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血清 PIVKA-Ⅱ和 AFP 联合检测对肝细胞癌的临床诊断价值研究 *

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摘要 目的:探讨血清异常凝血酶原(Protein induced by vitaminK absence or antagonist-Ⅱ,PIVKA-II)和甲胎蛋白(Alpha fetoprotein,AFP)联合检测对肝细胞癌(Hepatocellular carcinoma,HCC)的临床诊断价值。**方法:**选择 169 例 HCC 患者、141 例肝硬化患者、66 例慢性乙肝患者和 100 例健康体检者为研究对象,分别检测和比较其血清 AFP、PIVKA-Ⅱ含量。进一步分析血清 AFP、PIVKA-Ⅱ含量的相关性及其诊断 HCC 的敏感度、特异度、阳性预测值、阴性预测值、准确度,绘制 ROC 曲线,比较血清 AFP、PIVKA-Ⅱ及二者联合检测诊断 HCC 的 ROC。**结果:**(1)HCC 组血清 AFP、PIVKA-Ⅱ水平均显著高于肝硬化组、慢性乙肝组及健康对照组,差异具有统计学意义(*P* 值均*<0.05*)。(2)与参考线下面积相比,AFP、PIVKA-Ⅱ ROC 曲线下面积均显著增加,差异具有统计学意义(*P<0.01*);且 PIVKA-Ⅱ的曲线下面积(AUC=0.790)明显大于 AFP(AUC=0.708)。(3)PIVKA-Ⅱ在对 HCC 诊断的敏感度、特异度、阳性预测值、阴性预测值及准确度方面均优于 AFP;AFP+PIVKA-Ⅱ联合检测敏感度和阴性预测值最高(*P<0.05*),准确度维持在 75 %左右。(4)HCC 患者血清 AFP 和 PIVKA-Ⅱ水平并无显著相关性(*P>0.05*)。**结论:**血清 PIVKA-Ⅱ可作为临床辅助诊断原发性肝癌的重要指标,与 AFP 联合检测可提高原发性肝癌的检出率。

关键词:肝细胞癌;PIVKA-Ⅱ;AFP;诊断价值

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Diagnostic Value of Combined Detection of Serum PIVKA-Ⅱ and AFP in Hepatocellular Carcinoma*

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ABSTRACT Objective: To investigate the diagnostic value of combined detection of serum protein induced by vitaminK absence or antagonist-Ⅱ (PIVKA-Ⅱ) and alpha fetoprotein (AFP) in hepatocellular carcinoma (HCC). **Methods:** A total of 169 HCC patients,141 cirrhosis patients, 66 chronic hepatitis B patients and 100 healthy controls were collected. The levels of serum AFP and PIVKA- II were detected and compared. The correlation of serum AFP and PIVKA- II content and the sensitivity, specificity, positive predictive value, negative predictive value and accuracy for the diagnosis of HCC were further analyzed. ROC curves were plotted and the ROC of serum AFP, PIVKA- II and two combined detection for the diagnosis of HCC was compared. **Results:** (1) AFP level and PIVKA-Ⅱ level in HCC group was significantly higher than that in cirrhosisgroup, chronic hepatitis B group and healthy controls.The differences were statistically significant (*P<0.05*). (2) Compare to the areas underreference line, the areas under ROC curve of AFP and PIVKA-Ⅱ both significant increased and had statistical difference(*P<0.01*); the area under the ROC curve of PIVKA- II(AUC=0.790) is much larger than that of AFP(AUC=0.708). (3)The sensitivity, specificity, positive predictive value, negative predictive value and accuracy of PIVKA- II were superior to that of AFP in the diagnosis of HCC.The sensitivity and negative predictive value was the highest when AFP and PIVKA- II were combined detected (*P<0.05*), and the accuracy was about 75 %. (4) There was no significant correlation between the levels of serum AFP and PIVKA- II(*P>0.05*). **Conclusions:** Serum PIVKA-II can be used as an important indicator of clinical diagnosis of primary hepatocellular carcinoma. Combined detection with AFP can increase the detection rate of primary hepatocellular carcinoma.

