Clinical utility of tumor markers in early diagnosis of hepatocellular carcinoma

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<ABSTRACT>

Early diagnosis of hepatocellular carcinoma (HCC) is very important for a favorable prognosis. Some serologic tests including alpha-fetoprotein (AFP), protein induced by vitamin K absence-II (PIVKA-II), and lens culinaris agglutinin-reactive fraction of AFP (AFP-L3) have been studied as diagnostic markers of HCC; however, there is no consensus on which tumor markers are the most effective in detecting early HCC. In this study, we investigate the clinical utility of tumor markers in the early diagnosis of HCC. A total of 425 patients with liver cirrhosis (LC) (n =196) or HCC (n = 229) were studied from January 2012 to February 2013. Patients with LC had a mean age of 55.8 years and 58.7% were male, whereas the mean age of patients with HCC was 60.0 years and 76.0% were male. We analyzed the expression of tumor markers AFP, PIVKA-II, and AFP-L3 in these patients. All tumor markers were significantly elevated in HCC patients compared with LC patients (p <0.001). The area under the receiver operating characteristic curves (AUROC) of AFP, PIVKA-II, and AFP-L3 for distinguishing HCC from LC was 0.679 (95% confidence interval [CI], 0.626-0.732, p <0.001), 0.812 (95% CI, 0.770-0.854, p <0.001), and 0.690 (95% CI, 0.638-0.742, p <0.001), respectively. Moreover, PIVKA-II (AUROC = 0.705, 95% CI, 0.621-0.789, p <0.001) was superior to AFP (AUROC = 0.623, 95% CI, 0.527-0.719, p = 0.019) and AFP-

L3 (AUROC = 0.561, 95% CI, 0.453-0.668, *p* = 0.245) for diagnosis of early HCC, defined as a single tumor less than 3 cm in size. The low sensitivity (25.6%) of PIVKA-II (cut-off 40 mAU/ml) can be overcome by combining it with AFP (48.7%). Furthermore, with combined AFP (cut-off 20 ng/ml), PIVKA-II (cut-off 40 mAU/ml), and AFP-L3 (cut-off 10%), the sensitivity was enhanced to 56.4%. In patients with AFP <20 ng/ml, the AUROC for PIVKA-II (0.743, 95% CI, 0.678-0.807; p = <0.001) was superior to that of AFP-L3 (0.576, 95% CI, 0.500-0.653; p = 0.052). AFP-L3 was able to differentiate HCC patients from AFP-false negative patients in the logistic regression analysis (odds ratio 1.076, 95% CI, 1.037-1.116, p = < 0.001). All tumor marker levels including AFP, PIVKA-II, and AFP-L3 correlated with the size and stage of HCC with statistical significance. In conclusion, combined AFP and PIVKA-II can used for good screening tool of early HCC. Furthermore, AFP-L3 may have an additional role to differentiate between true HCC in AFP false-positive patients.

Key words: Hepatocellular carcinoma, AFP, PIVKA-II, AFP-L3, Tumor marker

Clinical utility of tumor markers in early diagnosis of hepatocellular carcinoma

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(Directed by Professor Do Young Kim)

I. INTRODUCTION

Hepatocellular carcinoma (HCC) is the sixth most common cancer in the world and the third most common cause of cancer-related death.[1] Because the prognosis of HCC is usually determined by stage at the time of diagnosis, early diagnosis of HCC is very important.

Serum alpha-fetoprotein (AFP) is the most representative tumor marker for HCC, and is used in the diagnosis and surveillance of HCC in combination with abdominal ultrasonography. A cutoff value of 20 ng/ml AFP is most commonly used.[2] Furthermore, patients with serum AFP greater than 400 ng/ml were reported to have greater tumor size, portal vein thrombosis, diffuse or massive types, and a lower survival rate.[3,4] However, because AFP level can also be increased in chronic hepatitis or liver cirrhosis without HCC[5,6] there is a need for markers with higher specificity.

Protein induced by vitamin K absence-II (PIVKA-II) is produced as the result of a defect in posttranslational carboxylation of the prothrombin precursor in cancer cells and shows abnormally increased expression in HCC patients. PIVKA-II has therefore been considered as an additional marker for the diagnosis of HCC, and some previous

studies have reported that PIVKA-II is more accurate than AFP in the diagnosis of HCC.[7-10]

Lens culinaris agglutinin-reactive fraction of AFP (AFP-L3) is an isoform of AFP that reflects changes in the carbohydrate chain and is also a specific marker for HCC.[11] Furthermore, AFP-L3 has been shown to be useful in early diagnosis,[11,12] prognosis after treatment,[13,14] or prediction of malignant potential in HCC.[15,16] Previous studies suggested 10% as the cutoff value of AFP-L3.[2] The analytical sensitivity of AFP-L3 is affected by total AFP level because AFP-L3 is described as a percentage of total AFP level. Until recently the percentage of AFP-L3 has been analyzed by liquid-phase binding assay (LBA); however, this method is affected by the total AFP level and its clinical utility is low in patients with low total AFP.[17,18] A highly sensitive AFP-L3 assay using micro-total analysis systems (μTAS) was suggested to overcome the limited value of the conventional method. This new method can measure AFP-L3 accurately in patients with very low AFP level,[19] and a recent study indicated that AFP-L3 measured by this highly sensitive technique was a more useful marker for diagnosis and predicting prognosis in HCC patients.[20]

Although AFP, PIVKA-II, and AFP-L3 are all recognized tumor markers for HCC, there is no consensus on which tumor marker is the most useful indicator of early diagnosis. Therefore, in this study we determined which tumor marker is the most useful for early diagnosis in HCC.

