

Des-gamma-carboxyprothrombin is a favorable biomarker for the early diagnosis of alfa-fetoprotein-negative hepatitis B virus-related hepatocellular carcinoma

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Abstract

Objectives: Des-gamma-carboxyprothrombin (DCP) is an important serum biomarker for the clinical screening of hepatocellular carcinoma (HCC). This study aimed to evaluate the value of DCP for the early diagnosis of alpha-fetoprotein (AFP)-negative hepatitis B virus (HBV)-related HCC.

Methods: We retrospectively enrolled patients with AFP-negative HBV-related HCC and benign liver disease. Serum DCP levels in all participants were measured by chemiluminescent enzyme immunoassay. The value of DCP for the early diagnosis of AFP-negative HBV-related HCC was evaluated by receiver operating characteristic curve (ROC) and area under the curve (AUC) analyses.

Results: A total of 210 patients, including 87 cases with AFP-negative HBV-related HCC and 123 control cases with chronic HBV infection (CHB) or liver cirrhosis (LC), were included. Serum DCP levels were significantly increased in patients with AFP-negative HBV-related HCC compared with those with CHB or LC. The AUC for DCP for distinguishing between the two groups was 0.731 (95% confidence interval (CI), 0.657 to 0.805) and that for early diagnosis was 0.685 (95%CI, 0.596 to 0.774).

Conclusions: DCP may be a favorable biomarker to improve the early diagnosis rate of AFP-negative HBV-related HCC.

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Keywords

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Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignant tumors in adults in Asia and the third leading cause of cancer-related death.^{1,2} There are about 470,000 new cases of HCC in China every year, accounting for 55% of the worldwide incidence.^{3,4} The occurrence of HCC in China is mainly related to hepatitis virus infection, hepatic fibrosis, and biliary cirrhosis,^{5,6} and about 1% to 5% of cases of liver cirrhosis per year develop into HCC.⁷ Treatments for HCC include surgical resection, interventional embolization, radiofrequency ablation, liver transplantation, and chemotherapy.⁸ However, the postoperative recurrence and metastasis rates remain high and the long-term survival rate for more than 5 years is low.⁹

Chronic hepatitis B virus (HBV) infection (CHB) is the major risk factor for HCC in China.¹ Although technologies such as ultrasound imaging, contrast-enhanced computed tomography, magnetic resonance imaging, and percutaneous transhepatic biopsy have greatly improved the rate of HCC diagnosis, these methods have some disadvantages, such as high cost, invasiveness, and a low efficiency for small lesions. Testing for serum tumor markers is thus an important complement for HCC diagnosis. Alpha-fetoprotein (AFP) is the most frequent biomarker used in the clinic; however, 30% to 40% of HCCs may present as AFP-negative.^{10,11} Furthermore, increased AFP levels may also be detected in patients with chronic active hepatitis.¹² It thus

remains difficult to improve the efficiency of the early diagnosis of AFP-negative HCC, especially HBV-related HCC.

Des-gamma-carboxyprothrombin (DCP; also known as protein induced by vitamin K deficiency or antagonist-II (PIVKA II)) is a prothrombin precursor synthesized by the liver. Liebman et al.¹³ first reported serum DCP in patients with liver cancer in 1984, and >90% of patients with HCC have since been shown to have strong positive expression of DCP.¹⁴ Sterling et al.¹⁵ found that more than half of patients with AFP-negative liver cancers had elevated DCP, while liver cancer could be ruled out in patients with normal DCP levels, despite positive nodules on ultrasound results. DCP is therefore considered to be a diagnostic marker for AFP-negative HCC and an independent prognostic indicator.¹⁶ This retrospective study aimed to evaluate the early diagnostic value of serum DCP for distinguishing AFP-negative HBV-related HCC.

