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Usefulness of Highly Sensitive AFP-L3 and DCP in Surveillance for Hepatocellular Carcinoma in Patients with a Normal Alpha-Fetoprotein

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Abstract

Background and aims: Early detection of Hepatocellular Carcinoma (HCC) is crucial for effective management. Incidence of HCC has increased in the United States largely attributed to hepatitis B and C virus. *Lens culinaris* agglutinin-reactive Alpha-Fetoprotein (AFP-L3) and Des-Gamma-Carboxy Prothrombin (DCP) are being recognized specific biomarkers for HCC.

Methods: We measured AFP-L3 and DCP in serial serum specimens of a cohort of chronic hepatitis patients on HCC surveillance and compared these markers to abdominal imaging. Among fifty patients who developed HCC during surveillance, 30 were included in the study with available sera 1-2 years before, at diagnosis and post ablation of HCC. For controls, three consecutive annual sera were examined from 106 chronic hepatitis patients without HCC during surveillance for 5-10 years. The μTASWako i30 auto analyzer was used for the assay that utilizes the microfluidics chip based assay platform. It can fractionate AFP-L3 glycoform and calculates AFP-L3% if AFP level is ≥ 0.6 ng/mL.

Results: Combination of AFP, AFP-L3 and DCP showed high sensitivity of 83% in all patients and 75% in patients with AFP<20 ng/mL. AFP-L3 and DCP assays were useful in patients with low levels of AFP (<20 ng/mL) and could detect significant AFP-L3% elevation in some patients more than one year before the diagnosis of HCC. Furthermore, AFP-L3 predicted recurrence of HCC.

Conclusions: This is the first study in the U.S. patients using the μTASWako i30 analyzer to test these HCC biomarkers. Our results suggest that combinations of these biomarkers are highly useful for early detection of HCC.

Keywords: Hepatocellular carcinoma; *Lens culinaris* agglutinin-reactive alpha-fetoprotein; Des-gamma-carboxyprothrombin; Surveillance; Risk assessment; Magnetic resonance imaging

Introduction

TIncidence of Hepatocellular Carcinoma (HCC) has been on the rise in the United States lately attributed to an increase of chronic hepatitis B and C [1]. Furthermore, obesity and diabetes may be additional risk factors to an increased rate of HCC [2]. American Association for the Study of Liver Disease (AASLD) recommends imaging studies for surveillance for patients at risk for HCC [3]. The Ultrasound (US) is used as the first line modality for HCC screening, followed by dynamic Computed Tomography (CT) or Magnetic Resonance Imaging (MRI). Imaging modalities for clinical diagnosis have changed medical practice in HCC surveillance in recent years mitigating the risks associated with liver biopsy. However, there are concerns for the cost and excessive radiation exposure using imaging study for surveillance. Therefore, the imaging techniques are usually applied annually or 6 monthly for screening. However, doubling time of liver cancer is approximately 6 months on average [4]. To detect HCC in treatable stage, AASLD recommends 3-6 months surveillance interval [3]. Also, National Comprehensive Cancer Network (NCCN) Clinical Practice Guideline on hepatobiliary cancer made similar recommendation [5]. NCCN clinical practice guideline also recommends Alpha-Fetoprotein (AFP) combined with imaging modalities. In general, the serum and imaging biomarkers are specific with high positive predictive value (PPV), but less sensitive, therefore having low Negative Predictive Value (NPV) for HCC surveillance [6,7].

Recently two novel serum biomarkers for HCC risk assessment, the Lectin-reactive Alpha-Fetoprotein (AFP-L3) and Des-Gamma-Carboxy Prothrombin (DCP) have been introduced into clinical practice. AFP-L3 is a glycosylation variant of AFP [8]. DCP is abnormal coagulation protein produced in the liver and a precursor of thrombin in the coagulation cascade [9]. These serum biomarkers are highly specific for HCC [10-12]. Recently, the second generation of the automated assays has been developed. This assay utilizes microfluidic chip-based assay technologies. Highly sensitive serum biomarkers are in great need for surveillance of HCC combined with high negative predictive value. Thus, only patients with positive results would need imaging study. Toyoda et al. showed that the high sensitivity of AFP-L3 is especially useful for patients with AFP <20 ng/mL with a lower cut-off at 5% [13]. Hanaoka et al. demonstrated that combination of AFP-L3 and DCP further enhanced the assay sensitivity [14]. In 2007, Carr et al. examined these markers in 98 patients with unresectable HCC in the U.S. using Liquid Phase Binding Immunoassay (LiBASys) and reported that the combination of AFP, AFP-L3% and DCP was superior to individual marker alone in the diagnosis of HCC [15]. They also found

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that AFP-L3% was significantly related to portal vain invasion and patient outcome suggesting AFP-L3% as a potential prognostic marker.

