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Risk factors and diagnostic biomarkers for nonalcoholic fatty liver disease-associated hepatocellular carcinoma: Current evidence and future perspectives

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Abstract

High rates of excessive calorie intake diets and sedentary lifestyles have led to a global increase in nonalcoholic fatty liver disease (NAFLD). As a result, this condition has recently become one of the leading causes of hepatocellular carcinoma (HCC). Furthermore, the incidence of NAFLD-associated HCC (NAFLD-HCC) is expected to increase in the near future. Advanced liver fibrosis is the most common risk factor for NAFLD-HCC. However, up to 50% of NAFLD-HCC cases develop without underlying liver cirrhosis. Epidemiological studies have revealed many other risk factors for this condition; including diabetes, other metabolic traits, obesity, old age, male sex, Hispanic ethnicity, mild alcohol intake, and elevated liver enzymes. Specific gene variants, such as single-nucleotide polymorphisms of patatin-like phospholipase domain 3, transmembrane 6 superfamily member 2, and membrane-bound O-acyl-transferase domain-containing 7, are also associated with an increased risk of HCC in patients with NAFLD. This clinical and genetic information should be interpreted together for accurate risk prediction. Alpha-fetoprotein (AFP) is the only biomarker currently recommended for HCC screening. However, it is not sufficiently sensitive in addressing this diagnostic challenge. The GALAD score can be calculated based on sex, age, lectin-bound AFP, AFP, and des-carboxyprothrombin and is reported to show better diagnostic performance for HCC. In addition, emerging studies on genetic and epigenetic biomarkers have also yielded promising diagnostic potential. However, further research is needed to establish an effective surveillance program for the early diagnosis of NAFLD-HCC.

Key Words: Nonalcoholic fatty liver disease; Hepatocellular carcinoma; Risk factors; Biomarkers; Tumor markers; Genetics

Core Tip: This review summarizes the risk factors and diagnostic biomarkers for nonalcoholic fatty liver disease (NAFLD)-associated hepatocellular carcinoma (HCC). The highlighted risk factors include liver fibrosis, diabetes, age, sex, race, alcohol intake, elevated liver enzymes, and specific genetic variants. Currently available diagnostic biomarkers include alpha-fetoprotein (AFP), des-carboxyprothrombin, and the AFP isoform L3. The combined use of these biomarkers may increase the diagnostic sensitivity of NAFLD-HCC detection. However, more discussion will be necessary on the cost-effectiveness of these approaches. This review also summarizes emerging means of discovering novel biomarkers using omics techniques. A better understanding of these risk factors and diagnostic biomarkers will facilitate the effective surveillance of NAFLD-HCC.

INTRODUCTION

Hepatocellular carcinoma (HCC) accounts for the majority of primary liver cancers[1]. It is the sixth most common form of cancer and the fourth leading cause of worldwide cancer-related deaths[2]. By contrast to the declining trends for other major cancers, mortality rates from liver cancer have increased by almost 3% *per* year[3]. Chronic liver infection from hepatitis B or C virus (HBV or HCV, respectively) and alcohol abuse are the most common causes of HCC[1,4]. However, there is a growing concern about the rapid increase in nonalcoholic fatty liver disease (NAFLD) as another cause of HCC.

NAFLD is a spectrum of chronic liver diseases characterized by excess fat accumulation in hepatocytes[5]. The prevalence of NAFLD is rapidly increasing worldwide, with recent studies reporting a global percentage of approximately 25%[6]. Unsurprisingly, the incidence of NAFLD-associated HCC (NAFLD-HCC) is also increasing in many areas of the world. In the United States, the proportion of NAFLD-HCC among all HCC patients has significantly increased from 9.3% to 13.6% over the past decade[7]. In a French cohort of patients who underwent liver resection, the prevalence of NAFLD-HCC increased from 2.6% (1995-1999) to 19.5% (2010-2014)[8]. Similar trends are also seen in Asian countries. According to a nationwide survey conducted in Japan, the proportion of nonalcoholic steatohepatitis (NASH)-related HCC has increased from 1.5% (2007 or before) to 7.2% (2014–2016)[9]. Universal HBV vaccination and widespread use of direct-acting antiviral agents for HCV are expected to decrease the number of virus-related HCC cases. Nonetheless, NAFLD-HCC is expected to continue to rise in the future[10,11].

