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ORIGINAL PAPER

# Diagnostic values of alpha-fetoprotein, dickkopf-1, and osteopontin for hepatocellular carcinoma

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**Abstract** The timely diagnosis and effective treatment are essential for improving the survival and prognosis of hepatocellular carcinoma (HCC) patients. Alpha-fetoprotein (AFP) is the most widely used biomarker for diagnosis of HCC, but the low sensitivity and specificity limits its clinical application. In this study, we evaluated the diagnostic capability of the combination of AFP with two novel potential biomarkers, dickkopf-1 (DKK1) and osteopontin (OPN), for HCC in 390 participants including 89 patients with HCC, 36 patients with liver cirrhosis, 65 patients with chronic hepatitis B, and 200 health controls. We found the combination of all three markers as a panel showed a better diagnostic performance than that of AFP alone, with increased AUC [0.948 (95 % CI 0.921–0.968) vs. 0.831 (95 % CI 0.790–0.867)] and sensitivity (88.76 vs. 71.91 %). Moreover, this combination showed a great improvement in diagnosing early-stage HCC patients. In conclusion, the combined use of AFP, DKK1, and OPN as a biomarker panel could enhance the diagnostic ability for HCC.

**Keywords** Hepatocellular carcinoma · Alpha-fetoprotein · Dickkopf-1 · Osteopontin · Diagnosis

**Abbreviations**

| | |
|---|---|
| AFP | Alpha-fetoprotein |
| DKK1 | Dickkopf-1 |
| OPN | Osteopontin |
| HCC | Hepatocellular carcinoma |
| LC | Liver cirrhosis |
| CHB | Chronic hepatitis B |
| HC | Health control |
| ROC | Curve receiver operating characteristic curve |
| AUC | Area under ROC curve |
| CI | Confidence interval |
| PPV | Positive predictive value |
| NPV | Negative predictive value |
| LR | Likelihood ratio |

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## Introduction

Hepatocellular carcinoma (HCC) is the sixth most common malignant tumor and the second leading cause of cancer-related deaths worldwide [1, 2]. Globally, there are approximately 750,000 new cases of liver cancer reported per year, 70–85 % of which are HCC [2, 3]. The incidence of HCC is still rising. HCC is more effectively treated when it is diagnosed at an early stage; however, only 30–40 % of patients with HCC are suitable for potentially curative treatments at the time of diagnosis, which is mainly due to the lack of effective methods for early detection [4–6].

The screen strategy for HCC proposed by the 2012 National Comprehensive Cancer Network (NCCN) guidelines recommends the measurement of serum alpha-fetoprotein (AFP) and liver ultrasound in high-risk patients every 6–12 months for timely diagnosis [2]. Although AFP serves as an important tool in screening HCC patients, its sensitivity is quite low (25–65 %), particularly in detection of early-stage HCC [7]. In addition, AFP is elevated in a considerable number of patients with chronic hepatitis and/or cirrhosis, which makes it unreliable in HCC surveillance [8]. Therefore, new serum biomarkers with high accuracy to complement AFP are urgently needed.

It has been described that some serologic biomarkers have potential to complement the deficiencies of AFP in diagnosing HCC. Des-γ-carboxy prothrombin (DCP) [9] and lens culinaris agglutinin-reactive fraction of AFP (AFP-L3) [10] have already been used in clinic in Japan, but whether they are superior to AFP remains controversial [11–13]. Recently, dickkopf-1 (DKK1) and osteopontin (OPN) as novel potential biomarkers for HCC have been reported. DKK1 was first identified in Xenopus as a necessary inducer of head formation [14]. It inhibits the canonical Wnt signaling pathway by binding to and antagonizing LRP5/6 [15]. Yu and colleagues [16] found that DKK1 was upregulated in HCC tissues by microarray analysis, which suggested that it could be used as a novel diagnostic and prognostic predictor for HCC patients, especially in patients with early-stage disease. A large-scale, multicenter study indicated that DKK1 had a better performance in HCC diagnosis than AFP with greater sensitivity of 69.1 % and specificity of 90.6 %, especially in early-stage HCC diagnosis, and it could improve the identification of patients with AFP-negative HCC (serum AFP $\leq$ 20 ng/mL) [17]. OPN, also known as SPP1 (secreted phosphoprotein 1), was initially characterized in 1979 as a phosphoprotein secreted by transformed, malignant epithelial cells [18]. It is a member of small integrin binding ligand N-linked glycoprotein (SIBLING) family and produced by cells of immune system and epithelial tissue, smooth muscle cells, osteoblasts, and tumor cells [19, 20]. Shang and co-workers [21] found that the levels of plasma OPN were significantly elevated in HCC patients, and it was more sensitive than AFP for the diagnosis of HCC.