In this study, utilizing the newly developed microfluidic chip platform (by Wako), we measured these biomarkers in the serum samples collected prospectively from 1999 to 2011 from a cohort of patients with chronic hepatitis without HCC, some of whom developed HCC during the follow-up period. This patient cohort has been on long term surveillance at the Liver Disease Prevention Center, Division of Gastroenterology and Hepatology, Thomas Jefferson University Hospital with 3-6 monthly AFP and biannual imaging. The majority had chronic hepatitis B with or without cirrhosis. We investigated the usefulness of these biomarkers for surveillance for HCC. Most subjects have had low AFP level <20 ng/mL. This is the first study in the U.S. using the novel and highly sensitive biomarkers assay on μTASWako i30 analyzer for patients who are on HCC surveillance.

Patients and Methods

Patients

Included in the study were patients most of whom had Chronic Hepatitis B (CHB) or Chronic Hepatitis C (CHC). The majority were Asian Americans. They were on HCC surveillance with 6 monthly AFP and 6 monthly abdominal imaging for minimum 5 years and longer between February 1999 and August 2011. Selection of HCC patients and controls for the study was based on the availability of the specimens. To be eligible for the study, HCC patients had to have sera obtained; 1-2 years before HCC diagnosis, at diagnosis and post-treatment follow up period. For controls with chronic hepatitis who did not develop HCC during surveillance, three consecutive annual serum samples were required. All had to have 6 monthly or annual imaging including ultrasound. Based on the criteria, 30 HCC patients and 106 controls were selected. HCC patients were diagnosed initially by imaging studies including US, CT or MRI. For the final diagnosis of HCC, the dynamic MRI was used as defined by AASLD guideline [16-18] and by our institution [19] that shows a mass demonstrating low to intermediate T1 signal on pre-contrast images, homogeneous, heterogeneous, or ring enhancement during the hepatic arterial phase and/or moderate hyperintensity on intermediate T2-weighted fat suppressed spin-echo images with washout relative to surrounding liver parenchyma on delayed post contrast images. To ensure that controls did not have HCC, they were followed for 2-3 years longer after the last serum sampling. Treatment for HCC was decided according to the treatment guidelines for HCC in the USA [3,20-22].

The study protocol was approved by the Institutional Review Board at Thomas Jefferson University and was in compliance with the declaration of Helsinki.

Measurements of AFP, AFP-L3 and DCP

AFP, AFP-L3 and DCP were measured in the serum sample obtained 1-2 years prior to the diagnosis of HCC, at the time of diagnosis and during the follow up period after tumor ablation (for HCC group) and from serum samples obtained annually during which time the absence of HCC was confirmed (for the control group).

AFP, AFP-L3 and DCP were measured using a microchip capillary electrophoresis and liquid-phase binding assay on a μTASWako i30 auto analyzer (Wako Diagnostics, Wako Life Sciences, Inc. Mountain View, California, USA). [23]. The measurement range was 0.3-1000 ng/mL for AFP and 0.1-950 ng/mL for DCP. The percentage of AFP-L3 on the i30 analyzer can be calculated when AFP is over 0.6 ng/mL compared to 10 ng/mL on the previous platform, LIBASys. The total precisions for nine concentration levels of the three biomarkers were less than 8% coefficient of variations in 21 days of evaluation. This new assay platform has enabled the accurate measurement of AFP-L3 with a high sensitivity and at low AFP concentrations. All processes were preformed automatically and followed the manufacture's instruction.

Statistical analyses

Receiver Operation Characteristic (ROC) analysis was used to evaluate the optimum cut-off values for AFP, AFP-L3 and DCP assays on μTASWako i30. To evaluate the diagnostic value of AFP-L3 and DCP, sensitivity, specificity Positive Predictive Value (PPV) and Negative Predictive Value (NPV) were estimated by using the cut-offs which are approved by FDA and currently used in medical practice in the USA. In addition, the optimum cut-offs were calculated. The sensitivity, specificity, PPV and NPV for patients who have low concentration of AFP (<20 ng/mL) were calculated. All analyses were performed using JMP5 statistical software (SAS Institute, Cary, NC, USA).

