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Diagnostic value of combined test of serum AFP and other three indexes in hepatocellular carcinoma related to hepatitis B virus

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Abstract: Objective To explore the clinical diagnostic value of combined serum alpha-fetoprotein (AFP), Protein induced by vitamin K absence or antagonist Ⅱ (PIVKA-Ⅱ), hepatic fibrosis 4 index (FIB-4) and tyrosine urine test (TUT) in hepatitis B virus (HBV)-related hepatocellular carcinoma (HCC). **Methods** Fifty patients with HBV-related HCC, 50 patients with HBV benign liver disease, and 50 health people in the First Hospital of Changsha from January 2021 to December 2022 were selected as the study subjects. The differences between serum AFP, PIVKA-Ⅱ, FIB-4, and the positive rates of TUT among three groups were compared. Area under the receiver operating characteristic curve (AUC) of individual detection and combined detection in the diagnosis of HCC were analyzed, and the clinical application value of combined test in the diagnosis of HBV-related HCC was evaluated. **Results** The levels of serum AFP, PIVKA-Ⅱ, FIB-4 and the positive rate of TUT among three groups were significantly different (P<0.05). The levels of AFP, PIVKA-Ⅱ, FIB-4 and the positive rate of TUT in the HBV-related HCC were higher than those in the healthy control group (P<0.05). The levels of AFP, PIVKA-Ⅱ and the positive rate of TUT in the HBV-related HCC group were higher than those in the HBV benign liver disease group (P<0.05), but there was no significant difference in FIB-4 (P>0.05). The levels of PIVKA-Ⅱ and FIB-4 in HBV benign liver disease group were significantly higher than those in healthy control group (P<0.05), while the levels of serum AFP and the positive rates of TUT were not significantly different (P>0.05). Receiver operating characteristic (ROC) curve analysis showed that the AUC of AFP, PIVKA-Ⅱ, FIB-4 and TUT were 0.812, 0.827, 0.722 and 0.761, respectively. The best cut-off values of serum AFP, PIVKA-Ⅱ and FIB-4 for diagnosing HCC were 21.1 ng/mL, 41.32 ng/mL and 3.27, respectively. In the single test for diagnosis of HBV-related HCC, the TUT had the highest sensitivity of 82.00% and the AFP had the highest specificity of 95.00%. In the combined test for diagnosis of HBV-related HCC, the serum AFP+PIVKA-Ⅱ+FIB-4+TUT had the maximum AUC of 0.935 and the highest sensitivity of 90.00%, while AFP+PIVKA-Ⅱ+FIB-4 had the highest specificity of 97.00%. **Conclusion** The combined test of serum AFP, PIVKA-Ⅱ, FIB-4 and TUT can improve the diagnostic efficacy of HCC and has a significant clinical value in the early diagnosis of HBV-related HCC.

Keywords: HBV-related hepatocellular carcinoma; alpha-fetoprotein; PIVKA-Ⅱ; Fibrosis 4 score; Urine p-hydroxyphenyl alanine test

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Liver hepatocellular carcinoma (HCC) is one of the common malignant tumors in China, with hepatitis B virus (HBV) infection being the leading cause. Approximately 80% of patients are diagnosed at an advanced stage, leading to a short 5-year survival period. Therefore, early diagnosis is crucial for improving patient prognosis [1]. Clinical early diagnosis of HCC mainly relies on the medical history of chronic liver disease, imaging examinations, and serological tests. Due to the atypical nature of early HCC on imaging, CT/MRI or other more sensitive imaging techniques have subjective and economic limitations. Alpha-fetoprotein (AFP) remains one of the most common and valuable diagnostic markers for early HCC screening in clinical practice due to its high specificity, albeit with low sensitivity [2]. Studies have shown that multi-marker combined detection can enhance the value of early HCC diagnosis. Protein induced by vitamin K absence or antagonist-II (PIVKA-II) is a novel serum biomarker that is synthesized and released into the blood in large quantities when hepatocytes undergo malignant transformation. Recent research has found its potential in liver cancer screening [3]. The fibrosis-4 index (FIB-4) is a commonly used non-invasive diagnostic model in clinical practice for liver fibrosis, with certain clinical predictive value for both cirrhosis and HCC [4]. Tyrosine urine test (TUT) detects specific metabolic products in urine that react with special components in reagents to indicate the presence of abnormal metabolic reactions associated with malignant tumors, providing a simple, non-invasive diagnostic method [5]. Currently, there is no related research on the diagnostic value of combined detection of serum AFP, FIB-4, PIVKA-II, and TUT for HBV-related HCC. This study aims to explore the clinical application value of these markers in the diagnosis of HBV-related HCC through combined detection.

1 Material and methods

1.1 General information

A total of 150 patients who visited the Department of Infectious Diseases and Outpatient Clinics at the First Hospital of Changsha from January 2021 to December 2022 were selected. There were 107 males and 43 females, with an average age of (55.85 ± 10.28) years. Among them, there were 50 patients with HBV-related HCC [41 males and 9 females, average age of (56.68 ± 10.41) years]; 50 patients with HBV benign lesions [including 38 patients with HBV-related cirrhosis and 12 patients with chronic hepatitis B, 36 males and 14 females, average age of (56.60 ± 10.00) years]; and 50 healthy individuals undergoing periodic health examinations [30 males and 20 females, average age of (54.28 ± 10.44) years].