Materials and methods

Study population and DCP measurement

This study retrospectively enrolled patients treated at Beijing YouAn Hospital between March 2010 and July 2018. The participants were divided into four subgroups: CHB group, liver cirrhosis (LC) group, early-stage HCC group, and advanced-stage HCC group. The inclusion criteria for the HCC groups in this study were: (1) diagnosis confirmed by histopathological examination,

(2) chronic HBV infection, (3) age 20 to 80 years, (4) serum collected preoperatively, and (5) serum AFP level ≤ 20 ng/mL. The exclusion were: (1) intrahepatic cholangiocarcinoma or mixed HCC, (2) hepatitis C virus, alcoholic liver disease, or biliary cirrhosis, and (3) other cancers. Early-stage HCC was defined as a single lesion or up to three lesions ≤ 3 cm. Advanced-stage HCC was defined as lesions > 3 cm or accompanied by metastasis. CHB and LC were diagnosed according to the Guideline of Prevention and Treatment for Chronic Hepatitis B (2015 update).¹⁷ This study was approved by the research ethics committee of Beijing YouAn Hospital. Written informed consent was obtained from all participants. Serum AFP and DCP levels were measured using an automated chemiluminescent enzyme immunoassay system (LUMIPULSE[®] G1200; Fujirebio Inc., Tokyo, Japan), according to the manufacturer's instructions.

Statistical analysis

The data were analyzed using SPSS version 22.0 (IBM, Armonk, NY, USA) and GraphPad Software version 7 (Graph Pad Software, San Diego, CA, USA). Differences within subgroups were compared using the Mann-Whitney test (for non-normally distributed data) and Student's *t*-test (for normally distributed data). The clinicopathological characteristics were compared with Pearson's χ^2 test. The diagnostic value of DCP was evaluated by analyzing the receiver-operating characteristic curves (ROC) and areas under the curves (AUC). A value of $P < 0.05$ was considered significant.

Results

Patient characteristics

The baseline characteristics of the study population are presented in Table 1. A total of

210 patients were enrolled, including 87 patients with AFP-negative HBV-related HCC and 123 with CHB or LC. There was a male predominance in both groups ($P = 0.008$) (Table 1). The Child-Pugh grade was well-matched between the groups. There were significant differences among the HCC, LC, and CHB groups in terms of alanine aminotransferase, aspartate aminotransferase, total bilirubin, and total protein (all $P < 0.05$) (Table 1). Serum DCP levels were significantly higher in patients with early- or advanced-stage HCC compared with the CHB and LC groups ($P = 0.032$) (Figure 1).

Diagnostic value of DCP

The diagnostic value of DCP for AFP-negative HBV-related HCC was evaluated by ROC curves. Based on the AUCs, DCP was able to distinguish between all HCCs and CHB or LC, all HCCs and LC, early-stage HCC and CHB or LC, early-stage HCC and LC (Table 2, Figure 2).

Discussion

The present study evaluated the performance of serum DCP for diagnosing AFP-negative HBV-related HCC in 87 patients with AFP-negative HBV-related HCC and 123 with CHB or LC. Serum DCP levels were significantly higher in the patients with HCC compared with the CHB and LC groups, indicating that DCP might be an excellent diagnostic biomarker for AFP-negative HBV-related HCC. The results of ROC curve analysis showed that DCP could effectively distinguish between all AFP-negative HCCs and CHB and LC (AUC 0.731) and between early-stage AFP-negative HBV-related HCC and CHB or LC (AUC 0.685). These results confirmed the value of DCP as a promising marker for diagnosing AFP-negative HBV-related HCC.

Table 1. Clinical features of the study population.

