JBUON 2017; 22(1): 6-20
ISSN: 1107-0625, online ISSN: 2241-6293 • www.jbuon.com
E-mail: editorial_office@jbuon.com

REVIEW ARTICLE

Hepatocellular carcinoma development in non-alcoholic fatty liver disease and non-alcoholic steatohepatitis. Is it going to be the "Plague" of the 21st century? A literature review focusing on pathogenesis, prevention and treatment

Chrysoula Argyrou[1]*, Demetrios Moris[2]*, Spiridon Vernadakis[1]

[1]Transplantation Unit, "Laikon" General Hospital, Athens, Greece; [2]Department of Immunology, Lerner Research Institute, Cleveland Clinic Foundation, Cleveland, OH, USA

*These authors contributed equally to this article

Summary

Liver carcinogenesis in non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH) is a subject of intense research nowadays, since NAFLD is the most common chronic liver disease, affecting a great percentage of the population worldwide, while hepatocellular carcinoma (HCC), which represents the most common primary liver malignancy, is the third leading cause of cancer-related mortality. The underlying pathogenic pathways of both NAFLD and HCC are not completely understood, but there is growing evidence that they share many common pathophysiologic mechanisms and risk factors. Due to lack of solid evidence, though, the ultimate goal of designing effective diagnostic tools, treatment options and screening policies remain unmet for the time being. This review article aims to present recent data available regarding pathogenesis, diagnosis and management of HCC and NAFLD, as well as to highlight the importance of the development of HCC in the setting of NAFLD and NASH.

Key words: *hepatocellular carcinoma, non-alcoholic fatty liver disease, non-alcoholic steatohepatitis*

Introduction

NAFLD has been recognized as one of the most common causes of chronic liver disease, progressively leading to cirrhosis and liver failure, and is estimated to affect an increasing number of people around the world to a percentage reaching almost 25% of the adult population worldwide [1]. It is true that there is much concern regarding the association of NAFLD with HCC, due to growing evidence that NAFLD is a risk factor for the development of HCC [2-5]. In fact, recent studies suggest that NAFLD is becoming an important cause of HCC, with NAFLD-related HCC individuals to suffer a worst prognosis than other causes of HCC [6,7]. It is estimated that almost 4-22% of HCC cases in the West are related to NAFLD [5]. To make matters even worse, patients with NAFLD are less likely to receive a liver transplant [6], despite the fact that NAFLD is currently the most rapidly growing indication of HCC-ralated liver transplantation (LT), after hepatitis C (HCV) infection, in the United States [8]. The detrimental effects of HCC are well established, since HCC is the third leading cause of cancer-related mortality, with poor prognosis, especially at later stages, when it is usually diagnosed [9-11].

According to the practice guidelines for the

Correspondence to: Demetrios Moris, MD, MSc, PhD, MACS. Lerner Research Institute, Cleveland Clinic, 9500 Euclid Ave., NE60, Cleveland, Ohio 44195, USA. Tel: +216 444 2574, Fax: +216 4454658, E-mail: dimmoris@yahoo.com
Received: 22/10/2016; Accepted: 14/11/2016

NAFLD published in 2012 in order to define NAFLD there must be evidence of hepatic steatosis diagnosed either by histology or by imaging, while secondary causes of hepatic fat accumulation like alcohol consumption, HCV infection, hepatotoxic medications and genetic causes, should be excluded [12]. NASH represents the most extreme form of NAFLD, defined by the presence of hepatic steatosis and inflammation with hepatic injury (called ballooning due to increase in cell size), while fibrosis is not a prerequisite for the diagnosis of NASH, although usually present [13]. Nevertheless, fibrosis can be developed even in the setting of NAFLD [14].

The prevalence of NAFLD and NASH is rather variable and highly depended on the modality used to confirm the diagnosis and on the population studied, while it is usually underreported due to the asymptomatic nature of the disease [15,16]. As a result, it may often be an incidental finding, and even then it can be underestimated and poorly treated [17]. It seems, though, that NAFLD presents in more than 25% of adult population globally [1,15]. Although it was so far considered that almost one third of NAFLD cases will progress to NASH [18], there is evidence that this percentage is even higher [1,19] (Figure 1).

Nevertheless, the severity of this chronic disease depends on the progression from simple steatosis to inflammation, fibrosis, cirrhosis and ultimately cancer and is not yet well defined. The great heterogeneity of the disease and the factors that lead to its progression or regression are not completely understood and the existing data remain vague and conflicting. The possible detrimental effects of such development highlight the fact that the correct diagnosis, treatment and prevention has become a necessity and new strategies should be developed to effectively stand up against this rapidly expanding epidemic.

The aim of this literature review is to present the latest information available regarding the development of HCC in the setting of NAFLD and NASH.

Methods

The MEDLINE/PubMed database was searched using "Non-alcoholic fatty liver disease" "Non-alcoholic steatohepatitis" "Hepatocellular Carcinoma" as keywords. Three independent reviewers (CA, DM, SV) performed the literature search, the study selection and the data extraction. All the references from the identified articles were searched for relevant information. The end date of the literature search was set to July 2016. Our search was focused on the latest published information regarding the pathogenesis, prevention and treatment of the HCC induced by NAFLD and NASH.

Results

Risk factors

There are already numerous studies that have investigated the connection between obesity, diabetes mellitus with NAFLD and HCC. Over the years, it became clear that some of the components of the metabolic syndrome, mainly obesity

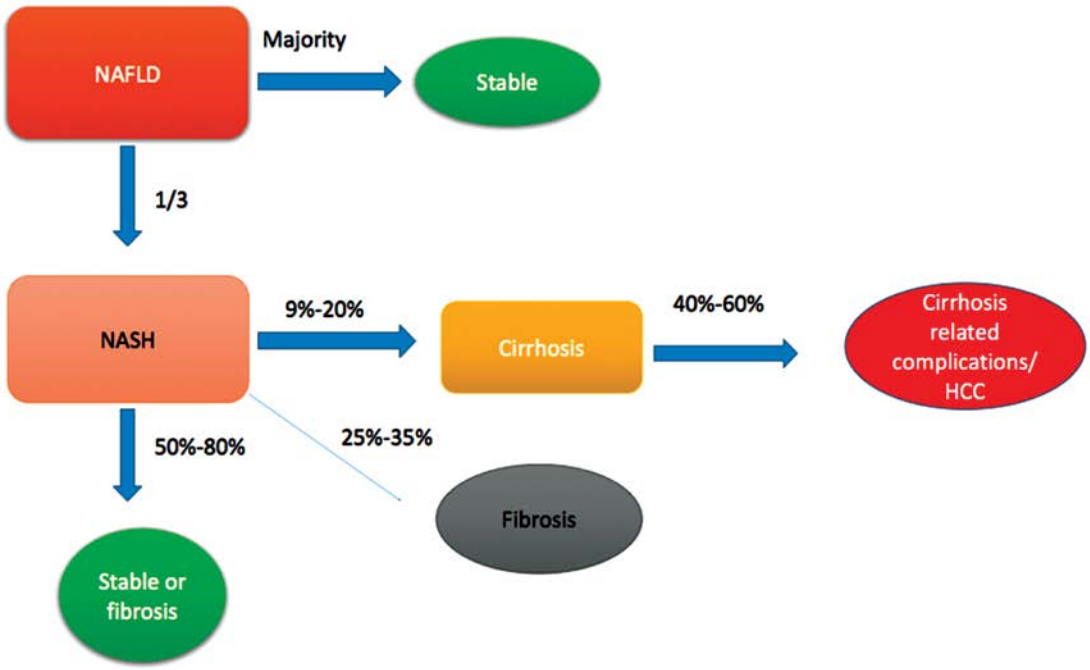

Figure 1. Natural history of nonalcoholic fatty liver disease.

Table 1. Risk factors for HCC in NASH

1. Obesity
2. Diabetes mellitus
3. Insulin resistance
4. Metabolic syndrome
5. Age
6. Genetic Factors
7. Fibrosis/Cirrhosis
8. Iron metabolism

and insulin resistance, are independent risk factors for NAFLD as well as for HCC [20-24].

Chronic liver disease and liver cirrhosis of any etiology remain the most common factors predisposing to primary liver cancer and more specifically to HCC [25]. Hepatitis B and hepatitis C infections are the most prevalent among them, followed by cirrhosis due to alcohol consumption and inherent liver diseases [25,26]. The real incidence of cirrhosis of other etiologies is not precisely known. However, HCC due to causes other than viral hepatitis has become rather common nowadays, with metabolic factors like obesity, diabetes, metabolic syndrome and NAFLD to stand out as the most important causes [6,7,29]. Obesity and diabetes mellitus (DM) have been recognized as independent risk factors for HCC. A meta-analysis conducted by Wang et al. in 2012, revealed that individuals suffering from DM have a 2-fold increased risk of HCC compared with non-diabetics [21]. Elevated alanine aminotransferase (ALT) to an abnormal extent, male gender and older age have also been acknowledged as significant risk factors for HCC when 905 Caucasian patients with non-viral cirrhosis were studied by Archambeaud et al. [22].