Inclusion criteria: All patients were clinically diagnosed with HBV-related HCC, HBV-related cirrhosis, or chronic hepatitis B according to clinical manifestations, laboratory tests, imaging examinations, and/or liver tissue pathology, in accordance with the guidelines [6-7]. **Exclusion criteria:** (1) Pregnant women, lactating women, and women within 3 days before or after menstruation; (2) Those who took sedatives, analgesics, antihypertensive drugs, hormones, central nervous system stimulants, health supplements, vitamin K, vitamin K antagonists, or cephalosporin antibiotics within the past month; (3) Those who consumed high-protein foods, alcohol, engaged in strenuous exercise, experienced trauma, or had active bleeding within the past week; (4) Other hepatitis virus infections, metabolic liver diseases, alcoholic liver disease, and malignant tumors in other parts of the body; (5) Those who have undergone liver cancer surgery, radiation therapy, or chemotherapy, those with incomplete data affecting specimen quality, or those unable to cooperate or refusing to participate. This study was approved by the Medical Ethics Committee of the First Hospital of Changsha (KX-2020063).

1.2 Serological tests

A fasting venous blood sample of 4 mL was collected, processed by centrifugation at 4000 r/min with a radius of 15 cm for 10 minutes, and stored in a -80 ℃ freezer for testing. Hematology was analyzed using the Shenzhen Mindray automatic five-category blood analyzer and corresponding reagents. Liver function tests were conducted using the Cil6200 automatic biochemical analyzer (Abbott Laboratories) and its reagents. AFP was detected using the DXI800 automated chemiluminescence immunoassay system (Beckman Coulter, Inc.) and its reagents (reference value < 20 ng/mL). PIVKA-II was measured using the MQ60 automated chemiluminescence immunoassay system (Beijing Hotgen Biotech Co., Ltd.) and its reagents (reference value <40 mAU/mL). FIB-4 was calculated as follows: FIB-4 = Age (years) × AST (U/L) / [PLT ($\times 10^9$ /L) × ALT (u/L) ×1/2].(AST: aspartate transaminase; ALT : alanine transaminase; PLT: platelet)

1.3 TUT test

The TUT test kit (Hunan Kang Licheng Biotechnology Co., Ltd. Xiangxiezhun 20182400087) was used. At room temperature, 3 mL of fresh clean morning urine was added to an ampoule containing the test reagent, shaken well, and allowed to stand for 3-5 minutes. The color of the precipitate was compared with a standard color chart by another laboratory technician to determine the result. Individuals with color blindness or color weakness were not suitable for conducting this experiment.

1.4 Statistical methods

SPSS 26.0 was used for statistical analysis. The Kolmogorov-Smirnov (K-S) test was initially used to analyze whether the data of each group followed a normal distribution. Normally distributed measurement data were presented as $\bar{x} \pm s$, and independent sample *t*-test was used for comparison between two groups, while one-way analysis of variance was used for comparison among multiple groups. Skewed distribution measurement data were presented as $M\ (P_{25},\ P_{75})$, and comparisons between two groups were conducted using Mann-Whitney U test. Categorical variables were presented as case (%), and chi-square tests were used for comparison. The receiver operating curve (ROC curve) and the area under the curve (AUC) were used to evaluate the diagnostic efficacy of serum AFP, PIVKA-II, FIB-4, and TUT for HCC, the optimal cutoff value, sensitivity and specificity were calculated. A significance level of $P < 0.05$ was considered statistically significant.

2 Results

2.1 Comparison of AFP, PIVKA-II, FIB-4 levels and TUT positivity rate

Significant differences were observed in serum AFP levels, PIVKA-II levels, FIB-4 scores, and TUT positivity rate among the three groups ($P<0.01$). The levels of AFP, PIVKA - Ⅱ, FIB-4, and TUT positivity in HCC group were higher than those in healthy examination group ($P<0.05$). The levels of AFP, PIVKA- Ⅱ, and TUT positivity in the HCC group were higher than those in benign liver disease group ($P<0.05$), while there was no significant difference in FIB-4 ($P>0.05$). The levels of PIVKA- Ⅱ, and FIB-4 in benign liver disease group were higher than those in healthy examination group ($P<0.05$), while there was no significant difference in AFP and TUT positivity ($P>0.05$). **See Table1.**

2.2 Diagnostic value of serum AFP, PIVKA-II, FIB-4, and TUT tests for HCC

ROC curves showed that the AUC of serum AFP, PIVKA-II, FIB-4, and TUT in diagnosing HCC was 0.812, 0.827, 0.722, and 0.761, respectively, with the optimal cutoff values of 21.1 ng/mL for AFP, 41.32 mAU/mL for

PIVKA-II, and 3.27 for FIB-4. Single marker test showed PIVKA-II with the highest AUC (0.827), TUT with the highest sensitivity (82.00%), and AFP with the highest specificity (95.00%). **See Figure 1 and Table 2.**

2.3 Diagnostic value of combined detection of makers

Combined test of AFP+PIVKA-II+FIB-4+TUT showed the highest AUC [0.935, 95%*CI*: 0.875–0.995)] and highest sensitivity (90.00%), while AFP+PIVKA-II+FIB-4 showed the highest specificity (97.00%). **Refer to Table 2 and Figure 2.**

Tab.1 Comparison of AFP, PIVKA-Ⅱ, FIB and TUT in each group [n=50, M (P_{25}, P_{75})]

Group	AFP (ng/mL)	PIVKA-Ⅱ (mAU/mL)	FIB-4	TUT positive [case(%)]
HCC group	29.50(3.92, 99.82)[ab]	69.45(34.98, 262.45)[ab]	5.42(2.19, 8.19)[a]	41(82)[ab]
Benign liver disease group	2.90(2.08, 5.08)	24.00(17.49, 33.92)[a]	3.44(1.99, 7.28)[a]	13(26)
Healthy examination group	2.90(2.00, 3.93)	29.97(26.43, 36.62)	1.66(1.29, 2.06)	11(22)
***H*/χ^2 value**	38.811	47.249	48.032	45.828
***P* value**	<0.001	<0.001	<0.001	<0.001

Note: Compared with healthy examination group, [a]P<0.05; compared with benign liver disease group, [b]P<0.05.