Feature	CHB	LC	Early-stage HCC	Advanced-stage HCC	P value
Number	51	72	62	25	
Sex					
Male	30	47	52	21	0.008
Female	21	25	10	4	
Age (year)					
≤55	45	44	39	10	<0.001
>55	6	28	23	15	
Child–Pugh					
A	51	51	52	21	0.183
B	0	17	10	3	
C	0	4	0	1	
ALT, U/L					
≤50	48	67	47	18	0.02
>50	3	5	15	7	
AST, U/L					
≤40	46	54	47	13	0.03
>40	5	18	15	12	
TBil, μmol/L					
≤21	41	37	44	19	0.04
>21	10	35	18	6	
TP, g/L					
≤65	3	27	18	9	0.01
>65	48	45	44	16	
Cirrhosis					
Yes	0	72	43	15	<0.001
No	51	0	19	10	
DCP(mAU/mL, median, range)	24 (13, 49)	24.5 (7, 12,391)	34 (11, 26,858)	406 (12, 75,000)	<0.001

HCC, hepatocellular carcinoma; LC, liver cirrhosis; CHB, chronic hepatitis B virus infection; TBil, total bilirubin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TP, total protein; DCP, des-γ-carboxy prothrombin.

Most previous research suggested that the diagnostic efficiency of DCP for HCC was inferior to that of AFP.^{18–20} For instance, Choi et al.²¹ conducted a longitudinal assessment of the diagnostic value of DCP for detecting HCC and reported an AUC for DCP of 0.71 for diagnosing HCC. However, regarding AFP-negative HCC, a large-scale multicenter study by Ji et al.²² showed that DCP was superior to AFP for the surveillance of early HCC, and that DCP had an AUC of 0.856 for differentiating between patients with

AFP-negative HCC and control subjects, including healthy individuals and patients with liver cirrhosis, liver hemangiomas, and chronic hepatitis B virus infection. The AUC for DCP in their study was higher than that in the current study, possibly because the former study included more patients with late-stage HCC and healthy controls. Another study reported by Wang et al.²³ showed an AUC for DCP of 0.731 among patients with AFP-negative HBV-related HCC, which was consistent with the current results.

The ratio of fucosylated to total serum paraoxonase 1 was previously reported to show superior potential compared with AFP-L3 and glypican-3 for distinguishing between AFP-negative HCC and LC.²⁴ In terms of other important markers, AFP-L3, as a specific type of AFP, was considered to be more specific than total AFP for diagnosing HCC, and might thus also improve the diagnosis of HCC in patients with low AFP levels.²⁵ Although we did not compare the diagnostic values of DCP and AFP-L3

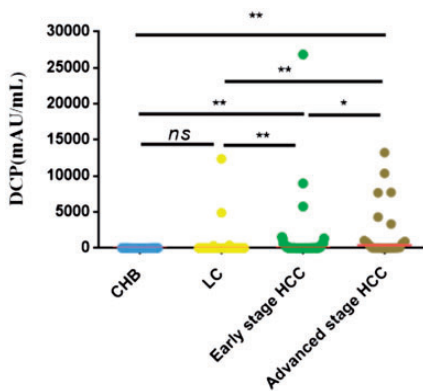


Figure 1. Serum DCP levels in patients with AFP-negative HBV-related HCC, LC, and CHB. ** $P < 0.001$; * $P < 0.01$. ns, not significant; HCC, hepatocellular carcinoma; HBV, hepatitis B virus; DCP, des- γ -carboxy prothrombin; AFP, alpha-feto-protein. Note: Extreme values were removed.

for differentiating HCC, Zhang et al.²⁶ revealed that the maximum AUC of AFP-L3 for diagnosing AFP-negative HCC was 0.609 (95% confidence interval (CI) 0.599 to 0.799), which was much lower than that for DCP in the present study. Furthermore, Mao et al.²⁷ showed that the AUCs of peripheral plasma Dickkopf-1 and Tie2-expressing monocytes for differentiating between AFP-negative HCC and LC and CHB were 0.649 (95%CI 0.542 to 0.755) and 0.708 (95%CI 0.605 to 0.812), respectively. However, the AUC for DCP in the current study was higher than these previous results, indicating that DCP was more promising than other potential biomarkers for diagnosing AFP-negative HBV-related HCC.