NAFLD-HCC is often diagnosed at an advanced stage due to a lack of efficient surveillance policies [11]. In the Veteran Affairs population, only 40% of NAFLD-HCC patients underwent HCC surveillance compared to more than 80% of patients with HCV-related HCC[12]. From the perspective of cost-effectiveness, HCC screening should be considered when the annual risk of HCC exceeds 0.4–2.0%[13,14]. However, in a large-scale retrospective cohort study, out of 2382289 person-years of follow-up, only 490 patients with NAFLD were diagnosed with HCC (0.21/1000 person-years)[15]. Thus, it is necessary to more effectively identify high-risk patients who will benefit from HCC surveillance. Additionally, the diagnostic performance of the implemented examination is important for effective surveillance. Although ultrasonography with or without alpha-fetoprotein (AFP) is recommended for HCC screening [16], the sensitivity and specificity of these examinations are insufficient in patients with NAFLD. The high prevalence of obesity in patients with NAFLD impairs ultrasonography performance[17]. Therefore, many researchers have been searching for alternative NAFLD-HCC surveillance tools to increase the chances of early diagnosis. Recent advances in omics technology have enabled easier genetic and epigenetic benchmark analysis. Thus, many potential biomarkers are currently under investigation.

In this review, we discuss the current evidence for clinical and genetic HCC risk factors. We also summarize previous reports on the diagnostic biomarkers of NAFLD-HCC, including those under development. Finally, we briefly address future perspectives on HCC surveillance for NAFLD patients.

CLINICAL RISK FACTORS

Liver fibrosis

Liver fibrosis is the most important risk factor for the development of HCC in patients with NAFLD[18,

19]. In a large retrospective cohort study, the annual HCC incidence rates amongst NAFLD patients with and without cirrhosis were 10.6 and 0.08 *per* 1000 person-years, respectively[15]. This indicates that the presence of liver cirrhosis increases the risk of NAFLD-HCC by more than 10-fold (Table 1).

Liver biopsy is the gold standard for evaluating the fibrotic stage of the liver[20]. However, noninvasive examinations are more widely used in clinical practice. Available serum biomarkers for assessing fibrosis include the aspartate transaminase (AST)-to-platelet ratio index, Fibrosis-4 (FIB-4) index, and enhanced liver fibrosis score[21]. The FIB-4 index is calculated using various factors; including age, AST, alanine aminotransferase (ALT), and platelet count. Furthermore, a significant association between the FIB-4 index and HCC risk in NAFLD patients has been reported[15,22]. The Mac-2-binding protein glycosylation isomer is a novel serum biomarker that shows relatively high efficacy at assessing liver fibrosis[23]. It may also be useful in predicting the risk of hepatocarcinogenesis among NAFLD patients[24].

Additionally, elastography techniques using ultrasound or magnetic resonance imaging are useful tools for noninvasive evaluation of degree of fibrosis[21]. A retrospective study from Japan suggested that shear wave velocity measurements can be used for HCC risk assessment in NAFLD patients[25].

Diabetes mellitus and other metabolic traits

Several studies have demonstrated that (mostly type 2) diabetes mellitus is associated with an increased risk of HCC development in NAFLD patients. The hazard ratio for HCC in diabetic patients has been reported to be around 2.2–4.2[22,26,27] (Table 1). In a nationwide Japanese study, the annual incidence of HCC was only 0.11% in diabetes patients overall. However, it increased to 1.0% when they had an FIB-4 index of 3.5 or more[14]. Therefore, the degree of liver fibrosis, together with presence or absence of diabetes, should be assessed to predict the risk of NAFLD-HCC.