Results

Demographics of the study population

The study cohort consisted of 136 chronic hepatitis patients with or without cirrhosis who have been under surveillance for HCC from 1999 to 2011.

Of the 136, 30 patients developed HCC during surveillance period and 106 did not.

Detailed characteristics of HCC patients and controls are shown in Table 1. In both Groups there were more men than women. HCC patients were 10 years older in average than the controls. The majority had chronic hepatitis B.

As shown in Table 2, of the 30 HCC patients, 26 had HBV, 3 with HCV and one without viral infection. Details of the underlying liver diseases including liver cirrhosis and the HBeAg status are given in the table.

Characteristics	Patients with HCC (n=30)	Patients without HCC (n=106)
Gender (%)		
Male/Female	25 (83) / 5 (17)	69 (65) / 37 (35)
Age		
Mean ± SD	59.1 ± 10.3	48.4 ± 10.8
Infection hepatitis virus (%) HBV/HCV/HBV+HCV/ none	26 (87)/3 (10)/0 (0)/1 (3)	105 (99)/0 (0)/0 (0)/1 (1)
Chronic Hepatitis/Liver cirrhosis (%)	N/A	87 (82) / 19 (18)
Tumor size		
≤ 2 cm	14	N/A
>2 cm and ≤ 3 cm	6	N/A
>3 cm and ≤ 5 cm	8	N/A
>5 cm	2	N/A
Number		
Single	23	N/A
Multiple	7	N/A

Table 1: Patient Characteristics.

	patients with HCC no=30	Patients without HCC no=106
Underlying liver disease		
Chronic hepatitis (%)	10 (33)	87 (82)
Cirrhosis (%)	20 (66)	19 (18)
Compensated (%)	16 (53)	13(12.3)
Decompensated (%)	4 (13)	6 (5.6)
HBsAg (+)	26 (87)	105 (99)
HBeAg (+)	5 (17)	32 (30)
HBeAg (-)	21 (70)	73 (69)
HCV	3 (10)	0 (0)
None	1 (3)	1 (1)

Table 2: Patients' liver disease status and viral markersa.

	Cut-off	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
AFP	200 ng/mL	13	98	67	80
AFP-L3	10%	47	98	88	87
DCP	7.5 ng/mL	13	99	80	80

Table 3: Sensitivity and Specificity of AFP, AFP-L3 and DCP in patients with and without HCC using the current cut-off values.

Clinical performance characteristics

Table 3 shows the sensitivity (true-positive rate), specificity (true-negative rate), Positive Predictive Value (PPV) and Negative Predictive Value (NPV) of the AFP, AFP-L3 and DCP using current recommended cut-offs for risk assessment in HCC surveillance from the manufacturers. The sensitivity of AFP, AFP-L3 and DCP is 13, 47, 13% respectively based on the cut-off value for AFP, AFP-L3 and DCP as 200 ng/mL, 10% and 7.5 ng/mL. It is noted that all three serum biomarkers demonstrated very high but comparable clinical specificity of greater than 98% with AFP-L3 having the highest or more than double the sensitivity of the other two biomarkers. This suggests that when used individually, the HCC biomarkers could be effective for rule in diagnosis since the PPV is favorable. However it may not be as effective for screening of patients at high risk of HCC.

Receiver Operating Characteristics (ROC) curve: The clinical performance characteristics of the serum biomarkers are compared in ROC analysis. Among the three HCC serum biomarkers, AFP has a slightly greater ROC of 0.836 compared to AFP-L3 of 0.820 and DCP of 0.728. The optimal cutoffs for AFP, AFP-L3 and DCP are 5.6 ng/mL, 4.5% and 0.57 ng/mL, respectively, and are lower than those currently applied for clinical diagnosis.

Optimal cut-off threshold for HCC surveillance: The current manufacturer's recommended cut-off value may not be suited for risk assessment in surveillance for different subgroup of chronic viral hepatitis patients. In our study group, most patients with chronic hepatitis and cirrhosis had HBV infection. From this data set, we calculated the optimal cut-off thresholds for the AFP-L3 and DCP for our study subjects.

Based on the ROC analysis of this data set, when optimal cut-offs of 4.5% for AFP-L3 and 0.57 ng/mL for DCP were used, the sensitivity of the AFP-L3 and DCP becomes 70% and 53.3%, respectively (Table 4). Therefore, clinical sensitivities of the AFP-L3 and DCP improve significantly with only minor decrease in the specificity. Therefore, these cut-offs (AFP-L3 4.5%, DCP 0.57 ng/ml) would be more useful for risk assessment in HCC surveillance.