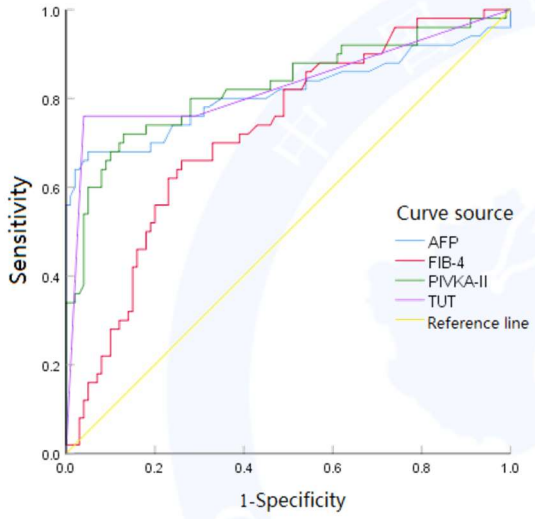

Fig.1 ROC curve of serum AFP, PIVKA-Ⅱ, FIB-4 and TUT for diagnosis of HCC

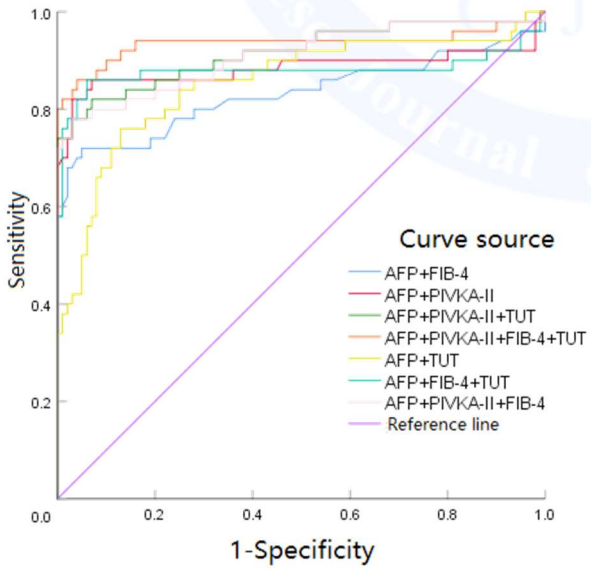

Fig. 2 ROC curve of serum AFP combined with PIVKA-Ⅱ, FIB-4 and TUT for diagnosis of HCC

Tab.2 Comparison of serum AFP, PIVKA-Ⅱ, FIB-4, TUT single and combined detection in the diagnosis of HCC

Indicator	AUC	95%*CI*	Sensitivity(%)	Specificity(%)	*P* value
AFP	0.812	0.724-0.901	68.00	95.00	<0.001
PIVKA-Ⅱ	0.827	0.748-0.905	72.00	87.00	<0.001
FIB-4	0.722	0.637-0.807	66.00	74.00	<0.001
TUT	0.761	0.649-0.873	82.00	76.00	<0.001
AFP+PIVKA-Ⅱ	0.883	0.802-0.964	86.00	93.00	<0.001
AFP+FIB-4	0.832	0.746-0.918	78.00	76.00	<0.001
AFP+TUT	0.853	0.781-0.926	76.00	83.00	<0.001
AFP+PIVKA-Ⅱ+FIB-4	0.910	0.851-0.969	80.00	97.00	<0.001
AFP+PIVKA-Ⅱ+TUT	0.916	0.859-0.974	82.00	93.00	<0.001
AFP+FIB-4+TUT	0.879	0.795-0.963	84.00	89.00	<0.001
AFP+ PIVKA Ⅱ + FIB-4+TUT	0.935	0.875-0.995	90.00	90.00	<0.001

3 Discission

In China, approximately 410,000 new cases of HCC are reported annually, with around 390,000 deaths, accounting for approximately 50% of global cases [8]. Although the overall incidence has shown a decreasing trend in recent years, most patients are diagnosed in the intermediate to advanced stages, resulting in a substantial disease burden [1,9]. Therefore, early detection is crucial. AFP remains one of the most important serum markers for early screening of HCC in clinical practice due to its high specificity, detecting cases up to 6-12 months before clinical symptoms appear. However, its sensitivity is limited, leading to potential missed diagnoses [10]. Studies have reported an AFP miss rate of 40% in early-stage HCC. Additionally, 15% to 30% of advanced HCC patients still have normal AFP levels, particularly those with tumors smaller than 3 cm, which delays early diagnosis and treatment opportunities for many HCC patients [10-11]. Multi-marker combined testing has been studied to enhance early HCC detection.

PIVKA-II, also known as des-γ-carboxy prothrombin, is a newly discovered serum marker in recent years. It is significantly elevated in the serum when hepatocytes undergo malignant transformation and necrosis, inhibiting the γ-carboxylation process of prothrombin precursor, which leads to its abundant synthesis and release into the blood [4]. Studies have found that PIVKA-II can significantly promote the proliferation and invasion of liver cancer cells, and its expression levels increase with tumor staging, serving as an independent risk factor for

assessing the prognosis of HCC patients [12-15]. Monitoring changes in PIVKA-II levels during follow-up of HCC patients is more valuable than AFP in predicting prognosis, with higher sensitivity but slightly lower specificity compared to AFP. Combined use of PIVKA-II and AFP can improve early HCC detection, especially in AFP-negative patients, complementing AFP in clinical HCC screening [16-19]. Most studies have found no significant correlation between PIVKA-II and serum AFP levels [15,17-19], consistent with the findings of this study. Furthermore, this study found that serum PIVKA-II levels in the HBV benign liver disease group were also higher than those in the healthy group, likely due to severe hepatocellular damage in enrolled patients. The maximum AUC for PIVKA-II in single marker testing was 0.827, demonstrating better sensitivity for diagnosing HCC compared to AFP. When combined with AFP, sensitivity increases, with the optimal cutoff value for diagnosing HCC being 41.32 mAU/mL, consistent with previous research results [13-19].

