

Value of AFP and PIVKA-II in Diagnosis of Primary Hepatocellular Carcinoma and Prediction of Vascular Invasion and Tumor Differentiation

Yuan-quan Si

Shandong Provincial Hospital

Xiu-Qin Wang

Shandong Provincial Hospital

Gang Fan

Shandong Provincial Hospital

Chang-Yin Wang

Shandong Provincial Hospital

Yuan-Wen Zheng

Shandong Provincial Hospital

Xie Song

Shandong Provincial Hospital

Cui-Cui Pan

Shandong Provincial Hospital

Fu-Lu Chu

Shandong Provincial Hospital

Zhan-Feng Liu

Shandong Provincial Hospital

Bing-Ru Lu

Shandong Provincial Hospital

Zhi-Ming Lu (✉ luzhiming@sdu.edu.cn)

Research Article

Keywords: Primary hepatocellular carcinoma, Alpha-fetoprotein, PIVKA-II, Vascular invasion

Posted Date: August 12th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-55985/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Version of Record: A version of this preprint was published on November 23rd, 2020. See the published version at <https://doi.org/10.1186/s13027-020-00337-0>.

Abstract

Background: To explore the value of alpha fetoprotein (AFP) and protein induced by vitamin K absence or antagonist-II (PIVKA-II) in diagnosis of primary hepatocellular carcinoma (HCC) and their relationship with vascular invasion, tumor differentiation and size.

Methods: A total of 433 participants were enrolled in this study including 266 cases with HCC, 87 cases with liver cirrhosis and 80 healthy individuals. Then we explored the correlation between AFP, PIVKA-II serum level and several pathological features such as vascular invasion, tumor differentiation and size. The value of these two markers used singly or jointly in diagnosing HCC was evaluated by receiver operating characteristic (ROC) curve. The ROC curve was also plotted to identify AFP, PIVKA-II serum cut-off values that would best distinguish HCC patients with and without vascular invasion.

Results: The level of AFP and PIVKA-II in HCC group was significantly higher (Z was 7.428, 11.243 respectively, all $P < 0.01$). When AFP and PIVKA-II were used as the individual tumor marker, the areas under the ROC curve (AUC) of HCC diagnosis were 0.765 (95% CI, 0.713–0.8170) for AFP, 0.901 (95% CI, 0.868–0.935) for PIVKA-II, and 0.917 (95% CI, 0.886–0.948) for AFP and PIVKA-II simultaneously.

The serum levels of AFP and PIVKA-II were positively correlated with tumor differentiation and size. High AFP and PIVKA-II expression was significantly associated with the presence of vascular invasion (P was 0.007 and 0.014 respectively). The AFP level $>64.4\text{ng/ml}$ or PIVKA-II level $>957.61\text{mAU/ml}$ was the best critical value to predict the presence of vascular invasion.

Conclusion: Our results validate that AFP and PIVKA-II play a significant role in the diagnosis of HCC. The diagnostic value of AFP and PIVKA-II combined detection or single assay of PIVKA-II is higher than that of separate assay of AFP. Moreover, their concentration has important clinical value in judging tumor size, tumor cell differentiation and vascular invasion.

Background

HCC is currently the fifth most common malignant tumor and the third leading cause of cancer related death worldwide[1]. It shows a poor prognosis and a high risk of recurrence.

For the lack of typical symptoms of HCC in early stage, it is generally not easy to diagnose. More than 80% HCC patients are diagnosed at the middle or late stages. Therefore, it is urgent to identify effective and specific biomarkers that provide early predictive potential for diagnosis of HCC[2].

AFP is a kind of glycoprotein that is often associated with primary hepatocellular carcinoma. However, AFP levels also increase in pregnancy and some benign diseases such as severe hepatitis and cirrhosis. AFP is not significantly increased in about 35% ~ 40% of the HCC patients, especially for small HCC[3, 4].

PIVKA-II is an abnormal form of prothrombin, which has been used as a good diagnostic biomarker for HCC[5-7]. There is now considerable evidence that PIVKA-II is an independent prognostic factor after liver

surgery, such as hepatic resection or liver transplantation[8]. In addition, PIVKA-II is influenced by many non-tumor factors, such as coagulation dysfunction, liver cirrhosis and so on[5].

There were few studies on the correlation between the above two indicators and vascular invasion, tumor differentiation and size. And the conclusions are controversial[9-11]. The aim of the study was to evaluate the diagnostic value of PIVKA-II and AFP in the diagnosis of HCC and further analysis the level of AFP and PIVKA-II in HCC patients with different clinicopathologic characteristics such as tumor differentiation and vascular invasion.