Combined use of the HCC serum biomarkers: The clinical sensitivities of the combined AFP-L3 and DCP assays are shown in Table 4. Notably, the sensitivity is increased to 83.3% with greater than 90% specificity for all the samples measured in the study. For patients with AFP <20 ng/mL (n=16 HCC; and n=102 CH controls), AFP-L3 has sensitivity of 50% and DCP 48.3%. In combination, AFP-L3 and DCP showed 75% of sensitivity and over 94% specificity. The ROC of different combinations of the HCC serum biomarkers shows that combination of AFP-L3 and DCP improve the AUC with 0.900 compared to AFP and AFP-L3 (0.846), AFP and DCP (0.874) and AFP, AFP-L3 and DCP (0.874).

Changes of AFP, AFP-L3, and DCP value in patient's overtime: High sensitivity indicated high risk of HCC development among the patients with chronic hepatitis and cirrhosis. Since the assays are more sensitive to the underlying pathological changes of HCC in the background of cirrhosis, it was important that prospectively collected serum specimens were available long before the HCC confirmation by MRI. Of the 30 HCC patients, 7 patients had multiple serum samples before HCC diagnosis. As shown in (Figure 1), 6 out of 7 patients (86%) with multiple specimens collected before the HCC diagnosis showed elevation of AFP-L3 level (≥ 5%) of a lead time of more than 1 year before diagnosis of HCC by MRI.

Predicting HCC recurrence: Of the 30 HCC patients, 8 patients had recurrent tumor after the initial successful ablation and had the markers examined in the serum collected after tumor ablation. Measurements for AFP-L3 and DCP are summarized in Table 5. Six of the 8 patients showed elevation of either AFP-L3 level of ≥ 5% or DCP of ≥ 0.57 ng/mL after treatment that predicted recurrence.

Discussion

Early detection of HCC at the treatable stage is imperative for effective HCC management. Sherman suggests that HCC <3 cm in diameter is critical for achieving successful treatment [6]. AFP, albeit having been used for HCC diagnosis in clinical settings, is a non-specific biomarker of HCC since it often increases in hepatic necro inflammation [24]. It is a poor indicator of HCC with low levels of AFP (<20 ng/mL).

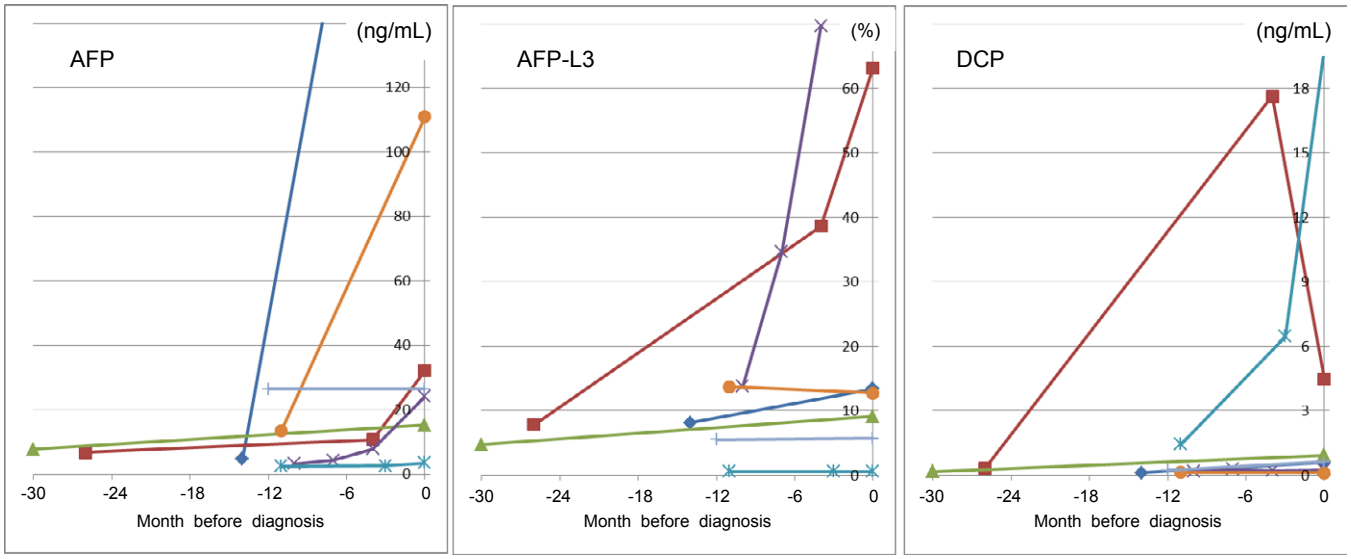
In HCC surveillance, highly sensitive serum biomarkers play important roles because of their high negative predictive values that can minimize the false negative results of less sensitive assays. Only patients with seropositive results may need imaging confirmation. While the imaging modalities provide confirmative diagnosis, they are expensive with limited access in physicians' clinic for surveillance.