The association of factors like obesity and insulin resistance with NAFLD is strong enough to characterize the disease as the "liver component of the metabolic syndrome". This observation is of great importance due to the potential hazards associated with metabolic syndrome. Thus, except its role in the pathogenesis of HCC, NAFLD is also a major predisposing factor for cardiovascular disease and the relevant morbidity and mortality [30,31], especially when metabolic syndrome is also present [32]. The magnitude of the problem becomes even more evident if we consider the fact that almost 80% of people with NAFLD are obese [1,33]. Metabolic syndrome itself, as well as its various components like hypertension and dyslipidemia, have also been shown to have higher prevalence among people with NAFLD and NASH [1,32] (Table 1).

Pathogenesis of NAFLD and HCC - Carcinogenesis in NAFLD

Heterogeneity in the pathogenesis of NAFLD has led to the distinction between two main types of NAFLD, the 'Metabolic NAFLD', caused by obesity and metabolic syndrome and the 'PNPLA3 NAFLD' (Patatin-like phospholipase domain-containing protein 3), reflecting the equal importance of the metabolic and the genetic background of the disease [34]. While different in a pathogenic point of view, both types of NAFLD increase the risk of NASH and HCC.

It is well established so far that NAFLD and HCC share many common risk factors [21,35]. The underlying mechanisms that link these two entities have been extensively investigated so far by a great number of researchers and recently reviewed [27,36,37]. The cornerstones of the mechanisms involved include the excessive fat accumulation and insulin resistance and the common endpoint of both is the production of oxidative stress, which reflects the inability of liver cells to cope with the lipid excess, and its effect on gene expression by inducing DNA alterations and damage leading to carcinogenesis [38].

The role of obesity

Adipose tissue is a rather vivid endocrine organ of the body with many functions. Of all the hormones secreted by adipose tissue, adiponectin and leptin are the most extensively investigated and have been associated with both NAFLD and liver cancer.

Adiponectin, which is the most abundant hormone of adipose tissue, is well-known for its many metabolic functions, including regulation of glucose and lipid metabolism through stimulation of fatty acid oxidation, suppression of hepatic glucose output, and increased insulin sensitivity in liver tissue [39]. Polymorphisms in adiponectin gene cause susceptibility to NAFLD, thus enhancing the pivotal role in the pathogenesis of the disease [40]. Low levels of adiponectin can lead to carcinogenesis through both direct and indirect mechanisms. The final result of this cascade of events is the production of free fatty acids (FFAs) and reactive oxygen species (ROS). Obesity alters normal adipocyte function in many ways. In the case of adiponectin, the levels of this protective cytokine are markedly diminished [39].

Another adipokine that holds a key role to the pathogenesis of obesity in hepatic steatosis, fibrosis and carcinogenesis is leptin [41]. Its

natural role is to act as an energy maintenance factor. Leptin is secreted by adipose tissue and acts, through the hypothalamus, as an hormone implicated in the process of satiety, by inhibiting orexigenic and upregulating anorexigenic neuropeptides [42]. Obese individuals have higher titles of leptin, but they also exhibit resistance to its action, a fact that is involved in the pathogenesis of metabolic syndrome, both directly and by regulating insulin sensitivity [43]. Leptin has also been shown to have a dual role, acting as an antisteatotic factor, but also as a proinflammatory cytokine and as a profibrogenic and proangiogenic factor, thus having an important role in the pathogenesis of NAFLD and NASH and also in HCC [44,45]. Involvement of leptin in a great variety of signaling pathways like STAT3 (signal transducer and activator of transcription 3) and therefore to the signaling of inflammatory cytokines like TNF (tumor necrosis factor), IL-6 (interleukin-6) as well as angiogenic factors, like VEGF (vascular endothelial growth factor) is critical to the HCC cell growth, angiogenesis and metastasis [46].

The role of insulin resistance

Insulin resistance is another key component of the metabolic syndrome that is directly linked to the pathogenesis of NAFLD. Along with obesity they create the circumstances for a chronic inflammatory state with the production of inflammatory cytokines and the reduction in protective antifibrotic and antiinflammatory mechanisms [47]. A recent study also highlights the role of insulin resistance and most importantly its connection with oxidative stress, which is the hallmark of carcinogenesis in the setting of NAFLD and NASH and also linked with the severity of the disease [48]. Another way that insulin resistance can interfere with liver metabolism is by interfering in regulation of the insulin-like growth factor (IGF) pathway [36]. The role of IGF, whose main site of production is the liver, is well established nowadays. It is known that IGF-I production is regulated mostly by the growth hormone (GH), whereas IGF-II is relatively independent and its role is more important during fetal development [49]. Dysregulation of the IGF axis is crucial in hepatocarcinogenesis as it is highlighted by the overexpression of IGF-II and reduction in serum IGF-I concentration observed in HCC [49]. Due to the major role of IGF signaling pathway dysregulation in the pathogenesis of HCC, extensive research is being held in order to find new therapies that will target the IGF axis [50,51].

The role of genetic factors

PNPLA3 is a gene located in chromosome 22. It is known for encoding a protein (named adiponutrin) which is a triacylglycerol lipase that mediates triacylglycerol hydrolysis in adipocytes [52]. NAFLD has been shown to have a strong genetic background, mainly attributed to genetic variations of the PNPLA3 gene [52,53]. These variations are relatively common, especially among people of European descent [53]. More specifically, the I148M variant of PNPLA3 gene has been linked to an increase in NAFLD risk by 1.4-fold [54]. The pathophysiologic pathway that leads to hepatic steatosis involves the impairment of intrahepatic lipolysis, thus increasing liver fat content. Except for the risk of NAFLD, it seems that the risk of progression to fibrosis, cirrhosis and HCC are also increased by this gene variation [55-57].

Recent evidence indicate that the presence of the E434K gene variant downregulates PNPLA3 and lessen the dominant negative action of I148M variant by hindering its accumulation on lipid droplets [58]. This finding is not yet of clinical use, but has added more information regarding the mechanism by which the PNPLA3 variations predispose to liver damage and liver cancer.

Moreover, another recognized gene variation which is implicated in the pathogenesis of NAFLD but also in the progression of fibrosis and disease severity, is the TM6SF2 rs58542926 gene variant [59,60].

On the other hand, other genes can have a protective role in the pathogenesis of NAFLD. For example, the presence of FNDC5 RS3480 gene variant can have a favorable effect on both steatosis and fibrosis in patients with NAFLD, as noticed by Anty et al.[61] in an experimental study and recently in a review article by Margini et al. [37].

The role of liver receptor homolog-1

The role of labile methyl groups (mainly methionine and choline) in liver metabolism and liver damage has been studied for a long time [62]. It has been shown that the ability of keeping methyl donors (SAMe, S-adenosylmethionine) balanced is highly important for normal liver metabolism and diet depleted in those substances can lead to hepatic injury [63]. The liver protective role of SAMe lies to its ability to improve membrane fluidity, to decrease the expression of TNF-a, to change the methylation of DNA and to protect the cells against apoptosis [64]. Wagner et al. recently elucidated