Materials And Methods

Patients

266 cases of primary hepatocellular carcinoma which were newly diagnosed in Shangdong Provincial Hospital from January 2017 to December 2018 were recruited as experimental group. All cases were confirmed by pathological examination. Pathological report of HCC included tumor diameter and differentiation. Vascular invasion was defined by the presence of tumor cells forming a thrombus in a vascular space lined by endothelial cells. 87 patients with benign liver disease (including 47 cases of hepatocirrhosis and 40 cases of Hepatitis B) and 80 healthy individuals were selected during the same period as control. Benign liver diseases were ascertained by image tests or ultrasonography detection. There was no evidence of any cancer or inflammatory disease from blood test results with healthy individuals. Participants with Haemocoagulatory disorders, vitamin K uptake disorders and intake of vitamin K blocking agents were excluded. Informed consents were obtained from all participants.

Measurement of AFP and PIVKA-II

The contents of AFP and PIVKA-II were detected in the same serum samples respectively. AFP was measured using the method of immunofluorescence on automatic electrophoresis fluorescence immunoassay analyzer (mTAS Wako i30, Japan). The cut-off value was set at 20ng/ml. Serum levels of PIVKA-II were detected by chemiluminescence enzyme immunoassay on automatic chemiluminescence immunoassay instrument (LUMIPULSE[®] G1200, Japan). The cut-off value was 40mAU/ml. The above reagents required were all original kit and the standard operation procedure was followed strictly during the test.

Confirmation of vascular invasion

Firstly, microscopic infiltration of the vessels at the periphery of the HCC nodules was searched in all native livers by hematoxylin-eosin (H&E) staining. Then, tumor vessel invasion was assessed by identifying neoplastic emboli within the tumor vessels stained by the mouse monoclonal antibody against CD34 protein. The stained slides were blindly and randomly examined by the same pathologist who had already evaluated H&E sections.

Statistical analysis

IBM SPSS statistical software (version 22.0, USA) and MedCalc (version 15.2.2.0, Belgium) were used for all statistical analysis. It was found that the concentration levels of AFP and PIVKA-II were skew distribution. Each variable was represented as median with interquartile range (IQR). Multi-numerical variables were compared by Kruskal-Wallis test, and the nonparametric Mann-Whitney test was applied to compare the differences between two numerical variables. Log transformation was used for AFP and PIVKA-II values to analyse the large range of values among the different groups for the markers. The transformed data were compared by box plots. Categorical variables were expressed in percentage and compared with the Pearson Chi-square test. ROC Curves and AUROC were calculated to evaluate the diagnostic value of AFP and PIVKA-II singly or jointly. Then Z tests were applied to compare the difference of AUROC. Two-tailed *P* value less than 0.05 was defined statistically significant.

Results

Patient demographics and tumor characteristics

The cut-off value of AFP and PIVKA-II commonly used was 20ng/ml and 40mAU/ml respectively. Based on this critical value, patient demographics and tumor characteristics were outlined in Table 1. As shown in Table 1, we found that AFP and PIVKA-II had strong correlation with tumor size (measured by diameter) and differentiation as well as vascular invasion.

AFP and PIVKA-II serum levels in experimental and control group

The median levels of AFP and PIVKA-II in HCC patients were 24.64 (IQR 4.38~528.82) and 334.08 (IQR 60.88~5095.10), while their concentrations of control group were 3.20 (IQR 1.90~9.60) and 22.17 (IQR 17.59~30.05) respectively. As shown in the Fig.1, the AFP and PIVKA-II levels were significantly higher in the HCC group than that in the control group (Z was 7.428, 11.243 respectively, *P* all <0.01). There was no significant difference of serum AFP and PIVKA-II level between patients with benign liver disease and healthy people in the control group. Above results validated that AFP and PIVKA-II played a significant role in the diagnosis of HCC

Comparison of AFP and PIVKA-II single and combined detection in HCC diagnosis

The ROC curve was plotted to compare diagnostic values of AFP and PIVKA-II detected singly or jointly in HCC and identify their cut-off values that would best distinguish patients with HCC from control group. As pictured in the Fig.2, the AUC of HCC diagnosis was 0.765 (95% CI, 0.713~0.8170) for AFP, and 0.901 (95% CI, 0.868~0.935) for PIVKA-II. The combination of AFP and PIVKA-II improved the diagnostic performance for HCC (AUC 0.917; 95% CI, 0.886~0.948). The diagnostic values of AFP and PIVKA-II combined detection or single assay of PIVKA-II were better than that of separate assay of AFP (Z was 5.927 and 4.51 respectively, *P* all <0.001). There was no significant difference of diagnostic accuracy between joint test of the two biomarkers and PIVKA-II detected singly (Z was 1.795, *P* = 0.0727). The optimal cut-off value of HCC diagnosis was 21.8ng/ml for AFP and 41.74mAU/ml for PIVKA-II. At the optimal cut-off value of AFP and PIVKA-II, sensitivity and specificity in diagnosis of HCC were reflected in Fig.2.