II. MATERIALS AND METHODS

1. Patients

Between January 2012 and February 2013, 229 consecutive patients who were diagnosed with HCC for the first time at Severance Hospital, Yonsei University College of Medicine, Seoul, Korea were enrolled in this study. HCC was diagnosed histologically or radiologically according to the guidelines of the American Association for the Study of Liver Disease (AASLD) or the European Association for Study of the Liver (EASL). The clinical diagnosis was made based on typical radiologic findings in dynamic computed tomography (CT), dynamic magnetic resonance imaging (MRI), or hepatic angiography in combination with increased AFP greater than 200 ng/ml in patients with chronic hepatitis or liver cirrhosis. If the AFP level was less than 200 ng/ml, at least two imaging findings should be consistent with HCC.[21-23] The exclusion criteria were as follows: age younger than 18 years; previously diagnosed HCC; previous history of liver transplantation or liver resection; any cancers other than HCC. Control samples were obtained from 196 consecutive cirrhotic patients without HCC between January 2012 and February 2013. Liver cirrhosis was defined by histology, clinical, biochemical, or imaging findings. This study protocol was approved by the institutional ethics review board and was in compliance with the Declaration of Helsinki.

2. Measurements of tumor markers

AFP, PIVKA-II, and AFP-L3 were measured in serum samples obtained from LC and HCC patients. For HCC patients, serum samples were collected at the time of HCC diagnosis before treatment. Measurements of AFP, PIVKA-II, and AFP-L3 were performed using the μTAS assay (Wako Pure Chemical Industries, Ltd, Osaka, Japan).[19]

3. HCC staging system

The American Joint Committee on Cancer (AJCC) TNM (tumor-node-metastasis) and Barcelona Clinic Liver Cancer (BCLC) systems were used for HCC staging.

4. Statistical analyses

Continuous variables were compared with *t*-tests or Mann-Whitney *U* tests, and categorical variables were compared using the chi-squared or Fisher's exact tests. Receiver operating characteristic (ROC) curves were constructed and the areas under the ROC curves (AUROC) were calculated. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated. The relationship between AFP-L3 and HCC diagnosis probability in patients with an AFP level greater than 20ng/ml were analyzed with a logistic regression model. A

probability (p) value of 0.05 was chosen for statistical significance. Statistical analyses were performed using SPSS version 18.0 (SPSS Inc., Chicago, IL, USA).

III. Results

1. Baseline characteristics of patients

A total of 425 patients with LC (n = 196) or HCC (n = 229) were enrolled. The patient's baseline characteristics are described in Table 1. Patients with LC had a mean age of 55.8 years and 58.7% were male. Patients with HCC had a mean age of 60.0 years and 76.0% were male. The 229 patients with HCC included 75 (32.3%) in stage I, 53 (23.1%) in stage II, 55 (24.0%) in stage III, and 45 (19.7%) in stage IV. Serum levels of AFP, PIVKA-II, and AFP-L3 were all significantly elevated in HCC patients compared with LC patients. Comparison of tumor markers in patients with LC and HCC is shown in Figure 1.

2. Diagnostic accuracy of tumor markers to detect overall HCC

The ROC curves of tumor markers for distinguishing HCC from LC are shown in Figure 2. AUROC for AFP, PIVKA-II, and AFP-L3 was 0.679 (95% CI, 0.626-0.732, p <0.001), 0.812 (95% CI, 0.770-0.854, p <0.001), and 0.690 (95% CI, 0.638-0.742, p <0.001), respectively. The sensitivity, specificity, NNP, and PPV for different cut-off

values are presented in Table 2. The three tumor markers combined resulted in an enhanced sensitivity of 80.8%.

Table 1. Baseline characteristics of the study population.

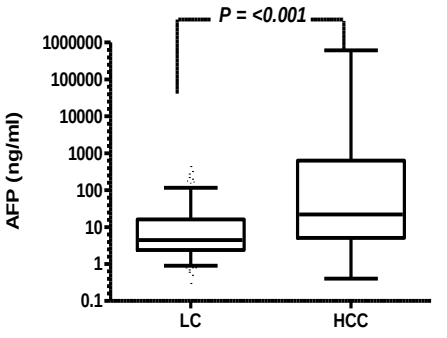
Variables	Patients with LC (n = 196)	Patients with HCC (n = 229)	*p*
Male gender (%)	115 (58.7%)	174 (76.0%)	
Age (years)	55.8 ± 10.7	60.0 ± 10.9	<0.001
Etiology			
HBV/HCV/	122 (62.2%) /33 (16.8%)	174 (76.0%) /23 (10.0%)	
Alcohol/Others, n (%)	/23 (11.7%) /18 (9.2%)	/12 (5.2%) /21 (9.2%)	
Child-Pugh class:	171 (87.2%) /17 (8.7%)	184 (80.3%) /41 (17.9%)	
A/B/C, n (%)	/8 (4.0%)	/5 (2.2%)	
Hb (g/dl)	13.6 (12.3-15.0)	13.3 (11.8-14.6)	0.176
Platelet count ($\times 10^3/mm^3$)	116.5 (80.5-149.7)	159.5 (100.8-205.3)	<0.001
AST (IU/L)	37.5 (28.0-56.0)	43.5 (29.0-81.0)	0.005
ALT (IU/L)	31.0 (20.0-44.8)	32.0 (20.8-54.3)	0.134
Albumin (g/dl)	4.2 (3.7-4.5)	3.8 (3.2-4.2)	<0.001
Total bilirubin (mg/dl)	0.9 (0.7-1.2)	0.9 (0.6-1.3)	0.327
PT INR	1.0 (1.0-1.1)	1.0 (1.0-1.1)	0.047
AFP (ng/ml)	4.5 (2.4-16.2)	22.0 (5.1-628.3)	<0.001
PIVKA-II (mAU/ml)	19.0 (14.3-27.0)	85.5 (24.0-2000.0)	<0.001
AFP-L3 (%)	2.1 (0.0-6.9)	8.7 (1.2-32.9)	<0.001
Vessel invasion (%)	NA	62 (27.1%)	
Portal vein thrombosis (%)	NA	34 (14.8%)	
Distant metastasis (%)	NA	20 (8.7%)	