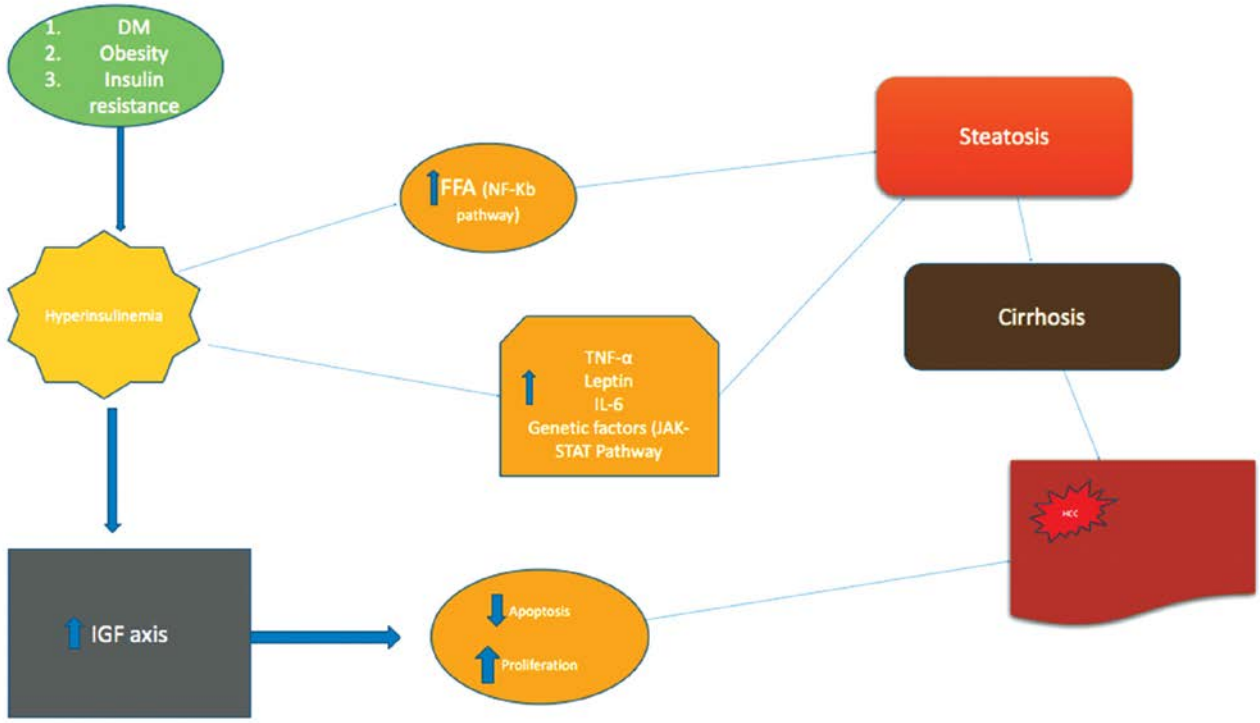

Figure 2. Proposed pathogenesis of HCC in NASH.

the regulatory system involved in the balance of labile methyl groups [65]. They proved that liver receptor homolog-1 (LRH-1) is a transcriptional factor that regulates liver methyltransferases that control and maintain labile methyl-groups in balance. GNMT (glycine N-methyltransferase) represents the majority of liver methyltransferases and is regulated by LRH-1. Its role is to maintain constant the cellular content of SAMe (S-adenosylmethionine). The fact that SAMe deficiency has been already shown to induce NASH and HCC emphasizes the importance of clarifying the relevant pathophysiologic pathway [66,67]. More research should be held towards this direction in order to find a way to exploit the possible therapeutic outcomes, as it is not yet clear what group of patients will profit from the activation or downregulation of the LRH-1 pathway [68].

Figure 2 illustrates the proposed pathogenesis of HCC in NASH.

Diagnosis of NAFLD and NASH

NAFLD and NASH can often be asymptomatic and can be an incidental finding in imaging performed for other reasons [17]. Mild elevation of ALT, with levels of ALT greater that aspartate aminotransferase (AST) and elevation of gamma-glutamyltransferase (GGT) and alkaline phosphatase (ALP) can all be serological manifestations of NAFLD [69]. In order to establish the diagnosis of NAFLD it is important to exclude other causes of chronic liver disease. Except for the common ones, like alcohol consumption or viral hepatitis, more rare causes should be also considered, if there is associated evidence (e.g. high serum ferritin and increased iron saturation should raise suspicion of hemochromatosis) [12].

Due to heterogeneity of NAFLD and NASH, it is rather difficult to use a diagnostic method that will apply to all patients. The gold standard of diagnosis remains liver biopsy and according to AASLD guidelines, it should be considered in patients with increased risk to have NASH and fibrosis [12].

The role of prediction scores and non-invasive biomarkers

Over the years, several scoring systems have been developed in order to assess inflammation and mostly fibrosis of the liver tissue, each of them using different parameters (e.g. NAFLD Fibrosis Score, APRI, BARD Score, FIB-4 etc.) [70,71]. The majority of the various scoring systems were initially designed to assess fibrosis in patients with HCV infection and then adopted for patients with NAFLD. The variety of different assessing systems reflects the inability to develop a single best method to stage fibrosis in all cases.

The most extensively tested among them is the NAFLD Fibrosis Score [70,72]. The parame-

ters measured in this scoring system include age, hyperglycemia, body mass index (BMI), platelet count, albumin, AST and ALT levels. The practice guidelines of AASLD recommend that the NAFLD Fibrosis Score may be used for identifying patients who are at risk for steatohepatitis and advanced fibrosis [12].

Of all the other systems proposed and used so far, FIB-4 Index appears to have the highest accuracy in diagnosing fibrosis in NAFLD patients [70]. Age, platelet count, AST and ALT are the parameters measured in FIB-4 Index [73]. In a recent study, Oral Glucose Insulin Sensitivity Index (OGIS) was shown to predict NAFLD better than NAFLD Fibrosis Score in non-diabetic patients [74]. Another scoring and predicting system that is based on detecting insulin resistance that is commonly present in NAFLD, is Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) [75]. The combination of these noninvasive tests with radiological findings increases the accuracy of assessment of liver fibrosis [56].

Nowadays, it has become a necessity to develop new noninvasive diagnostic methods for NASH. Towards this direction, Kamada et al. were recently able to isolate two glycobiomarkers, fucosylated haptoglobin (Fuc-Hpt) and Mac-2 binding protein (Mac2bp), which can be used independently for the diagnosis of NASH [76]. Other noninvasive biomarkers that have been used in NASH diagnosis are platelet count and serum hyaluronic acid, which are currently included in several scoring systems [77-79]. Plasma cytokeratin-18 fragments have also been evaluated for their ability to diagnose NASH [70,80]. Furthermore, newer methods based on glycated proteins have been recently developed but more research is warranted in order to become clinically useful [71].

The major role of leptin and adiponectin in the pathogenesis of NAFLD and HCC led scientists to investigate their potential use as biomarkers of the severity of the disease [81,82]. Although they cannot yet be used as noninvasive biomarkers for NAFLD or HCC, a recent meta-analysis suggested that there is an association between circulating levels of leptin and the severity of NAFLD [83].

Moreover, some biomarkers can be used in order to define prognosis in NASH-related HCC. One of them is the well- known cell-cycle regulator p27. Recent findings suggest that p27 can be useful in predicting tumor recurrence in NASH patients [82,84].

The role of imaging methods

Since the presence of hepatic fibrosis is one of the most important predictors for the risk of progression of NASH to cirrhosis and the related mortality, it is evident that there is a necessity to quantify hepatic fibrosis.

Ultrasound (US) is the most common modality used in daily practice either as a programmed procedure or in the acute setting. Its ability to detect intrahepatic fat, depicted as hyper-echogenic regions within the liver parenchyma, makes it suitable modality to detect moderate to severe hepatic steatosis [85]. Although cost-effective, US is highly dependent on several factors, like obesity and operator's experience.

Of the other radiological methods available, magnetic resonance imaging (MRI) and spectroscopy (MRS), as well as transient elastography (TE) and MR elastography have been shown to have high accuracy and ability to quantify the amount of fat in the liver [86-89].

Elastography is a noninvasive imaging modality used to measure liver stiffness (Liver Stiffness Measurement-LSM) and subsequently fibrosis. Elastography can be performed either by US or MRI imaging. There have been described several US elastography techniques (TE, acoustic radiation force impulse etc.) [86]. Among them TE has been shown that it is an excellent modality in diagnosing fibrosis stage 3 and 4 (F3, F4) but has moderate accuracy in F2 stage [51]. TE though, has its limits and can be influenced by many factors, like BMI or marked ALT elevations [90]. MR elastography seems to be superior to TE, but further studies designed specifically for NAFLD patients need to be performed [86].

Conclusively, since there is no consensus regarding the diagnosis of NAFLD and NASH, further investigation is needed in order to find a single or

Table 2. Evaluation of imaging modalities in NAFLD and NASH

Imaging modality	*Steatosis*	*Cost-effectiveness*	*Accuracy*	*Comment*
US	++	+++	+	1. Operator-dependent 2. Obesity as an obstacle
Transient elastography	++	++	+	1. Moderate accuracy in F2 stage
MRI-elastography	+++	+	++	1. Lack of validation

a combination of cost-effective and noninvasive methods, both radiological modalities and serological biomarkers, that could successfully replace liver biopsy in terms of accuracy of diagnosis (Table 2).

Management and therapeutic approach of NAFLD and NASH (Table 3)

The possibility of progression of NAFLD to NASH, cirrhosis and HCC highlights the importance of early intervention in the course of disease. However, since it is not possible yet to predict which simple steatosis individuals will develop a progressive disease, several pharmacological agents have been administered in different stages of disease with various and contrasting results regarding their efficacy and safety [91-94]. For the time being, the majority of therapies currently tested apply to patients with proven NASH, whereas treatments that target risk factors of NAFLD are indicated for the rest of NAFLD patients.