Correlations between AFP, PIVKA-II serum levels and tumor differentiation and size (measured by diameter) of HCC

The AFP, PIVKA-II concentration showed significant differences in well/moderate/poor differentiation as well as tumor size of HCC. Generally, the serum levels of AFP and PIVKA-II were positively correlated with tumor differentiation and size, just as shown in Fig.3.

Predictive value of AFP and PIVKA-II for HCC vascular invasion

The positive results of H&E and immunohistochemical staining in HCC tissues with vascular invasion were shown in Fig.4 (A and B). The median levels of AFP and PIVKA-II in HCC patients without vascular invasion were 15 (IQR 3.4~69) and 174.71 (IQR 46.76~1156.6), while their concentrations of patients with vascular invasion were 45 (IQR 6.37~1039.2) and 549.3 (IQR 65.13~7805.3) respectively. As shown in the Fig.4 (C and D), the AFP and PIVKA-II level in HCC with or without vascular invasion was obviously different. High AFP and PIVKA-II expression was significantly associated with the presence of vascular invasion (Z was 2.683, 2.463 respectively, $P = 0.007$ and 0.014). The AFP level $>64.4\text{ng/ml}$ or PIVKA-II level $>957.61\text{mAU/ml}$ was the predictor of vascular invasion.

Discussion

The incidence of hepatocellular carcinoma is increasing annually, thus early diagnoses and treatments are particularly important. AFP is a glycoprotein that is the most common biomarker for diagnosing HCC. But it has not yet become an optimal biomarker for diagnostic purposes because it lacks high sensitivity and specificity[12]. PIVKA-II also known as des-gamma-carboxyprothrombin (DCP) is an immature form of prothrombin without any coagulative function[13]. PIVKA-II is a specific marker for HCC, which is poorly related to AFP and exhibits higher sensitivity and specificity than AFP in diagnosing HCC[14-16].

In this study, we evaluated the performance of AFP and PIVKA-II for the diagnosis of HCC and explored the relationship between them and different clinicopathologic features, such as vascular invasion, tumor differentiation and size. Compared with control group, the level of AFP and PIVKA-II in experimental group was significantly higher. Our results validated that AFP and PIVKA-II played a significant role in the diagnosis of HCC. The optimal cut-off values of HCC diagnosis in our study were 21.8ng/ml for AFP and 41.74mAU/ml for PIVKA-II respectively, which were in close agreement with their cutoff values used currently. The combination of AFP and PIVKA-II slightly improved the diagnostic performance, and the serum PIVKA-II level had a better diagnostic value than AFP. These findings were in accordance with conclusions from other studies[17-21]. The serum levels of AFP and PIVKA-II were positively correlated with tumor differentiation and size. High AFP and PIVKA-II expression was significantly associated with the presence of vascular invasion. The AFP level $>64.4\text{ng/ml}$ or PIVKA-II level $>957.61\text{mAU/ml}$ was the best critical value to predict the presence and absence of vascular invasion.

The conclusions about the relation between AFP, PIVKA-II and clinicopathologic features (such as vascular invasion, tumor differentiation and size) were different, even controversial[9-11, 22-25]. There

were two main reasons for the different conclusions of previous studies. One important factor was research cancer object which was not strictly screened. Hence they did not distinguish the amount and source of tumors. The other reason was related to detection instruments and methods, the levels of AFP and PIVKA-II varied according to instruments and methods used. Based on the above two reasons caused the contradiction between different research. Tumor subjects in our study were primary liver cancer, then immunofluorescence assay and chemiluminescence immunoassay were used to measure AFP and PIVKA-II respectively. The methods were accurate which had the advantages of good repeatability and high test efficiency. At the same time, when studying the relationship between AFP, PIVKA-II level and tumor size, all the selected HCC cases were all single tumors and multiple tumors were excluded by imaging examinations. Accordingly, the conclusions were more persuasive. However, data of this study were obtained in a single hospital, and the number of participants was small. Further research should spend more time and get more detailed information, for instance, we were not sure whether PIVKA-II correlates with size or grade better than AFP.

Conclusions

In conclusion, PIVKA-II is the most useful single biomarker to diagnose the presence of HCC. Combining the AFP and PIVKA-II further improves the diagnostic performance. Moreover, their concentrations play an important role in judging tumor size, tumor cell differentiation and vascular invasion.