Tumor number, ≥2 (%)	NA	90 (39.3%)
Tumor size, ≥3 cm (%)	NA	147 (64.2%)
TNM staging:	NA	74 (32.3%) /53 (23.1%)
I/II/III/IV, n (%)		/55 (24.0%) /45 (19.7%)
BCLC staging:	NA	88 (38.4%) /43 (18.8%)
A/B/C/D, n (%)		/92 (40.2%) /7 (3.1%)

Data are expressed as the number (percentage), mean±SD, and median (interquartile range).

HBV, Hepatitis B virus; HCV, Hepatitis C virus; AST, Aspartate aminotransferase; ALT, Alanine aminotransferase; PT INR, Prothrombin time international normalized ratio; AFP, Alpha-fetoprotein; PIVKA-II, Protein induced by vitamin K absence-II; AFP-L3, Lens culinaris agglutinin-reactive fraction of alpha-fetoprotein; TNM, Tumor-node-metastasis; BCLC, Barcelona Clinic Liver Cancer

(a)



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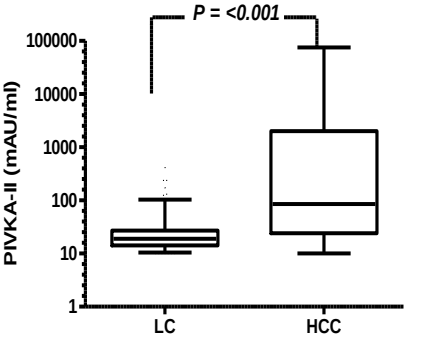

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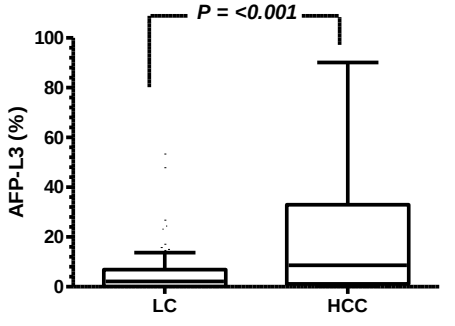

Figure 1. Comparison of serum AFP, PIVKA-II, and AFP-L3 values in patients with HCC and LC. The values of AFP (a), PIVKA-II (b), and AFP-L3 (c) are shown as rectangles, in which the line represents the median.

LC, Liver cirrhosis; HCC, Hepatocellular carcinoma; AFP, Alpha-fetoprotein; PIVKA-II, Protein induced by vitamin K absence-II; AFP-L3, Lens culinaris agglutinin-reactive fraction of alpha-fetoprotein