Different from previous studies, we also evaluated the value of DCP for the early diagnosis of AFP-negative HBV-related HCC, and showed that serum DCP might be a suitable biomarker to aid the early diagnosis of AFP-negative HCC.

This study had some limitations. First, the total number of included participants was relatively small. Second, this was a retrospective study from single center and not a prospective study. Third, we only focused on patients with HBV-related HCC.

In conclusion, DCP shows relative high efficiency for differentiating between

Table 2. Diagnostic values of DCP for AFP-negative HBV-related HCC.

Groups compared	DCP					
	Cutoff (mAU/mL)	AUC (95%CI)	Sen (%)	Spe (%)	PPV (%)	NPV (%)
All HCC vs LC or CHB	45	0.731 (0.657–0.805)	50.6	94.3	86.3	73.0
All HCC vs LC	41	0.728 (0.649–0.806)	50.6	91.7	88.0	60.6
Early-stage HCC vs LC, CHB	45	0.685 (0.596–0.774)	43.5	94.3	79.4	76.8
Early-stage HCC vs LC	41	0.683 (0.591–0.775)	43.5	91.7	81.8	65.3

HCC, hepatocellular carcinoma; LC, liver cirrhosis; CHB, chronic hepatitis B virus infection; DCP, des- γ -carboxy prothrombin; AUC, area under curve; Sen, sensitivity; Spe, specificity; PPV, positive predictive value; NPV, negative predictive value.

