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Alpha-fetoprotein, Des-gamma Carboxyprothrombin, and Lectin-Bound Alpha-fetoprotein in Early Hepatocellular Carcinoma

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Abstract

Background and Aims—Alpha-fetoprotein (AFP) is widely used as a surveillance test for Hepatocellular carcinoma (HCC) among patients with cirrhosis. Des-gamma carboxy-prothrombin (DCP) and lectin-bound AFP (AFP-L3) are potential surveillance tests for HCC. The aims of this study were to determine performance of DCP and AFP-L3 for the diagnosis of early HCC, whether they complement AFP, and what factors affect DCP, AFP-L3 or AFP levels.

Methods—We conducted a large phase 2 biomarker case-control study in 7 academic medical centers in the United States. Controls were patients with compensated cirrhosis and cases were patients with HCC. AFP, DCP and AFP-L3 levels were measured blinded to clinical data in a central reference laboratory.

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Results—A total of 836 patients were enrolled, 417 (50%) were cirrhosis controls and 419 (50%) were HCC cases, of which 208 (49.6%) had early stage HCC (n=77 very early, n=131 early). AFP had the best area under the ROC curve (0.80, 95%CI: 0.77–0.84) followed by DCP (0.72, 95%CI: 0.68–0.77) and AFP-L3% (0.66, 95%CI: 0.62–0.70) for early stage HCC. The optimal AFP cutoff value was 10.9 ng/ml leading to a sensitivity of 66%. When only those with very early HCC were evaluated, the AUC for AFP was 0.78 (95%CI: 0.72–0.85) leading to a sensitivity of 65% at the same cutoff.

Conclusion—AFP was more sensitive than DCP and AFP-L3 for the diagnosis of early and very early stage HCC at a new cutoff of 10.9 ng/mL.

INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common tumor and the third most common cause of cancer-related deaths worldwide (1,2). According to the World Health Organization, the burden of HCC is expected to continue to increase until 2030, and it is the tumor with the second highest increase in overall death rates (3). The incidence and mortality rates for HCC are virtually identical, reflecting the overall poor survival of patients with this tumor. Curative therapy exists for HCC if diagnosed early (4). Hepatitis C virus (HCV) is the causative agent largely responsible for the increase in incidence of HCC in the U.S. (5). However, HBV is the leading cause of HCC worldwide, particularly in Asia and Africa (6).

Cirrhosis is the most important risk factor in the development of HCC and represents an opportunity for surveillance (7). Therefore, it is recommended that patients with cirrhosis undergo surveillance (4). The diagnosis of HCC is made by typical findings on cross-sectional imaging (CT or MRI scan): a liver lesion showing arterial vascularization, followed by washout of contrast in the lesion in the delayed phases (8). If the cross-sectional imaging is atypical, then histopathological assessment is needed to confirm HCC.

The National Cancer Institute's Early Detection Research Network (EDRN) has developed guidelines on phases of evaluating an early detection biomarker for cancer, analogous to the degrees of evidence seen in the various phases of drug discovery (9). Phase 1 are biomarker discovery studies, Phase 2 are large case control studies on clinically diagnosed patients to evaluate biomarker performance, Phase 3 are retrospective evaluation of prospectively collected specimens from cohorts of patients at risk to determine the ability of a biomarker to diagnose pre-clinical disease, Phase 4 are prospective studies in which biomarker positivity triggers work up for the diagnosis of the tumor, and Phase 5 are prospective randomized studies with the end point of survival.

AFP is the most utilized surveillance biomarker for HCC worldwide. Recent systematic reviews of the literature show that the quality of evidence supporting the use of AFP as a diagnostic and screening test for HCV-related HCC is limited (10,11). Given the significant concerns about the validity of the data generated by these previous studies, the authors could not calculate conclusive summary estimates of the sensitivity and specificity of AFP as a diagnostic test for HCC. The problems encountered included verification bias, the lack of application of cross-sectional imaging (CT or MRI) to both cases and controls to appropriately determine who has cancer, lack of blinding, and inclusion of patients with a heterogeneous risk of HCC. Another systematic review indicated that studies evaluating AFP as a surveillance test suffered from variable study design, patient characteristics, sample size and verification bias (12). Better studies are needed to determine AFP's performance in early stage HCC.