The combination of biomarkers is recommended in diagnosis of human cancers and is the tendency of future studies [12, 13, 22]. Previous studies have proven that a combination of AFP, AFP-L3, and DCP had a better diagnostic performance for HCC [11–13], and the Japanese evidence-based clinical practice guidelines also recommend screening HCC from high-risk population using the combination of these three markers [23]. The potential use of DKK1 and OPN in the complement of AFP has been revealed in recent studies; however, the diagnostic value of combination of these three markers in HCC diagnosis is unclear. Therefore, we first investigated and evaluated the diagnostic capability of the combination of DKK1, OPN, and AFP as a biomarker panel for HCC in this study.

## Materials and methods

### Study design

The HCC patients and health controls (HCs) enrolled in this study were collected from December 2008 to June 2009 and from May to June, 2013, respectively, from the Liver Cancer Institute, Zhongshan Hospital, Fudan University, Shanghai, China. The chronic hepatitis B virus (HBV) carriers and liver cirrhosis (LC) patients were recruited from January to April, 2013, from the Department of Infectious Disease, First Affiliated Hospital of Soochow University, Suzhou, China.

The diagnosis of HCC was based on American Association for the Study of Liver Diseases (AASLD) Practice Guidelines [8], verified by ultrasound, CT scan, or MRI and biochemistry (AFP serology and liver function enzymes) findings, and was confirmed by histopathology. Staging was defined according to the Barcelona Clinic Liver Cancer (BCLC) staging system [6]. We defined BCLC stage 0+A HCC as early-stage HCC in this study. For comparison of tumor markers in HCC patients, we used non-malignant liver disease patients with either chronic HBV infection or cirrhosis and healthy donors as controls. Diagnosis of chronic HBV infection was based on the guidelines of prevention and treatment of chronic HBV infection [24], including the presence of HBsAg for previous 6 months and HBV DNA concentrations higher than $10^3$ copies per mL. Cirrhosis was defined on the basis of both histopathology of liver biopsy samples and imaging evidence, such as nodular liver contour, portal hypertension, varices and enlargement of the caudate lobe. The healthy controls were blood donors without liver diseases and any tumors. The concerning

informed consent was obtained from each participant, and the study was approved by institutional ethics review committees of both study centers.

### Collection, storage, and measurements of serum samples

Serum samples of HCC patients were collected at the time of diagnosis, prior to the surgery or any other treatments. All the serum samples were centrifuged and stored at −80 °C until they were tested.

Concentrations of serum DKK1 and OPN were measured by ELISA with commercial kits (R&D Systems, Minneapolis, MN, USA). Concentrations of serum AFP was measured by the same method with another commercial kit (Raygene Biotechnology Company, Shanghai, China). The assays were conducted according to the manufacturer's instructions, and all specimens were performed blindly and in duplicate.

### Statistical analysis

Statistical analyses were performed with SPSS 19 and MedCalc software. The significant level was 0.05. Mann–Whitney *U* tests were performed to distinguish the differences between each independent group. Receiver operating characteristics (ROC) curves were performed to determine the optimal cutoff values of AFP, DKK1, and OPN, for diagnosing HCC. Area under ROC curve (AUC) with 95 % confidence interval (CI) was also calculated, respectively, to compare the ability of each marker for diagnosing. The correlation between marker concentrations and clinicopathological characteristics was analyzed with Pearson's $\chi^2$ test or Fisher's exact test. To assess the diagnostic performance of the combination of biomarkers, logistic regression models including two or three markers as covariates were performed.

## Results

### Patient characteristics

A total of 390 participants were enrolled in this study, of which 89 were HCC cases, 36 were LC patients, 65 were chronic HBV carriers, and 200 were health individuals. Clinicopathological characteristics of HCC patients were shown in Table S1.