The role of lifestyle interventions

It is true that NAFLD patients are usually obese individuals with lack of healthy habits, like healthy diet and regular exercise [95]. Therefore, it is not surprising that the mainstay of treatment of NAFLD is lifestyle modifications. Weight loss and physical activity have been shown to reduce hepatic steatosis and improve the metabolic profile of patients [12,16]. The beneficial results of these alterations are mainly attributed to the improvement of NAFLD major risk factors, like DM, insulin resistance, hypertension and dyslipidemia [95]. Thus, it is important to inspire patients to adopt a healthier lifestyle due to the multiple beneficial effects.

The role of pharmacological therapies

Several insulin sensitizers, antioxidants and lower-lipid substances have been suggested over the years for the treatment of NAFLD and NASH. Some of them are currently used in NASH, as in this stage the disease is more likely to progress even more, and thus the drug therapy will be of profit.

Table 3. Summary of the treatment options in NAFLD and NASH

Lifestyle changes
Medications 1. Vitamin E (antioxidant) 2. Metformin (insulin-sensitizer) 3. TZDs (insulin-sensitizers) 4. Bile acid analogues (effective against liver steatosis) 5. Pentoxifylline (antiinflammatory and antioxidant functions) 6. Polyphenols (antiinflammatory and antioxidant functions) 7. Nicotinic acid and hydroquinone (effective against liver steatosis) 8. GLP-1 analogue (effective against liver steatosis)
Bariatric surgery

Vitamin E is already in use for non-diabetic, non-cirrhotic patients with biopsy-proven NASH and is considered as first-line treatment [12]. Vitamin E has been shown to improve both liver function and histology [97,98]. Nonetheless, it should be given cautiously due to potential hazards, mostly associated with the risk of hemorrhagic stroke [99,100].

Metformin has been extensively studied as a therapeutic agent against NAFLD and NASH, due to its massive use in diabetic patients. Despite initial promising results, metformin failed to display therapeutic results and it is therefore not recommended for the treatment of NASH [101]. It is however successfully used in type 2 diabetes mellitus, which is a major risk factor for NAFLD and NASH [12].

Thiazolidinediones (TZDs) belong to insulin-sensitizers and have been largely investigated in the treatment of NASH. The most widely used TZD is pioglitazone, which is currently used in the treatment of established NASH [12]. Pioglitazone seems to improve all parameters of liver histology and proves better outcomes than placebo, but failed to be proved superior against vitamin E [101].

Bile acid analogues also have their place in the treatment of NASH. Obeticholic acid is the most important among them [102]. This agent seems to ameliorate the histological profile of NASH, namely steatosis, inflammation, ballooning and fibrosis, mostly by decreasing hepatic lipogenesis, though not widely administered yet [103].

Antiinflammatory and antioxidant functions of Pentoxifylline have been proved beneficial in the treatment of NASH [104]. Of note, a recent metaanalysis indicated that pentoxyfilline and obeticholic acid improve fibrosis, while vitamin E, TZDs and obeticholic acid improve ballooning degeneration in NASH patients [105].

Natural polyphenols include a wide variety of phytochemicals and are classified as flavonoids and non-flavonoids. Recent review articles emphasize the role of polyphenols in hepatic fibrosis and analyze the underlying pathogenic pathways [106,107]. The hepatoprotective role of these compounds arises from their antioxidant and antiinflammatory properties and their ability to pro-

mote hepatic lipolysis and to inhibit lipogenesis. Consumption of fruits and vegetables rich in polyphenols, as well as coffee and green tea, can be beneficial in both treatment and prevention of the disease progression.

Omega- 3 fatty acids have also been considered as possible therapeutic factors [108,109]. Their use has not yet been established in NAFLD and NASH, but they are strongly recommended to be used in hypertriglyceridemia which often accompanies NAFLD [12]. Statins, which also are important for the treatment of NAFLD risk factors, have been proposed as possible drug candidates, but larger studies are needed to confirm current findings, especially due to their well-established hepatic adverse effects [110].

Finally, new agents have been emerged and appear to have liver protective properties as well. Nicotinic acid and hydroquinone have been studied and appear to be effective against liver steatosis [111]. Of note, nicotinic acid seems to have marked effects on both steatosis and transaminase levels. Although nicotinic acid has already been investigated as a lower-lipid agent, its role in NAFLD needs to be further investigated, in order to be recruited for the treatment and prevention of NAFLD and NASH. Other agents, like liraglutide, a glucagon-like peptide-1 (GLP-1) analogue, are recently under investigation with very promising results, as shown by the LEAN study, a large multicenter phase 2 clinical trial [112].

The role of bariatric surgery

The effects of bariatric surgery in NASH are highly debatable, since the type of bariatric surgery performed can lead to different results in the level of hepatic steatosis. Restrictive procedures, like gastric sleeve gastrectomy, and mixed procedures, mainly gastric bypass have been shown to lead to the resolution of hepatic steatosis [15]. In contrast, malabsorptive procedures, like intestinal bypass, were thought to worsen fibrosis, while is not yet defined if this increase in fibrosis is clinically significant [12]. Overall, it seems that NASH individuals suffering from severe obesity can be substantially improved [113]. More research, though, needs to be done in order to elucidate the role of bariatric surgery in the treatment of NAFLD and NASH (Table 3).

Prevention

The multifactorial nature of the pathogenesis of NAFLD makes the design of universal preventive measures rather challenging. Despite all the progress that has been made over the years in revealing the underlying pathophysiologic pathways, along with environmental factors and genetic predisposition that take part in this process, it is still largely unknown to us why some individuals with NAFLD will progress to NASH, fibrosis and cancer and others will not. Except for the uncertainty regarding the pathogenesis and natural history, our diagnostic tools and therapeutic strategies also exhibit important gaps. Thus, screening, which is the most important preventive strategy that can be used to detect individuals at risk, is not yet well defined for NAFLD.

Screening is currently been used for the population at risk for HCC development, which are considered practically all the individuals suffering from cirrhosis, but also those with hepatitis B and C infection [26,114]. Many scientific societies in North America (AALSD), in Europe (EASL) and Asia (APASL and JSH) have released recommendations regarding HCC surveillance [26,115-117]. Despite several differences in the target population and recall policies, it seems that surveillance in high risk groups should be performed by US and the optimum time interval used for screening currently is 6 months [26].

Screening for people at risk for NAFLD is not yet in clinical practice though. The fact that the prevalence of the disease is high and continuously rising has dual importance. It highlights the imperative need for screening but, on the other hand, it is not cost-effective to screen a population of that size. Screening for NASH patients, though, is something that may be considered for further evaluation. The use of US and liver function blood tests can be very helpful towards this direction. TE has also been proposed as a screening tool in community in specific subgroups of people [118]. Populations at risk for NASH and hepatic fibrosis, except for patients who have been diagnosed with NAFLD, are considered people suffering from obesity, DM and metabolic syndrome [119,120]. Other groups of patients that are considered to be at greater risk of developing NAFLD, NASH and fibrosis are those with obstructive sleep apnea and screening these patients might be of profit [121].

Patients undergoing cholecystectomy are a group that might profit by liver biopsy in certain cases. If there is perioperative evidence of insulin resistance or fatty liver on US, these patients should be considered for needle biopsy during cholecystectomy [57,122]. Another patient group that intraoperative liver biopsy should be con-

sidered is patients undergoing bariatric surgery, since they often have NAFLD [12].

Moreover, given the role of PNPLA3 variations in the pathogenesis of NAFLD and most importantly to HCC, using genetic testing to specific populations, like those with NAFLD but not characteristics of the metabolic syndrome might be wise [27,34].

Diagnosis of HCC

Given the poor prognosis of HCC and the need for early intervention in order to improve survival, it is evident that early and definitive diagnosis is crucial. The diagnosis of HCC is principally based on radiological imaging techniques.

Contrast-enhanced studies are highly diagnostic for HCC. For tumors larger than 2cm, one 4-phase study is required (unenhanced, arterial, venous and delayed phases) and the typical appearance with arterial enhancement followed by "washout" is sufficient to establish the diagnosis of HCC [26,123]. Contrast enhanced US, multiphasic enhanced CT (computer tomography) and various MRI techniques, like DW-MRI (Diffusion weighted MRI) have been used in the diagnostic process over the years [123,124]. Contrast enhanced US is no longer considered adequate for HCC diagnosis according to AASLD guidelines, due to difficulty in distinguishing HCC from intrahepatic cholangiocarcinoma [26]. When the results of the combination of various imaging modalities are inconclusive, invasive methods like liver biopsy should be performed [26].