In this study, we measured serial serum specimens from a cohort of 136 patients with chronic viral hepatitis and cirrhosis. These patients have been closely monitored for HCC development with 3-6 monthly

	Cut-off	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
All samples					
AFP-L3	4.5%	70.0	92.5	72.4	91.6
DCP	0.57	53.3	98.1	88.9	88.1
AFP-L3 + DCP		83.3	91.3	73.5	95.1
AFP <20 ng/mL (HCC: n=16, Control: n=102)					
AFP-L3	4.5%	50.0	94.1	57.1	92.3
DCP	0.57	43.8	100.0	100.0	91.9
AFP-L3 + DCP		75.0	94.1	66.7	96.0

The clinical sensitivities would be significantly improved by using the optimal cut-offs derived from this study data set and further increased by combining all three biomarkers.

Table 4: Optimal Cut-off Values Derived from ROC Analysis for the current study.



The unit on x-axis is month, 0 indicates the time of HCC diagnosis; on y-axis for AFP is ng/mL; AFP-L3 is %; and DCP is ng/mL. Six of the 7 patients with multiple serum specimens showing elevated AFP-L3 >5% one year prior to MRI confirmation, while only 2 with AFP >20 ng/mL and 1 with DCP >7.5 ng/mL (AFP-L3 superior to others).

Figure 1: Change of AFP, AFP-L3 and DCP Value Overtime before HCC Diagnosis.

No.	Sex	Etiology	AFP	U%	DCP	HCC /Non-HCC	Imaging	Size of tumor (cm)	Number of tumor
1	M	HBV	8.4	74.2	0.89	HCC	MRI	3.5x2.4	1
2	F	HBV	2419.3	9.6	0.26	HCC	MRI	1.7x1.8	1
3	M	HBV	3.1	10.7	0.24	Suspious	MRI	0.7	1
4	M	HBV	206.1	2.5	79.88	HCC	MRI	1.2	1
5	M	HBV	2.3	0.5	0.38	HCC	MRI	1.6x1.4	1
6	M	HBV	9.3	28.8	0.37	HCC	MR1	3.0, 4.0	2
7	M	HBV	3.8	0.5	0.10	HCC	MRI	2.0	1
8	M	HBV	4.3	7.0	0.11	Suspious	MRI	2.0x1.8	1

In 5 among 8 patients with specimens after successful treatment, the AFP-L3 values were elevated before the recurrence (by MRI) in comparison to only 2 each for AFP and DCP (cut-offs for L3% 4.5 and for DCP 0.57).

Table 5: AFP, AFP-L3, and DCP in Predicting HCC Recurrence.

AFP and 6 monthly imaging for HCC for a minimum of 5-10 years. Thirty patients who developed HCC during surveillance (26/30 with had HBV) and 106 controls (105/106 with HBV) were enrolled. Our study of HCC biomarkers demonstrated extremely high specificity. Individual HCC biomarker's sensitivity using current recommended cut-off level is relatively low from 13% to 47%. Among these, the sensitivity of AFP-L3 is the highest. In addition, HCC patients detected by the individual HCC biomarkers do not overlap suggesting the heterogeneities of HCC. The relative low sensitivity of individual biomarkers is to be expected if used independently because each subtype of the HCC may be defined by different biomarkers.

In the past study using the previous platform (LiBASys), DCP had highest sensitivity and were the most accurate tumor marker, and were suggested to be used for HCC surveillance [25]. However, AFP-L3 did not show adequate sensitivity to be considered a surveillance tool for HCC although it had high specificity.

In the current study, AFP-L3 on the μTASWako i30 had the highest sensitivity using lower cut-off (5%) compared to AFP and DCP [26]. In focusing on the patients with less than 20 ng/mL of AFP, AFP-L3 levels were significantly higher in HCC than in non-HCC and the sensitivity of AFP-L3 for HCC detection was more than 70% with cut-off of 5%. In addition of this study, the combination of AFP-L3 and DCP improved the sensitivity reaching the level of close to 90% and showed significantly increased AUC even in patients with low AFP concentration.