### Biomarker levels

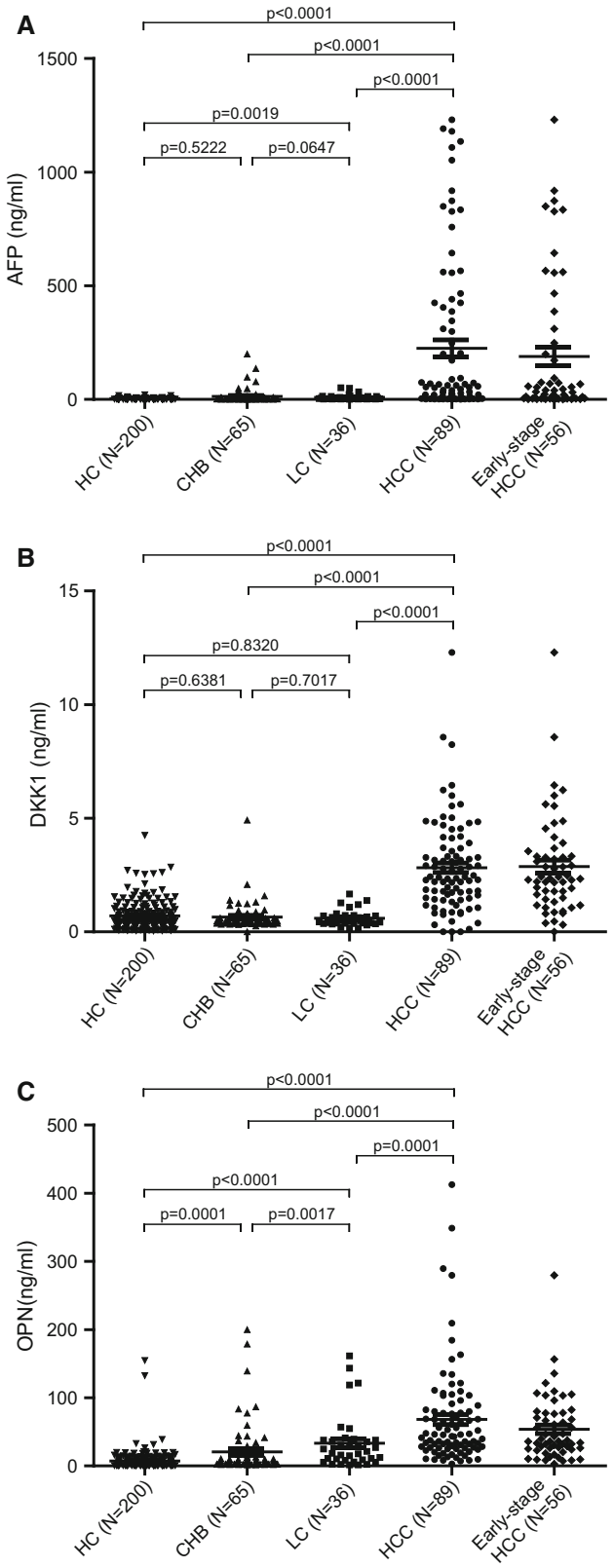
Serum levels of AFP, DKK1, and OPN were significantly elevated in patients with HCC when compared to all three control groups (Fig. 1). The mean concentration of AFP in serum of patients with HCC was 224.69 ng/mL, higher than that in healthy individuals (3.30 ng/mL, $p < 0.0001$), chronic HBV infection (12.99 ng/mL, $p < 0.0001$), and cirrhosis controls (8.87 ng/mL, $p < 0.0001$). Similar results were found in DKK1 and OPN measurements (Table S2). In contrast to AFP and OPN, there is no significant difference in DKK1 concentrations among three control groups.

### Optimum cutoff values

To determine the optimum diagnostic cutoff value for AFP, DKK1, and OPN, ROC curves were performed. The optimum cutoff for AFP was 6.79 ng/mL with a AUC of 0.831 (95 % CI 0.790–0.867), a sensitivity of 71.91 %, and a specificity of 88.04 %. The optimum cutoff for DKK1 was 1.31 ng/mL (AUC 0.889, 95 % CI 0.854–0.919, sensitivity 79.78 %, specificity 89.37 %) and for OPN was 15.11 ng/mL (AUC 0.908, 95 % CI 0.875–0.935, sensitivity 89.89 %, specificity 82.06 %). When using the currently recommended clinical cutoff for AFP (20 ng/mL), the sensitivity was 58.40 % and the specificity was 95.70 %. To be consistent and comparable with DKK1 and OPN, we chose 6.79 ng/mL as the cutoff value for AFP in this study. Based on these cutoffs, predictive values and likelihood ratios for all three markers in the diagnosis of HCC were also calculated in Table 1.

### Combination performance of three biomarkers in diagnosis of HCC

When all the HCC and control participants were enrolled, the AUC for OPN (AUC 0.908, 95 % CI 0.875–0.935) was the largest, followed by DKK1 (AUC 0.889, 95 % CI 0.854–0.919), and both of them were larger than AFP (AUC 0.831, 95 % CI 0.790–0.867). In order to evaluate the diagnostic value when these markers were combined, we used a binary logistic regression model to assess combinatorial ROC curves and determine whether better diagnostic accuracy can be achieved. The new variable predicted probability ($p$) for HCC was created on the basis of the equation obtained by the binary logistic regression (all HCC vs. all three control groups). The equations used in this study were as follows: for combination of AFP and DKK1, $\ln\left(\frac{p}{1-p}\right) = -3.926 + 0.028 \times \text{AFP} + 1.506 \times \text{DKK1}$; for combination of AFP and OPN, $\ln\left(\frac{p}{1-p}\right) = -2.508 + 0.023 \times \text{AFP} + 0.023 \times \text{OPN}$; and for combination of all three markers, $\ln\left(\frac{p}{1-p}\right) = -4.181 + 0.019 \times \text{AFP} + 1.444 \times \text{DKK1} + 0.016 \times \text{OPN}$. The cutoff