FIB-4 is a commonly used serological method in clinical practice for assessing liver fibrosis diagnosis and staging. It is based on patient age, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and platelet count. FIB-4 data are noted for their simplicity, ease of access, objective quantifiability, and good reproducibility. Importantly, it holds clinical value in assessing the condition and prognosis of patients with cirrhosis and hepatocellular carcinoma (HCC) [3,20]. Current clinical research has focused extensively on the correlation between FIB-4 and HCC. A large retrospective study indicated that FIB-4 can identify chronic HBV-infected individuals at lower risk of HCC [21]. Research suggested that FIB-4 was a predictive factor for HCC in a majority (85%) of untreated chronic hepatitis B surface antigen (HBsAg) carriers [22]. Meta-analyses by He *et al.* [23] also demonstrated that baseline and post-antiviral treatment FIB-4 levels could predict HCC. Furthermore, researchers have found that in patients with cirrhosis, higher Child-Pugh grades correlated with higher FIB-4 levels, corresponding to an increased risk of future HCC development [24]. This study found that FIB-4 levels were higher in both HCC group and benign liver disease group compared to healthy group. There was no significant difference in FIB-4 levels between the HCC group and benign liver disease group, likely due to a predominance of cirrhotic patients in the study, many of whom were in the decompensated stage. ROC curve analysis indicated that combined detection of FIB-4 and AFP improved sensitivity in diagnosing HCC, with an AUC of 0.722 and an optimal cutoff value of 3.27, consistent with the initially proposed cutoff for predicting significant liver fibrosis by Sterling *et al.* [25]. The combined detection of FIB-4 and AFP will increase the AUC for diagnosing HCC to 0.832.

Tumor screening via urine analysis has gained widespread attention due to its convenience and simplicity. Studies have found that levels of tyrosine, a metabolite in urine, are significantly elevated in nearly all patients with malignant tumors. Monitoring changes in the concentration of 3-hydroxyphenylalanine (tyrosine) in urine can indicate the metabolic activity of malignant tumor cells, thereby assessing the risk of malignancy. This method can be used for early detection of primary tumors, screening high-risk populations, evaluating treatment efficacy, monitoring, and prognostic assessment [26-27]. The TUT reagent reacts with tyrosine to produce a brick-red precipitate, indicating abnormal metabolic reactions in the subject's body and thus diagnosing malignant tumors. Domestic scholars have found that TUT positivity rates are significantly higher in digestive tract tumors, breast tumors, urological tumors, and lung cancer, demonstrating good sensitivity for tumor screening [27-30]. This study found that TUT positivity rates were markedly higher in HBV-related HCC compared to benign lesion and healthy control groups, while there was no difference in positivity rates among non-tumor patients. Single-test sensitivity for HCC diagnosis was highest with TUT at 82.00%, though its specificity was relatively low. However, when combined with other indicators, sensitivity for diagnosing HCC significantly increased without a substantial decrease in specificity, thereby reducing missed diagnoses and improving diagnostic accuracy.

In summary, AFP demonstrated the highest specificity when tested individually, while TUT showed the highest sensitivity. AUC values for AFP combined with other tests were higher than those for individual tests, with the combination of AFP, PIVKA-II, FIB-4, and TUT yielding the highest AUC of 0.935. This combination exhibited good sensitivity and specificity, underscoring its practical value in HCC diagnosis and providing guidance for early clinical diagnosis. This study has limitations, including its single-center origin, small sample size, focus primarily on HBV-related HCC, lack of tumor staging, absence of long-term follow-up and outcomes assessment in cirrhotic patients, and absence of dynamic monitoring of indicators. Future research should expand the sample size and monitor changes in various indicators across different tumor stages and fibrosis stages in non-HCC patients to comprehensively evaluate their value in early HCC detection and prognosis.

The authors report no conflict of interest

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·论　著·

血清甲胎蛋白等四项指标联合检测对乙型肝炎病毒相关肝癌的诊断价值

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摘要：目的　检测肝病患者血清甲胎蛋白（AFP）、维生素 K 缺乏或拮抗剂Ⅱ诱导蛋白（PIVKA-Ⅱ）、肝纤维化 4 因子指数（FIB-4）和对羟基苯丙氨酸尿液检测（TUT）水平，探讨其联合检测对乙型肝炎病毒（HBV）相关肝细胞癌（HCC）的临床诊断价值。**方法**　选取 2021 年 1 月至 2022 年 12 月在长沙市第一医院就诊的 HBV 相关 HCC 患者 50 例、HBV 相关良性肝病患者 50 例以及同期健康体检者 50 例作为研究对象，比较 3 组血清 AFP、PIVKA-Ⅱ、FIB-4 及 TUT 阳性率之间的差异，分析各指标单项检测及联合检测诊断 HCC 的受试者工作特征曲线下面积（AUC），评价联合检测在诊断 HBV 相关 HCC 中的临床应用价值。**结果**　HBV 相关 HCC 组、HBV 相关良性肝病组和健康体检组三组对象血清 AFP、PIVKA-Ⅱ、FIB-4 及 TUT 阳性率比较差异均有统计学意义（$P<0.05$）。HBV 相关 HCC 组血清 AFP、PIVKA-Ⅱ、FIB-4 及 TUT 阳性率均高于健康体检组（$P<0.05$）。HBV 相关 HCC 组血清 AFP、PIVKA-Ⅱ及 TUT 阳性率均高于 HBV 相关良性肝病组（$P<0.05$），而 FIB-4 两组间差异无统计学意义（$P>0.05$）。HBV 相关良性肝病组 PIVKA-Ⅱ、FIB-4 明显高于健康体检组（$P<0.05$），而 AFP、TUT 阳性率两组间差异无统计学意义（$P>0.05$）。ROC 曲线分析显示血清 AFP、PIVKA-Ⅱ、FIB-4、TUT 单项检测的 AUC 分别为0.812、0.827、0.722、0.761。血清 AFP、PIVKA-Ⅱ、FIB-4 诊断 HCC 的最佳截断值分别为 21.1 ng/mL、41.32 mAU/mL、3.27。单项检测 TUT 的灵敏度最高（82.00%），AFP 的特异度最高（95.00%）。联合检测以血清 AFP+PIVKA-Ⅱ+FIB-4+TUT 的 AUC 最大（0.935），灵敏度最高（90.00%），以 AFP+PIVKA-Ⅱ+FIB-4 特异度最高（97.00%）。**结论**　血清 AFP、PIVKA-Ⅱ、FIB-4 及 TUT 联合检测可提高对 HCC 的诊断效能，在 HCC 的早期诊断中具有重要的临床价值。