There have been conflicting reports regarding the association between other metabolic traits such as hypertension, dyslipidemia, with NAFLD-HCC[28]. However, a recent study showed that these conditions were also risk factors for NAFLD-HCC (Table 1). Furthermore, the hazard ratio for HCC was 5.6 in patients with diabetes, hypertension, and dyslipidemia[27,29].

Obesity

Regardless of underlying liver diseases, obesity is associated with an increased risk of HCC[30]. This relationship has also been confirmed in patients with NAFLD[29,31]. In a cohort of NAFLD-HCC patients treated with curative radiofrequency ablation, the degree of visceral fat accumulation, but not body mass index (BMI), was independently associated with an increased risk of HCC recurrence[32]. A propensity score-matched study showed that the incidence of both NASH and HCC were significantly lower in patients who underwent bariatric surgery, indicating a protective role of sustained weight loss by bariatric surgery[33].

Age, sex, and race

Old age, male sex, and Hispanic ethnicity are also known risk factors for NAFLD-HCC (Table 1); the details are described in another review article[31]. In the United States, a multicenter case-control study showed that old age (*per* year) and male sex were independently associated with a higher NAFLD-HCC risk [odds ratios (OR), 1.08 and 4.34, respectively][34]. In another study, age ≥ 65 years was associated with a 1.83 times higher NAFLD-HCC risk compared to age < 65 years[15]. Regarding ethnicity, a previous study showed that Hispanic patients had a lower NAFLD-HCC risk (OR, 0.3). By contrast, another study showed the opposite result (hazard ratio, 1.59)[15,34]. Several other studies have shown that Hispanic ethnicity is, in itself, a risk factor for NAFLD, partly because of the high prevalence of patatin-like phospholipase domain 3 (*PNPLA3*) variants among Hispanic people[35,36].

Alcohol intake and smoking

NAFLD is defined as hepatic steatosis without harmful alcohol intake (more than 30 g/day for men and 20 g/day for women). However, mild alcohol intake can also be a risk factor for NAFLD-HCC[37]. Previous studies have shown that mild drinking habits were associated with 3.6–4.8 times higher risk of HCC in NAFLD patients compared to no habits of drinking[38,39] (Table 1). No study has specifically investigated the association between smoking and NAFLD-HCC. However, smoking is associated with an increased risk of HCC. Additionally, it has been reported to be associated with advanced liver fibrosis in NAFLD patients[31,40]. Because these factors are modifiable, NAFLD patients should be informed that cessation of alcohol intake and smoking may reduce the risk of future HCC development.

Elevated liver enzymes

Patients with NASH are thought to have a higher risk of HCC than those with NAFLD[18]. However, liver biopsy is required to accurately distinguish these conditions. Therefore, elevated liver enzyme levels are often used as surrogate markers for NASH in clinical practice. Several studies have shown that elevated liver enzymes in NAFLD patients are significantly associated with an increased risk of HCC (hazard ratio, 2.07–8.20)[41-43] (Table 1). At the same time, however, normal transaminase levels do not exclude the possibility of advanced liver fibrosis[44].

Table 1 Clinical risk factors for nonalcoholic fatty liver disease-associated hepatocellular carcinoma

Risk factors	Reported evidence	Ref.
Liver fibrosis	The annual incidence rate of HCC in NAFLD patients with cirrhosis was more than 10 times higher than in those without	[15,22,24,25]
	Non-invasive fibrosis markers (*e.g.*, FIB-4 index, M2BPGi, and shear wave velocity in VTQ) also had significant associations with the risk of NAFLD-HCC	
Diabetes	Associated with increased risk of HCC in NAFLD patients (hazard ratio: 2.2–4.2)	[22,26,27]
Hypertension	May be an independent risk factor for NAFLD-HCC	[29]
Dyslipidemia	May be an independent risk factor for NAFLD-HCC	[29]
Age	Increased age was an independent risk factor for HCC in patients with NASH-related cirrhosis	[15,34]
	NAFLD patients aged ≥ 65 had 1.83 times higher risk of HCC than those aged < 65	
Male sex	Male patients with NASH-related cirrhosis had 4.34 times higher risk of HCC than female patients	[34]
Ethnicity	Hispanic ethnicity was associated with 1.59 times higher risk of HCC in NAFLD patients compared to white ethnicity (however, there have been conflicting results)	[15]
Mild alcohol intake	Associated with increased risk of HCC in NAFLD patients (hazard ratio: 3.6–4.8)	[38,39]
Elevated liver enzymes	Associated with increased risk of HCC in NAFLD patients (hazard ratio: 2.1–8.2)	[41-43]

