Pathology & Oncology Research (2020) 26:599–603
https://doi.org/10.1007/s12253-019-00585-5

REVIEW

Current Status and Perspective Biomarkers in AFP Negative HCC: Towards Screening for and Diagnosing Hepatocellular Carcinoma at an Earlier Stage

Ping Luo[1,2] · Sanyun Wu[2] · Yalan Yu[2] · Xinliang Ming[1] · Shuo Li[1] · Xuelan Zuo[2] · Jiancheng Tu[1]

Received: 28 February 2018 / Accepted: 15 January 2019 / Published online: 19 January 2019

Abstract
Hepatocellular carcinoma (HCC) is one of the most malignant cancer with high morbidity and mortality which lead to a serious burden to society. AFP (alpha-fetoprotein) is the most widely used serum biomarker to detect HCC worldwide. However, no AFP elevation have been found in many HCC and AFP analysis can't be used to screen HCC in these cases. Currently, many studies have been carried out to find reliable biomarker in diagnosing AFP-negative HCC. Such biomarker would help the diagnosis of AFP-negative HCC, ensuring the timely initiation of treatment. In this review, we highlight the important role of biomarkers that can differentiate AFP-negative HCCs, and discuss their potential clinical applications as biomarkers for the diagnosis of AFP-negative HCC.

Keywords HCC · Biomarker · AFP-negative · Diagnose

Introduction

Hepatocellular carcinoma (HCC) is one of the most malignant cancer with high morbidity and mortality [1] and it may cause about over 700,000 deaths worldwide each year [2]. HCC is the most common type of liver cancer accounts for about 90% of primary liver cancers [3], and it's prevalent in Asian countries, accounting for 75–80% cases reported globally [4]. Despite the cancer death rate has dropped significantly since 1991, the death caused by liver cancer is still increasing according to the cancer statistics 2016 [5]. It has been proved that cirrhosis is the major risk factor for HCC [6], and hepatitis B virus (HBV) infection represents the most of cirrhosis cases [7]. Although surgical resection and liver transplantation can treat HCC validly, the 5-year overall survival is still only 7% [8]. It's reported that in the last 20 years, the mortality of HCC has been increased significantly and the burden of medicine and economy will still significantly increase during the next decades [9]. The poor progress of HCC patients is also related to the fact that most HCCs can't be diagnosed at an early stage. It is generally accepted that accurate and early diagnosis of HCC can improve the clinical curative effect significantly and alleviate the suffering of patients [1]. However, clinical techniques such as imaging and histology methods are only able to detect HCC patients who are at relatively late stage [10]. Thus it is urgently needed to extend the window for early detection of HCC and a great deal of efforts should be made to test HCC with AFP negative.

Currently, imaging examination and serological tests are mostly used for the early diagnosis of HCC. Although

✉ Jiancheng Tu
jianchengtu@whu.edu.cn

Ping Luo
luop@whu.edu.cn

Sanyun Wu
wusanyun@126.com

Yalan Yu
yuyalan@znhospital.cn

Xinliang Ming
1599381035@qq.com

Shuo Li
904733306@qq.com

Xuelan Zuo
zuoxuelan2004@126.com

[1] Department of Clinical Laboratory Medicine & Center for Gene Diagnosis, Zhongnan Hospital of Wuhan University, Wuhan, China

[2] Department of Hematology, Zhongnan Hospital of Wuhan University, Wuhan, China