关键词：乙型肝炎病毒相关性肝癌；甲胎蛋白；维生素 K 缺乏或拮抗剂Ⅱ诱导蛋白；肝纤维化 4 因子指数；对羟基苯丙氨酸尿液检测
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Diagnostic value of the combined detection of AFP and other three indexes in hepatocellular carcinoma related to hepatitis B virus

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Abstract: Objective　To explore the clinical diagnostic value of combined serum alpha-fetoprotein (AFP), protein induced by vitamin K absence or antagonist-Ⅱ (PIVKA-Ⅱ), hepatic fibrosis 4 index (FIB-4) and tyrosine urine test (TUT) in hepatitis B virus (HBV)-related hepatocellular carcinoma (HCC). **Methods**　Fifty patients with HBV-related HCC, 50 patients with HBV-related benign liver diseases and 50 healthy people in the First Hospital of Changsha from January 2021 to December 2022 were selected as the study subjects. The differences of serum AFP, PIVKA-Ⅱ,

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FIB-4, and the positive rates of TUT among three groups were compared. The area under the receiver operating characteristic curve (AUC) of individual detection and combined detection in the diagnosis of HCC were analyzed, and the clinical application value of combined detection in the diagnosis of HBV-related HCC was evaluated. **Results** The levels of serum AFP, PIVKA-Ⅱ, FIB-4 and the positive rate of TUT among three groups were significantly different (P<0.05). The levels of AFP, PIVKA-Ⅱ, FIB-4 and the positive rate of TUT in the HBV-related HCC were higher than those in the healthy control group (P<0.05). The levels of AFP, PIVKA-Ⅱ and the positive rate of TUT in the HBV-related HCC group were higher than those in the HBV-related benign liver disease group (P<0.05), but there was no significant difference in FIB-4 (P>0.05). The levels of PIVKA-Ⅱ and FIB-4 in HBV-related benign liver disease group were significantly higher than those in healthy control group (P<0.05), while the levels of serum AFP and the positive rates of TUT were not significantly different (P>0.05). Receiver operating characteristic (ROC) curve analysis showed that the AUC of AFP, PIVKA-Ⅱ, FIB-4 and TUT were 0.812, 0.827, 0.722 and 0.761, respectively. The best cut-off values of serum AFP, PIVKA-Ⅱ and FIB-4 for diagnosing HCC were 21.1 ng/mL, 41.32 mAU/mL and 3.27, respectively. In the single test for diagnosis of HBV-related HCC, the TUT had the highest sensitivity of 82.00% and the AFP had the highest specificity of 95.00%. In the combined test for diagnosis of HBV-related HCC, the serum AFP+PIVKA-Ⅱ+FIB-4+TUT had the maximum AUC of 0.935 and the highest sensitivity of 90.00%, while AFP+PIVKA-Ⅱ+FIB-4 had the highest specificity of 97.00%. **Conclusion** The combined test of serum AFP, PIVKA-Ⅱ, FIB-4 and TUT can improve the diagnostic efficacy of HCC and has a significant clinical value in the early diagnosis of HBV-related HCC.

Keywords: HBV-related hepatocellular carcinoma; Alpha-fetoprotein; Protein induced by vitamin K absence or antagonist-Ⅱ; Hepatic fibrosis 4 index; Tyrosine urine test

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肝细胞癌(hepatocellular carcinoma, HCC)是我国常见的恶性肿瘤之一，乙型肝炎病毒(hepatitis B virus, HBV)感染是我国 HCC 发生的最主要病因,近 80%患者确诊时已处于中晚期,5 年生存期短,因此,早期诊断对于改善患者的预后至关重要[1]。临床上对 HCC 的早期诊断主要依靠慢性肝病的病史、影像学检查和血清学检测等。由于早期 HCC 影像学检查不典型,CT/MRI 或其他更为灵敏的影像学检查,存在主观性及条件和经济限制,甲胎蛋白(alpha-fetoprotein, AFP)仍是目前临床上早期筛查 HCC 的最常见最有价值的诊断指标,其特异性高,但灵敏度较低[2]。研究表明,多指标联合检测可以提升各单项对 HCC 的早期诊断效能。维生素 K 缺乏或拮抗剂Ⅱ诱导蛋白(protein induced by vitamin K absence or antagonist-Ⅱ, PIVKA-Ⅱ)是因维生素 K 缺乏诱生的异常凝血酶原,当肝细胞发生癌变时,大量合成并释放入血,近年来的研究发现可用于肝癌筛查[3]。肝纤维化 4 因子指数(fibrosis-4 index, FIB-4)是临床上常用的非侵入性肝纤维化诊断指标,对肝硬化和 HCC 预测评估均有一定临床指导价值[4]。对羟基苯丙氨酸尿液检测(tyrosine urine test, TUT)利用试剂中的特殊成分与恶性肿瘤患者尿液中升高的异常代谢产物酪氨酸发生特异性反应,根据产物颜色不同判定被检者体内是否存在异常的代谢反应,从而诊断恶性肿瘤,具有简单快捷、无创等特点[5]。目前,尚无血清 AFP、FIB-4、PIVKA-Ⅱ及 TUT 联合检测对 HBV 相关 HCC 诊断价值的研究,本研讨旨在探讨上述指标联合检测在 HBV 相关 HCC 诊断中的临床应用价值。