◀**Fig. 1** Serum concentrations of AFP, DKK1, and OPN in HCC, LC, CHB, and HC groups. **a** Concentration of AFP in serum. **b** Concentration of DKK1 in serum. **c** Concentration of OPN in serum. HCC group had higher level ($p < 0.0001$) compared with LC, CHB, and HC groups in all three markers. *Black horizontal lines* are means, and *error bars* are SEs. *AFP* alpha-fetoprotein, *DKK1* dickkopf-1, *OPN* osteopontin, *HCC* hepatocellular carcinoma, *LC* liver cirrhosis, *CHB* chronic hepatitis B, *HC* health control

values calculated from the combinational ROC curves were 0.135, 0.122, and 0.147, respectively.

The AUC for the combination of AFP and DKK1 (0.931, 95 % CI 0.901–0.954) was larger than that of AFP or DKK1 alone ($p < 0.05$), and a similar result was found in the combination of AFP and OPN (0.937, 95 % CI 0.908–0.959, $p < 0.05$). The combination of the three markers showed the largest AUC (0.948, 95 % CI 0.921–0.968) when compared with any single marker alone ($p < 0.05$; Fig. 2b; Table 1), which meant the panel of these three markers could improve the diagnostic value in distinguishing patients with HCC from all participants including health individuals, LC patients, and chronic hepatitis B virus carriers.

Since early detection is one of the key approaches to improving the survival of cancer patients, we further evaluate the diagnostic performance of these three markers in 56 early-stage HCC patients (BCLC stage 0+A) in this study. As shown in Fig. 2c and Table 1, DKK1 had the best AUC (0.901, 95 % CI 0.865–0.930), followed by OPN (AUC 0.890, 95 % CI 0.853–0.920), and both of them were better than AFP (AUC 0.820, 95 % CI 0.776–0.858). The AUC of the combination of three markers was larger than the combined use of AFP and DKK1 or the use of AFP and OPN. All combinations were better than individual marker used alone ($p < 0.05$; Fig. 2d; Table 1).

Among 89 HCC patients, 25 were AFP-negative patients when the cutoff of AFP was 6.79 ng/mL. A total of 18 (72 %) of 25 AFP-negative patients had positive DKK1 results (DKK1 >1.31 ng/mL), and 21 (84 %) of them had positive OPN results (OPN >15.11 ng/mL). The probability of patients with either positive DKK1 results or positive OPN results accounted for 96 % (24/25) in AFP-negative HCC patients. Furthermore, we stratified the AFP-negative HCC patients according to the BCLC stage (0+A, B, C, D), tumor size (<3, 3–5, >5 cm), and tumor number (single and multiple tumor) (Table 2). The sensitivities of DKK1 and OPN alone in AFP-negative HCC patients at early stage (BCLC stage 0+A) ($n = 18$) were 72 and 78 %, respectively, while the combination of them increased the sensitivity up to 94 %. Likewise, the combination of two markers increased the sensitivity in all subgroups (Table 2).