advances in computed tomography (CT) and magnetic resonance imaging (MRI) technology have greatly improved the diagnostic performance of HCC, these procedures are too expensive for widespread screening at the present time [11]. NCCN Clinical Practice Guidelines recommend that patients with high-risk to develop into HCC (namely, patients with cirrhosis of any etiology) should be screened by abdominal ultrasonography (US) and AFP twice per year for surveillance [10]. Ultrasound, the primary radiologic screening modality under current use, is limited by its operator dependence and poor ability to differentiate malignant from benign nodules in the small cirrhotic liver [12]. Alpha-fetoprotein (AFP) was first introduced as a serological marker for HCC in the1960s [13] and is currently the most widely used biomarker for HCC worldwide. However, even if a low-level cutoff is used (ie, 10–20 ng/mL), the sensitivity value of AFP to diagnose HCC is around 60% and the specificity is still inadequate [14]. Furthermore, serum AFP levels remain normal in 15–30% advanced HCC, [15], and it's reported that the elevation of AFP can also occur in benign liver diseases, such as hepatitis and cirrhosis [16]. Thus the American Association for the Study of Liver Diseases (AASLD) Practice Guidelines Committee no longer recommended AFP for the early detection of HCC [17] .

Circulation biomarkers can facilitate the screening of cancer because they are easily accessible and noninvasive. For serological tests, AFP is known to be the most widely used biomarker for HCC worldwide. However, about 30% of early-stage HCC can't be detected using AFP analysis [12], and then cause treatment delaying. So it is of great importance to find biomarkers that is able to identify HCC patients with AFP negative. Thus far, many other serum biomarkers have been proposed to detect AFP negative HCCs. This review will briefly outline recent findings about those biomarkers with a focus on serum, and discuss their potential clinical application as diagnostic and/or prognostic biomarkers for HCC.

The Diagnostic Value of some Well-Known Biomarkers in AFP-Negative HCC

Many biomarkers including MicroRNAs, PIVKA-II, AFP-L3 have been researched to diagnose AFP-negative HCC from a long time ago. Thus far, AFP-L3, and PIVKA- II have also been recommended for the surveillance of high-risk populations in Japan [18]. Studies demonstrated that the detection rate of early HCC and patients' 5-year survival in Japan was 68% and 45.2% [19, 20], which are much higher than those in the China and US, suggesting the promising value of biomarkers apart from AFP in early HCC detection.

PIVKA-II to Detect AFP-Negative HCC

Protein Induced by Vitamin K deficiency or antagonist-II (PIVKA-II), also known as Des-γ -carboxy-prothrombin (DCP) was first reported in 1984 and is believed to be a suitable serum biomarker specific for HCC [21]. It is an abnormal prothrombin molecule products caused by the acquired defect in the post translational carboxylation of the prothrombin precursor in malignant cells [22]. The expression level of PIVKA-II have been identified to be elevated in HCC patients and an increasing number of studies indicate that PIVKAII has high sensitivity and specificity for differentiating HCC at all stages from patients with cirrhosis or chronic hepatitis [23, 24]. In addition, several studies have shown that PIVKAII is a favorable biomarkers to detect AFP-negative HCC. For example, Ji et al. [25] found that the AUC of DCP to differentiate AFP-negative HCC from all control subjects was 0.856 (95% CI 0.798–0.914, sensitivity: 76.3%, specificity: 89.1%). As to differentiate AFP-negative HCC from the CHB and LC who is in high risk to develop into HCC, a similar AUC of 0.834 was revealed. In 2017, to assess the diagnostic accuracy of PIVKA-II as marker for HCC, another research was conducted. The data concluded that the area under ROC curve for PIVKA-II to discriminate AFP-negative HCC patients from patients in CHB group was 0.73 (95%CI 0.640–0.815) with sensitivity of 51.02% and specificity of 84.47% when the cut-off value of PIVKA-II was set as 32.09 mAU/ml [26].