HCC: Hepatocellular carcinoma; NAFLD: Nonalcoholic fatty liver disease; FIB-4: Fibrosis-4; M2BPGi: Mac-2-binding protein glycosylation isomer; VTQ: Virtual touch quantification; NASH: Nonalcoholic steatohepatitis.

Combined risk assessment

As we described above, many factors influence the risk of HCC in NAFLD patients. Therefore, integrating multiple factors will improve the accuracy of risk assessment. Lee *et al*[45] proposed a risk prediction model consisting of age, platelet count, and liver stiffness. This showed relatively good prediction performance in the validation cohort (*i.e.*, area under the receiver operating characteristic curve (AUROC), 0.78)[45]. Ioannou *et al*[46] reported another prediction model with a similar predictive value (AUROC, 0.75), consisting of age, sex, diabetes, BMI, platelet count, serum albumin, and AST/$\sqrt{}$ ALT ratio[46].

GENETIC RISK FACTORS

Although NAFLD development and progression is largely determined by environmental factors, several genetic factors are involved in NAFLD pathogenesis. These genes included *PNPLA3*, transmembrane 6 superfamily member 2 (*TM6SF2*), glucokinase regulator (*GCKR*), membrane-bound O-acyl-transferase domain-containing 7 (*MBOAT7*), and 17-beta-hydroxysteroid dehydrogenase 13 (*HSD17B13*)[5,47]. Epigenetic factors, such as DNA methylation, histone modification, and non-coding RNAs, also play roles in the progression of NAFLD and hepatocarcinogenesis[48]. The risk of severe liver fibrosis has been reported to be 12.5-fold higher in patients with first-degree relatives with NAFLD-related cirrhosis than those without[49].

Single-nucleotide polymorphisms (SNPs) of *PNPLA3* have been the most studied for its association with HCC[50]. In a cohort of European Caucasian patients with NAFLD, the *PNPLA3* rs738409 polymorphism was significantly associated with HCC risk. GG carriers were found to evince a 5-fold increased risk compared to CC carriers[51]. This association was confirmed by subsequent studies[52,53]. The *TM6SF2* rs58542926 polymorphism has been reported to be another risk factor for HCC and advanced fibrosis/cirrhosis[54,55]. In addition, the *MBOAT7* rs641738 variant has also been reported as a risk factor for NAFLD-HCC. Furthermore, in an Italian cohort, the rs6417338 C-to-T variant was associated with a 1.65-fold increased risk of HCC[56]. Other reported SNPs associated with NAFLD-HCC risk include Toll-like receptor 5 rs5744174[57], signal transducer transcription 6 rs167769[58] activator, yes-associated protein 1 rs11225163[58], *HSD17B13* rs72613567[55], and dystrophy-associated fer-1-like protein rs17007417[59] (Table 2).

Zhang *et al*[55] reported a combined risk assessment model consisting of *PNPLA3*, *TM6SF2*, and *HSD17B13* variants[55]. In this study, patients with the highest score had a 29-fold higher risk of developing HCC. Bianco *et al*[60] reported another polygenic risk assessment model consisting of *PNPLA3*, *TM6SF2*, *GCKR*, *MBOAT7*, and *HSD17B13*[60]. The sensitivity and specificity for diagnosing HCC were 0.43 and 0.79%, respectively (AUROC, 0.65).