Despite the accuracy in diagnosis of HCC provided by imaging techniques, especially by DW-MRI, there is still much concern regarding the diagnosis of tumors under 1cm, as well as diagnosis in the setting of liver fibrosis and cirrhosis, which are known confounding factors for many imaging modalities [125]. For tumors less than 1cm, close surveillance (every 3 months) and monitoring for changes in size and shape are currently recommended [26].

Histological staining and immunohistochemistry are equally important to the diagnosis of HCC as the morphologic features recognized by imaging studies. Heat Shock Protein 70 (HSP70), glypican 3, and glutamine synthase are included in the markers that are usually detected, while other markers, like cytokeratin 7 (CK7) should be negative in order to make the diagnosis of HCC more likely [126,127]. A recent study also identified PARP 1 (Poly [ADP-ribose] polymerase 1) as a novel biomarker for the diagnosis and prognosis of HCC, as higher titles of PARP 1 were associated with larger tumor size and poorer survival [128]. Keratin 19 (K19) has also been associated with tumor aggressiveness and is another protein that could be used mostly in the prognosis of survival [129].

Non-invasive biomarkers that can be measured in the serum could be very useful in both diagnosis and prognosis of the disease. Alpha-fetoprotein (AFP) used to be one of the biomarkers that was used both in diagnosis and screening, but this is no longer the case [114]. Des-gamma-carboxy prothrombin (DCP) has also been investigated for its potential role as a diagnostic tool, but the results are yet inconclusive [123,130]. Moreover, as discussed above, the cell-cycle regulator p27 is a molecule that can serve as a prognostic biomarker in the investigation of recurrence in NASH-induced HCC [84].

It is true that a direct consequence of the constant improvement of the diagnostic tools available and regular surveillance of population at risk is the identification of smaller HCC's and dysplastic nodules, making the distinction between malignant and benign lesions even more challenging. The combination of various radiologic and histopathologic methods available, along with close monitoring in cases of uncertainty, ensures the early detection and therefore intervention.

Staging and management of HCC

It is well established so far that optimal treatment of HCC is tightly connected to prognosis, which in turn depends on many factors, like tumor stage, patient's liver function and performance status. Therefore, there is a need of a staging system that will combine these parameters and provide the best treatment option in each case. Although a great variety of staging systems has been used so far by clinicians all over the world, up until now the Barcelona Clinic Liver Cancer (BCLC) is considered to be the most appropriate and therefore is being widely accepted [131-133].

According to BCLC staging system and treatment algorithm, in early stages of HCC (very early-BCLC stage 0 and early-BCLC stage A) the treatment of choice is liver resection for patients without cirrhosis or with cirrhosis with well-preserved liver function. Orthotopic Liver Transplantation (OLT) is also an alternative option in early stages of the disease for the patients corresponding to the Milan criteria [26,132]. Percutaneous local radiofrequency (RFA) is also recommended for patients with early stage HCC who cannot un-

dergo surgery or as a bridge to OLT. Preoperative portal vein embolization (PVE) is gaining acceptance worldwide, in order to avoid postoperative liver failure, but also for patients who cannot undergo surgery due to lack of sufficient normal parenchyma after liver resection [134].

Equally important to the efficient treatment is the proper and regular monitoring of recurrence. Imaging methods, either CT or MRI, as well as serological markers like AFP, while not always adequately reliable if used alone, are usually evaluated in close intervals [26].

Inoperable stages of HCC (intermediate-BCLC stage B) can be treated successfully with transarterial chemoembolization (TACE) and radioembolization (TARE) [26,132,135]. In cases of vascular invasion or extrahepatic spread of the tumor (advanced-BCLC stage C), administration of sorafenib, a tyrosine kinase inhibitor, is the treatment of choice [26,132]. In terminal stage HCC (terminal-BCLC stage D) there is no effective treatment to date except for palliative care, mainly adequate pain management and psychological support.

Despite the wide acceptance of the BCLC staging system, not every scientific society around the world is in accordance with that. Differences exist among the current published guidelines in different countries, reflecting regional differences in the biology and prevalence of the disease and the availability of diagnostic and therapeutic tools. For example, Asian guidelines tend to have more expanded criteria regarding tumor resection and liver transplantation and emphasize the role of TACE [136,137].

Discussion

Liver carcinogenesis in the setting of NAFLD and NASH has become increasingly common nowadays. NAFLD seems to affect almost one third or more of the global adult population and HCC is the fifth most common cancer and the second leading cause of death related to cancer [1,11]. As NAFLD has been recognized as a risk factor for the development of HCC it is of no surprise the amount of research that is being done to elucidate the mechanisms involved in this process, as these two entities share many common risk factors and pathogenic pathways [2-6]. Obesity, DM and metabolic syndrome are some of the disorders that are strongly associated with both diseases [6,7,21,27-29].

Deep knowledge and understanding of the underlying mechanisms of liver carcinogenesis is the key to design effective diagnostic tools and therapies. Obesity and insulin resistance are the cornerstones in the pathogenesis of the disease and share many molecular pathways, ultimately leading to the activation of the inflammatory and oxidative processes that cause DNA damage and carcinogenesis [27,36,37]. The role of other factors, though, especially that of genetic predisposition, should not be obscured.

The usually asymptomatic nature of NAFLD makes diagnosis rather challenging. Diagnostic tools for NAFLD are limited so far and a combination of serological and radiological methods should be used. Liver biopsy remains the gold-standard and should be performed in patients at increased risk of fibrosis [12]. From all the prediction scores available, it seems that the NAFLD Fibrosis Score is the most suitable in identifying patients at greater risk of fibrosis [70,72]. Elastography is a non-invasive modality that can accurately measure fibrosis, but its inability to detect fibrosis in early stages limits its use [90].

On the other hand, diagnosis of HCC is more well-defined and a contrast enhanced imaging technique with the classic appearance of arterial enhancement followed by "washout" is sufficient to establish the diagnosis [26]. A wide variety of novel biomarkers for NAFLD and HCC, either serological or immunohistochemical are currently under extensive research with promising results.

Treatment options for NAFLD include lifestyle modifications, as well as the use of various pharmacological agents that are recommended for the treatment of NASH. Vitamin E and TZDs are the most extensively tested among them [105]. Modification of risk factors like dyslipidemia, hypertriglyceridemia and DM are equally important and the use of natural antioxidants should be encouraged. Bariatric surgery can have a therapeutic role in cases of extreme obesity [113]. Nonetheless, current evidence is scarce and further investigation is needed towards NAFLD therapy with larger prospective well-designed studies.

The management of HCC is more clarified, especially after the introduction of the BCLC staging system and treatment algorithm that is being applied in many countries [131-133]. According to BCLC, in early stages a combination of liver resection, orthotopic liver transplantation and RFA can be used. Intermediate stages can be treated by TACE or TARE and advanced disease with the administration of sorafenib.

Prevention is of utmost importance due to

the high prevalence of both diseases. Due to the multifactorial nature of NAFLD and the lack of radiological imaging techniques or serological markers that can detect individuals at risk, screening has not yet been applied. It is, though, crucial to identify individuals at risk and figure out a cost-effective way to apply screening policies. Is it possible to screen all obese patients or type 2 DM patients? Will that be enough? What is the correct combination that will lead to the best screening policy? What is the right modality that can point out which patients will develop a progressive disease? These are only some of the questions that remain to be answered. Therefore, further investigation and establishment of non-invasive and cost-effective biomarkers and imaging techniques that will be massively applied to population at risk, in order to design the best screening policy, is imperative.

Conclusion

Finally, we are aware of the limitations of the present review. There is a great amount of studies that are being published every day, each of them of different design and purpose, investigating various aspects of the relationship between NAFLD and HCC. Therefore, the comparison of the relevant data is puzzling and solid conclusions are difficult to reach. Further well-designed prospective studies are needed in all areas of research in order to shed more light and provide robust data in pathogenesis, diagnosis, treatment and prevention of HCC in NAFLD and NASH in order to avoid the devastating results of that rapidly expanding global epidemic.

Conflict of interests

The authors declare no confict of interests.