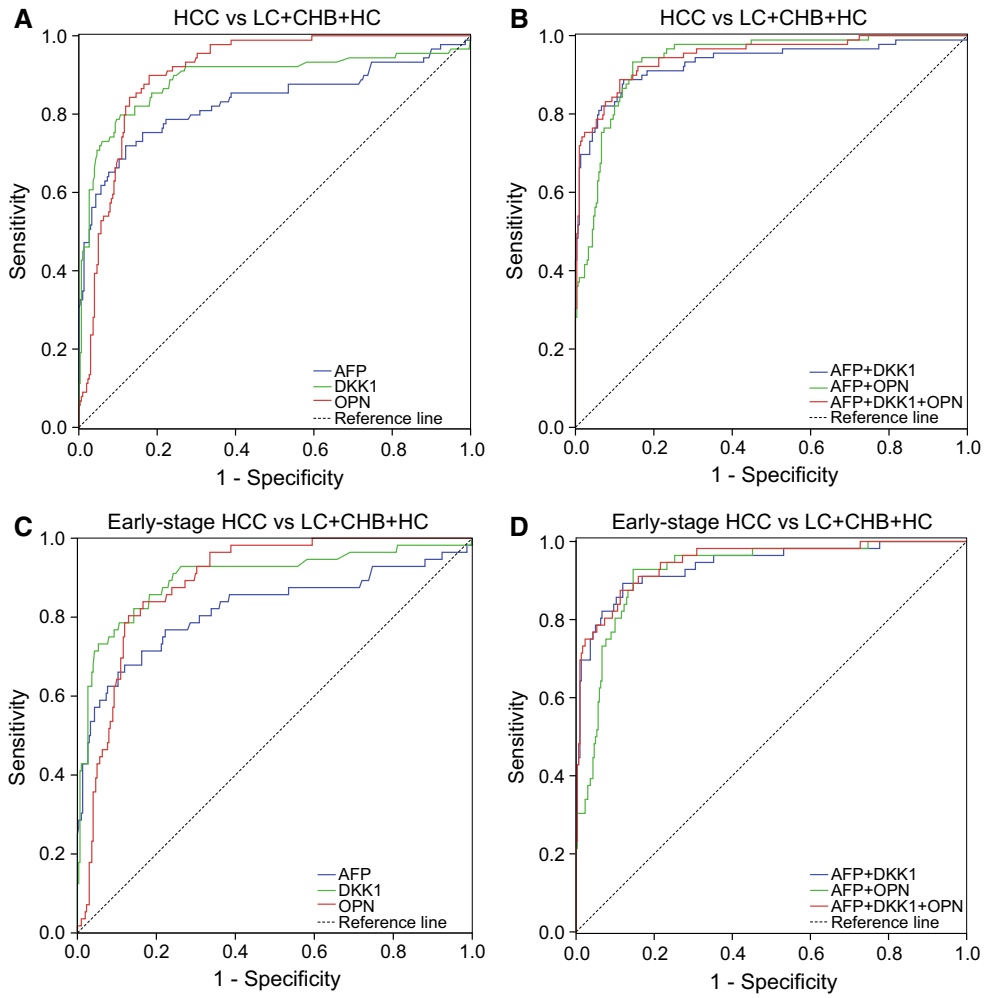



**Fig. 2** ROC curves for AFP, DKK1, OPN, and their combinations in the diagnosis of HCC and early-stage HCC. **a** ROC curves for AFP, DKK1, and OPN for patients with HCC versus all controls. **b** Combination of three markers for patients with HCC versus all controls. **c** ROC curves for AFP, DKK1, and OPN for patients with early-stage HCC versus all controls. **d** Combination of three markers for patients with early-stage HCC versus all controls. Both DKK1 and OPN showed a greater AUC than AFP. Combination of three markers showed a significantly improved AUC than AFP alone (0.948 vs. 0.831 in HCC group and all three control groups, $p < 0.05$; 0.949 vs. 0.820 in early-stage HCC and all three control groups, $p < 0.05$). *ROC* receiver operating characteristic, *AUC* area under ROC curve, *AFP* alpha-fetoprotein, *DKK1* dickkopf-1, *OPN* osteopontin, *HCC* hepatocellular carcinoma, *LC* liver cirrhosis, *CHB* chronic hepatitis B, *HC* health control

The best chance for early diagnosis comes from the surveillance of patients known to be at high risk. In this study, we classified LC patients and chronic HBV carriers as patients at high risk. We evaluated the performance of three markers in distinguishing HCC patients from high-risk patients. We found that DKK1 had the best diagnostic performance with the greatest AUC, specificity, and PPV, whereas the AUC of OPN was similar to that of AFP (Fig. 3a; Table 3). The combination of all three markers got the largest AUC (Fig. 3b). When these three markers were, respectively, used to distinguish early-stage HCC from cirrhosis and chronic HBV patients, DKK1 still showed the best performance with the largest AUC (0.914, 95 % CI 0.858–0.952) compared with AFP (0.778, 95 % CI 0.705–0.841) and OPN (0.780, 95 % CI 0.707–0.842), suggesting that DKK1 was better in distinguishing HCC, especially patients at an early stage, from high-risk patients (Fig. 3c). The combination of three markers had no significantly different AUC, sensitivity, and specificity values when compared with the combination of AFP and DKK1 in differentiating patients with early-stage HCC from high-risk controls (Fig. 3d; Table 3).

## Correlation analysis

Lastly, we evaluated the relationship between several factors with three markers by Chi-square test. Serum AFP level was found to be significantly associated with