Table 2 Genetic risk factors for nonalcoholic fatty liver disease-associated hepatocellular carcinoma		
Risk factors	**Reported evidence**	**Ref.**
PNPLA3	Carriage of rs738409 GG polymorphism is associated with 5.1–6.4-fold increased risk of HCC in NAFLD patients	[51-53,60]
	PNPLA3 G variant (GG *vs* CG *vs* CC) was not significantly associated with the risk of cardiovascular events extrahepatic cancers or overall death, but was associated with HCC (HR: 2.66) and liver-related death (HR: 2.42)	
	Used for developing polygenic risk scores	
TM6SF2	*TM6SF2* minor allele carriage (rs58542926 C>T) was associated with advanced fibrosis/cirrhosis and HCC (OR, 2.8) in NAFLD patients	[54,55,60]
	Combined assessment with *PNPLA3* and *HSD17B13* variants were useful for risk stratification of NAFLD-HCC	
	Used for developing polygenic risk scores	
MBOAT7	*MBOAT7* rs641738 C>T variants were associated with higher risk of HCC in NAFLD patients (OR, 1.65–2.10)	[56,60]
	Used for developing polygenic risk scores	
TLR5	*TLR5* rs5744174 TT genotype was a risk factor of HCC in patients with steatohepatitis-related cirrhosis (OR, 1.9)	[57]
STAT6	*STAT6* rs167769 CC genotype was inversely associated with the risk of HCC in NASH patients (OR, 0.015)	[58]
YAP1	Carriage of *YAP1* rs11225163 C allele was inversely associated with the risk of HCC in NASH patients (OR, 0.047)	[58]
HSD17B13	Combined assessment with *PNPLA3* and *TM6SF2* variants were useful for risk stratification of NAFLD-HCC	[55]
DYSF	*DYSF* rs17007417 T allele carriage was associated with increased risk of HCC in NAFLD patients (OR, 2.74)	[59]
GCKR	*GCKR* rs1260326 T allele carriage was associated with increased risk of HCC in NAFLD patients (OR, 1.38)	[59,60]
	Used for developing polygenic risk scores	

PNPLA3: Patatin-like phospholipase domain 3; HCC: Hepatocellular carcinoma; NAFLD: Nonalcoholic fatty liver disease; HR: Hazard ratio; TM6SF2: Transmembrane 6 superfamily member 2; HSD17B13: 7-beta-Hydroxysteroid dehydrogenase 13; OR: Odds ratio; MBOAT7: Membrane-bound O-acyl-transferase domain-containing 7; TLR5: Toll-like receptor 5; STAT6: Signal transducer activator of transcription 6; NASH: Nonalcoholic steatohepatitis; YAP1: Yes-associated protein 1; DYSF: Dystrophy-associated fer-1-like protein; GCKR: Glucokinase regulator.

DIAGNOSTIC BIOMARKERS

Currently available biomarkers

The currently available serum biomarkers for diagnosing HCC include AFP, des-carboxyprothrombin (DCP), and AFP isoform L3 (AFP-L3) (Table 3). Only AFP is recommended for HCC surveillance as *per* the major guidelines[31]. However, patients with NAFLD-HCC tend to have lower AFP levels than those with viral HCC[12,61]. Thus, the combined use of multiple tumor markers may be considered to increase the detection rate of HCC[62]. The GALAD score is a scoring system for the diagnosis of HCC. This score can be calculated by sex, age, lectin-bound AFP, AFP, and DCP. In a German cohort of NASH patients with and without HCC, the GALAD score exhibited excellent diagnostic performance (AUROC, 0.96). This was significantly better than AFP (AUROC, 0.88), DCP (AUROC, 0.87), and AFP-L3 (AUROC, 0.86) alone[63]. However, it must be noted that that only 20% of the HCC patients included in this study were within the Milan criteria. In addition, further research is needed from the perspective of cost effectiveness to determine whether this scoring system should be recommended for routine HCC screening in patients with NAFLD.