Current guidelines recommended ultrasound (US) as the main surveillance test in patients with cirrhosis (4). However, the level of recommendation was low, indicating that it was supported by retrospective cohort studies and not prospective studies. Moreover, a systematic review has

shown that the level of evidence for US as a surveillance test is weak (Grade C), and the strength of the data was limited by sample size, variable surveillance frequency, extent of liver disease and verification bias (12). Another systematic review of US as a surveillance test concluded that US is insufficiently sensitive to detect HCC in many cirrhotics or to support an effective surveillance program (10). Therefore, the current recommendation of surveillance by US is not supported by rigorous data.

Des-gamma carboxyprothrombin (DCP) and lectin-bound AFP (AFP-L3) are other potential serum biomarkers for HCC (13,14). Most data on DCP and AFP-L3 have been from underpowered, single center case-controlled studies (15–20). A multicenter prospective study compared AFP to AFP-L3 in North America, but one center dominated recruitment leading to potential selection bias; the diagnostic criteria for HCC were different among centers leading to verification and selection biases, and the follow up of the cohort was short (21). Therefore, well designed biomarker studies to compare the performance of AFP, DCP, and AFP-L3 for the diagnosis of early stage HCC are urgently needed. The paucity of high-quality data calls for more rigorous studies of AFP and other tests for detecting HCC (11).

The aims of this study were to determine (i) the sensitivity and specificity of DCP and AFP-L3 for the diagnosis of early HCC, (ii) whether they complement AFP, and (iii) to determine whether demographic factors or etiology of underlying liver disease alter the expression of DCP or AFP in an EDRN-defined phase 2 biomarker study.

METHODS

Study Design

We performed a large EDRN-defined Phase 2 biomarker case-control study. Cases included consecutive adult patients with HCC seen between February 2005 and August 2007 at 7 medical centers in the United States (University of Michigan, Mayo Clinic-Minnesota, Mayo Clinic-Florida, Mt. Sinai Medical Center-New York, University of Pennsylvania, Saint Louis University, and Stanford University). Assays were performed at the University of California at Los Angeles in a blinded fashion to clinical data, and the Fred Hutchinson Cancer Research Center served as the Data Management and Coordinating Center. The study was performed in compliance and after approval from the respective institutional review boards of all sites. Each participant signed a consent form for participation into the study and prior to data and blood collection.

HCC was defined by histological examination or by the appropriate imaging characteristics as defined by accepted guidelines (22). Staging was determined by the Barcelona Clinic Liver Cancer staging system (BCLC) (4). BCLC stage A (Early stage) is defined by a single lesion between 2–5 cm or ≤ 3 lesions each < 3 cm, without portal vein thrombosis or extrahepatic metastasis. BCLC stage 0 (Very early HCC) was defined as a single lesion < 2 cm without vascular involvement or metastasis. Late stage was defined as the combination of intermediate (BCLC stage B)/advanced (BCLC stage C) HCC (4). Patients with HCC were excluded if they were younger than 18 years of age, had prior treatment of their tumor or history of other solid tumors. Controls were patients with cirrhosis seen during the same period as the cases at these centers. The presence of cirrhosis was defined by histology or non-histologically by evidence of portal hypertension in the presence of chronic liver disease. Evidence of portal hypertension included: 1) a cirrhotic-appearing liver on ultrasound, CT or MRI examinations with splenomegaly and no vascular thrombosis, 2) thrombocytopenia with a platelet count $< 120 \text{ mm}^{-3}$, and/or 3) presence of esophagogastric varices on endoscopic examination. Controls must have an ultrasound, CT or MRI showing no evidence of hepatic mass within 6 months prior to enrollment. Patients with an elevated AFP ($> 20 \text{ ng/mL}$) at enrollment were required to have a CT or MRI showing no mass suggestive of HCC at least 3 months prior to enrollment.

or up to 2 weeks after consent. To assure that controls did not have HCC, all controls were assessed by AFP and an imaging test (ultrasound, CT or MRI) 6 months after enrollment. Controls were excluded if there was clinical evidence of significant hepatic decompensation (refractory ascites, grade 3–4 encephalopathy, or hepatorenal syndrome), Child class C or MELD score > 15, detection of HCC at initial evaluation or at follow up, need for long-term immunosuppressive therapy, prior solid organ transplant, previous or current cancer history (excluding non-melanoma skin cancer), and significant medical comorbidities in which survival was predicted to be less than a year.