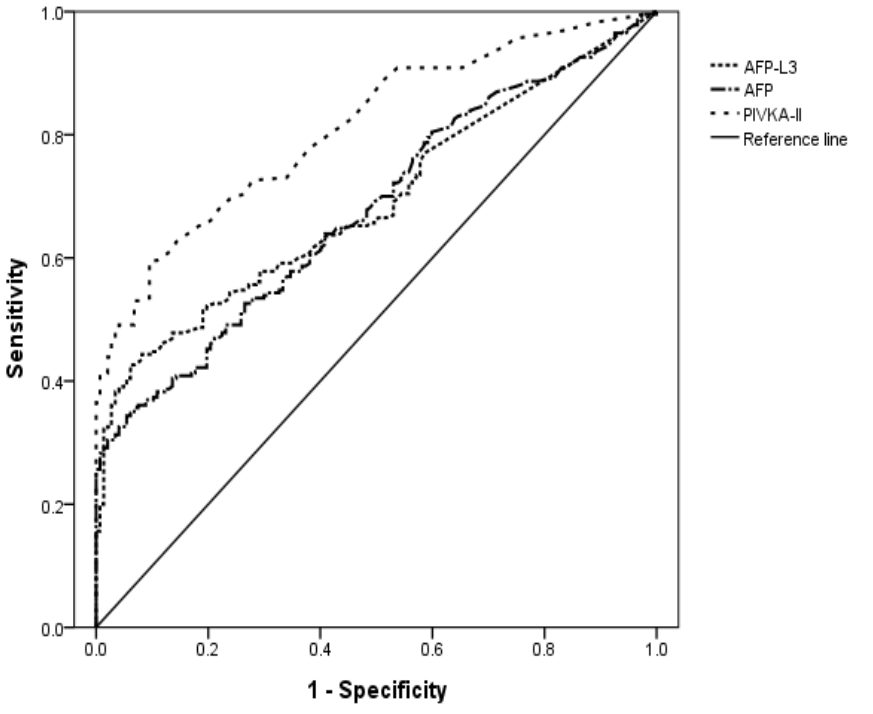

Figure 2. ROC curves of AFP, PIVKA-II, and AFP-L3 for distinguishing HCC from LC. AUROC was 0.679 (95% CI, 0.626-0.732, p <0.001) for AFP, 0.812 (95% CI, 0.770-0.854, p <0.001) for PIVKA-II, and 0.690 (95% CI, 0.638-0.742, p <0.001) for AFP-L3.

ROC, Receiver operating characteristic; LC, Liver cirrhosis; HCC, Hepatocellular carcinoma; AUROC, The area under the receiver operating characteristic curves; AFP, Alpha-fetoprotein; PIVKA-II, Protein induced by vitamin K absence-II; AFP-L3, Lens culinaris agglutinin-reactive fraction of alpha-fetoprotein

Table 2. Sensitivity, specificity, PPV, and NPV for different cut-off values of tumor markers in distinguishing overall HCC from LC

Variables	Cut-off value	Sensitivity	Specificity	NPV	PPV
AFP	20 ng/ml	52.6%	78.6%	58.6%	74.2%
	200 ng/ml	30.4%	98.0%	54.5%	94.6%
PIVKA-II	40 mAU/ml	59.6%	91.9%	66.0%	89.5%
	100 mAU/ml	49.1%	96.0%	61.6%	93.3%
AFP-L3	5%	60.8%	65.3%	58.7%	67.3%
	10%	46.1%	89.7%	58.5%	84.1%
AFP +PIVKA-II	20ng/ml for AFP or 40mAU/ml for PIVKA-II	76.4%	71.9%	72.3%	76.0%
PIVKA-II+AFP-L3	40mAU/ml for PIVKA-II or 10% for AFP-L3	71.3%	85.2%	71.7%	85.0%
AFP + PIVKA –II + AFP-L3	20ng/ml for AFP, 40mAU/ml for PIVKA-II, or 10% for AFP-L3	80.8%	67.3%	75.0%	74.3%

PPV, Positive predictive value; NPV, Negative predictive value; AFP, Alpha-fetoprotein; PIVKA-II, Protein induced by vitamin K absence-II; AFP-L3, Lens culinaris agglutinin-reactive fraction of alpha-fetoprotein

3. Diagnostic accuracy of tumor markers to distinguish early HCC

To evaluate the diagnostic accuracy of tumor markers in distinguishing early HCC from LC, we analyzed ROC curves as shown in Figure 3. Early HCC was defined as a single tumor less than 3 cm in size. The number of patients with the early HCC was 39. The AUROC that diagnosed patients with early HCC was 0.623 (95% CI, 0.527-0.719, p = 0.019) for AFP, 0.705 (95% CI, 0.621-0.789, p = 0.001) for PIVKA-II, and 0.561 (95% CI, 0.453-0.668, p = 0.245) for AFP-L3. The Sensitivity, specificity, PPV, and NPV for different cut-off values of tumor markers in distinguishing early HCC from LC is described in table 3. The low sensitivity (25.6%) of PIVKA-II (cut-off 40 mAU/ml) can be overcome by combining it with AFP (48.7%). Furthermore, with combination of three tumor markers, the sensitivity was enhanced to 56.4%.

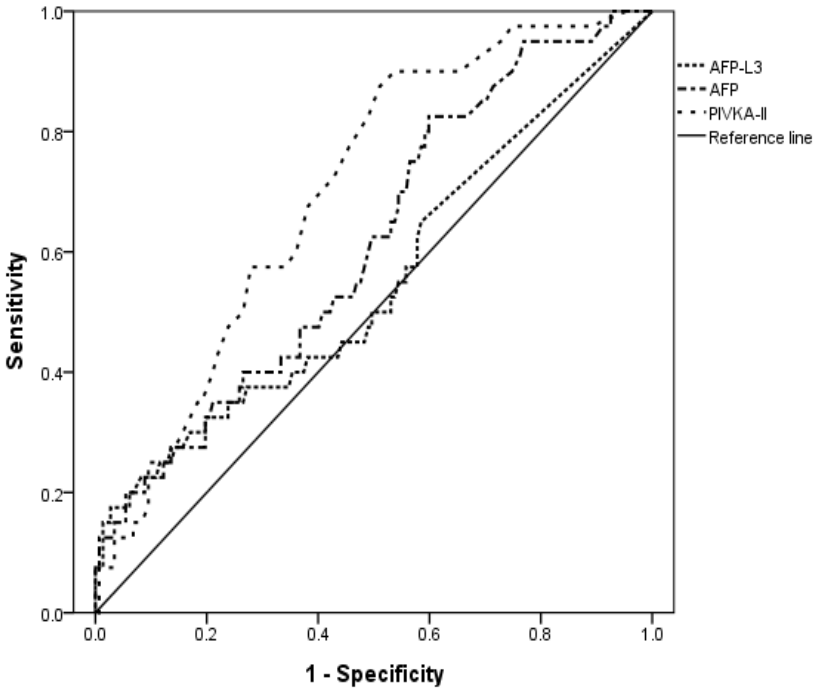

Figure 3. ROC curves of AFP, PIVKA-II, and AFP-L3 for distinguishing early HCC (single tumor less than 3 cm in size) from LC. AUROC was 0.623 (95% CI, 0.527-0.719, $p = 0.019$) for AFP, 0.705 (95% CI, 0.621-0.789, $p < 0.001$) for PIVKA-II, and 0.561 (95% CI, 0.453-0.668, $p = 0.245$) for AFP-L3.