Key words: Hepatocellular carcinoma; PIVKA-Ⅱ; AFP; Diagnostic value

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前言

肝细胞癌(Hepatocellular carcinoma,HCC)是全球最为常见的恶性肿瘤之一,发病率位居全球所有恶性肿瘤第六位,而死

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亡率则居第二位[1]。亚太地区是 HCC 的高发区,很大程度上与乙型肝炎病毒(Hepatitis B virus ,HBV)或丙型肝炎病毒(Hepatitis C virus,HCV)的感染有关,在中国主要与 HBV 的慢性感染相关[2-4]。目前,HCC 的临床治疗虽取得了一些进步,但其预后并不尽如人意,五年生存率低于 10%[5]。甲胎蛋白(Alpha fetoprotein,AFP) 作为 HCC 的经典肿瘤标志物已普遍用于全球临床实践,但其临床应用价值存在一定的局限[6]。

近年来,在 HCC 诊断领域出现了一些新的肿瘤标志物,如异常凝血酶原 (Protein induced by vitaminK absence or antagonist-Ⅱ,PIVKA-II)/ 脱 -γ- 羧基凝血酶原 (Des-γ-carboxy prothrombin,DCP)、磷脂酰肌醇蛋白聚糖 3(Glypican,GPC3)、甲胎蛋白异质体(Alpha-fetoproteinvariants,AFP-L3)等[7-9]。其中,PIVKA-II 在日本已经得到了成熟广泛的应用,目前国内也逐渐开始了 PIVKA-II 的应用价值评估研究。本研究旨在探讨血清 PIVKA-Ⅱ和 AFP 联合检测对 HCC 的诊断价值,以期为提高 HCC 的早期诊断率提供参考依据。

1 材料与方法

1.1 研究对象

收集 2017 年 2 月～2018 年 2 月来本院就诊的住院患者及健康体检者 476 例,包括 HCC 患者 169 例,肝硬化患者 141 例,慢性乙肝患者 66 例,健康体检者 100 例。所有 HCC 患者均经病理证实或临床诊断确诊,肝硬化和慢性乙肝患者通过临床特征、超声、CT 等确诊并排除 HCC。HCC 患者均未接受肝癌相关治疗如肝动脉化疗栓塞、射频消融、肝脏切除等。所有研究对象近三个月均未使用维生素 K 和华法林等药物治疗。

1.2 试剂、仪器

AFP 采用瑞士罗氏 E601 型电化学发光分析仪进行检测,罗氏原装试剂,应用伯乐肿瘤标志物复合质控,校准品为罗氏原装。PIVKA-Ⅱ采用日本 Lumipulse G1200 全自动化学发光酶免分析仪检测,试剂由珠海丽珠股份有限公司提供,质控品、校准品均为原装配套试剂。

1.3 方法

1.3.1 标本收集方法 受检者均于入院后第 2 天清晨空腹静脉采血 3～4 mL,3000 r/min 离心 10 min, 分离血清于 -70℃冻存待测。对照组采集清晨空腹静脉血 3～4 mL,同样按上述方法处理。所有标本均无乳糜及溶血。

1.3.2 指标阳性判断标准 AFP＞20 U/mL,PIVKA-Ⅱ＞40 mAU/mL 为阳性,小于上述值为阴性;两项联合检测以一项或两项均阳性,为阳性标准,三项联合检测中两项或三项同时阳性,为阳性标准。

1.3.3 检测方法 AFP 采用电化学发光法检测;PIVKA-Ⅱ采用化学发光酶免法检测。

1.4 统计学分析

应用 SPSS 17.0 统计软件进行统计分析。计量资料因样本浓度均呈偏态分布,故以 $M(P_{25}, P_{75})$表示,组间比较采用非参数 Kruskal-Wallis 检验,两两比较采用 MannWhitney U 检验。计数资料的比较采用卡方检验。绘制受试者工作特征(ROC)曲线,计算曲线下面积(AUC)。对受检指标的检测结果进行直线相关分析。以 $P<0.05$ 为差异有统计学意义。