Consecutive patients with HCC (cases) and cirrhosis (controls) who met eligibility criteria were enrolled at each site. The following data elements were obtained: demographics (age, gender, ethnicity, race, weight, height), medical history, etiology of liver disease, family history, history of alcohol and tobacco exposure, medication use, laboratory data including AFP, and imaging results (ultrasound, CT or MRI). For controls, AFP, imaging results, survival status (dead or alive) and cause of death (if applicable) were obtained 6 months after enrollment. Etiology of liver disease was based on the judgment of the treating physician. For patients with viral hepatitis, anti-HCV antibody, serum HCV RNA, hepatitis B (HBV) surface antigen, HBV e antigen and HBV DNA levels were recorded.

Serum Sample Collection, Storage and Assays

Peripheral blood was collected from each participant at the time of the office visit prior to treatment. Sera were stored at -80°C . All aliquots were shipped to a centralized storage facility at the University of Michigan. One aliquot was sent to a centralized laboratory at the University of California, Los Angeles, where the DCP, AFP, and AFP-L3 assays were performed blinded to clinical data and identifiers. Sera from 10% of the participants were assayed at a different facility for quality control purposes. DCP was measured using a sandwich ELISA (Eisai Co, Japan) as previously done (14). AFP and AFP-L3 were simultaneously determined in serum by automated systems (Wako USA). All samples were performed in duplicates. Samples with AFP value exceeding 1000 ng/ml (upper limit of standard curve), were diluted 10, 100, and 1000-fold and re-measured. Samples from 554 (65%) patients had total AFP values of < 10 ng/mL with non-detectable (ND) AFP-L3%. For this analysis, ND values were assigned a value of 0.5% which is the lower limit of detection. Another 66 (8%) samples had AFP-L3% values that were non-reportable (NR), the results of these samples were non-reliable because the total AFP was between 10 ng/mL and 20 ng/mL. For this analysis, NR values were set as missing data.

Statistical Analysis

The study was designed to have above 90% power at one-sided 5% type-I error for comparing the joint sensitivity and specificity for differentiating early stage HCC from cirrhotic patients between DCP and AFP at current clinical cutoff points of 20 ng/mL for AFP, 10% for AFP-L3 and 150 mAU/mL for DCP. Sample sizes of 200 for early stage HCC and 400 for cirrhotics were required to achieve the stated power. We used Chi-Squared test to compare the distributions of demographic and clinical variables among disease groups (cirrhosis, early HCC, late HCC) for discrete variables and T-test for continuous variables. The sensitivity and specificity and their 95% confidence intervals were calculated for AFP, AFP-L3 and DCP. The differences of sensitivities and specificities for differentiating early stage HCC and cirrhotic controls between DCP, AFP-L3 and AFP and their 95% confidence intervals were calculated using 1,000 bootstrap samples. Since the optimal cutoffs for DCP, AFP-L3 and AFP for early stage HCC were not available, we calculated new optimal cutoffs in two ways. One cutoff aimed to maximize the sum of sensitivity and specificity and another cutoff corresponded to 95% sensitivity for detecting early HCC. To summarize test performance on the whole range of thresholds, Receiver Operating Characteristic (ROC) curve was plotted for each biomarker

test. Area under the ROC curve (AUC) was calculated and its 95% confidence interval was calculated via 1,000 bootstrap samples. The complementary property of DCP to AFP for the diagnosis of HCC was illustrated by comparing ROC curves using the linear score of a logistic regression with DCP and AFP in the model to that with only AFP in the model. Similar analyses were performed for AFP-L3 and all analyses were repeated after stratifying for viral etiology (viral or non-viral).