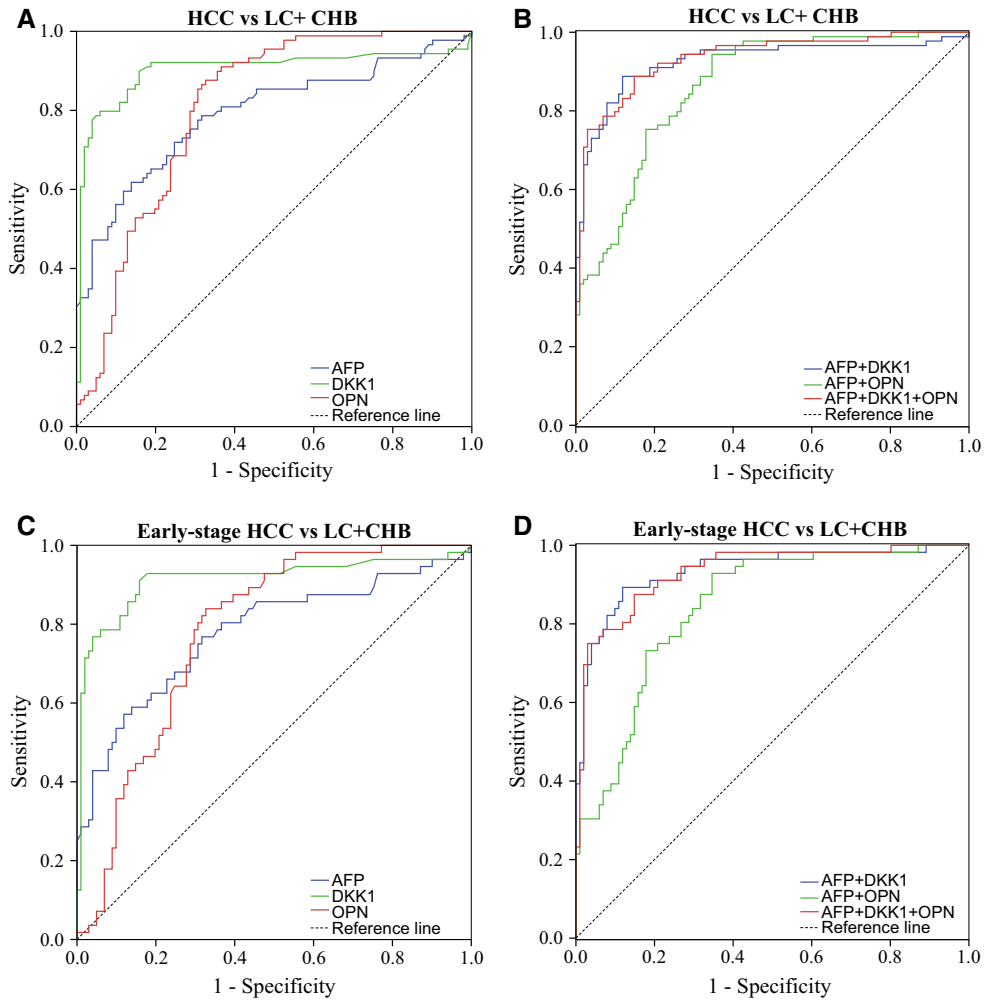



**Fig. 3** ROC curves for AFP, DKK1, OPN, and their combinations for distinguishing HCC from high-risk patients. **a** ROC curves for AFP, DKK1, and OPN for patients with HCC versus high-risk patients. **b** Combination of three markers for patients with HCC versus high-risk patients. **c** ROC curves for AFP, DKK1, and OPN for patients with early-stage HCC versus high-risk patients. **d** Combination of three markers for patients with early-stage HCC versus high-risk patients. In this study, we defined LC and CHB infection patients as high-risk patients. The combination of DKK1 and AFP showed the largest AUC in distinguishing early-stage HCC from high-risk patients. *ROC* receiver operating characteristic, *AUC* area under ROC curve, *AFP* alpha-fetoprotein, *DKK1* dickkopf-1, *OPN* osteopontin, *HCC* hepatocellular carcinoma, *LC* liver cirrhosis, *CHB* chronic hepatitis B, *HC* health control

cirrhosis, and serum OPN level was found to be significantly associated with HBsAg copies, while DKK1 had no statistically significant correlation with all these pathology parameters (Table S1).

## Discussion

Nowadays, diagnosis of HCC mainly relies on radiological appearances and histology, such as ultrasound, CT scanning, MRI, and biopsy [25]. All their accuracies are highly depended on the equipment used and the experience of the operators [26]. Moreover, methods such as ultrasound, CT, and MRI cannot distinguish between malignant and benign nodules, and the high expenses also limit their wide application. Biopsy may result in higher recurrence rates because of the risk of tumor cells seeding along the needle track [27, 28].

AFP is the most widely used tumor marker for diagnosis of HCC; however, it is deficient in sensitivity and specificity. DKK1 was found specifically overexpressed in cancer cells as a secreted protein [29, 30], and it had potential to be used as a tumor-specific serum biomarker for various human cancers [29], especially for HCC. We previously designed a multicenter study to evaluate the diagnostic accuracy of DKK1 as a serological biomarker for HCC [17], and found that the performance of DKK1 was better than AFP in distinguishing HCC patient. The combination of these two markers in diagnosis of HCC was better than either alone. Similar results were obtained by