Declarations

Acknowledgments

We appreciated the contributions of Department of Hepatobiliary Surgery, Shandong Provincial Hospital affiliated to Shandong University.

Funding

None.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Author contributions

ZL conceived the project and designed the experiments. CP, FC, ZL, BL performed the data extraction and analyzation; YS, XW, wrote and revised the manuscript. GF, CW, YZ, XS clinically managed the patients. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

This study was approved by Ethics Committee of the Shandong Provincial Hospital affiliated to Shandong University. In this study, written informed consent was obtained from the patients or their families, and patient anonymity was preserved.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interest.

References

1. Siegel RL, Miller KD and Jemal A. Cancer statistics, 2016. *CA Cancer J Clin.* 2016;66(1):7-30
2. Li S, Yao J, Xie M, Liu Y and Zheng M. Exosomal miRNAs in hepatocellular carcinoma development and clinical responses. *J Hematol Oncol.* 2018;11(1):54
3. Afshar M, Fletcher P, Bardoli AD, Ma YT and Punia P. Non-secretion of AFP and neutrophil lymphocyte ratio as predictors for survival in hepatocellular carcinoma patients treated with sorafenib: a large UK cohort. *Oncotarget.* 2018;9(24):16988-95
4. Hiraoka A, Ishimaru Y, Kawasaki H, Aibiki T, Okudaira T, Toshimori A, et al. Tumor Markers AFP, AFP-L3, and DCP in Hepatocellular Carcinoma Refractory to Transcatheter Arterial Chemoembolization. *Oncology.* 2015;89(3):167-74
5. Dauti F, Hjaltalin Jonsson M, Hillarp A, Bentzer P and Schott U. Perioperative changes in PIVKA-II. *Scand J Clin Lab Invest.* 2015;75(7):562-7
6. Eguchi S, Takatsuki M, Hidaka M, Soyama A, Tomonaga T, Muraoka I, et al. Predictor for histological microvascular invasion of hepatocellular carcinoma: a lesson from 229 consecutive cases of curative liver resection. *World J Surg.* 2010;34(5):1034-8
7. Fujiki M, Takada Y, Ogura Y, Oike F, Kaido T, Teramukai S, et al. Significance of des-gamma-carboxy prothrombin in selection criteria for living donor liver transplantation for hepatocellular carcinoma. *Am J Transplant.* 2009;9(10):2362-71
8. Shirabe K, Itoh S, Yoshizumi T, Soejima Y, Taketomi A, Aishima S, et al. The predictors of microvascular invasion in candidates for liver transplantation with hepatocellular carcinoma-with special reference to the serum levels of des-gamma-carboxy prothrombin. *J Surg Oncol.* 2007;95(3):235-40
9. Saitta C, Raffa G, Alibrandi A, Brancatelli S, Lombardo D, Tripodi G, et al. PIVKA-II is a useful tool for diagnostic characterization of ultrasound-detected liver nodules in cirrhotic patients. *Medicine (Baltimore).* 2017;96(26):e7266
10. Wang BL, Tan QW, Gao XH, Wu J and Guo W. Elevated PIVKA-II is associated with early recurrence and poor prognosis in BCLC 0-A hepatocellular carcinomas. *Asian Pac J Cancer Prev.*