RESULTS

Patient Characteristics

A total of 859 patients were approached. Five cases and 18 controls were excluded due to the sample not being collected according to protocol or because they did not meet eligibility criteria. A total of 836 patients were included in this study, of which 417 (50%) were cirrhosis controls without HCC and 419 (50%) were HCC cases. Of the cases, 208 (49.6%) were considered Early stage HCC: BCLC stage A (n=131, 63%) and BCLC stage 0 (n=77, 37%). The characteristics of these patients are shown in Table 1. The cirrhosis controls were younger than those with early HCC ($p<0.0001$) and those with intermediate-advanced stage HCC ($p<0.0001$). There was a male predominance in all groups and a predominance of Caucasian ethnicity in cirrhotic controls and HCC cases. The majority of cases and controls had a viral etiology of their liver disease, with HCV in 254 (61%) controls and 215 (51%) HCC cases of which 120 (58%) had early stage HCC (BCLC stage 0 and BCLC stage A). HBV was the underlying etiology of liver disease in 22 (5%) cirrhosis controls and 66 (16%) HCC cases of which 33 (16%) were early stage (BCLC stage 0 and BCLC stage A).

Biomarker Levels

Serum levels of total AFP, DCP and AFP-L3 were significantly elevated in early stage HCC (BCLC stage 0 and BCLC stage A) when compared to controls (Figure 1). The geometric mean AFP level for Early stage HCC was greater than in cirrhotic controls (37.3 vs. 3.6 ng/mL, $p<0.001$). The geometric mean DCP level for Early stage HCC was greater than in cirrhosis controls (303.8 vs. 86.2 mAU/mL, $p<0.001$). Finally, the geometric mean AFP-L3% level for patients with Early HCC were also greater than cirrhotic controls (2.0% vs. 0.6%, $p<0.001$).

Area under the ROC Curves

When all patients with HCC were evaluated, the AUC for total AFP (0.83, 95%CI: 0.80–0.85) was similar to that for DCP (0.81, 95%CI: 0.78–0.84), but higher than AFP-L3% (0.72, 95%CI: 0.69–0.75). However, when only Early stage HCC (BCLC stage 0 and BCLC stage A) was compared to cirrhosis controls, AFP had the best AUC (0.80, 95%CI: 0.77–0.84) followed by DCP (0.72, 95%CI: 0.68–0.77) and then AFP-L3% (0.66, 95%CI: 0.62–0.70) (AFP vs. DCP: $p=0.006$; DCP vs. AFP-L3: $p=0.014$; AFP vs. AFP-L3: $p<0.0001$) as shown in Figure 2. When intermediate-advanced stage HCC was compared to cirrhotic controls, DCP had the highest AUC (0.89, 95%CI: 0.86–0.92) compared to total AFP (0.84, 95%CI: 0.81–0.88) ($p=0.01$), indicating that DCP was more predictive of late stages HCC than of early stage HCC.

Performance of Biomarkers

As shown in Table 2, when using the currently recommended clinical cutoffs for AFP (20 ng/mL), DCP (150 mAU/mL) and AFP-L3 (10%), DCP had the best performance with a sensitivity of 61% for Early stage HCC (BCLC stage 0 and BCLC stage A). However, as shown in Table 3, when the cutoffs were determined for the point in the ROC curve that maximizes sensitivity + specificity, AFP (cutoff of 10.9 ng/mL) had the best performance for Early stage HCC with a sensitivity of 66% and specificity of 82%.

When AFP, AFP-L3 and DCP were combined in a logistic regression model (after log10 transformation), AFP (OR 4.2, 95% CI: 3.0–5.9) and DCP (OR 3.0, 95% CI: 2.1–4.2) were independent markers of Early HCC (BCLC stage 0 and BCLC stage A), while AFP-L3% did not contribute significantly (OR 1.1, 95% CI: 0.8–1.7); consequently AFP-L3% was not included in further analysis of the combination of the markers. As shown in Figure 2, the AUC for the combination of AFP and DCP (either marker elevated) mildly improved to 0.83 (95% CI: 0.79–0.86) from 0.80 for AFP alone and 0.72 for DCP alone. Figure 3 shows a scatter plot of AFP and DCP for Early stage HCC (BCLC stage 0 and BCLC stage A) and all cirrhosis controls; it is evident that the two markers do not completely overlap and in cases of Early stage HCC with low AFP levels, DCP can add to the diagnosis. Table 3 shows that the combination of AFP and DCP improves the sensitivity for Early stage HCC (BCLC stage 0 and BCLC stage A) to 70% when the cutoffs that maximize sensitivity+specificity were utilized, but was not statistically significant to the single makers.