AFP-L3 to Detect AFP-Negative HCC

AFP-L3 is a specific type of AFP that binds to lectin *Lens culinaris* agglutinin. It was originally reported as an HCC-specific tumor marker. Recently, an increasing number of evidence has revealed that AFP-L3 can predict the biological malignancy potential of HCC regardless of the tumor size and/or serum AFP concentration [27]. Studies have reported that AFP-L3 isoform seems to be more specific than the total AFP level for diagnosing HCC [28]. In 2011, Hidenori et al. [29] proved that the diagnostic sensitivity and specificity of hs-AFP-L3 were 41.5% and 85.1% at a cut-off level of 5%. In 2016, Best J and his colleagues combined DCP and AFP-L3 to detect AFP-negative HCCs, as we predicted, the detection rate of them to detect HCC that was AFP-negative is 68.4% [30]. Another retrospective study which evaluated the diagnostic accuracy of GP73 and AFP-L3 in AFP-negative HCC showed that maximum area under the curve for GP73 was 0.7811, and the sensitivity, specificity were 66%, 96.2%, respectively. For AFP-L3, the maximum area under the curve to distinguish AFP-negative HCC was 0.6094 and the sensitivity, specificity were 50%, 97.5%, respectively [31].

MicroRNAs to Detect AFP-Negative HCC

MicroRNAs (miRNAs) are a type of small noncoding RNAs that contain 20–25 nucleotides, which regulate the expression of target genes at the post-transcriptional level [32]. Increasing number of studies have shown that microRNAs were aberrantly expressed in HCC [33–35]. Particularly, microRNAs are highly stable in circulation and expression patterns seem to be tissue-specific, which suggests that circulating microRNAs may be ideal biomarkers to test early-stage HCC [36]. To assess the feasibility of miR-125b to discriminate AFP-negative HCC patients to HBV infection cases, a study was carried out in 2016. The results revealed that plasma miR-125b yielded an AUC of 0.943 (95% CI, 0.907–0.980) to discriminate HBV-HCC patients with AFP levels below 200 ng/mL from those HCC-free HBV infection cases with AFP levels also below 200 ng/mL (sensitivity = 100%; specificity = 75.5%) [16]. In another study exploring the diagnostic value of miR-4651 in HCC involving 279 HCC patients, among which 38.7% are negative for AFP, results showed that miR-4651 demonstrated high accuracy in discriminating individuals with AFP-negative HCC from controls (AUC = 0.80; 95% CI, 0.75–0.86) with a sensitivity of 70% and specificity of 90% respectively [37]. Guo et al. [38] revealed that serum levels of miR-21 were positive in 45 of 58 patients in AFP-negative HCC group and positive in 102 of 123 patients in AFP- positive HCC group. Additionally, the expression level of miR-21 showed no significance in AFP-positive and AFP-negative HCC subgroups (23.52 ± 1.04 vs. 25.90 ± 1.15 folds, $P < 0.0001$). The ROC curves showed that miR-21 had a great performance (AUC = 0.831, 95% CI 0.756–0.905,) with a sensitivity of 81.2% and a specificity of 83.2% to diagnose AFP-negative HCCs.

The combination of multiple biomarkers may improve diagnostic value. Thus, Zuo and his colleagues conducted a study to evaluate the combined effect of four miRNAs to differentiate HCC from controls in 2016. In his study, all the subjects, including HCC, CHB, and healthy controls, with serum AFP levels <20 μg/L were considered to be the AFP-negative subjects. They found that the combination of miR-125b, miR- 223, miR-27a, and miR-26a are valuable biomarkers in distinguishing HCC from non-cancer group in AFP subjects with an AUC of 0.874 [39]. In another study, 19 candidate serum miRNAs were identified to be increased in six patients with hepatocellular carcinoma compared with eight control patients with chronic hepatitis B in discovery set. Then through using a training cohort of patients with HCC and controls, they built a serum miRNA classifier (Cmi) containing seven differentially expressed miRNAs (miR-29a, miR-29c, miR-133a, miR-143, miR-145, miR-192, and miR-505) to detect HCC. The data showed that AUC for Cmi was 0.825 (0·779–0·871) for detection of AFP-negative HCC and the sensitivity of Cmi to discriminate individuals with AFP-negative HCC from all non-HCC controls was 78.8% in the training cohort, 75.8% in validation cohort 1, and 80.0% in validation cohort 2, specificity was 86.3%, 88.2%, and 91.1%, respectively [40]. All the above results indicated that the measurement of the miRNA panel could detect AFP-negative HCC effectively.