1　资料与方法

1.1　资料来源　选取 2021 年 1 月至 2022 年 12 月在长沙市第一医院感染科和门诊就诊的 HBV 相关 HCC 患者、HBV 相关良性肝病患者和同期健康体检者各 50 例为研究对象。其中 HBV 相关 HCC 患者(HCC 组)50 例,男性 41 例,女性 9 例,年龄(56.68±10.41)岁;HBV 相关良性肝病患者(良性肝病组)50 例(乙肝肝硬化 38 例,慢性乙型肝炎 12 例),男性 36 例,女性 14 例,年龄(56.60±10.00)岁;同期健康体检组 50 例,男性 30 例,女性 20 例,年龄(54.28±10.44)岁。三组一般资料比较差异无统计学意义(P>0.05)。纳入标准:所有患者经临床表现、实验室检查、影像学检查和/或肝组织病理学检查明确诊断为 HBV 相关 HCC、乙肝肝硬化以及慢性乙型肝炎,诊断符合《原发性肝癌诊疗指南(2022 年版)》、《慢性乙型肝炎防治指南(2022 年版)》[6-7]。排除标准:(1) 孕妇、哺乳期妇女、经期及经期前后 3 天;(2) 检测前 1 个月内服用镇静、止痛、降压、激素、

神经系统兴奋、维生素 K、维生素 K 拮抗剂和头孢类抗菌药物及保健品者;(3) 检测前 1 周摄入高蛋白食物、饮酒、剧烈运动、创伤以及活动性出血者;(4) 其他肝炎病毒感染、代谢性肝病、酒精性肝病以及全身其他部位恶性肿瘤者;(5) 接受过肝癌手术及放化疗、资料不全等影响获取标本质量以及不能配合完成实验或拒绝参与者。本研究经长沙市第一医院医学伦理委员会批准(KX-2020063)。

1.2　血清学检查　采集所有患者的空腹静脉血 4 mL,设置 4 000 r/min、离心半径 15 cm,对血液标本进行 10 min 的离心处理,置于-80 ℃冰箱保存待检。血常规使用深圳迈瑞全自动五分类血液分析仪及配套试剂。肝功能使用美国雅培 Cil6200 全自动生化分析仪及其配套试剂。AFP 检测采用 DXI800 全自动化学发光免疫分析仪及其配套试剂(贝克曼公司)(参考值为<20 ng/mL)。PIVKA-Ⅱ采用 MQ60 全自动化学发光免疫分析仪及其配套试剂(北京热景生物技术)(参考值为<40 mAU/mL)。计算 FIB-4,FIB-4=年龄(岁)×AST(u/L)/[PLT(×10^9/L)×ALT(u/L)1/2](AST:门冬氨酸氨基转移酶;ALT:丙氨酸氨基转移酶;PLT:血小板)。

1.3　TUT 检测　采用湖南康立成生物科技研制提供的 TUT 试剂(湘械注准 20182400087),在室温下,取新鲜清洁晨尿 3 mL 加入装有检测试剂的安瓿瓶中,摇匀后静放 3~5 min,将沉淀物颜色与标准色板对照以判定结果。所配的标准色板,由另外一名检验人员审核结果,色盲或色弱者不适合从事本实验。

1.4　统计学方法　采用 SPSS 26.0 对数据进行统计分析。采用 Kolmogorov-Smirnov(K-S)检验初步分析各组数据是否呈正态性分布。正态分布的计量资料以 $\bar{x}\pm s$ 表示,两组间比较采用独立样本 t 检验,多组间比较采用单因素方差分析。偏态分布的计量资料以 $M(P_{25},P_{75})$ 表示,多组间比较及两两比较采用 Kruskal-Wallis H 检验。计数资料用例(%)表示,行 χ^2 检验。采用受试者工作特征曲线(ROC 曲线),计算曲线下面积(AUC),以约登指数对应的检测值为最佳截断值,分别评价血清 AFP、PIVKA-Ⅱ、FIB-4 及 TUT 诊断 HCC 的效能,并确定诊断的灵敏度和特异度。P<0.05 为差异有统计学意义。

2　结　果

2.1　各组 AFP、PIVKA-Ⅱ、FIB-4 及 TUT 阳性率的比较　三组血清 AFP 水平、PIVKA-Ⅱ水平、FIB-4 及 TUT 阳性率比较差异有统计学意义(P<0.01)。HCC 组血清 AFP 水平、PIVKA-Ⅱ水平、FIB-4 及 TUT 阳性率均高于健康体检组(P<0.05)。HCC 组血清 AFP 水平、PIVKA-Ⅱ水平及 TUT 阳性率均高于良性肝病组(P<0.05),而 FIB-4 比较差异无统计学意义。良性肝病组 PIVKA-Ⅱ水平、FIB-4 明显高于健康对照组(P<0.05),而 AFP 水平、TUT 阳性率比较差异无统计学意义(P>0.05)。见表 1。