We evaluated the performance of AFP, DCP and AFP-L3 in only those at the BCLC stage A (Table 3). The AUC for AFP was 0.82 (95% CI: 0.77–0.86) compared to 0.76 for DCP (95% CI: 0.71–0.80) and 0.66 for AFP-L3 (95% CI: 0.63–0.72). AFP had a significantly better AUC compared to DCP ($p=0.03$) and AFP-L3 ($p<0.0001$), and DCP was significantly better than AFP-L3 ($p=0.02$).

Lastly, we evaluated the performance of AFP, DCP and AFP-L3 in those with BCLC stage 0 ($n=77$). The AUC for AFP was 0.78 (95% CI: 0.72–0.85) compared to 0.67 for DCP (95% CI: 0.60–0.73) and 0.63 for AFP-L3 (95% CI: 0.57–0.68). As shown in Table 3, AFP had a significantly better AUC compared to DCP ($p=0.01$) and AFP-L3 ($p<0.0001$), while there was no difference between DCP and AFP-L3 ($p=0.33$). AFP seemed to be a better marker for very early stage HCC at a cutoff of 10.9 ng/mL.

Confounders

We evaluated whether several factors affected the performance of AFP, DCP and AFP-L3% by examining the statistical significance of each factor (e.g. gender) by marker (e.g. AFP) with an interaction term in a logistic regression model. Gender ($p=0.4$ for AFP, $p=0.2$ for DCP and $p=0.6$ for AFP-L3), race ($p=0.8$ for AFP, $p=0.8$ for DCP and $p=0.35$ for AFP-L3) and age ($p=0.9$ for AFP, $p=0.1$ for DCP and $p=0.8$ for AFP-L3) were not significant factors. However, etiology appeared to be significant for DCP but not for the other markers ($p=0.3$ for AFP, $p=0.04$ for DCP and $p=0.2$ for AFP-L3). Figure 3 shows a scatter plot of AFP and DCP according to viral etiology, and DCP adds to the diagnosis of HCC among those with low AFP level.

We then evaluated the performance of the markers comparing viral (Early stage HCC = 156 and cirrhosis controls=278) versus non-viral etiology (Early stage HCC = 52 and cirrhosis controls = 154). As shown in Figure 4, there were significant differences in the AUC among the viral and non viral etiology for early stage HCC (BCLC stage 0 and BCLC stage A).

For those with viral etiology, the AUC for AFP, DCP and AFP-L3% was 0.78 (95% CI: 0.74–0.83), 0.76 (95% CI: 0.71–0.81) and 0.64 (95% CI: 0.60–0.68), respectively for Early stage HCC. In contrast for non-viral etiology, the AUC for AFP, DCP and AFP-L3% was 0.81 (95% CI: 0.74–0.88), 0.65 (95% CI: 0.56–0.73) and 0.70 (95% CI: 0.63–0.78), respectively. The AUC for AFP was similar for viral and non-viral etiology, while DCP had a better AUC in patients with viral etiology. When comparing Early stage HCC ($n=156$) and cirrhotic controls ($n=278$) with viral etiology, the combined markers had a 78% sensitivity but it was not statistically better than the markers alone (Table 3). The performance of AFP or the combination of AFP and DCP did not differ much in those with non-viral etiology.

DISCUSSION

In the largest study of biomarkers for the diagnosis of early stage HCC, we showed that AFP had a sensitivity of 66% and specificity of 81%, at a new cutoff of 10.9 ng/mL. AFP had the best AUC in tumors at the BCLC stage 0 and BCLC state A when compared to other markers. Importantly, AFP had the best performance of all the markers for BCLC stage 0 HCC, the detection of which is the main goal of a surveillance program.