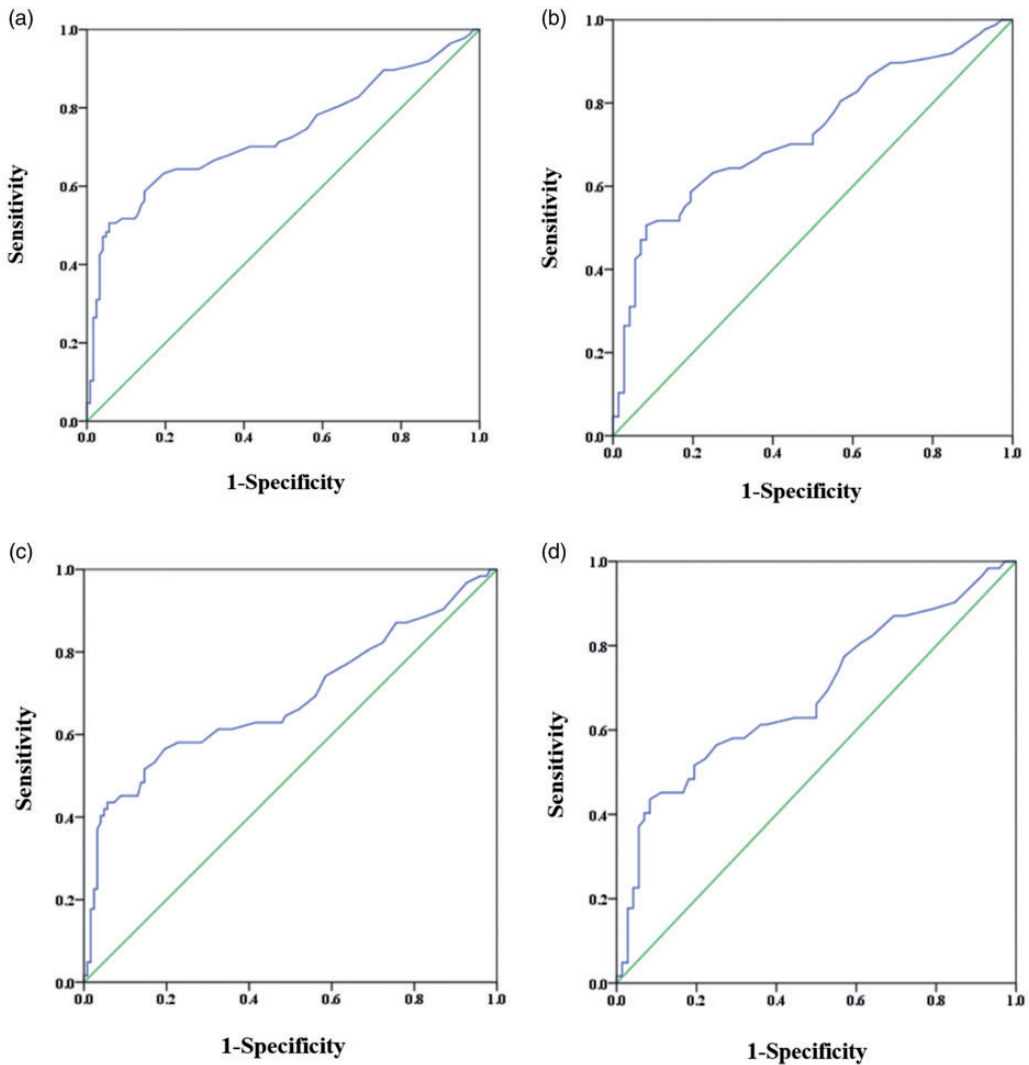


Figure 2. Values of DCP for early diagnosis of AFP-negative HBV-related HCC. (a) All HCC vs CHB and LC; (b) all HCC vs LC; (c) early-stage HCC vs CHB and LC; (d) early-stage HCC vs LC. HCC, hepatocellular carcinoma; LC, liver cirrhosis; CHB, chronic hepatitis B virus infection.

AFP-negative HBV-related HCC and CHB or LC. DCP may thus be a suitable biomarker to improve the rate of early diagnosis of AFP-negative HBV-related HCC.

Availability of data and materials

The datasets supporting the conclusion of this study are included in the article.


Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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References

