contributes to improve the accuracy of screening of hepatic malignancies.

The role of *a*FP levels in the diagnosis of HCC remains controversial; in fact, despite of elevated *a*FP levels in 60% - 80% of patients with HCC and its usefulness in monitoring of HCC-treated patients, serum *a*FP values may be influenced by non-neoplastic factors, such as viral infections and cirrhosis [120] [121] [122]; moreover, low levels of *a*FP may be detectable in more than 50% of HCC patients, highlighting the poor sensitivity of this biomarker [123].

PIVKA-II is considered a suitable serum marker of HCC due to its high specificity. Thanks to the development of accurate measuring methods, Japanese Society Hepatology (JSH) recommends it as a marker of surveillance for HCC [124] [125] [126]. PIVKA-II, also known as des-γ-carboxy-prothrombin, is an abnormal product of decarboxylation detected in HCC patients, which explicates a mitogenic effect on liver cells, inducing cancerogenesis. Rentao *et al.* enrolled 2070 PIVKA-II positive patients including 1016 (49.1%) HCC. Serum levels of PIVKA-II resulted significantly higher in HCC, especially in advanced stage, as compared with no-malignant diseases (including patients with cirrhosis, chronic hepatitis, steatosis, benign nodules, liver abscess, pregnancy) [127]. Furthermore,

an Italian observational cohort study in patients with US evidence of liver nodules, shows a higher specificity and positive predictive value (PPV) of PIVKA-II as compared to *α*FP; furthermore, the combined use of PIVKA-II and *α*FP improves the diagnostic accuracy as compared to either PIVKA-II or *α*FPalone (**Table 1**) [128].

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