AFP has been the most widely utilized serologic test for HCC. A previous case control study involving 170 patients with HCC, only 68 with early HCC, and 170 matched cirrhosis controls showed that the cutoff of 20 ng/mL maximizes the sensitivity and specificity (23). However, the sensitivity of AFP at this cutoff has ranged from 41–65% with specificity from 80–94% in multiple prior studies performed (24). Our study eliminated a large portion of the pitfalls and limitations from previous studies. Our study accounted for verification bias, as we utilized histology and imaging as diagnosis of HCC based on the latest definition of HCC and AFP was not used as the definition of a case, there was appropriate blinding of operator when assays were performed, appropriate controls were used with 6 month post-enrollment follow up to eliminate false negatives, and an appropriately powered multicenter study design. The goal of a surveillance program is the detection of early stage tumor, in particular very early stage tumor, where it allows curative intervention to improve outcomes, and our study shows that AFP has the best performance in those with BCLC stage 0 and BCLC stage A.

Our current study shows that the performance of DCP, but not that of AFP, is significantly affected by the etiology of liver disease for the diagnosis of early stage HCC. For the diagnosis of early stage HCC, AFP-L3 is not useful and not recommended likely due to the need for an elevated total AFP thereby, limiting its effectiveness. The sensitivity of DCP in patients with viral etiology is 79% for early stage HCC, whereas the combination of AFP and DCP in viral etiology was not better than each of the serum markers alone (Table 3). For non-viral etiology AFP performed better than the other markers alone and adding other markers to it improved little. DCP appears to perform better in those with viral etiology but the combination of AFP and DCP did not improve the overall performance.

Our study determined a lower cut point for AFP that would improve the early detection of this tumor while maintaining an adequate specificity of 82%. Our data suggest that for cirrhotic patients with viral etiology, an AFP > 10.9 ng/mL should trigger a CT/MRI for the diagnosis of early stage HCC. The specificity of this cutoff would be 82% alone and 80% in combination with DCP, therefore, less than 20% patients would be getting cross-sectional imaging to evaluate for HCC. While these sensitivities and specificities are reasonable, they are not optimal for HCC surveillance as the costs will be prohibitive if 20% of cirrhotic patients have to undergo repeated CT or MRI to rule out the diagnosis of HCC. This suggested new cutoff needs to be tested in large phase 3 biomarker studies (retrospective evaluation of prospectively collected specimens from cohorts of patients with cirrhosis to determine the ability of a biomarker to diagnose pre-clinical disease) to determine its applicability in HCC surveillance.

Ultrasound (US) of the liver has been recommended in the surveillance for HCC based on cohort studies (4). US have been reported to have a sensitivity ranging from 60% to 80% in patients with HCC (24). The drawbacks to US screening among patients with cirrhosis is that it is operator dependent, its performance may be affected by body habitus, the nodularity in cirrhosis of the liver may lead to difficulty in interpretation, agreement among radiologists has not been studied, and it is not widely available. In a large randomized trial among hepatitis B carriers in China, AFP was the sole test utilized for surveillance of HCC. The sensitivity and specificity was 80.0% and 80.9%, respectively. A third (29.6%) of the patients had stage I HCC (25). In another large randomized study of hepatitis B carriers, HCC surveillance with AFP

and US was shown to decrease mortality (26). These 2 randomized studies indicate that AFP is an important part of the surveillance strategy of hepatitis B patients at risk for HCC. These results cannot be generalized to patients with cirrhosis, in whom systematic reviews overwhelmingly showed that the performance of US is poor (10,12). We did not include US in our biomarker study because it was utilized in the definition of HCC, potentially leading to verification bias, and our goal was to determine the best serum biomarker for HCC. An EDNRN-defined Phase 3 study, prospective cirrhotic cohort to identify preclinical HCC, would be the best design to address the performance of US (with or without serum biomarkers).

Our current study has some limitations. It is a case-control study. A Phase 3 biomarker study is needed to determine whether biomarkers can detect preclinical HCC in a prospective manner or its performance in those with dysplastic nodules. More importantly, the prospective specimen and data collection in a Phase 3 study will eliminate potential biases because HCC status will be unknown at the time of specimen and data collection. Another limitation is that the patients enrolled were receiving attention at tertiary medical centers, and our results may not be generalizable to all patients with cirrhosis and HCC. Nevertheless, this is large multicenter study that was adequately powered, etiology of liver disease was accounted for (viral vs. non-viral), assays were performed blindly, and all geographic regions in the United States were accounted for. In addition, it provides the benchmark for studying the performance of biomarkers for the diagnosis of early HCC.