2 结果

2.1 HCC 组、肝硬化组、慢性乙肝组及健康对照组血清 AFP,PIVKA-Ⅱ水平的比较

HCC 组血清 AFP、PIVKA-Ⅱ水平均显著高于肝硬化组、慢性乙肝组及健康对照组,差异具有统计学意义(P 值均<0.05),见表 1。

表 1 HCC 组、肝硬化组、慢性乙肝组及健康对照组血清 AFP、PIVKA-Ⅱ水平比较

Table 1 Comparison of the level of serum AFP and PIVKA- II between HCC group,cirrhosis group, chronic hepatitis B group and healthy control group

Groups	n	AFP (ng/mL)	PIVKA-Ⅱ (mAU/mL)
HCC group	169	22.12(4.07,516.65)*#△	121.00(28.00,2456.00)*#△
Cirrhosis Group	141	4.02(2.55,10.00)	27.00(20.50,35.00)
Chronic Hepatitis B Group	66	3.61(2.50,12.61)	26.00(19.00,30.00)
Healthy Control Group	100	3.01(1.89,11.33)	24.00(18.60,31.00)

注:与健康对照组相比,*$P<0.05$;与肝硬化相比,#$P<0.05$;与慢性乙肝组相比,△ $P<0.05$。

Note: compared withthe control group,*$P<0.05$; compared withthe cirrhosis group ,#$P<0.05$; compared with the chronic hepatitis B group,△ $P<0.05$.

2.2 HCC 患者血清 AFP 和 PIVKA-Ⅱ水平的相关性分析

对 HCC 患者血清 AFP 和 PIVKA-Ⅱ进行相关性分析,2 项指标的 Pearson 相关系数为 0.081(P=0.370),二者无显著相关性。

2.3 血清 AFP、PIVKA-Ⅱ单项与联合检测对 HCC 诊断的敏感度、特异度、阳性预测值、阴性预测值及准确度

单项检测时,PIVKA-Ⅱ在对 HCC 诊断的敏感度、特异度、阳性预测值、阴性预测值及准确度方面均优于 AFP;AFP+PIVKA-Ⅱ联合检测敏感度和 NPV 最高($P<0.05$),准确度维持在 75%左右,见表 2。

2.4 血清 AFP、PIVKA-Ⅱ用于评价 HCC 诊断价值的 ROC 曲线分析

血清 AFP、PIVKA-Ⅱ用于评价 HCC 诊断价值的 ROC 曲线分析显示:与参考线下面积相比,血清 AFP、PIVKA-Ⅱ诊断 HCC 的 ROC 曲线下面积均显著增加, 差异具有统计学意义($P<0.05$), 且血清 PIVKA-Ⅱ的曲线下面积明显大于血清 AFP,见图 1 和表 2。

表 2 AFP,PIVKA-Ⅱ单项与联合检测对 HCC 诊断的敏感度、特异度、阳性预测值、阴性预测值及准确度(%)

Table 2 The sensitivity, specificity, positive predictive value, negative predictive value and accuracy for the diagnosis of HCC when AFP and PIVKA- II were deteced singly and jointly(%)

Items	Sen	Spe	PPV	NPV	Accuracy
AFP	69.70	84.54	72.41	67.31	68.88
PIVKA-Ⅱ	72.72	87.92	80.92	74.29	76.60
AFP+PIVKA-Ⅱ	82.78	78.26	73.21	77.88	75.80

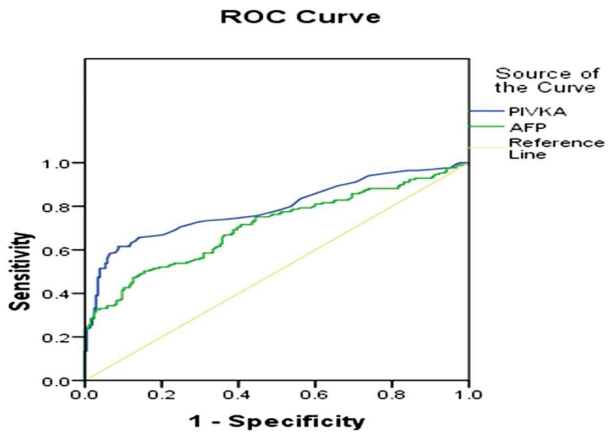

图 1 ROC 曲线评价各指标单独对 HCC 的诊断价值

Fig.1 Evaluation of the diagnostic value of each index to HCC by ROC curve

PIVKA-Ⅱ水平在 HCC 中升高的生物学机制尚未完全明确,其过度表达的机制可能与 γ- 谷氨酰羧化酶活性下降、还原型维生素 K 缺乏及凝血酶原前体的过度表达相关[11-15]。