2014;15(16):6673-8

11. Wu J, Xiang Z, Bai L, He L, Tan L, Hu M, et al. Diagnostic value of serum PIVKA-II levels for BCLC early hepatocellular carcinoma and correlation with HBV DNA. *Cancer Biomark*. 2018;23(2):235-42
12. Lim TS, Kim DY, Han KH, Kim HS, Shin SH, Jung KS, et al. Combined use of AFP, PIVKA-II, and AFP-L3 as tumor markers enhances diagnostic accuracy for hepatocellular carcinoma in cirrhotic patients. *Scand J Gastroenterol*. 2016;51(3):344-53
13. Svobodova S, Karlikova M, Topolcan O, Pecen L, Pestova M, Kott O, et al. PIVKA-II as a Potential New Biomarker for Hepatocellular Carcinoma - A Pilot Study. *In Vivo*. 2018;32(6):1551-4
14. Abdel-Aziz MM, Elshal MF, Abass AT, El-Kafrawy S, Ezzat S and Abdel-Wahab M. Comparison of AFP-L3 and p53 Antigen Concentration with Alpha-Fetoprotein as Serum Markers for Hepatocellular Carcinoma. *Clin Lab*. 2016;62(6):1121-9
15. Sauzay C, Petit A, Bourgeois AM, Barbare JC, Chauffert B, Galmiche A, et al. Alpha-fetoprotein (AFP): A multi-purpose marker in hepatocellular carcinoma. *Clin Chim Acta*. 2016;463(39-44)
16. Seo SI, Kim HS, Kim WJ, Shin WG, Kim DJ, Kim KH, et al. Diagnostic value of PIVKA-II and alpha-fetoprotein in hepatitis B virus-associated hepatocellular carcinoma. *World J Gastroenterol*. 2015;21(13):3928-35
17. Durazo FA, Blatt LM, Corey WG, Lin JH, Han S, Saab S, et al. Des-gamma-carboxyprothrombin, alpha-fetoprotein and AFP-L3 in patients with chronic hepatitis, cirrhosis and hepatocellular carcinoma. *J Gastroenterol Hepatol*. 2008;23(10):1541-8
18. Li C, Zhang Z, Zhang P and Liu J. Diagnostic accuracy of des-gamma-carboxy prothrombin versus alpha-fetoprotein for hepatocellular carcinoma: A systematic review. *Hepatol Res*. 2014;44(10):E11-25
19. Marrero JA, Su GL, Wei W, Emick D, Conjeevaram HS, Fontana RJ, et al. Des-gamma carboxyprothrombin can differentiate hepatocellular carcinoma from nonmalignant chronic liver disease in american patients. *Hepatology*. 2003;37(5):1114-21
20. Pote N, Cauchy F, Albuquerque M, Voitot H, Belghiti J, Castera L, et al. Performance of PIVKA-II for early hepatocellular carcinoma diagnosis and prediction of microvascular invasion. *J Hepatol*. 2015;62(4):848-54
21. Inagaki Y, Tang W, Makuuchi M, Hasegawa K, Sugawara Y and Kokudo N. Clinical and molecular insights into the hepatocellular carcinoma tumour marker des-gamma-carboxyprothrombin. *Liver Int*. 2011;31(1):22-35
22. Agopian VG, Harlander-Locke MP, Markovic D, Zarrinpar A, Kaldas FM, Cheng EY, et al. Evaluation of Patients With Hepatocellular Carcinomas That Do Not Produce alpha-Fetoprotein. *JAMA Surg*. 2017;152(1):55-64
23. Carr BI, Guerra V, Giannini EG, Farinati F, Ciccarese F, Rapaccini GL, et al. Significance of platelet and AFP levels and liver function parameters for HCC size and survival. *Int J Biol Markers*. 2014;29(3):e215-23

24. Huang GQ, Zhu GQ, Huang S, You J, Shi KQ, Hu B, et al. Combined AFP-CRUT with microvascular invasion accurately predicts mortality risk in patients with hepatocellular carcinoma following curative liver resection. *Expert Rev Gastroenterol Hepatol.* 2015;9(8):1127-38
25. Kurokawa T, Yamazaki S, Mitsuka Y, Moriguchi M, Sugitani M and Takayama T. Prediction of vascular invasion in hepatocellular carcinoma by next-generation des-r-carboxy prothrombin. *Br J Cancer.* 2016;114(1):53-8

Tables

“Due to technical limitations, table 1 is only available as a download in the Supplemental Files section.”