ROC, Receiver operating characteristic; LC, Liver cirrhosis; HCC, Hepatocellular carcinoma; AFP, Alpha-fetoprotein; PIVKA-II, Protein induced by vitamin K absence-II; AFP-L3, Lens culinaris agglutinin-reactive fraction of AFP; AUROC, The area under the receiver operating characteristic curves

Table 3. Sensitivity, specificity, PPV, and NPV for different cut-off values of tumor markers in distinguishing early HCC (single tumor less than 3 cm in size) from LC

Variables	Cut-off value	Sensitivity	Specificity	NPV	PPV
AFP	20 ng/ml	41.0%	78.6%	87.0%	27.6%
	200 ng/ml	12.8%	98.0%	85.0%	55.6%
PIVKA-II	40 mAU/ml	25.6%	91.8%	86.1%	38.5%
	100 mAU/ml	12.8%	95.9%	84.7%	38.4%
AFP-L3	5%	43.6%	65.3%	85.3%	20.0%
	10%	25.6%	85.8%	85.8%	33.3%
AFP +PIVKA-II	20ng/ml for AFP or 40mAU/ml for PIVKA-II	48.7%	71.9%	87.6%	25.7%
PIVKA-II+AFP-L3	40mAU/ml for PIVKA-II or 10% for AFP-L3	41.0%	85.2%	87.9%	35.6%
AFP + PIVKA–II + AFP-L3	20ng/ml for AFP, 40mAU/ml for PIVKA-II, or 10% for AFP-L3	56.4%	67.3%	88.6%	25.9%

PPV, Positive predictive value; NPV, Negative predictive value; AFP, Alpha-fetoprotein; PIVKA-II, Protein induced by vitamin K absence-II; AFP-L3, Lens culinaris agglutinin-reactive fraction of AFP

4. Diagnostic utility of PIVKA-II and AFP-L3 in patients with AFP <20 ng/ml

The ROC curves of PIVKA-II and AFP-L3 in patients with AFP <20 ng/ml is given in Figure 4. The number of LC and HCC patients with AFP <20 ng/ml was 197 and 66, respectively. The diagnostic accuracy of PIVKA-II was superior to that of AFP-L3 in patients with AFP <20 ng/ml. The AUROC of PIVKA and AFP-L3 was 0.743 (95% CI, 0.678-0.807, p <0.001) and 0.576 (95% CI, 0.500-0.653, p = 0.052), respectively. The sensitivity, specificity, PPV, and NPV for PIVKA-II and AFP-L3 in patients with an AFP <20 ng/ml is presented in Table 4. The sensitivity of PIVKA-II, with a cut-off value of 40 mAU/ml, was 48.6%, and PIVKA-II and AFP-L3 combined showed an enhanced sensitivity of up to 57.8%.

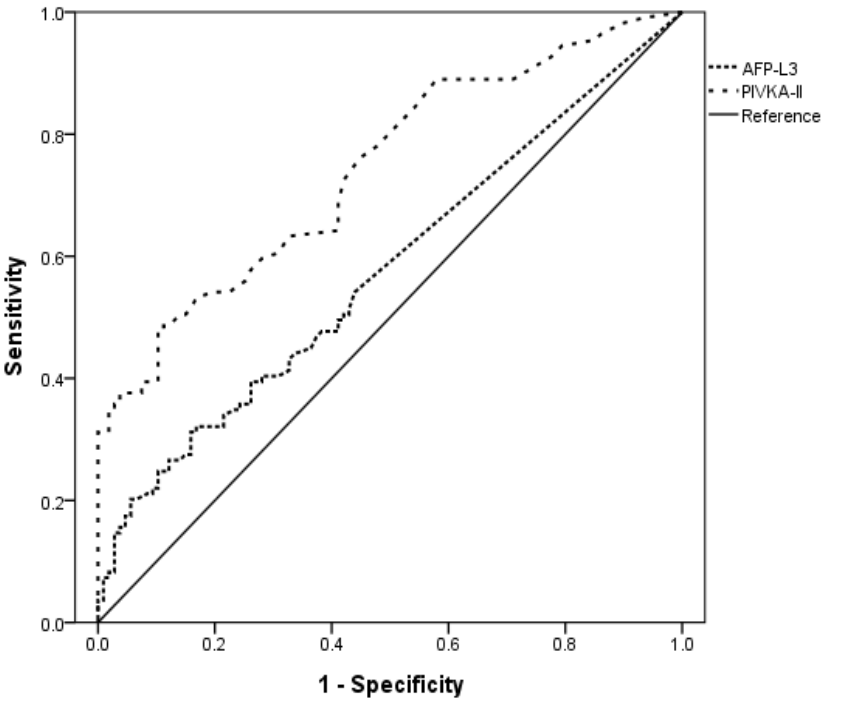

Figure 4. ROC curves of PIVKA-II and AFP-L3 for distinguishing HCC from LC in patients with AFP <20 ng/ml. AUROC was 0.743 (95% CI, 0.678-0.807, p <0.001) for PIVKA-II and 0.576 (95% CI, 0.500-0.653, p = 0.052) for AFP-L3.