1. Yuen MF, Hou JL, Chutaputti A, et al. Hepatocellular carcinoma in the Asia Pacific region. *J Gastroenterol Hepatol* 2009; 24: 346–353.
2. Moore MA, Ariyaratne Y, Badar F, et al. Cancer epidemiology in South Asia - past, present and future. *Asian Pac J Cancer Prev* 2010; 11: 49–66.
3. Wang FS, Fan JG, Zhang Z, et al. The global burden of liver disease: the major impact of China. *Hepatology* 2014; 60: 2099–2108.
4. Siegel RL, Miller KD and Jemal A. Cancer statistics, 2018. *CA Cancer J Clin* 2018; 68: 7–30.
5. Pungpapong S, Kim WR and Poterucha JJ. Natural history of hepatitis B virus infection: an update for clinicians. *Mayo Clin Proc* 2007; 82: 967–975.
6. Nagaratnam N, Nagaratnam K and Cheuk G. Hepatocellular Carcinoma. *Geriatric Diseases* pp.1–4.
7. Ganem D and Prince AM. Hepatitis B virus infection—natural history and clinical consequences. *N Engl J Med* 2004; 350: 1118–1129.
8. Marquardt JU, Galle PR and Teufel A. Molecular diagnosis and therapy of hepatocellular carcinoma (HCC): an emerging field for advanced technologies. *J Hepatol* 2012; 56: 267–275.
9. Ju YC, Jun DW, Choi J, et al. Long term outcome of antiviral therapy in patients with hepatitis B associated decompensated cirrhosis. *World J Gastroenterol* 2018; 24: 4606–4614.
10. Chen S, Chen H, Gao S, et al. Differential expression of plasma miR-125b in hepatitis B virus related liver diseases and diagnostic potential for hepatitis B virus induced hepatocellular carcinoma. *Hepatol Res* 2017; 47: 312–320.
11. Han LL, Lv Y, Guo H, et al. Implications of biomarkers in human hepatocellular carcinoma pathogenesis and therapy. *World J Gastroenterol* 2014; 20: 10249–10261.
12. Males S, Gad RR, Esmat G, et al. Serum alpha-foetoprotein level predicts treatment outcome in chronic hepatitis C. *Antivir Ther* 2007; 12: 797–803.
13. Liebman HA, Furie BC, Tong MJ, et al. Des-gamma-carboxy (abnormal) prothrombin as a serum marker of primary hepatocellular carcinoma. *N Engl J Med* 1984; 310: 1427–1431.
14. Gao J, Feng X, Inagaki Y, et al. Des- γ -carboxy prothrombin and c-Met were concurrently and extensively expressed in hepatocellular carcinoma and associated with tumor recurrence. *Biosci Trends* 2012; 6: 153–159.
15. Sterling RK, Jeffers L, Gordon F, et al. Utility of *Lens culinaris* agglutinin-reactive fraction of alpha-fetoprotein and des-gamma-carboxy prothrombin, alone or in combination, as biomarkers for hepatocellular carcinoma. *Clin Gastroenterol Hepatol* 2009; 7: 104–113.
16. Song P, Feng X, Zhang K, et al. Perspectives on using des-gamma-carboxyprothrombin (DCP) as a serum biomarker: facilitating early detection of hepatocellular carcinoma in China. *Hepatobiliary Surg Nutr* 2013; 2: 227–231.
17. Hou J, Wang G, Wang F, et al. Guideline of prevention and treatment for chronic hepatitis B (2015 update). *J Clin Transl Hepatol* 2017; 5: 297–318.
18. Park SJ, Jang JY, Jeong SW, et al. Usefulness of AFP, AFP-L3, and PIVKA-II, and their combinations in diagnosing hepatocellular carcinoma. *Medicine (Baltimore)* 2017; 96: e5811.
19. Zhao Q, Liu YY, Lei X, et al. PIVKA-II as a screening marker for hepatocellular carcinoma in patients with hepatitis B virus infection. *World Chinese Journal of Digestology* 2017; 9: 803.
20. Yoon YJ, Han KH and Kim DY. Role of serum prothrombin induced by vitamin K absence or antagonist-II in the early detection of hepatocellular carcinoma in patients with chronic hepatitis B virus infection. *Scand J Gastroenterol* 2009; 44: 861–866.
21. Choi J, Kim GA, Han S, et al. Longitudinal assessment of three serum biomarkers to

- detect very early-stage hepatocellular carcinoma. *Hepatology* 2019; 69: 1983–1994.
22. Ji J, Wang H, Li Y, et al. Diagnostic evaluation of des-gamma-carboxy prothrombin versus α -fetoprotein for hepatitis B virus-related hepatocellular carcinoma in China: a large-scale, multicentre study. *PLoS One* 2016; 11: e0153227.
 23. Wang X, Zhang W, Liu Y, et al. Diagnostic value of prothrombin induced by the absence of vitamin K or antagonist-II (PIVKA-II) for early stage HBV related hepatocellular carcinoma. *Infect Agent Cancer* 2017; 12: 47.
 24. Shu H, Li W, Shang S, et al. Diagnosis of AFP-negative early-stage hepatocellular carcinoma using Fuc-PON1. *Discov Med* 2017; 23: 163–168.
 25. Luo P, Wu S, Yu Y, et al. Current status and perspective biomarkers in AFP negative HCC: towards screening for and diagnosing hepatocellular carcinoma at an earlier stage. *Pathol Oncol Res* 2019. <https://doi.org/10.1007/s12253-019-00585-5>.
 26. Zhang Z, Zhang Y, Wang Y, et al. Alpha-fetoprotein-L3 and Golgi protein 73 may serve as candidate biomarkers for diagnosing alpha-fetoprotein-negative hepatocellular carcinoma. *Onco Targets Ther* 2015; 9: 123–129.
 27. Mao L, Wang Y, Wang D, et al. TEMs but not DKK1 could serve as complementary biomarkers for AFP in diagnosing AFP-negative hepatocellular carcinoma. *PLoS One* 2017; 12: e0183880.