Novel Biomarkers in Detecting AFP-Negative HCC

Recently, many researches have been done to probe novel biomarkers that can diagnose AFP-negative HCC timely. Heat shock protein 90alpha (Hsp90α), a conserved and essential molecular chaperone, was reported to be significantly increased in cancer patients, and correlate positively with tumor malignancy and metastatic ability [41]. Fu and his colleagues designed a large-scale, multicenter clinical trial to assess the diagnostic accuracy of plasma Hsp90α for AFP-negative HCC. Their data showed that plasma Hsp90α achieved an incredible high performance in the diagnosis of AFP-negative HCC with sensitivity of 93.9% and specificity of 91.3% [42]. Liquid chromatography-mass spectrometry (LC-MS)-based metabolomics is a useful tool that can be used to discover novel circulating biomarkers for many diseases [43]. In Luo's study, by using LC-MS, they found that 80.6% of the AFP false-negative HCC patients were diagnosed correctly by using serum metabolite biomarker panel including phenylalanyl-tryptophan and glycocholate identified by their study [44]. The finding of novel biomarkers with high sensitivity and specificity to detect AFP-negative HCC will surely benefit cancer patients.

The Diagnostic Value of AFP Combined with Other Serum Biomarkers

Although AFP has its limitations as a biomarker to screening HCC, we can't deny the fact that it is the mostly used biomarkers currently. The combination of it with other serum biomarkers will certainly improve the diagnostic sensitivity and specificity of liver HCC. In 2017, a study containing 200 participants in total revealed that the combined detection of AFP and exosomal hnRNPH1 mRNA improves their diagnostic value for HCC with AUC of 0.891 (95% CI = 0.873–0.939, $P = 0.005$) and the sensitivity and specificity was 87.5%, 84.8% respectively [45]. A recent clinical study demonstrated that the diagnostic performance of combination of AFP, AFP-L3 and PIVKA II is superior to a single biomarker in HCC detection with the AUC high to 0.947 [46]. In another study which also assessed the performance of these three biomarkers to distinguish early stage HCC, Der et al. showed that utilization of the biomarker-based GALAD score resulted in a

superior specificity of 93.3% and sensitivity of 85.6% [30]. A large amount of studies have provided a glimpse into using combined markers detect HCC early, but the studies were small in scale. Larger scale, multi-center researches are need to be conducted. Considering this, a study consisted of five groups of 1153 patients from three hospitals in China were performed. Results showed that combined testing of DCP and AFP got a sensitivity of approximately 90% in the diagnosis of HCC, which was significantly higher than that for AFP or DCP alone [47].

Conclusions and Future Perspectives

HCC is still the major threat to human, for decades, a great number of researchers were undertook to explore the biomarker for HCC [48]. AFP, as a conventional biomarker for HCC surveillance has been widely used in clinical practice. The elevation of serum AFP provided critical clues for the diagnosis of HCC. However, AFP has its own limitations since many advanced HCC patients are AFP-negative. Biomarkers that can detect HCC patients who are AFP negative are urgently needed to be discovered. Our study assed the ability of some traditional biomarkers to diagnose AFP-negative HCC including PIVKA-II, AFP-L3 and miRNAs and some novel biomarkers, such as Hsp90α and metabolite biomarker. They showed an incredible high performance in the diagnosis of AFP-negative HCC. The combination of them with AFP will certainly diagnose HCC at an earlier stage and improve the survival time of HCC. In the future the identification of new noninvasive biomarkers for early diagnosis of HCC with larger scale will be one of the most promising fields of biomarker research.

Acknowledgements This work was supported by the National Basic Research Program of China (973 Program) (2012CB720605).

Compliance with Ethical Standards

Conflict of Interest The authors declare that there are no conflicts of interest.

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