The significant improvement of clinical sensitivity of the biomarkers observed in this study is largely due to the novel assay technologies that utilized microfluidics chip based assay platform. Compared to the older version assays performed on Liquid Phase Binding Immunoassay (LiBASys) platform, the current analyzer can fractionate AFP-L3 glycoform and calculate the percentage of AFP-L3 at the AFP level of ≥ 0.6 ng/mL, the amount which virtually cover the entire reportable range of AFP. Sherman et al reported that a large portion of patients (20-80%) with liver cancer would not have AFP >20 ng/mL depending on the size of HCC at diagnosis [27]. AFP has low sensitivity for surveillance at the current cut-off level that is designed for rule-in HCC diagnosis. Therefore, AFP has not been recommended for HCC surveillance by AASLD guideline [3]. Changing the cutoff threshold to lower range would improve sensitivity but decrease specificity thereby increasing false positivity.

In our study, we demonstrated that AFP-L3 had higher sensitivity in all patients as well as in patients with AFP level less than 20 ng/

mL-70% and 50%, respectively. The combination of AFP-L3 and DCP improved the sensitivities to 83% and 75%, respectively. The seropositive HCC biomarkers of high sensitivity could indicate the high risk for HCC. For surveillance, a fixed cut-off is less significant than the changes of HCC biomarkers overtime. The relatively low sensitivity of individual serum biomarker in this study is not unexpected since HCC is probably not a single disease entity but of a group of liver malignancies having subtypes with different clinical outcomes [28]. AFP, AFP-L3, and DCP may be representing different disease subtypes. In our study, we are able to confirm the distribution patterns of the biomarker expression as reported previously [29,30]. We also observed that not all three biomarkers are expressed in HCC patients at the same time. AFP-L3 has been also reported as a biomarker for aggressive HCC, while DCP is a marker of intra hepatic metastasis [8,31]. Patients with primary malignant hepatic tumors seropositive for AFP-L3 and low AFP concentrations present unique clinicopathologic features. These cancers are reported having a higher incidence of non-HCC primary liver cancer derived from cholangiocytes. They also had a high frequency of poorly differentiated tumors and sarcomatous changes, and showed a poor prognosis [32]. Patient's positive for AFP-L3 and negative for DCP demonstrated histopathologic features of more advanced HCC while those positive for DCP alone presented infiltrative and poorly differentiated HCC [33]. Okuda et al. found that a subgroup of Intrahepatic Cholangiocarcinoma (ICC) seropositive for AFP-L3, and patients with combined HCC and ICC have features more like HCC. They suggest that these liver cancers are different from the ICC which is seropositive for CA19-9 [34]. This supports that AFP-L3 seropositive HCC is a subtype with aggressive behavior.

The limitation of our study is the small number of patients. While several hundreds of patients with chronic hepatitis B have been under surveillance for HCC only 136 met the eligibility criteria for the study. Nonetheless, the trending of these two HCC biomarkers even before MRI confirmation is encouraging and presents a testable hypothesis that these highly sensitive HCC biomarkers can be included as important markers for HCC surveillance. Due to the biological heterogeneity, some HCC was not detected by the 3 biomarkers. We expect this performance gap would be narrowed with additional new HCC serum biomarkers in the future. In this regard, Shen et al. reported that a new serum biomarker of Dickkopf-1 (DKK1) could complement AFP for patients with negative AFP [35]. Other new potential serum biomarkers have been reported as well [36,37].

Conclusions

Our study suggests that AFP-L3 and DCP are highly specific for HCC, and the clinical sensitivity can significantly improve if AFP, AFP L3 and DCP are used together for HCC surveillance. Serial testing would also greatly improve clinical sensitivity in surveillance of risk of HCC development. The highly sensitive AFP-L3 and DCP assays would be useful in patients with AFP in reference range <20 ng/mL and can detect significant AFP-L3% elevation in some HCC cases more than one year prior to confirmative HCC diagnosis. In addition, AFP-L3 may be useful in predicting the recurrence of HCC after treatment. Combination of AFP-L3 and DCP and serial sampling for parallel testing can improve clinical sensitivity of the overall testing results while maintaining clinically acceptable high specificity.