AFP 作为 HCC 的经典肿瘤标志物已普遍用于临床，但约 40%早期 HCC 患者血清 AFP 水平可能处于正常范围，而在慢性肝炎或肝硬化患者中却可能出现假性增高的现象,其临床应用价值存在一定的局限[6]。本研究结果显示 AFP 对 HCC 诊断的敏感度和特异度分别为 69.70 %和 84.54 %,PPV 和 NPV 分别为 72.41 %和 67.31 %,准确度 68.88 %。结果与既往报道一致[16-20]。可见,单项 AFP 对于 HCC 的诊断其结果并不理想。已有研究报道 PIVKA-Ⅱ可作为 HCC 新的血清标志物[21-23]。本研究结果显示与 AFP 相比,PIVKA-Ⅱ在 HCC 诊断的敏感度、特异度、阳性预测值、阴性预测值及准确度方面均优于 AFP,并且 ROC 曲线下面积也大于 AFP。这些结果表明单项指标诊断 HCC 中,PIVKA-Ⅱ的诊断价值要高于 AFP。

表 3 ROC 曲线参数评价各指标单独对 HCC 的诊断价值

Table 3 Evaluation of the diagnostic value of each index to HCC by ROC curve parameters

Test Result Variables	Area	Std. Error	Asymptotic Sig.	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
AFP	0.708	0.027	.000	0.654	0.762
PIVKA-Ⅱ	0.790	0.024	.000	0.743	0.838

3 讨论

正常无活性的凝血酶原前体其谷氨酸结构区中有 10 个谷氨酸残基，谷氨酸残基在还原型维生素 K 的作用下被羧化为 γ- 羧基谷氨酸,γ- 羧基谷氨酸中的羧基是钙结合的功能区,其与钙离子结合后产生正常的凝血功能。异常凝血酶原是指维生素 K 缺乏或拮抗剂Ⅱ诱导的蛋白质 (Protein induced by VitaminK Absence or Antagonist-Ⅱ,PIVKA-Ⅱ),又称为脱 -γ- 羧基凝血酶原(Des-γ-carboxy prothrombin,DCP),是肝脏合成的无凝血活性的异常凝血酶原。异常凝血酶原的产生是基于恶性肿瘤细胞中凝血酶原前体转译后羧化的缺失，羧化过程的任何一个环节发生障碍均可引起前体蛋白羧化不全,产生异常凝血酶原。

1984 年,Liebman 等[10]首次提出 PIVKA-Ⅱ可能是原发性肝细胞癌的肿瘤标志物。在经活检证实为 HCC 的 76 例患者中,69 例患者的血清异常凝血酶原水平平均在 900 ng/mL,而慢性活动性肝炎患者或正常人群则很低。本研究通过对 HCC 组、肝硬化组、慢性乙肝组及健康对照组血清 AFP,PIVKA-Ⅱ水平进行比较，发现 HCC 患者血清 AFP、PIVKA-Ⅱ水平均显著高于肝硬化组、慢性乙肝组及健康对照组。但目前对于血清

有研究表明 PIVKA-Ⅱ和 AFP 具有相对独立性,血清 PIVKA-Ⅱ水平的升高不受 AFP 的影响，二者联合检测可相互补充,提高 HCC 的检出率[24,25]。本研究中,对 HCC 患者血清 AFP 和 PIVKA-Ⅱ的相关性分析显示 2 项指标的 Pearson 相关系数为 0.081(P=0.370),二者无显著相关性。AFP+PIVKA-Ⅱ的联合检测表明二者联合检测的敏感度为 82.78 %，比 AFP、PIVKA-Ⅱ单独检测时的敏感度分别提高了 13.08 %、10.06 %，差异具有统计学意义($P<0.05$)。

综上所述,PIVKA-Ⅱ是可与 AFP 相媲美的原发性肝癌的标志物,具有良好的辅助诊断价值,与 AFP 联合检测可提高原发性肝癌的检出率。血清 PIVKA-Ⅱ可作为临床辅助诊断原发性肝癌的重要指标。

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