In summary, our study shows that AFP is currently the best serum biomarker for the diagnosis of early stage HCC at a new cutoff of 10.9 ng/mL. DCP performs better in those with viral etiology of liver disease. Our study helps determine the performance of AFP and DCP for early stage HCC, however these results are not optimal by themselves as surveillance tests and either new or complimentary markers are needed. An EDNRN-defined Phase 3 study is needed to evaluate the performance of AFP, at its new cutoff of 10.9 ng/mL, in the detection of preclinical HCC in combination with US, DCP or other new markers.

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Abbreviations

AFP	alpha-fetoprotein
DCP	des-gamma carboxyprothrombin
AFP-L3	lectin-bound alpha-fetoprotein
HCC	Hepatocellular carcinoma

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Figure 1.

Serum levels of alpha-fetoprotein (AFP), des-gamma carboxyprothrombin (DCP) and lectin-bound AFP among those with cirrhosis, early stage Hepatocellular carcinoma (HCC) and late stage of HCC (the latter defined as Intermediate and Advanced stages based on the Barcelona staging classification). The box refers to the 25th to and 75th percentile values with a line indicating the median levels, while the interquartile range extend outside the box. Points outside the interquartile range are outliers. Median levels are shown for each marker at the bottom of each. Both early and late stage HCC had higher levels ($p < 0.0001$) compared to cirrhosis. Late stage HCC had higher levels compared to early stage HCC ($p < 0.05$).

Figure 2.

Receiver operating characteristics (ROC) curve evaluating those with early stage HCC (n=208) and cirrhosis controls (n=417). The area under the ROC curve is shown with its 95% confidence intervals. DCP is black, AFP is red, AFP-L3 is blue and combination of AFP and DCP is pink.

Figure 3. Scattered plot for alpha-fetoprotein (AFP) and des-gamma carboxyprothrombin (DCP) according viral and non-viral etiology. Red = early stage HCC, green = cirrhosis controls.

Figure 4.

Receiver operating characteristics (ROC) curve according to etiology of liver disease in those with early stage HCC (n=208) and cirrhosis controls (n=417). Panel A shows those with viral etiology and Panel B shows those with non-viral etiology. The area under the ROC curve is shown with its 95% confidence intervals. DCP is black, AFP is red, AFP-L3 is blue and combination of AFP and DCP is pink.

Table 1

Characteristics of patients enrolled in the study

Variable	Cirrhosis Controls (n=417)	Early HCC (n=208)	Late HCC (n=211)	P value*
Age, mean (SD), y	55 (8.8)	60 (9.9)	61 (10.3)	<0.0001
Gender (M:F) (%)	69.5:30.5	72.5:27.5	86.8:13.2	<0.0001
Race (%)				<0.0001
C	80	57	71.4	
AA	4.1	14	6.3	
As	7.3	19.5	19.9	
Other	8.5	9.5	2.4	
Ethnicity (%)	12.7	15	5.2	0.003
Hispanic				
Etiology (%)				
Viral:Non-Viral	65:35	74:26	61:39	0.003
HCV	60	58	45	0.001
HBV	5	16	16	
Alcohol	12	11	9	
Cryptogenic	13	5	18	
Others	10	10	12	
Child Class (%)				0.0001
A	55.9	70.4	66.5	
B	44.1	27.7	29.2	
C	0	1.9	4.2	
Maximum Tumor Diameter, mean (SD), cm	NA	2.9 (1.0)	7.0 (3.8)	<0.0001
No. of Lesions, mean (SD)	NA	1.3 (0.6)	2.3 (1.5)	<0.0001
1 (%)		79.2	41	<0.0001
2 (%)		12.1	24.6	
3 (%)		8.7	11.4	
4 (%)		0	9.5	
≥5		0	13.2	
Portal Vein Thrombosis (%)	NA	0	24.5	<0.0001
Metastasis (%)	NA	0	13.2	<0.0001

Variable	Cirrhosis Controls (n=417)	Early HCC (n=208)	Late HCC (n=211)	P value [*]
BCLC Stage n (%)	NA			
Very early (0)		77 (37)	0	<0.0001
Early (A)		131 (63)	0	
Intermediate (B)		0	130 (62)	
Advanced (C)		0	81 (38)	
Terminal (D)		0	0	

HCC=Hepatocellular carcinoma. C=Caucasian, AA=African American, As=Asian. MELD= model for endstage liver disease.