Figures

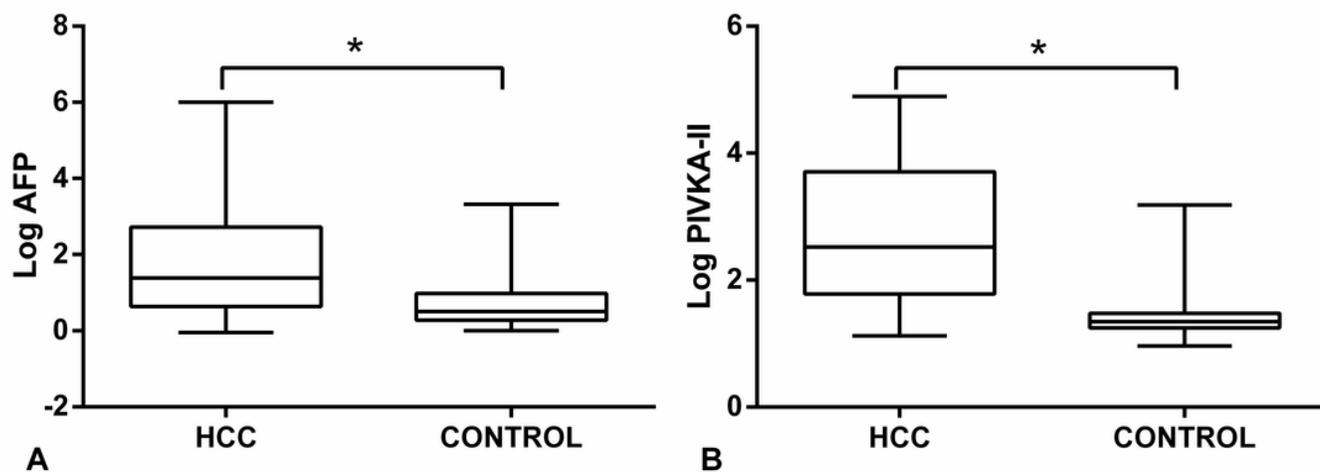


Figure 1

Log transformation was used on the AFP and PIVKA-II values to account for the large range of values for both markers. Log values of serum levels of AFP (A) and PIVKA-II (B) in HCC and control group were compared by box plots. AFP: alpha fetoprotein, PIVKA-II: prothrombin induced by vitamin K absence- II. *P < 0.01.

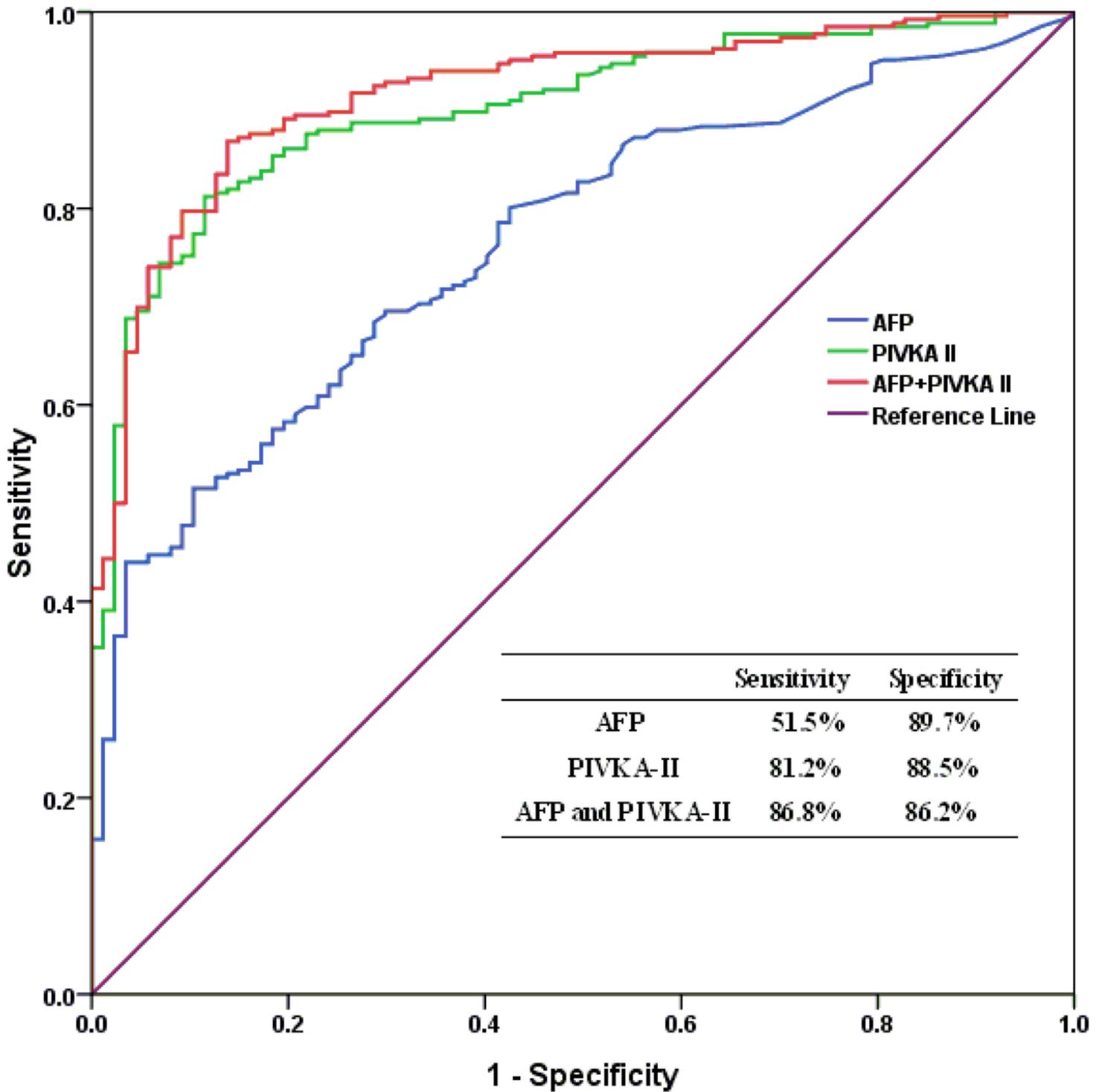


Figure 2

ROC's curve comparing value of AFP, PIVKA-II and the combination of them as diagnostic markers for HCC. Sensibility and specificity of the two kind of markers in detecting HCC was also listed. AFP: alpha fetoprotein, PIVKA-II: protein induced by vitamin K absence-II.