2.2　血清 AFP、PIVKA-Ⅱ、FIB、TUT 单项检测对 HCC 的诊断价值　ROC 曲线示,血清 AFP、PIVKA-Ⅱ、FIB-4、TUT 诊断 HCC 的 AUC 分别为 0.812、0.827、0.722、0.761。血清 AFP、PIVKA-Ⅱ、FIB-4 诊断 HCC 的最佳截断值分别为 21.1 ng/mL、41.32 mAU/mL 和 3.27。单项检测以 PIVKA-Ⅱ的 AUC 最大(0.827),TUT 的灵敏度最高(82.00%),AFP 的特异度最高(95.00%)。见图 1、表 2。

2.3　各指标联合检测对 HCC 诊断的价值　联合检测以 AFP+PIVKA-Ⅱ+FIB-4+TUT 的 AUC 最大[0.935(95%*CI*:0.875~0.995)]、灵敏度最高(90.00%);以 AFP+ PIVKA-Ⅱ+FIB-4特异度最高(97.00%)。见表 2、图 2。

表 1　各组 AFP、PIVKA-Ⅱ、FIB 及 TUT 阳性率的比较　[n=50,$M(P_{25},P_{75})$]

Tab. 1　Comparison of AFP, PIVKA-Ⅱ, FIB and TUT in each group　[n=50, $M(P_{25}, P_{75})$]

组别	AFP(ng/mL)	PIVKA-Ⅱ(mAU/mL)	FIB-4	TUT 阳性(例)
HCC 组	29.50(3.92, 99.82)[a]	69.45(34.98, 262.45)[a]	5.42(2.19, 8.19)[a]	41[a]
良性肝病组	2.90(2.08, 5.08)	24.00(17.49, 33.92)[a]	3.44(1.99, 7.28)[a]	13
健康体检组	2.90(2.00, 3.93)	29.97(26.43, 36.62)	1.66(1.29, 2.06)	11
H/χ^2 值	38.811	47.249	48.032	45.828
P 值	<0.001	<0.001	<0.001	<0.001

注:与健康体检组比较,[a]P<0.05。

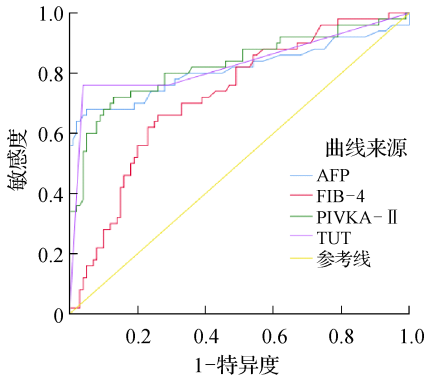

图 1　血清 AFP、PIVKA-Ⅱ、FIB-4、TUT 诊断 HCC 的 ROC 曲线

Fig. 1　ROC curve of serum AFP, PIVKA-Ⅱ, FIB-4 and TUT for diagnosis of HCC

表 2　AFP、PIVKA-Ⅱ、FIB-4、TUT 单项及联合检测对 HCC 诊断价值

Tab. 2　Diagnostic value of single and combined detection of AFP, PIVKA-Ⅱ, FIB-4 and TUT in HCC

指标	AUC	95%*CI*	灵敏度（%）	特异度（%）	*P* 值
AFP	0.812	0.724～0.901	68.00	95.00	<0.001
PIVKA-Ⅱ	0.827	0.748～0.905	72.00	87.00	<0.001
FIB-4	0.722	0.637～0.807	66.00	74.00	<0.001
TUT	0.761	0.649～0.873	82.00	76.00	<0.001
AFP+PIVKA-Ⅱ	0.883	0.802～0.964	86.00	93.00	<0.001
AFP+FIB-4	0.832	0.746～0.918	78.00	76.00	<0.001
AFP+TUT	0.853	0.781～0.926	76.00	83.00	<0.001
AFP+PIVKA-Ⅱ+FIB-4	0.910	0.851～0.969	80.00	97.00	<0.001
AFP+PIVKA-Ⅱ+TUT	0.916	0.859～0.974	82.00	93.00	<0.001
AFP+FIB-4+TUT	0.879	0.795～0.963	84.00	89.00	<0.001
AFP+PIVKA-Ⅱ+FIB-4+TUT	0.935	0.875～0.995	90.00	90.00	<0.001

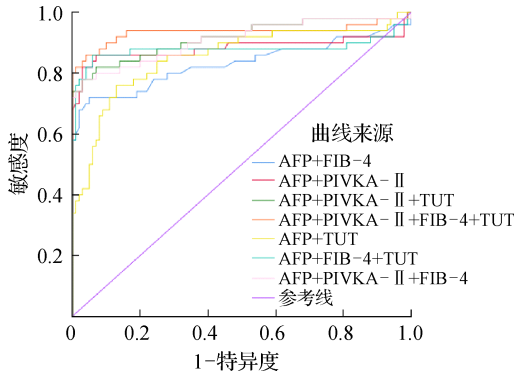

图 2　AFP、PIVKA-Ⅱ、FIB-4、TUT 联合检测诊断 HCC 的 ROC 曲线

Fig. 2　ROC curve of combined detection of AFP, PIVKA-Ⅱ, FIB-4 and TUT for diagnosing HCC