Potential biomarkers under investigation

Many serum biomarkers are under investigation for more accurate diagnosis of NAFLD-HCC without imaging. These biomarkers include iron and transferrin saturation[64], glypican-3 and adiponectin[65], midkine[66], apoptosis inhibitor of macrophages[67,68], glycosylation patterns of glycoproteins[69], and specific types of glycopeptides[70,71] (Table 3). Recently, Kozumi *et al*[72] showed that intrahepatic and serum thrombospondin 2 (*THBS2*) expression levels are strongly associated with advanced fibrosis in patients with NASH[72]. In their study, HCC was observed only in patients with high serum levels of

Table 3 Currently available and potential diagnostic biomarkers for nonalcoholic fatty liver disease-associated hepatocellular carcinoma		
Biomarkers	**Reported evidence**	**Ref.**
Currently available		
AFP	Modest diagnostic ability for HCC in NAFLD patients (AUROC, 0.71–0.88)	[63,84]
DCP	The diagnostic ability for NAFLD-HCC was similar to that of AFP	[63,84]
	Combined use with AFP improved the diagnostic performance	
	Used for calculation of GALAD score	
AFP-L3	The diagnostic ability for NAFLD-HCC was similar to that of AFP	[63]
	Used for calculation of GALAD score	
Under development		
Iron status	Elevations of serum iron levels and transferrin saturation were associated with increased risk of HCC in NAFLD patients (HR, 2.91 and 2.02, respectively)	[64]
Proteins	Midkine increased the diagnostic yield in AFP-negative HCC in NAFLD patients; 59.2% of AFP-negative NAFLD-HCC patients had elevation of serum midkine levels	[65-67,72]
	IgM-free AIM had better diagnostic performance for NASH-HCC than AFP or DCP (AUROC, 0.905–0.929)	
	Serum TSP-2 levels were significantly associated with advanced fibrosis in NASH patients. Among 164 patients with NAFLD, HCC occurred only in patients with high serum levels of TSP-2	
Glycoprotein	Glycosylation patterns of alpha-1 acid glycoprotein may serve as a diagnostic biomarker for AFP-negative HCC in NAFLD patients	[69]
Proteoglycan	Glypican-3 had modest diagnostic ability (AUROC, 0.759), similar to AFP (AUROC, 0.763). When combined with age, sex, DCP and adiponectin, the AUROC increased to 0.948	[65]
Glycopeptide	Site-specific N-glycopeptides from vitronectin may serve as diagnostic biomarkers for NASH-HCC. When used together with AFP, the AUROC were 0.834 and 0.847, compared to 0.791 of AFP alone	[70,71]
	Site-specific N-glycopeptides from serum haptoglobin showed better diagnostic accuracy for NASH-HCC than AFP	
Cytokine (adipokine)	Adiponectin had slightly better diagnostic ability (AUROC, 0.770) than AFP (AUROC, 0.763). When combined with age, sex, DCP and glypican-3, the AUROC increased to 0.948	[65]
Cell-free DNA	*TERT* promoter mutation (C228T) in serum cfDNA showed better diagnostic ability for early NAFLD-HCC than AFP and DCP	[76,78]
	Methylation biomarkers in cfDNA improved the diagnostic performance when combined with AFP	
microRNA	The expression levels of exosomal miR-182, miR-301a and miR-373 in both serum and ascetic fluid were higher in NASH-cirrhosis patients with HCC than in those without HCC	[77]

AFP: Alpha-fetoprotein; HCC: Hepatocellular carcinoma; NAFLD: Nonalcoholic fatty liver disease; AUROC: Area under receiver operating characteristic curve; DCP: Des-carboxyprothrombin; AFP-L3: AFP isoform L3; HR: Hazard ratio; IgM: Immunoglobulin M; AIM: Apoptosis inhibitor of macrophages; TSP-2: Thrombospondin-2; NASH: Nonalcoholic steatohepatitis; TERT: Telomerase reverse transcriptase; cfDNA: Cell-free DNA; miR: microRNA.

thrombospondin-2 (TSP-2). Thus, serum TSP-2 testing may also be useful for NAFLD-HCC surveillance.