ROC, Receiver operating characteristic; LC, Liver cirrhosis; HCC, Hepatocellular carcinoma; AFP, Alpha-fetoprotein; AUROC, The area under the receiver operating characteristic curves; PIVKA-II, Protein induced by vitamin K absence-II; AFP-L3, Lens culinaris agglutinin-reactive fraction of alpha-fetoprotein

Table 4. Sensitivity, specificity, PPV, and NPV for PIVKA-II and AFP-L3 in patients with AFP < 20ng/ml

Variables	Cut-off value	Sensitivity	Specificity	NPV	PPV
PIVKA-II	40 mAU/ml	48.6%	91.6%	71.6%	80.3%
	100 mAU/ml	37.6%	96.1%	68.5%	87.2%
AFP-L3	5%	42.2%	70.1%	63.2%	50.0%
	10%	24.7%	91.5%	63.0%	67.5%
PIVKA-II+AFP-L3	40mAU/ml for PIVKA-II or 10% for AFP-L3	57.8%	85.7%	74.2%	74.1%

PPV, Positive predictive value; NPV, Negative predictive value; AFP, Alpha-fetoprotein; PIVKA-II, Protein induced by vitamin K absence-II; AFP-L3, Lens culinaris agglutinin-reactive fraction of alpha-fetoprotein

5. HCC diagnosis probability in patients with AFP ≥ 20ng/ml

We present HCC diagnostic probability in patients with AFP ≥ 20ng/ml in Figure 5. HCC diagnostic probability of AFP-L3 was calculated with univariate logistic regression analysis (Table 5). The number of LC and HCC patients with AFP ≥ 20ng/ml was 42 and 121, respectively. Among the LC patients with a false-positive AFP (AFP ≥ 20ng/ml), 35 (83.3%) LC patients had an AFP-L3 less than 10%. HCC

diagnosis probability and AFP-L3 level were correlated with statistical significance ($p < 0.001$).

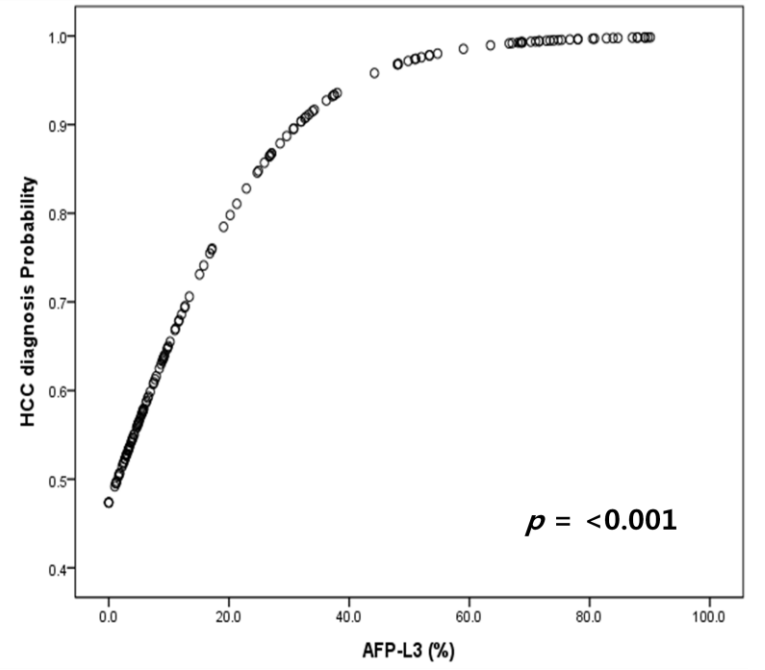

Figure 5. The graph of HCC diagnosis probability as AFP-L3 level in patients with AFP ≥ 20ng/ml

HCC, Hepatocellular carcinoma; AFP, Alpha-fetoprotein; AFP-L3, Lens culinaris agglutinin-reactive fraction of alpha-fetoprotein

Table 5. Logistic regression analysis for HCC diagnosis probability in patients with AFP ≥ 20ng/ml

	OR	95% CI	*p*
AFP-L3 (%)	1.076	1.037 - 1.116	<0.001

HCC, Hepatocellular carcinoma; AFP-L3, Lens culinaris agglutinin-reactive fraction of alpha-fetoprotein; OR, Odds ratio; CI, Confidence interval

6. Correlation between tumor-related variables and serum level of tumor markers

We investigated the correlation between tumor markers and HCC staging and found that levels of AFP, PIVKA, and AFP-L3 were significantly elevated in HCC larger than 3 cm, in the presence of vascular invasion or distant metastasis, and in disease with AJCC stage III and IV (Table 6). All tumor markers correlated with tumor size and staging with statistical significance.