References

1. Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. Hepatology 2016;64:73-84.
2. White DL, Kanwal F, El-Serag HB. Association between nonalcoholic fatty liver disease and risk for hepatocellular cancer, based on systematic review. Clin Gastroenterol Hepatol 2012;10:1342-1359.e2.
3. Zoller H, Tilg H. Nonalcoholic fatty liver disease and hepatocellular carcinoma. Metabol Clin Experim 2016;65:1151-1160.
4. Perumpail RB, Wong RJ, Ahmed A, Harrison SA. Hepatocellular Carcinoma in the Setting of Non-cirrhotic Nonalcoholic Fatty Liver Disease and the Metabolic Syndrome: US Experience. Digest Dis Sci 2015;60:3142-3148.
5. Michelotti GA, Machado MV, Diehl AM. NAFLD, NASH and liver cancer. Nat Rev 2013;10:656-665.
6. Younossi ZM, Otgonsuren M, Henry L et al. Association of nonalcoholic fatty liver disease (NAFLD) with hepatocellular carcinoma (HCC) in the United States from 2004 to 2009. Hepatology 2015;62:1723-1730.
7. Dyson J, Jaques B, Chattopadyhay D et al. Hepatocellular cancer: the impact of obesity, type 2 diabetes and a multidisciplinary team. J Hepatol 2014;60:110-117.
8. Wong RJ, Cheung R, Ahmed A. Nonalcoholic steatohepatitis is the most rapidly growing indication for liver transplantation in patients with hepatocellular carcinoma in the U.S. Hepatology 2014;59:2188-2195.
9. Bruix J, Llovet JM. Major achievements in hepatocellular carcinoma. Lancet 2009;373:614-616.
10. GLOBOCAN. Estimated cancer incidence: mortality and prevalence worldwide 2012. 2012. Available from: http://globocan.iarc.fr/Pages/fact_sheets_cancer.aspx.
11. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA: Cancer J Clin 2015;65:87-108.
12. Chalasani N, Younossi Z, Lavine JE et al. The diagnosis and management of non-alcoholic fatty liver disease: Practice guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. Am J Gastroenterol 2012;107:811-826.
13. Takahashi Y, Fukusato T. Histopathology of nonalcoholic fatty liver disease/nonalcoholic steatohepatitis. World J Gastroenterol 2014;20:15539-15548.
14. Singh S, Allen AM, Wang Z, Prokop LJ, Murad MH, Loomba R. Fibrosis progression in nonalcoholic fatty liver vs nonalcoholic steatohepatitis: a systematic review and meta-analysis of paired-biopsy studies. Clin Gastroenterol Hepatol 2015;13:643-654.e1-9; quiz e39-40.
15. Vernon G, Baranova A, Younossi ZM. Systematic review: the epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. Aliment Pharmacol Ther 2011;34:274-285.
16. Yki-Jarvinen H. Diagnosis of non-alcoholic fatty liver disease (NAFLD). Diabetologia 2016;59:1104-1111.
17. Wells M, Li ZJ, Addeman B et al. Computed tomography measurement of hepatic steatosis: Prevalence of hepatic steatosis in a Canadian population. Can J Gastroenterol Hepatol 2015, Jun 30, pii 16940 (Epub ahead of print).
18. Williams CD, Stengel J, Asike MI et al. 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.

19. McPherson S, Hardy T, Henderson E, Burt AD, Day CP, Anstee QM. Evidence of NAFLD progression from steatosis to fibrosing-steatohepatitis using paired biopsies: implications for prognosis and clinical management. J Hepatol 2015;62:1148-1155.
20. Souza MR, Diniz Mde F, Medeiros-Filho JE, Araujo MS. Metabolic syndrome and risk factors for non-alcoholic fatty liver disease. Arquivos de gastroenterologia. 2012;49:89-96.
21. Wang C, Wang X, Gong G et al. Increased risk of hepatocellular carcinoma in patients with diabetes mellitus: a systematic review and meta-analysis of cohort studies. Int J Cancer 2012;130:1639-1648.
22. Archambeaud I, Auble H, Nahon P et al. Risk factors for hepatocellular carcinoma in Caucasian patients with non-viral cirrhosis: the importance of prior obesity. Liver Int 2015;35:1872-1876.
23. Trichopoulos D, Bamia C, Lagiou P et al. Hepatocellular carcinoma risk factors and disease burden in a European cohort: a nested case-control study. J Natl Cancer Inst 2011;103:1686-1695.
24. Asrih M, Jornayvaz FR. Metabolic syndrome and nonalcoholic fatty liver disease: Is insulin resistance the link? Mol Cell Endocrinol 2015;418(Pt 1):55-65.
25. McGlynn KA, London WT. The global epidemiology of hepatocellular carcinoma: present and future. Clin Liver Dis 2011;15:223-243, vii-x.
26. Bruix J, Sherman M. Management of hepatocellular carcinoma: An update. Hepatology 2011;53:1020-1022.
27. Reeves HL, Zaki MY, Day CP. Hepatocellular Carcinoma in Obesity, Type 2 Diabetes, and NAFLD. Digest Dis Sci 2016;61:1234-1245.
28. Marengo A, Rosso C, Bugianesi E. Liver Cancer: Connections with Obesity, Fatty Liver, and Cirrhosis. Ann Rev Med 2016;67:103-117.
29. Jinjuvadia R, Patel S, Liangpunsakul S. The association between metabolic syndrome and hepatocellular carcinoma: systemic review and meta-analysis. J Clin Gastroenterol 2014;48:172-177.
30. Mavrogiannaki AN, Migdalis IN. Nonalcoholic Fatty liver disease, diabetes mellitus and cardiovascular disease: newer data. Int J Endocrinol 2013;2013:450639.
31. Fotbolcu H, Zorlu E. Nonalcoholic fatty liver disease as a multi-systemic disease. World J Gastroenterol 2016;22:4079-4090.
32. Kanwar P, Kowdley KV. The Metabolic Syndrome and Its Influence on Nonalcoholic Steatohepatitis. Clin Liver Dis 2016;20:225-243.
33. Milic S, Lulic D, Stimac D. Non-alcoholic fatty liver disease and obesity: biochemical, metabolic and clinical presentations. World J Gastroenterol 2014;20:9330-9337.
34. Yki-Järvinen H, Luukkonen PK. Heterogeneity of non-alcoholic fatty liver disease. Liver Int 2015;35:2498-2500.
35. Streba LA, Vere CC, Rogoveanu I, Streba CT. Nonalcoholic fatty liver disease, metabolic risk factors, and hepatocellular carcinoma: an open question. World J Gastroenterol 2015;21:4103-4110.
36. Scalera A, Tarantino G. Could metabolic syndrome lead to hepatocarcinoma via non-alcoholic fatty liver disease? World J Gastroenterol 2014;20:9217-9228.
37. Margini C, Dufour JF. The story of HCC in NAFLD: from epidemiology, across pathogenesis, to prevention and treatment. Liver Int 2016;36:317-324.
38. Toffanin S, Friedman SL, Llovet JM. Obesity, inflammatory signaling, and hepatocellular carcinoma-an enlarging link. Cancer Cell 2010;17:115-117.
39. Dalamaga M, Diakopoulos KN, Mantzoros CS. The role of adiponectin in cancer: a review of current evidence. Endocrine Rev 2012;33:547-594.
40. Li HJ, Li CP, Zhang C, Zhong XL, Shi L. Association of Adiponectin gene polymorphisms and nonalcoholic fatty liver disease. Int J Clin Experim Med 2015;8:16676-16681.
41. Duan XF, Tang P, Li Q, Yu ZT. Obesity, adipokines and hepatocellular carcinoma. Int J Cancer 2013;133:1776-1783.
42. Jequier E. Leptin signaling, adiposity, and energy balance. Ann New York Acad Sci 2002;967:379-388.
43. de Courten M, Zimmet P, Hodge A et al. Hyperleptinaemia: the missing link in the metabolic syndrome? Diabetic Med 1997;14:200-208.
44. Polyzos SA, Kountouras J, Mantzoros CS. Leptin in nonalcoholic fatty liver disease: a narrative review. Metabol Clin Experim 2015;64:60-78.
45. Wang SN, Lee KT, Ker CG. Leptin in hepatocellular carcinoma. World J Gastroenterol 2010;16:5801-5809.
46. Jiang N, Sun R, Sun Q. Leptin signaling molecular actions and drug target in hepatocellular carcinoma. Drug Design Develop Ther 2014;8:2295-2302.
47. Hashimoto E, Tokushige K. Hepatocellular carcinoma in non-alcoholic steatohepatitis: Growing evidence of an epidemic? Hepatol Res 2012;42:1-14.
48. Koroglu E, Canbakan B, Atay K, Hatemi I, Tuncer M, Dobrucali A. Role of oxidative stress and insulin resistance in disease severity of non-alcoholic fatty liver disease. Turk J Gastroenterol 2016;27:361-366.
49. Scharf JG, Dombrowski F, Ramadori G. The IGF axis and hepatocarcinogenesis. Mol Pathol 2001;54:138-144.
50. Enguita-German M, Fortes P. Targeting the insulin-like growth factor pathway in hepatocellular carcinoma. World J Hepatol 2014;6:716-737.
51. El Tayebi HM, Abdelaziz AI. Epigenetic regulation of insulin-like growth factor axis in hepatocellular carcinoma. World J Gastroenterol 2016;22:2668-2677.
52. Romeo S, Kozlitina J, Xing C et al. Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. Nat Genetics 2008;40:1461-1465.
53. Chen LZ, Xin YN, Geng N, Jiang M, Zhang DD, Xuan SY. PNPLA3 I148M variant in nonalcoholic fatty liver disease: demographic and ethnic characteristics and the role of the variant in nonalcoholic fatty liver fibrosis. World J Gastroenterol 2015;21:794-802.
54. Park JH, Cho B, Kwon H et al. I148M variant in PNPLA3 reduces central adiposity and metabolic disease

risks while increasing nonalcoholic fatty liver disease. Liver Int 2015;35:2537-2546.