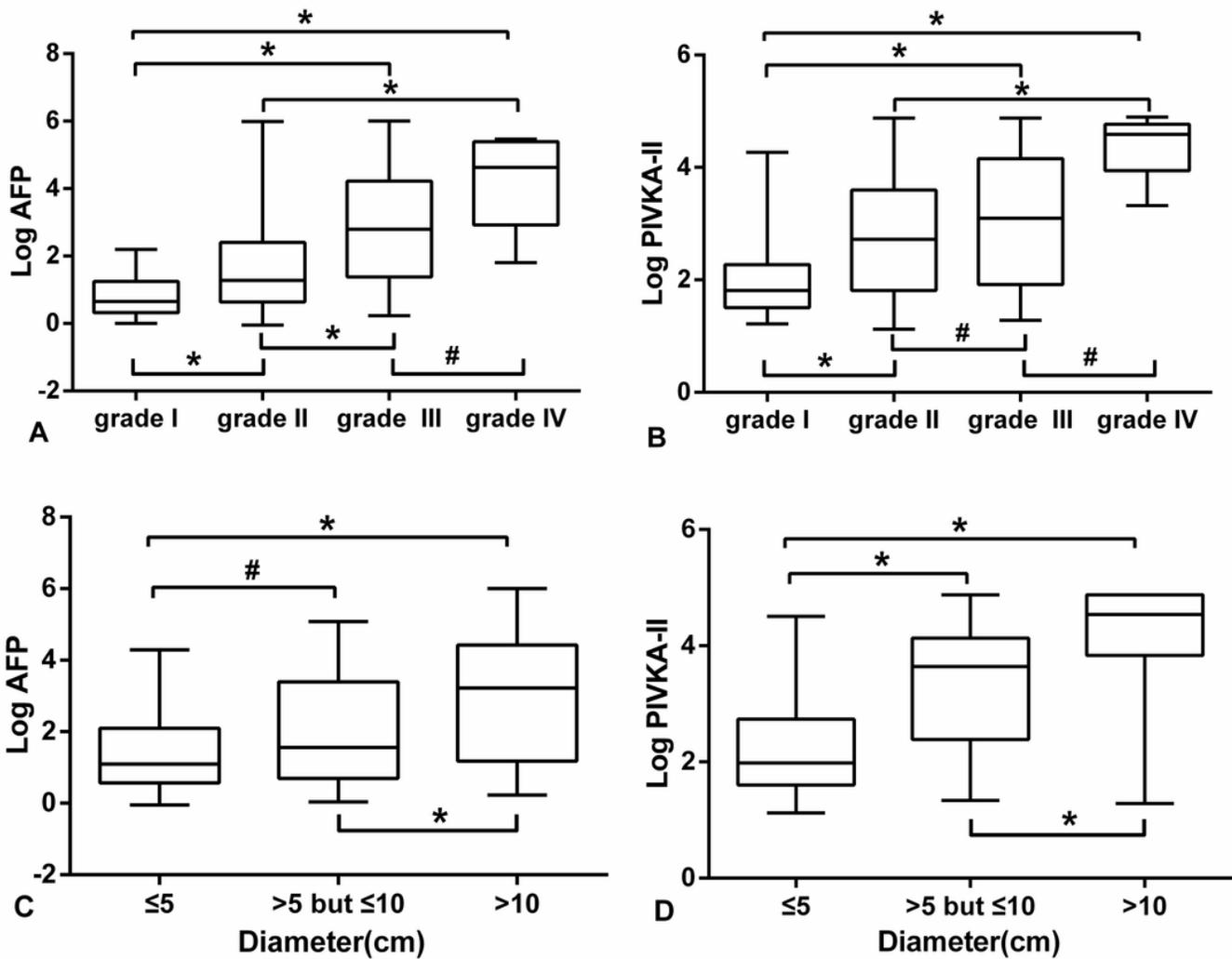


Figure 3

Log values of serum levels of AFP (A and C) and PIVKA-II (B and D) were used to compare difference between their serum levels and tumor differentiation and size (measured by diameter) of HCC. Among them, grade I and grade II were considered as well and moderate differentiation respectively while grade III or IV were regarded as poor differentiation. Based on the HCC diameter, the experimental group were divided into the ≤ 5 cm, >5 but ≤ 10 cm and >10cm group. AFP: alpha fetoprotein, PIVKA-II: protein induced by vitamin K absence-II. *P < 0.05 but #P > 0.05.