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References

1. Colvin HM, Mitchell AE (2010) Hepatitis and liver cancer a national strategy for prevention and control hepatitis B and C. Institute of Medicine of National Academies. The National Academies Press 500 Fifth Street, N.W. Washington, D.C. 20001.
2. Polesel J, Zucchetto A, Montella M, Dal Maso L, Crispo A, et al. (2009) The impact of obesity and diabetes mellitus on the risk of hepatocellular carcinoma. Ann Oncol 20: 353-357.
3. Bruix J, Sherman M; American Association for the Study of Liver Diseases (2011) Management of hepatocellular carcinoma: an update. Hepatology 53: 1020-1022.
4. O'Malley ME, Takayama Y, Sherman M (2005) Outcome of small (10-20 mm) arterial phase-enhancing nodules seen on triphasic liver CT in patients with cirrhosis or chronic liver disease. Am J Gastroenterol 100: 1523-1528.
5. National Comprehensive Cancer Network (NCCN). Clinical Practice Guideline on hepatobiliary cancer
6. Sherman M (2011) Hepatocellular carcinoma: screening and staging. Clin Liver Dis 15: 323-334.
7. Sherman M (2010) Hepatocellular carcinoma: epidemiology, surveillance, and diagnosis. Semin Liver Dis 30: 3-16.
8. Li D, Mallory T, Satomura S (2001) AFP-L3: a new generation of tumor marker for hepatocellular carcinoma. ClinChimActa 313: 15-19.
9. Weitz IC, Liebman HA (1993) Des-gamma-carboxy (abnormal) prothrombin and hepatocellular carcinoma: a critical review. Hepatology 18: 990-997.
10. Taketa K, Hirai H (1989) Lectin affinity electrophoresis of alpha-fetoprotein in cancer diagnosis. Electrophoresis 10: 562-567.
11. Taketa K, Endo Y, Sekiya C, Tanikawa K, Koji T, et al. (1993) A collaborative study for the evaluation of lectin-reactive alpha-fetoproteins in early detection of hepatocellular carcinoma. Cancer Res 53: 5419-5423.
12. Sato Y, Nakata K, Kato Y, Shima M, Ishii N, et al. (1993) Early recognition of hepatocellular carcinoma based on altered profiles of alpha-fetoprotein. N Engl J Med 328: 1802-1806.
13. Toyoda H, Kumada T, Tada T, Kaneoka Y, Maeda A, et al. (2011) Clinical utility of highly sensitive Lens culinaris agglutinin-reactive alpha-fetoprotein in hepatocellular carcinoma patients with alpha-fetoprotein <20 ng/mL. Cancer Sci 102: 1025-1031.
14. Hanaoka T, Sato S, Tobita H, Miyake T, Ishihara S, et al. (2011) Clinical significance of the highly sensitive fucosylated fraction of Î±-fetoprotein in patients with chronic liver disease. J Gastroenterol Hepatol 26: 739-744.
15. Carr BI, Kanke F, Wise M, Satomura S (2007) Clinical evaluation of lens culinaris agglutinin-reactive alpha-fetoprotein and des-gamma-carboxyprothrombin in histologically proven hepatocellular carcinoma in the United States. Dig Dis Sci 52: 776-782.
16. Bruix J, Sherman M; American Association for the Study of Liver Diseases (2011) Management of hepatocellular carcinoma: an update. Hepatology 53: 1020-1022.
17. Burrel M, Llovet JM, Ayuso C, Iglesias C, Sala M, et al. (2003) MRI angiography is superior to helical CT for detection of HCC prior to liver transplantation: an explant correlation. Hepatology 38: 1034-1042.
18. Yu JS, Kim KW, Kim EK,Lee JT, Yoo HS (1999) Contrast enhancement of small hepatocellular carcinoma: usefulness of three successive early image acquisitions during multiphase dynamic MR imaging. AJR Am J Roentgenol 173: 597-604.
19. Mitchell DG (2010) MRI for detection and evaluation of hepatocellular carcinoma, Carr BI, (2ndedn) Hepatocellular carcinoma. Humana Press.
20. Llovet JM, Real MI, Montaña X, Planas R, Coll S, et al. (2002) Arterial embolization versus symptomatic treatment in patients with unresectable hepatocellular carcinoma: a randomized controlled trial. Lancet 359: 1734-1739.
21. Lo CM, Ngan H, Tso WK, Liu CL, Lam CM, et al. (2002) Randomized controlled trial of transarteriallipiodol chemoembolization for unresectable hepatocellular carcinoma. Hepatology 35: 1164-1171.
22. Brown DB, Chapman WC, Cook RD, Kerr JR, Gould JE, et al. (2008)

Chemoembolization of hepatocellular carcinoma: patient status at presentation and outcome over 15 years at a single center. AJR Am J Roentgenol 190: 608-615.