Table 6. Relationship between tumor-related variables and tumor marker levels

Variables	AFP (ng/ml)	PIVKA-II (mAU/ml)	AFP-L3 (%)
Tumor size (cm)			
≤3	15.0 (5.1-77.8)	27.0 (20.0-57.0)	4.9 (0.0-12.1)
>3	31.1 (5.1-1663.0)	775.0 (44.0-2528.0)	13.7 (2.4-48.4)
p value	0.014	<0.001	<0.001
Vascular invasion			
No	20.6 (5.1-170.3)	42.0 (22.0-401.3)	6.2 (0.0-22.6)
Yes	115.2 (5.0-4784.5)	2000.0 (149.0-2801.5)	27.0 (5.7-55.1)
p value	0.013	<0.001	<0.001
Distant metastasis			
No	19.7 (4.8-257.7)	58.5 (23.0-1485.8)	7.5 (0.0-28.8)
Yes	4338.2 (251.1-48473.6)	20000.0 (1844.0-9441.3)	33.3 (10.4-71.5)
p value	<0.001	<0.001	0.001
TNM stage			
I+II	15.2 (5.1-189.9)	32.0 (20.3-134.5)	6.0 (0.0-21.0)
III+IV	44.3 (5.2-1772.5)	1792.0 (126.0-3343.0)	16.4 (2.7-56.9)
p value	0.033	<0.001	<0.001

AFP, Alpha-fetoprotein; PIVKA-II, Protein induced by vitamin K absence-II; AFP-L3, Lens culinaris agglutinin-reactive fraction of alpha-fetoprotein; TNM, Tumor-node-metastasis

IV. DISCUSSION

AFP, PIVKA-II, and AFP-L3 are commonly used as tumor markers for HCC. Of these, AFP is the most widely used marker for monitoring HCC development, and AFP assessment and liver ultrasonography are the most commonly used tools for HCC surveillance. However, elevated AFP is not known to be reliable in patients with early HCC or a small tumor,[24-28] and the performance of ultrasonography depends on several factors such as the examiner's experience, the technology used, the patient's body habitus, the presence of cirrhosis, and tumor size.[29] Ultrasonography has a particularly low sensitivity for detecting tumor nodules in a cirrhotic liver.[30-32] Despite these limitations, there are no reliable prospective data on other tumor markers such as PIVKA-II and AFP-L3, therefore AFP and ultrasonography are still being commonly used for HCC surveillance.[29]

PIVKA-II was first described as a tumor marker of HCC by Liebman et al. in 1984.[33] PIVKA-II is a more specific marker than total AFP in the diagnosis for HCC because other liver diseases rarely give rise to elevated PIVKA-II.[10,34] Although an American study suggested that PIVKA-II was significantly better than AFP or AFP-L3 in differentiating HCC from cirrhosis for total HCC and small HCC,[35] a Japanese study demonstrated that PIVKA-II has limited value in detecting small HCC. In the latter study, the efficacy of PIVKA-II was lower than that of AFP in the diagnosis of HCC smaller than 3 cm, whereas the opposite result was obtained for tumors larger than 5 cm.[36] In the present study, PVIKA-II was superior to AFP and AFP-L3 for not only detecting overall HCC, but also for detecting early stage HCC. This finding is

consistent with a recent Korean study, which reported that PIVKA-II was a more useful marker than AFP for differentiating HCC from liver cirrhosis, especially in cases with small HCC.[37] Although PIVKA-II has been reported to be more sensitive than AFP in HCC diagnosis in North America and East Asian countries,[38-40] European studies have shown different results. These discrepancies may be related to etiologic factors in addition to racial factors.[29] Furthermore, the control group in our study was limited to patients with liver cirrhosis. Two studies suggesting that PIVKA-II was better than AFP included a control group limited to patients with liver cirrhosis,[35,37] whereas one study showing that PIVKA-II was not useful for detecting early HCC included patients with chronic liver disease, with or without LC.[36] Although the mechanism is currently unknown, PIVKA-II may be the most specific marker for detecting early HCC especially with respect to liver cirrhosis, and subgroup analysis including chronic hepatitis without liver cirrhosis will be needed.

Ultrasonography has a limited role in the detection of early HCC especially in cirrhotic liver;[30-32] therefore, increasing the sensitivity of tumor markers is very important to diagnose early HCC. Although PIVKA-II showed the best diagnostic accuracy for the detection of early HCC in our study, its sensitivity (cut-off 40mAU/ml) was only 25.6%. This weak point can be overcome when it is combined with AFP. The sensitivity of combined PIVKA-II (cut-off 40mAU/ml) and AFP (cut-off 20ng/ml) was enhanced to 48.7%, and the sensitivity of the three tumor markers combined was enhanced to 56.4%. In this study, PPV to detect early HCC was only

25.9%, despite combining the three tumor markers. We think this is due to our small sample size of early HCC patients (n = 39).

In the present study, PIVKA-II was also superior to AFP-L3 for the diagnosis of HCC in patients with AFP <20 ng/ml. The AUROC of AFP-L3 was 0.576 without statistical significance. This finding is not consistent with recent Japanese studies that reported the usefulness of AFP-L3 in patients with serum AFP less than 20 ng/ml.[20,41] In Japan, hepatitis C virus is the most common etiology, while hepatitis B virus is the most common cause of HCC in Korea. Moreover, these Japanese studies defined the control group as chronic liver disease regardless of liver cirrhosis. We think that discrepancies between our study and recent Japanese studies are due to different etiologies and control groups.