Genetic and epigenetic biomarkers have attracted increasing attention in recent years (Table 3). For example, telomerase reverse transcriptase (*TERT*) promoter mutations, the most common form of HCC genetic alteration[73-75], can be analyzed using cell-free DNA (cfDNA). Akuta *et al*[76] reported that *TERT* C228T mutation could be detected in 63.9% of NAFLD-HCC patients by analyzing cfDNA[76]. Notably, it can be positive, even in patients with normal AFP and DCP levels. Another example of circulating biomarkers is non-coding RNAs, especially microRNAs. In a preliminary study, the serum expression levels of miR-182, miR-301a, and miR-373 were significantly higher in NASH-HCC patients than in NASH patients without HCC[77]. DNA methylation can also be analyzed using peripheral blood. In a recent study, methylation cfDNA biomarkers provided a modest diagnostic value for NAFLD-HCC with a sensitivity of 87.5% and specificity of 39.4%[78]. The combination of this DNA methylation panel and AFP led to a better cohort wide diagnostic performance than either of them used

alone. Although the utility of these biomarkers is yet to be validated, in the near future, advances in omics technology will surely provide better diagnostic tools for NAFLD-HCC than conventional tumor markers.

FUTURE PERSPECTIVES

A more effective surveillance approach is strongly desired to reduce the number of deaths due to NAFLD-HCC. As advanced liver fibrosis is the most common risk factor for NAFLD-HCC, current screening recommendations primarily focus on patients with cirrhosis. However, in a French study, only 37% of patients undergoing liver resection for NAFLD-HCC had bridging fibrosis or cirrhosis; with a frequency significantly lower than that of other etiologies[8]. Similar results were observed in a Japanese multicenter study; in which only 49% of patients with NAFLD-HCC had underlying cirrhosis [79]. Therefore, more than half of patients with NAFLD-HCC will lose the chance of early detection if no other risk factors are considered. As discussed above, other clinical factors and specific genetic variants have robust associations with NAFLD-HCC risk. We should make the best use of this evidence to enhance effective HCC surveillance. In addition, more accurate diagnostic biomarkers for NAFLD-HCC are needed. Among the currently available biomarkers, the GALAD score seems to be the most reliable. However, advances in omics technology will certainly provide more powerful diagnostic tools in the future. For instance, a recent study showed that fragmentomic cfDNA analysis offered excellent accuracy in detecting primary liver tumors (AUROC, 0.995), regardless of the underlying liver disease [80]. Moreover, it showed a sensitivity of over 95%, even for stage I tumors. Thus, further research on genetic and epigenetic biomarkers is very much needed.

Novel conceptional criteria for metabolic dysfunction-associated fatty liver disease (MAFLD) were proposed in 2020[81]. Although MAFLD has been reported as a more practical definition for identifying patients with fatty liver disease with high risk of disease progression[82], it remains unclear whether the evidence of risk factors and diagnostic markers for NAFLD-HCC can be extended to MAFLD-HCC. A nationwide cohort study conducted in Taiwan revealed that patients with NAFLD/MAFLD overlap had similar risk of HCC compared to those with NAFLD alone[83]. Nonetheless, further studies are warranted on this topic.

CONCLUSION

The incidence of NAFLD-HCC is increasing rapidly worldwide. Thus, an effective surveillance approach is required to reduce the number of deaths due to this condition. Although advanced fibrosis is the most important risk factor for NAFLD-HCC, other clinical risk factors such as diabetes, old age, male sex, and elevated liver enzymes should also be considered. Recent advances in omics technology have revealed that genetic factors such as the SNP of *PNPLA3* also affect the risk of hepatocarcinogenesis in patients with NAFLD. With regards to the diagnostic biomarkers for NAFLD-HCC, only AFP is recommended for surveillance as *per* major guidelines. A recent study showed that the GALAD score evinced a more effective diagnostic performance than when AFP was used alone. However, clinical implementation should be discussed from the perspective of cost-effectiveness. Specific gene mutations and DNA methylation can be detected in cfDNA and are expected to be novel biomarkers for the early NAFLD-HCC diagnosis, as well as specific proteins, glycopeptides, and adipokines.

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FOOTNOTES

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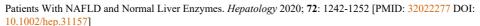
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