**Table 1** Performance of AFP, DKK1, OPN, and the combination for the diagnosis of HCC and early-stage HCC

| | AUC | 95 % CI | Sensitivity (%) | Specificity (%) | PPV (%) | NPV (%) | Positive LR | Negative LR |
|---|---|---|---|---|---|---|---|---|
| HCC versus LC + CHB + HC | | | | | | | | |
| AFP | 0.831 | 0.790–0.867 | 71.91 | 88.04 | 64.00 | 91.38 | 6.01 | 0.32 |
| DKK1 | 0.889 | 0.854–0.919 | 79.78 | 89.37 | 68.93 | 93.73 | 7.50 | 0.23 |
| OPN | 0.908 | 0.875–0.935 | 89.89 | 82.06 | 59.70 | 96.48 | 5.01 | 0.12 |
| AFP + DKK1 | 0.931 | 0.901–0.954 | 88.76 | 87.71 | 68.10 | 96.35 | 7.22 | 0.13 |
| AFP + OPN | 0.937 | 0.908–0.959 | 93.26 | 85.38 | 65.35 | 97.72 | 6.38 | 0.08 |
| AFP + DKK1 + OPN | 0.948 | 0.921–0.968 | 88.76 | 88.70 | 69.91 | 96.39 | 7.86 | 0.13 |
| Early-stage HCC versus LC + CHB + HC | | | | | | | | |
| AFP | 0.820 | 0.776–0.858 | 67.86 | 88.04 | 51.35 | 93.64 | 5.67 | 0.37 |
| DKK1 | 0.901 | 0.865–0.930 | 78.57 | 89.04 | 57.14 | 95.71 | 7.17 | 0.24 |
| OPN | 0.890 | 0.853–0.920 | 83.93 | 81.73 | 46.08 | 96.47 | 4.59 | 0.20 |
| AFP + DKK1 | 0.940 | 0.910–0.962 | 89.29 | 87.38 | 56.82 | 97.77 | 7.07 | 0.12 |
| AFP + OPN | 0.925 | 0.892–0.950 | 92.86 | 85.38 | 54.17 | 98.47 | 6.35 | 0.08 |
| AFP + DKK1 + OPN | 0.949 | 0.921–0.969 | 87.50 | 88.37 | 58.33 | 97.44 | 7.53 | 0.14 |

*AFP* alpha-fetoprotein, *DKK1* dickkopf-1, *OPN* osteopontin *AUC* area under curve, *PPV* positive predictive value, *NPV* negative predictive value, *LR* likelihood ratio

other two studies [31, 32]. OPN was identified relevant to HCC metastasis and patient survival, and it was proved to be both a diagnostic marker and a potential therapeutic target for HCC [33]. Its high sensitivity but low specificity limited the clinical diagnostic value due to OPN was elevated in many chronic inflammatory diseases and about 30 different types of cancer [34, 35]. Recent studies showed that OPN was more sensitive than AFP and it could complement the measurement of AFP in the diagnosis of HCC [21].

In the present study, serum concentrations of AFP, DKK1, and OPN were significantly elevated in HCC group when compared to all three control groups (Fig. 1). Only DKK1 had no significant difference between three control groups, which meant it could perform well in distinguishing HCC from non-malignant chronic liver diseases and health individuals; however, the serum concentrations of OPN were upregulated in CHB and LC, both of which were chronic inflammatory diseases. The combination of all three markers had the largest AUC, with a sensitivity of 88.76 % and specificity of 88.70 % (Table 1). Among 25 AFP-negative patients (AFP $< 6.79$ ng/mL) with HCC, 24 (96 %) had increased serum DKK1 or OPN concentrations (Table 2). It showed the combination of DKK1 and OPN could improve the sensitivity even in patients with low AFP.

The diagnosis of HCC at an early stage has a high clinical relevance since it can be more effectively treated [8]. In this study, more than half of patients belonged to early-stage HCC [56 (63 %) of 89], which was defined as stages 0 and A according to the BCLC staging system. As a result, DKK1 showed the best diagnostic accuracy with greater AUC, sensitivity, and specificity than AFP. OPN also had a greater AUC and sensitivity than AFP, but less in specificity. The combination of AFP, DKK1, and OPN resulted in the largest AUC and a sensitivity of 87.50 % and specificity of 88.37 % (Fig. 2; Table 1).

**Table 2** Sensitivity of DKK1 and OPN in AFP-negative HCC patients

| | *n* (%) | DKK1 (%) | OPN (%) | Combination of DKK1 and OPN (%) |
|---|---|---|---|---|
| Tumor stage (BCLC) | | | | |
| 0+A | 18 | 13 (72.2) | 14 (77.8) | 17 (94.4) |
| B | 5 | 5 (100) | 5 (100) | 5 (100) |
| C | 2 | 0 (0) | 2 (100) | 2 (100) |
| D | NA | NA | NA | NA |
| Tumor size (cm) | | | | |
| <3 | 11 | 7 (63.6) | 8 (72.7) | 10 (90.9) |
| 3–5 | 6 | 4 (66.7) | 6 (100) | 6 (100) |
| >5 | 8 | 7 (87.5) | 7 (87.5) | 8 (100) |
| Tumor number | | | | |
| Single | 19 | 12 (63.2) | 15 (78.9) | 18 (94.7) |
| Multiple | 6 | 6 (100) | 6 (100) | 6 (100) |