* p value comparing controls vs. early stage HCC except tumor size, number of lesions, portal vein thrombosis and metastasis, all denoting early vs. late HCC.

NA = not applicable. BCLC= Barcelona Clinic Liver Cancer stage. HCV = hepatitis C, HBV = hepatitis B.

Table 2

The sensitivity and specificity of AFP, DCP and AFP-L3% using the current clinical cutoffs.

Marker	Cutoff	Sensitivity % (95%CI)	Specificity % (95%CI)
All HCC (n=419)			
AFP	20	59 (55–64)	90 (86–93)
DCP	150	74 (70–79)	70 (65–74)
AFP-L3	10	42 (37–47)	97 (93–100)
AFP+DCP	AFP=20 or DCP=150	86 (82–89)	63 (58–67)
Early Stage HCC (n=208)*			
AFP	20	53 (46–59)	90 (87–93)
DCP	150	61 (55–68)	70 (65–74)
AFP-L3	10	28 (22–34)	97 (93–100)
AFP+DCP	AFP=20 or DCP=150	78 (72–83)	62 (58–67)

DCP= des-gamma carboxyprothrombin in mAU/mL, AFP= alpha-fetoprotein in ng/mL, AFP-L3 = % of AFP that is fucosylated. CI= confidence intervals.

* Very early (BCLC stage 0) + early stage (BCLC stage A) HCC based on Barcelona staging classification.

Table 3
Cutoffs for AFP, DCP, and AFP-L3 at the maximum sensitivity+specificity in the receiver operating characteristic curve.

Marker	Cutoff	Sensitivity % (95 CI)	Specificity % (95 CI)
All HCC (n=419)			
AFP	10.3	70 (56–77)	82 (76–94)
DCP	298.5	66 (53–75)	82 (73–94)
AFP-L3	1.7	50 (44–55)	94 (92–97)
AFP+DCP	AFP=27 or DCP=619	74 (68–81)	87 (80–92)
Early HCC* (n=208)			
AFP	10.9	66 (56–77)	82 (71–90)
DCP	221.5	56 (47–87)	77 (46–86)
AFP-L3	1.7	37 (31–45)	94 (91–96)
AFP+DCP	AFP=11 or DCP=598	70 (62–82)	80 (69–88)
BCLC stage A (n=131)			
AFP	10.9	67 (58–75)	82 (78–85)
DCP	221.5	62 (53–69)	77 (73–81)
AFP-L3	1.7	41 (32–49)	94 (92–96)
AFP+DCP	AFP=11 or DCP=598	77 (69–84)	76 (72–80)
BCLC Stage 0 (n=77)			
AFP	10.9	65 (55–75)	82 (78–85)
DCP	221.5	47 (36–57)	77 (72–81)
AFP-L3	1.7	32 (20–43)	94 (92–96)
AFP+DCP	AFP=11 or DCP=598	65 (54–75)	76 (72–80)
Viral Etiology and Early HCC* (n=156)			
AFP	14.1	67 (54–77)	79 (71–91)
DCP	69.5	79 (46–88)	60 (53–92)
AFP-L3	1.7	35 (26–43)	93 (89–96)
AFP+DCP	AFP=16 or DCP=330	78 (64–86)	74 (67–88)
Non-Viral Etiology and Early HCC* (n=52)			
AFP	5.5	65 (48–81)	87 (78–99)
DCP	124	69 (33–90)	56 (37–91)
AFP-L3	2.5	45 (30–59)	97 (93–99)
AFP+DCP	AFP=6 or DCP=877	67 (54–90)	83 (62–95)

DCP= des-gamma carboxyprothrombin in mAU/mL, AFP= alpha-fetoprotein in ng/mL, AFP-L3 = % of AFP that is fucosylated.

* A combination of very early (BCLC stage 0) + early stage (BCLC stage A) HCC based on Barcelona staging classification.