AFP is the most commonly used tumor marker in high-risk patients of HCC, but it has limited value due to its low specificity.[5,6] In comparison with AFP, AFP-L3 has been known as a very specific marker for HCC.[42] In this study, the proportion of LC patients with AFP-false positive (cut-off 20ng/ml) was 21.4% (42/196). In patients with AFP ≥ 20ng/ml, the probability of HCC diagnosis was significantly increased as AFP-L3 levels. This finding can suggest that AFP-L3 can differentiate between true HCC in AFP false-positive patients.

In this study, serum levels of the three tumor markers all showed a correlation with tumor size and staging with statistical significance. Although serum AFP is markedly elevated in patients with distant metastasis, the median value of AFP was less than 200 ng/ml not only in early-stage disease, but also in patients with tumors larger than

3 cm, vascular invasion, and advanced TNM stage. This is interesting because clinical diagnosis was defined as AFP greater than 200 ng/ml. Our findings suggest that other tumor markers such as PIVKA-II or AFP-L3 might be needed to evaluate treatment response in both early and advanced stage HCC.

V. CONCLUSION

Combined AFP and PIVKA-II can used for good screening tool of early HCC. Furthermore, AFP-L3 may have an additional role to differentiate between true HCC in AFP false-positive patients.

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ABSTRACT (IN KOREAN)

간세포암 조기진단을 위한 종양표지자의 임상적 유용성

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임태섭

간세포암의 조기진단은 그 예후를 결정하는데 매우 중요한 역할을 한다. Alpha-fetoprotein (AFP), Protein induce by vitamin K absence-II (PIVKA-II), and Lens culinaris agglutinin-reactive fraction of AFP (AFP-L3)는 간세포암을 진단할 수 있는 표지자자로 연구되어 왔다. 하지만, 어떠한 종양표지자가 간세포암의 조기진단에 가장 좋은 도구인지는 아직 의견이 일치된 바가 없다. 따라서 본 연구에서는, 간세포암의 조기진단에서 종양표지자들의 임상적 유용성에 대하여 알아보고자 한다. 환자군은 2012 년 1 월부터 2013 년 2 월까지 총 425 명의 환자 (간경변 196 명, 간세포암 229 명)가 포함되었고 종양표지자로 AFP, PIVKA-II, AFP-L3 를 측정하였다. 본 환자들에서 간경변환자 중 58.7%, 간세포암중 76.0%가 남성이었고 간경변과 간암 환자들의 평균나이는 각각 55.8 세와 60.0 세였다. 모든 종양표지자들은 통계학적으로 유의하게 간경변 환자들과 비교하여 간암환자들에서 증가되어 있었다 (p = <0.001). 간경변 환자들로부터 간암을 진단하는데 있어 Area under receiver operating characteristic curves (AUROC)는 AFP, PIVKA-II, AFP-L3 가 각각 0.679 (95% confidence interval [CI], 0.626-0.732, p = <0.001), 0.812 (95%

CI, 0.770-0.854, p = <0.001), 0.690 (95% CI, 0.638-0.742, p = <0.001)였다. 3cm 미만의 단일종양의 조기 간세포암을 진단하는데 있어서도 PIVKA-II (AUROC = 0.705, 95% CI, 0.621-0.789, p = <0.001)가 AFP (AUROC = 0.623, 95% CI, 0.527-0.719, p = <0.019) 나 AFP-L3 (AUROC = 0.561, 95% CI, 0.453-0.668, p = 0.245)보다 우월한 것으로 나타났다. 하지만, 조기간암을 진단하는데 있어 PIVKA-II (cut-off 40mAU/ml)는 25.6%의 낮은 민감도를 보였는데 이것은 AFP 를 조합함으로서 48.7%의 향상된 민감도를 보였고, AFP (cut-off 20ng/ml), PIVKA-II (cut-off 40 mAU/ml), AFP-L3 (cut-off 10%)를 조합할 때에는 56.4%까지 민감도의 향상을 보였다. AFP 이 20ng/ml 미만인 환자들에서는 PIVKA-II 의 AUROC (0.743, 95% CI, 0.678-0.807; p = <0.001) 가 AFP-L3 (0.576, 95% CI, 0.500-0.653; p = 0.052)보다 우월한 것으로 나타났다. 또한, 본 연구에서는 로지스틱회귀분석을 통하여 AFP-L3 가 AFP 가 20ng/ml 이상인 AFP 위양성 간경화환자들로부터 간세포암환자를 구분하는데 유용한 지표임을 보여주었다. (odds ratio 1.076, 95% CI, 1.037-1.116, p = < 0.001). AFP, PIVKA, AFP-L3 등 모든 종양표지자들은 통계학적으로 유의하게 간세포암의 크기 및 병기와 연관되어 증가하는 것으로 나타났다. 결론적으로 AFP 와 PIVKA-II 를 조합하는 것이 조기간세포암을 진단하는데 유용한 도구로 사용될 수 있다고 생각되며, AFP-L3 는 AFP 위양성 환자들로부터 간세포암환자를 구분하는데 추가적인 역할을 할 수 있을 것으로 생각된다.

핵심되는 말: 간세포암, AFP, PIVKA-II, AFP-L3, 종양표지자