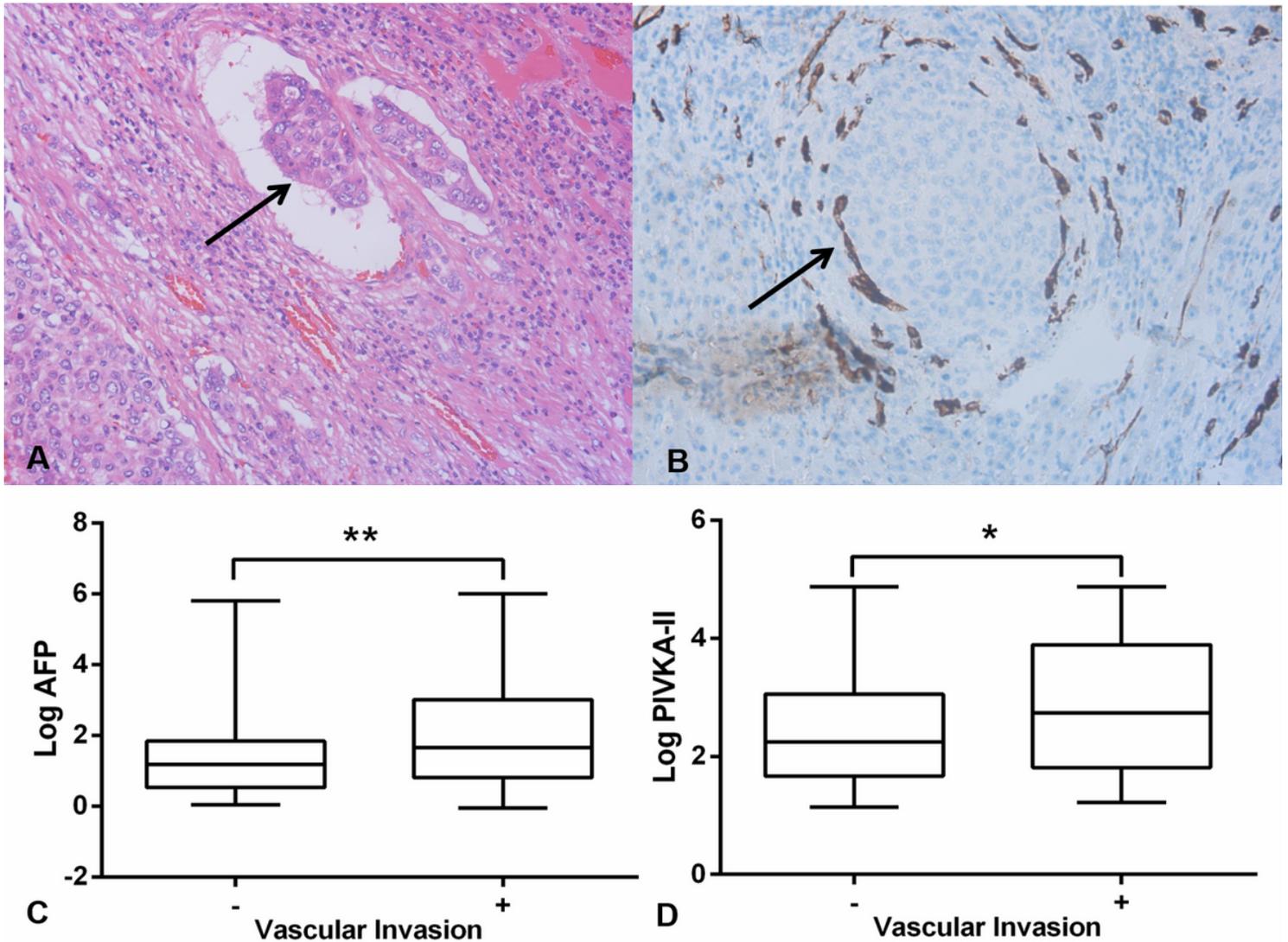


Figure 4

The arrow in Fig.4 (A) showed patent microscopic invasion of a vessel by neoplastic cells (H&E staining; magnification X100). With anti-CD34 immunohistochemistry(B), vascular invasion was confirmed by the presence of small nests of malignant cells inside the vessels (magnification X200). Log values of serum levels of AFP (C) and PIVKA-II (D) in HCC with and without vascular invasion were compared by box plots. AFP: alpha fetoprotein, PIVKA-II: protein induced by vitamin K absence-II. *P < 0.05 and **P < 0.01.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Table1.bmp](#)