55. 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.
56. Valenti L, Dongiovanni P, Ginanni Corradini S, Burza MA, Romeo S. PNPLA3 I148M variant and hepatocellular carcinoma: a common genetic variant for a rare disease. Digest Liver Dis 2013;45:619-624.
57. Garcia-Monzon C, Vargas-Castrillon J, Porrero JL et al. Prevalence and risk factors for biopsy-proven non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in a prospective cohort of adult patients with gallstones. Liver Int 2015;35:1983-1991.
58. Donati B, Motta BM, Pingitore P et al. The rs2294918 E434K variant modulates patatin-like phospholipase domain-containing 3 expression and liver damage. Hepatology 2016;63:787-798.
59. Liu YL, Reeves HL, Burt AD et al. TM6SF2 rs58542926 influences hepatic fibrosis progression in patients with non-alcoholic fatty liver disease. Nat Commun 2014;5:4309.
60. Sookoian S, Castano GO, Scian R et al. Genetic variation in transmembrane 6 superfamily member 2 and the risk of nonalcoholic fatty liver disease and histological disease severity. Hepatology 2015;61:515-525.
61. Petta S, Valenti L, Marra F et al. MERTK rs4374383 polymorphism affects the severity of fibrosis in non-alcoholic fatty liver disease. J Hepatol 2016;64:682-690.
62. Kinsell LW, Harper HA, Barton HC, Michaels GD, Weiss HA. Rate of Disappearance From Plasma of Intravenously Administered Methionine in Patients With Liver Damage. Science 1947;106:589-590.
63. Mato JM, Martinez-Chantar ML, Lu SC. Methionine metabolism and liver disease. Ann Rev Nutr 2008;28:273-293.
64. Lu SC, Mato JM. S-adenosylmethionine in liver health, injury, and cancer. Physiol Rev 2012;92:1515-1542.
65. Wagner M, Choi S, Panzitt K et al. Liver receptor homolog-1 is a critical determinant of methyl-pool metabolism. Hepatology 2016;63:95-106.
66. Martinez-Chantar ML, Corrales FJ, Martinez-Cruz LA et al. Spontaneous oxidative stress and liver tumors in mice lacking methionine adenosyltransferase 1A. FASEB J 2002;16:1292-1294.
67. Lu SC, Alvarez L, Huang ZZ et al. Methionine adenosyltransferase 1A knockout mice are predisposed to liver injury and exhibit increased expression of genes involved in proliferation. Proc Natl Acad Sci USA 2001;98:5560-5565.
68. Mato JM, Lu SC. Liver receptor homolog 1 and transmethylation fluxes in nonalcoholic steatohepatitis. Hepatology 2016;63:17-19.
69. Dowman JK, Tomlinson JW, Newsome PN. Systematic review: the diagnosis and staging of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis. Aliment Pharmacol Ther 2011;33:525-540.
70. Kwok R, Tse YK, Wong GL et al. Systematic review with meta-analysis: non-invasive assessment of non-alcoholic fatty liver disease--the role of transient elastography and plasma cytokeratin-18 fragments. Aliment Pharmacol Ther 2014;39:254-269.
71. Enomoto H, Bando Y, Nakamura H, Nishiguchi S, Koga M. Liver fibrosis markers of nonalcoholic steatohepatitis. World J Gastroenterol 2015;21:7427-7435.
72. Angulo P, Hui JM, Marchesini G et al. The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. Hepatology 2007;45:846-854.
73. Sterling RK, Lissen E, Clumeck N et al. Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. Hepatology 2006;43:1317-1325.
74. Rosso C, Mezzabotta L, Gaggini M et al. Peripheral insulin resistance predicts liver damage in nondiabetic subjects with nonalcoholic fatty liver disease. Hepatology 2016;63:107-116.
75. Sesti G, Fiorentino TV, Hribal ML, Sciacqua A, Perticone F. Association of hepatic insulin resistance indexes to nonalcoholic fatty liver disease and related biomarkers. Nutr Metabol Cardiovasc Dis 2013;23:1182-187.
76. Kamada Y, Ono M, Hyogo H et al. A novel noninvasive diagnostic method for nonalcoholic steatohepatitis using two glycobiomarkers. Hepatology 2015;62:1433-1443.
77. Kaneda H, Hashimoto E, Yatsuji S, Tokushige K, Shiratori K. Hyaluronic acid levels can predict severe fibrosis and platelet counts can predict cirrhosis in patients with nonalcoholic fatty liver disease. J Gastroenterol Hepatol 2006;21:1459-1465.
78. Orasan OH, Ciulei G, Cozma A, Sava M, Dumitrascu DL. Hyaluronic acid as a biomarker of fibrosis in chronic liver diseases of different etiologies. Clujul Med 2016;89:24-31.
79. Tanwar S, Trembling PM, Hogan BJ et al. Biomarkers of Hepatic Fibrosis in Chronic Hepatitis C: A Comparison of 10 Biomarkers Using 2 Different Assays for Hyaluronic Acid. J Clin Gastroenterol 2016, Jul 1 (Epub ahead of print).
80. Wieckowska A, Zein NN, Yerian LM, Lopez AR, McCullough AJ, Feldstein AE. In vivo assessment of liver cell apoptosis as a novel biomarker of disease severity in nonalcoholic fatty liver disease. Hepatology 2006;44:27-33.
81. Sadik NA, Ahmed A, Ahmed S. The significance of serum levels of adiponectin, leptin, and hyaluronic acid in hepatocellular carcinoma of cirrhotic and noncirrhotic patients. Hum Experim Toxicol 2012;31:311-321.
82. Voiculescu M, Nanau RM, Neuman MG. Non-invasive biomarkers in non-alcoholic steatohepatitis-induced hepatocellular carcinoma. J Gastrointest Liver Dis 2014;23:425-429.
83. Polyzos SA, Aronis KN, Kountouras J, Raptis DD, Vasiloglou MF, Mantzoros CS. Circulating leptin in non-alcoholic fatty liver disease: a systematic review and meta-analysis. Diabetologia 2016;59:30-43.