3　讨　论

我国每年新发 HCC 例数约 36.8 万人，死亡例数约 31.7 万人，新发病例数及死亡病例数均占全球 50%左右[8]，近年来整体发病率虽呈下降趋势，但确诊时多数患者已处于中晚期，疾病负担仍较重[1,9]，因此早期发现是关键。AFP 仍是目前临床上早期筛查 HCC 的一种最重要的血清学诊断指标，其特异性高，假阳性率仅为 2%左右，可先于临床症状前 6～12 个月做出诊断，但不足是灵敏度低，容易漏诊[10]。有研究报道，在 HCC 的早期阶段，AFP 漏诊率达 40%。在晚期 HCC 患者中，仍有 15%～30%患者 AFP 处于正常水平，尤其对于肿瘤小于 3 cm 的患者，使许多 HCC 患者失去早期诊断和治疗机会[10-11]。有研究认为多指标联合检测可以提升其对 HCC 早期诊断价值。

PIVKA-Ⅱ是近年来发现的一种新型血清标志物，又称脱-γ-羧基凝血酶原，当肝细胞发生癌变变性、坏死时，凝血酶原前体的谷氨酸残基 γ 羧化过程受阻，最终导致其大量合成并释放入血[4]。有研究发现，PIVKA-Ⅱ能够显著促进肝癌细胞的增殖与侵袭，表达水平随着肿瘤分期的增加而升高，是影响 HCC 患者预后的独立危险因素[12-15]。在 HCC 患者预后的随访中，监测 PIVKA-Ⅱ水平变化较监测 AFP 更有价值，其诊断 HCC 的灵敏度优于 AFP，特异度略低于 AFP，两者联合检测可以提高对 HCC 的早期诊断率，尤其对于 AFP 阴性 HCC 患者，可作为与 AFP 互补的 HCC 筛查手段应用于临床[16-19]。大多数研究认为 PIVKA-Ⅱ与血清 AFP 两者之间无明显相关性[15,17-19]，本研究结果与之类似。同时，本研究还发现，良性肝病组血清 PIVKA-Ⅱ水平也高于健康对照组，分析与入组患者多为肝硬化肝细胞损害较重有关。单项检测时 PIVKA-Ⅱ的 AUC 最大，为 0.827，对 HCC 诊断的灵敏度优于 AFP，与 AFP 联合应用时灵敏度提高，其诊断 HCC 的最佳截断值为 41.32 mAU/mL，与既往研究报道结果基本一致[13-19]。

FIB-4 是临床上常见的血清学肝纤维化诊断和分期的评价方法，其基于患者年龄、ALT、AST 和 PLT，不但数据简单易取、可客观定量、重复性好，并且对肝硬化和 HCC 患者病情及预后评估均有一定临床指导价值[3,20]。目前临床关于 FIB-4 与 HCC 相关性的研究较多。来自台湾的一项大型回顾性研究表明，FIB-4 可以识别 HCC 风险较低的慢性 HBV 感染者[21]，Suh 等[22]的研究表明，FIB-4 是大多数（85%）未治疗的慢性乙型肝炎表面抗原携带者发生 HCC 的预测因子。来自贺超等[23]的 Meta 分析还表明基线和抗病毒治疗后 FIB-4 均可预测 HCC。此外，还有学者发现，肝硬化患者 Child-Puge 分级越高，FIB-4 也越

高,其将来发生 HCC 的风险也相应增加[24]。本研究发现,HCC 组、良性肝病组 FIB-4 均高于健康对照组,而前两组间 FIB-4 无明显差异,分析可能与入组的患者多为肝硬化,且多处于失代偿期有关。ROC 曲线分析显示,FIB-4 诊断 HCC 的 AUC 为 0.722,最佳截断值为 3.27,与 Sterling 等[25]提出的预测肝显著纤维化的截断值基本一致。FIB-4 与 AFP 联合检测将诊断 HCC 的 AUC 提高为 0.832。

通过尿液进行肿瘤筛查,具有取材方便、操作简单等优点。有研究发现,几乎所有恶性肿瘤患者尿液中的酪氨酸含量均明显升高,检测尿液中对羟基苯丙氨酸(酪氨酸)浓度变化,可以判断人体内恶性肿瘤细胞的代谢活跃程度,从而评估恶性肿瘤的风险,可以用于原发肿瘤的早期发现、肿瘤高危人群的筛选、肿瘤治疗效果的评价、监测以及预后评估等[26-27]。TUT 试剂能够与酪氨酸发生螯合反应,并产生砖红色沉淀,根据沉淀物颜色不同即可判定被检者体内是否存在异常的代谢反应,从而诊断恶性肿瘤。国内诸多学者发现,在消化道肿瘤、乳腺肿瘤、泌尿道肿瘤以及肺癌等肿瘤中 TUT 阳性率明显升高,灵敏度较好,适用于肿瘤筛查[27-30]。本研究发现,TUT 阳性率在 HBV 相关 HCC 中明显高于良性肝病组及健康对照组,而在非肿瘤研究对象中其阳性率无差异。单项检测中 TUT 诊断 HCC 的灵敏度最高,达 82.00%,其不足是特异度偏低,联合其他指标后在特异度不下降情况下能显著提高灵敏度,降低漏诊率,提高 HCC 诊断的准确性。

综上所述,对 HBV 相关 HCC 的诊断,单项检测时 AFP 的特异度最高,TUT 的灵敏度最高,各指标与 AFP 联合检测的 AUC 均比各指标单项检测时大,以 AFP、PIVKA-Ⅱ、FIB-4、TUT 联合检测的 AUC 最大(0.935),且灵敏度及特异度均较好,充分说明联合检测在 HCC 诊断中的实用价值,可为临床早期诊断提供参考。本研究尚有局限性,病例来自单中心,样本量较少,HCC 患者仅涉及 HBV 相关,且未涉及肿瘤分期,肝硬化患者未涉及长远随访及病情转归,也未对各项指标进行动态监测,有待进一步增加病例数,通过在不同肿瘤分期 HCC 患者以及不同纤维化分期非 HCC 患者中监测各项指标的变化,更加深入地综合性评估其在 HCC 早期监测和预后中的价值。

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