23. Kagebayashi C, Yamaguchi I, Akinaga A, Kitano H, Yokoyama K, et al. (2009) Automated immunoassay system for AFP-L3% using on-chip electrokinetic reaction and separation by affinity electrophoresis. Anal Biochem 388: 306-311.

24. Colli A, Fraquelli M, Casazza G, Massironi S, Colucci A, et al. (2006) Accuracy of ultrasonography, spiral CT, magnetic resonance, and alpha-fetoprotein in diagnosing hepatocellular carcinoma: a systematic review. Am J Gastroenterol 101: 513-523.

25. Marrero JA, Henley KS (2011) The role of serum biomarkers in hepatocellular carcinoma surveillance. Gastroenterol Hepatol (N Y) 7: 821-823.

26. Durazo FA, Blatt LM, Corey WG, Lin JH, Han S, et al. (2008) Des-gamma-carboxyprothrombin, alpha-fetoprotein and AFP-L3 in patients with chronic hepatitis, cirrhosis and hepatocellular carcinoma. J Gastroenterol Hepatol 23: 1541-1548.

27. Choi JY, Jung SW, Kim HY, Kim M, Kim Y, et al. (2013) Diagnostic value of AFP-L3 and PIVKA-II in hepatocellular carcinoma according to total-AFP. World J Gastroenterol 19: 339-346.

28. Sherman M (2010) The resurrection of alphafetoprotein. J Hepatol 52: 939-940.

29. Thomas MB, Jaffe D, Choti MM, Belghiti J, Curley S, et al. (2010) Hepatocellular carcinoma: consensus recommendations of the National Cancer Institute Clinical Trials Planning Meeting. J Clin Oncol 28: 3994-4005.

30. Toyoda H, Kumada T, Kiriyama S, Sone Y, Tanikawa M, et al. (2006) Prognostic significance of simultaneous measurement of three tumor markers in patients with hepatocellular carcinoma. ClinGastroenterol Hepatol 4: 111-117.

31. Sterling RK, Jeffers L, Gordon F, Venook AP, Reddy KR, et al. (2009) Utility of Lens culinaris agglutinin-reactive fraction of alpha-fetoprotein and des-gamma-carboxyprothrombin, alone or in combination, as biomarkers for hepatocellular carcinoma. ClinGastroenterol Hepatol 7: 104-113.

32. Yuan LW, Tang W, Kokudo N, Sugawara Y, Karako H, et al. (2004) Measurement of des-gamma-carboxyprothrombin levels in cancer and non-cancer tissue in patients with hepatocellular carcinoma. Oncol Rep 12: 269-273.

33. Okuda H, Saito A, Shiratori K, Yamamoto M, Takasaki K, et al. (2005) Clinicopathologic features of patients with primary malignant hepatic tumors seropositive for alpha-fetoprotein-L3 alone in comparison with other patients seropositive for alpha-fetoprotein-L3.J Gastroenterol Hepatol20: 759-764.

34. Okuda H, Nakanishi T, Takatsu K,Saito A, Hayashi N, et al. (2002) Clinicopathologic features of patients with hepatocellular carcinoma seropositive for alpha-fetoprotein-L3 and seronegative for des-gamma-carboxyprothrombin in comparison with those seropositive for des-gamma-carboxyprothrombin alone. J Gastroenterol Hepatol17: 772-778.

35. Okuda H, Shiratori K, Yamamoto M, Takasaki K, Nakano M (2006) Clinicopathologic features of patients with intrahepatic cholangiocarcinoma who are seropositive for alpha-fetoprotein-L3 and those with combined hepatocellular and cholangiocarcinoma. J Gastroenterol Hepatol 21: 869-873.

36. Shen Q, Fan J, Yang XR, Tan Y, Zhao W, et al. (2012) Serum DKK1 as a protein biomarker for the diagnosis of hepatocellular carcinoma: a large-scale, multicentre study. Lancet Oncol 13: 817-826.

37. Malaguarnera G, Giordano M, Paladina I, Berretta M, Cappellani A, et al. (2010) Serum markers of hepatocellular carcinoma. Dig Dis Sci 55: 2744-2755.