84. Matsuda Y, Wakai T, Hirose Y, Osawa M, Fujimaki S, Kubota M. p27 is a critical prognostic biomarker in non-alcoholic steatohepatitis-related hepatocellular carcinoma. Int J Mol Sci 2013;14:23499-23515.
85. Lee SS, Park SH. Radiologic evaluation of nonalcoholic fatty liver disease. World J Gastroenterol 2014;20:7392-7402.
86. Koplay M, Sivri M, Erdogan H, Nayman A. Importance of imaging and recent developments in diagnosis of nonalcoholic fatty liver disease. World J Hepatol 2015;7:769-776.
87. Chan T-T, Wong VW-S. In search of new biomarkers for nonalcoholic fatty liver disease. Clin Liver Dis 2016;8:19-23.
88. Idilman IS, Keskin O, Celik A et al. A comparison of liver fat content as determined by magnetic resonance imaging-proton density fat fraction and MRS versus liver histology in non-alcoholic fatty liver disease. Acta Radiol 2016;57:271-278.
89. Bannas P, Kramer H, Hernando D et al. Quantitative magnetic resonance imaging of hepatic steatosis: Validation in ex vivo human livers. Hepatology 2015;62:1444-1455.
90. Myers RP, Elkashab M, Ma M, Crotty P, Pomier-Layrargues G. Transient elastography for the noninvasive assessment of liver fibrosis: a multicentre Canadian study. Can J Gastroenterol 2010;24:661-670.
91. Corey KE, Rinella ME. Medical and Surgical Treatment Options for Nonalcoholic Steatohepatitis. Digest Dis Sci 2016;61:1387-1397.
92. Ganesh S, Rustgi VK. Current Pharmacologic Therapy for Nonalcoholic Fatty Liver Disease. Clin Liver Dis 2016;20:351-364.
93. Hameed B, Terrault N. Emerging Therapies for Nonalcoholic Fatty Liver Disease. Clin Liver Dis 2016;20:365-385.
94. Munteanu MA, Nagy GA, Mircea PA. Current Management of NAFLD. Clujul Med 2016;89:19-23.
95. Zelber-Sagi S, Ratziu V, Oren R. Nutrition and physical activity in NAFLD: An overview of the epidemiological evidence. World J Gastroenterol 2011;17:3377-3389.
96. Tilg H, Moschen A. Weight loss: cornerstone in the treatment of non-alcoholic fatty liver disease. Minerva Gastroenterol Dietolog 2010;56:159-167.
97. Sato K, Gosho M, Yamamoto T et al. Vitamin E has a beneficial effect on nonalcoholic fatty liver disease: a meta-analysis of randomized controlled trials. Nutrition 2015;31:923-930.
98. Ji HF, Sun Y, Shen L. Effect of vitamin E supplementation on aminotransferase levels in patients with NAFLD, NASH, and CHC: results from a meta-analysis. Nutrition 2014;30:986-991.
99. Pacana T, Sanyal AJ. Vitamin E and Non-alcoholic Fatty Liver Disease. Current Opin Clin Nutr Metabol Care 2012;15:641-648.
100. Schurks M, Glynn RJ, Rist PM, Tzourio C, Kurth T. Effects of vitamin E on stroke subtypes: meta-analysis of randomised controlled trials. BMJ 2010;341:c5702.
101. Shyangdan D, Clar C, Ghouri N et al. Insulin sensitisers in the treatment of non-alcoholic fatty liver disease: a systematic review. Health Technol Assess 2011;15:1-110.
102. Yuan L, Bambha K. Bile acid receptors and nonalcoholic fatty liver disease. World J Hepatol 2015;7:2811-2818.
103. Neuschwander-Tetri BA, Loomba R, Sanyal AJ et al. Farnesoid X nuclear receptor ligand obeticholic acid for non-cirrhotic, non-alcoholic steatohepatitis (FLINT): a multicentre, randomised, placebo-controlled trial. Lancet 2015;385:956-965.
104. Du J, Ma YY, Yu CH, Li YM. Effects of pentoxifylline on nonalcoholic fatty liver disease: a meta-analysis. World J Gastroenterol 2014;20:569-577.
105. Singh S, Khera R, Allen AM, Murad MH, Loomba R. Comparative effectiveness of pharmacological interventions for nonalcoholic steatohepatitis: A systematic review and network meta-analysis. Hepatology 2015;62:1417-1432.
106. Salomone F, Godos J, Zelber-Sagi S. Natural antioxidants for non-alcoholic fatty liver disease: molecular targets and clinical perspectives. Liver Int 2016;36:5-20.
107. Pisonero-Vaquero S, Gonzalez-Gallego J, Sanchez-Campos S, Garcia-Mediavilla MV. Flavonoids and Related Compounds in Non-Alcoholic Fatty Liver Disease Therapy. Curr Medic Chem 2015;22:2991-3012.
108. Popescu LA, Virgolici B, Lixandru D et al. Effect of diet and omega-3 fatty acids in NAFLD. Rom J Morphol Embryol 2013;54(3 Suppl):785-790.
109. Scorletti E, Byrne CD. Omega-3 fatty acids, hepatic lipid metabolism, and nonalcoholic fatty liver disease. Annu Rev Nutr 2013;33:231-248.
110. Eslami L, Merat S, Malekzadeh R, Nasseri-Moghaddam S, Aramin H. Statins for non-alcoholic fatty liver disease and non-alcoholic steatohepatitis. Cochrane database of Systematic Reviews 2013(12):Cd008623.
111. von Schonfels W, Patsenker E, Fahrner R et al. Metabolomic tissue signature in human non-alcoholic fatty liver disease identifies protective candidate metabolites. Liver Int 2015;35:207-214.
112. Armstrong MJ, Gaunt P, Aithal GP et al. Liraglutide safety and efficacy in patients with non-alcoholic steatohepatitis (LEAN): a multicentre, double-blind, randomised, placebo-controlled phase 2 study. Lancet 2016;387:679-690.
113. Mummadi RR, Kasturi KS, Chennareddygari S, Sood GK. Effect of bariatric surgery on nonalcoholic fatty liver disease: systematic review and meta-analysis. Clin Gastroenterol Hepatol 2008;6:1396-1402.
114. Della Corte C, Triolo M, Iavarone M, Sangiovanni A. Early diagnosis of liver cancer: an appraisal of international recommendations and future perspectives. Liver Int 2016;36:166-176.
115. EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma. J Hepatol 2012;56:908-943.
116. Omata M, Lesmana LA, Tateishi R et al. Asian Pacific Association for the Study of the Liver consensus rec-

ommendations on hepatocellular carcinoma. Hepatol Int 2010;4:439-474.

117. Kudo M, Izumi N, Kokudo N et al. Management of hepatocellular carcinoma in Japan: Consensus-Based Clinical Practice Guidelines proposed by the Japan Society of Hepatology (JSH) 2010 updated version. Digest Dis 2011;29:339-364.

118. Cheng PN, Chiu YC, Chiu HC, Chien SC. The Application of Liver Stiffness Measurement in Residents Without Overt Liver Diseases Through a Community-Based Screening Program. Medicine 2016;95:e3193.

119. Koehler EM, Plompen EP, Schouten JN et al. Presence of diabetes mellitus and steatosis is associated with liver stiffness in a general population: The Rotterdam study. Hepatology 2016;63:138-1347.

120. Ahmed A, Perumpail RB, Harrison SA. High prevalence of hepatic fibrosis in the setting of coexisting diabetes and hepatic steatosis: A case for selective screening in the general population? Hepatology 2016;63:20-22.

121. Musso G, Cassader M, Olivetti C, Rosina F, Carbone G, Gambino R. Association of obstructive sleep apnoea with the presence and severity of non-alcoholic fatty liver disease. A systematic review and meta-analysis. Obesity Rev 2013;14:417-431.

122. Rinella ME, Loomba R, Caldwell SH et al. Controversies in the Diagnosis and Management of NAFLD and NASH. Gastroenterol Hepatol 2014;10:219-227.

123. Cristea CG, Gheonea IA, Săndulescu LD, Gheonea DI, Ciurea T, Purcarea MR. Considerations regarding current diagnosis and prognosis of hepatocellular carcinoma. J Med Life 2015;8:120-128.

124. Sun H, Song T. Hepatocellular carcinoma: Advances in diagnostic imaging. Drug Discov Ther 2015;9:310-318.

125. Gluskin JS, Chegai F, Monti S, Squillaci E, Mannelli L. Hepatocellular Carcinoma and Diffusion-Weighted MRI: Detection and Evaluation of Treatment Response. J Cancer 2016;7:1565-1570.

126. Di Tommaso L, Destro A, Seok JY et al. The application of markers (HSP70 GPC3 and GS) in liver biopsies is useful for detection of hepatocellular carcinoma. J Hepatol 2009;50:746-754.

127. Shafizadeh N, Kakar S. Diagnosis of well-differentiated hepatocellular lesions: role of immunohistochemistry and other ancillary techniques. Adv Anat Pathol 2011;18:438-445.

128. Xu X, Liu Z, Wang J et al. Global proteomic profiling in multistep hepatocarcinogenesis and identification of PARP1 as a novel molecular marker in hepatocellular carcinoma. Oncotarget 2016;7:13730-13741.

129. Fatourou E, Koskinas J, Karandrea D et al. Keratin 19 protein expression is an independent predictor of survival in human hepatocellular carcinoma. Eur J Gastroenterol Hepatol 2015;27:1094-1102.

130. Chaiteerakij R, Addissie BD, Roberts LR. Update on biomarkers for hepatocellular carcinoma. Clin Gastroenterol Hepatol 2015;13:237-245.

131. Forner A, Reig ME, de Lope CR, Bruix J. Current strategy for staging and treatment: the BCLC update and future prospects. Semin Liver Dis 2010;30:61-74.

132. Rahbari NN, Mehrabi A, Mollberg NM et al. Hepatocellular carcinoma: current management and perspectives for the future. Ann Surg 2011;253:453-469.

133. Maida M, Orlando E, Camma C, Cabibbo G. Staging systems of hepatocellular carcinoma: a review of literature. World J Gastroenterol 2014;20:4141-4150.

134. Loffroy R, Favelier S, Chevallier O et al. Preoperative portal vein embolization in liver cancer: indications, techniques and outcomes. Quant Imag Medicine Surg 2015;5:730-739.

135. Murata S, Mine T, Sugihara F et al. Interventional treatment for unresectable hepatocellular carcinoma. World J Gastroenterol 2014;20:13453-13465.

136. Yu SJ. A concise review of updated guidelines regarding the management of hepatocellular carcinoma around the world: 2010-2016. Clin Mol Hepatol 2016;22:7-17.

137. 2014 KLCSG-NCC Korea Practice Guideline for the Management of Hepatocellular Carcinoma. Gut Liver 2015;9:267-317.