The diagnostic cutoff values for DKK1 and OPN were 1.31 and 15.11 ng/mL, respectively

*AFP* alpha-fetoprotein, *DKK1* dickkopf-1, *OPN* osteopontin, *NA* Not available

Most HCC cases result from cirrhotic livers and chronic liver diseases from viral hepatitis, alcohol abuse, and/or nonalcoholic steatohepatitis (NASH) [2, 13], and surveillance of patients known to be at high risk which include cirrhosis and chronic HBV carriers is important in

**Table 3** Performance of AFP, DKK1, OPN, and the combination for distinguishing HCC from high-risk patients

| | AUC | 95 % CI | Sensitivity (%) | Specificity (%) | PPV (%) | NPV (%) | Positive LR | Negative LR |
|---|---|---|---|---|---|---|---|---|
| HCC versus LC + CHB | | | | | | | | |
| AFP | 0.792 | 0.727–0.847 | 71.91 | 75.25 | 71.91 | 75.25 | 2.91 | 0.37 |
| DKK1 | 0.902 | 0.851–0.941 | 79.78 | 93.07 | 91.03 | 83.93 | 11.51 | 0.22 |
| OPN | 0.807 | 0.744–0.861 | 89.89 | 64.36 | 68.97 | 87.84 | 2.52 | 0.16 |
| AFP + DKK1 | 0.925 | 0.878–0.958 | 88.76 | 88.12 | 86.81 | 89.90 | 7.47 | 0.13 |
| AFP + OPN | 0.863 | 0.806–0.909 | 93.26 | 65.35 | 70.34 | 91.67 | 2.69 | 0.10 |
| AFP + DKK1 + OPN | 0.934 | 0.889–0.965 | 88.76 | 85.15 | 84.04 | 89.58 | 5.98 | 0.13 |
| Early-stage HCC versus LC + CHB | | | | | | | | |
| AFP | 0.778 | 0.705–0.841 | 67.86 | 75.25 | 60.32 | 80.85 | 2.74 | 0.43 |
| DKK1 | 0.914 | 0.858–0.952 | 78.57 | 93.07 | 86.27 | 88.68 | 11.34 | 0.23 |
| OPN | 0.780 | 0.707–0.842 | 83.93 | 64.36 | 56.63 | 87.84 | 2.35 | 0.25 |
| AFP + DKK1 | 0.935 | 0.885–0.968 | 89.29 | 88.12 | 80.65 | 93.68 | 7.51 | 0.12 |
| AFP + OPN | 0.844 | 0.777–0.897 | 92.86 | 65.35 | 59.77 | 94.29 | 2.68 | 0.11 |
| AFP + DKK1 + OPN | 0.933 | 0.882–0.967 | 87.50 | 85.15 | 76.56 | 92.47 | 5.89 | 0.15 |

*AFP* alpha-fetoprotein, *DKK1* dickkopf-1, *OPN* osteopontin, *AUC* area under curve, *PPV* positive predictive value, *NPV* negative predictive value, *LR* likelihood ratio, *HCC* hepatocellular carcinoma, *CHB* chronic hepatitis B virus infection, *LC* liver cirrhosis

reducing mortality of this disease [36]. So, we assessed the diagnostic performance of all three markers for distinguishing early-stage HCC from LC and CHB patients. When OPN was calculated at a cutoff of 15.11 ng/mL, results were positive in 36 % patients with chronic HBV infection and cirrhosis. It resulted in the highest sensitivity of 83.93 % but the lowest specificity of 64.36 % (Table 3). The less specificity due to its wide expression of various immune cells, those cells include macrophages, dendritic cells (DCs), neutrophils, NK cells, and T and B lymphocytes. So, the serum concentration of OPN was increased in a variety of acute and chronic inflammatory conditions [37, 38], such as LC and CHB in this study. Therefore, the AUC of the combination of three markers was no better than the combination of AFP and DKK1 (0.933 vs. 0.935) in differentiating early-stage HCC from non-malignant chronic liver diseases. This study also revealed DKK1 was better than OPN and AFP in distinguishing early-stage HCC form high-risk patients. Measurement of combination of DKK1 and AFP in serum can help to make a differential diagnosis of HCC in these high-risk populations (Fig. 3; Table 3).

This study, however, had some limitations. The sample size was small, and we just included patients with background of chronic HBV infected. Patients with other non-malignant liver diseases and HCV carriers were not included. So, the results are still needed to be validated in further study with larger sample size, and whether the combination of AFP, DKK1, and OPN is useful in HCV-related HCC is also needed to be confirmed.

## Conclusion

This study validated the diagnostic capability of DKK1 and OPN, and assessed the combination of AFP, DKK1, and OPN as a panel for the diagnosis of HCC. These findings suggest that the combination of three markers could enhance the sensitivity in the diagnosis of HCC and may provide a new diagnostic strategy for HCC patients.

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