



AFP-L3  
& DCP

# A Bibliography for HCC Biomarkers: AFP-L3 and DCP

## BACKGROUND

Alpha-fetoprotein (AFP) has been used as a biomarker in surveillance for hepatocellular carcinoma (HCC) along with ultrasound. Several studies have shown that lectin-reactive AFP (AFP-L3) and des-gamma-carboxy prothrombin (DCP) are aids in the clinical assessment for early detection and that combined use of both markers should be performed for HCC early detection. Conventional AFP-L3 assays do not give accurate results in patients having AFP levels less than 20 ng/mL. A newly developed immunoassay system,  $\mu$ TASWako i30, shows improved analytical sensitivity for AFP-L3 for this patient population. Therefore, published  $\mu$ TASWako i30 studies have focused on clinical utility of AFP-L3 in patients with AFP less than 20 ng/mL.

## RECENT STUDIES

The titles listed below were published between 2009 and September 2011. All clinical studies [1-7] were performed using the immunoassay system  $\mu$ TASWako i30. Reference [8] provides technical information about the system.

### **(1) Highly sensitive AFP-L3% assay is useful for predicting recurrence of hepatocellular carcinoma after curative treatment pre- and postoperatively. Kobayashi M, et al. Hepatol Res. 2011 Aug 26.**

**Aim:** The micro-total analysis system ( $\mu$ TAS), a fully automated immunoassay system using microchip capillary electrophoresis, is highly sensitive and able to quickly assay the AFP-L3%. The clinical usefulness of this system was studied. **Methods:** We retrospectively enrolled 250 patients who underwent curative treatment for primary hepatocellular carcinoma (HCC) (93 patients underwent hepatic resection and 157, radiofrequency ablation [RFA]). **Results:** The sensitivity for  $\mu$ TAS AFP-L3% was 40.3% at the cutoff value of 5% in a range of AFP less than 20 ng/mL where the conventional method was unable to determine AFP-L3%. The sensitivity for AFP-L3% remained high even at stage I and at tumor size less than 2 cm (42.5% and 46.0%, respectively). Recurrence rate of patients with AFP-L3% greater than 5% was significantly higher than that of patients with less than 5% ( $P = 0.001$ ). Furthermore, in resected patients, the postoperative AFP-L3% remained elevated with value greater than 5% was related to HCC recurrence ( $P = 0.001$ ). Multivariate analysis revealed that multiple tumors ( $P = 0.004$ ), preoperative AFP-L3% greater than 5% ( $P = 0.003$ ), albumin less than 3.5 g/dL ( $P = 0.008$ ), and RFA ( $P = 0.003$ ) were significant prognostic factors of recurrence. **Conclusions:** The  $\mu$ TAS was found to be a highly sensitive assay for AFP-L3% in patients with curative treatment of HCC. A cutoff value of 5% was useful for predicting recurrence after the curative treatment and detecting small tumors and early stage HCC. Additionally, postoperative AFP-L3% was found to be a prognostic factor of HCC recurrence.

**(2) Highly sensitive lens culinaris agglutinin-reactive  $\alpha$ -fetoprotein is useful for early detection of hepatocellular carcinoma in patients with chronic liver disease. Oda K, et al. *Oncol Rep.* 2011;26:1227-33.**

The fucosylated fraction of  $\alpha$ -fetoprotein (AFP-L3) is a specific marker for hepatocellular carcinoma (HCC). However, conventional AFP-L3% (c-AFP-L3%) has not always been reliable in cases with low serum  $\alpha$ -fetoprotein (AFP) levels. In this study, we evaluated the clinical utility of a newly developed assay, highly sensitive AFP-L3% (hs-AFP-L3%). Subjects included 74 patients with benign liver disease (BLD), including chronic hepatitis and cirrhosis, and 94 with HCC. Serum hs-AFP-L3% was significantly higher than c-AFP-L3% in patients with early-stage HCC (solitary or <20 mm in diameter). Additionally, hs-AFP-L3% was significantly increased in patients with well-differentiated HCC. In patients with serum AFP <20 ng/ml, the sensitivities of c-AFP-L3% and hs-AFP-L3% were 12.5 and 44.6%, respectively, at a cut-off value of 5%. In 59 BLD patients with serum AFP <20 ng/ml, the HCC-positive rate in patients with hs-AFP-L3%  $\geq$ 5% was significantly higher compared to those with hs-AFP-L3% <5% during the follow-up period (median, 35 months; range, 5-48 months). Importantly, none of the BLD patients with both serum AFP <20 ng/ml and hs-AFP-L3% <5% developed HCC. These results indicated that hs-AFP-L3% is useful for early detection of HCC in BLD patients, even for those with serum AFP <20 ng/ml. Furthermore, since hs-AFP-L3% increases before HCC is detectable by various advanced imaging modalities, this assay may help identify BLD patients with a higher risk of HCC.

**(3) Novel Lens culinaris agglutinin-reactive fraction of  $\alpha$ -fetoprotein: a biomarker of hepatocellular carcinoma recurrence in patients with low  $\alpha$ -fetoprotein concentrations. Morimoto M, et al. *Int J Clin Oncol.* 2011 Aug 17.**

Background: *Lens culinaris* agglutinin-reactive fraction of  $\alpha$ -fetoprotein (AFP-L3) is a specific marker used to detect hepatocellular carcinoma (HCC). However, its clinical utility is not sufficient in patients with low total AFP concentrations because of limitations in instrument sensitivity. Recent advances have led to the introduction of a highly sensitive AFP-L3% assay (sensitive AFP-L3%), provided by a novel on-chip, liquid-phase binding assay. This cross-sectional study was conducted to evaluate the clinical significance of the sensitive AFP-L3% in determining HCC recurrence in patients with low total AFP concentrations. Methods: A total of 370 consecutive patients with HCC were screened within 1-3 months of locoregional treatment, and 215 of the 370 patients showed serum AFP <20 ng/ml. Total AFP, sensitive AFP-L3%, and des-gamma-carboxy prothrombin (DCP) were measured in those 215 patients and HCC recurrence was evaluated by radiological findings. Optimal cutoff values of the markers for detecting HCC recurrence were obtained on the basis of receiver operating characteristic (ROC) curve. Results: The area under the ROC curve of the total AFP, sensitive AFP-L3%, and DCP in HCC patients with serum AFP <20 ng/ml were 0.638, 0.724, and 0.779, respectively. The diagnostic accuracies of the total AFP, sensitive AFP-L3%, and DCP were 60.9% (cutoff value 5 ng/ml), 67.7% (cutoff value 7%), and 64.6% (cutoff value 40 ng/ml), respectively. Conclusions: The new sensitive AFP-L3% assay provides great utility in determining HCC recurrence in patients with low AFP concentrations. Further studies focusing on the combinatorial use of the markers (total AFP, sensitive AFP-L3%, and DCP) are required.

**(4) Prognostic importance of fucosylated alpha-fetoprotein in hepatocellular carcinoma patients with low alpha-fetoprotein. Nouse K, et al. *J Gastroenterol Hepatol.* 2011;26:1195-200.**

Background and Aim: Fucosylated alpha-fetoprotein (AFP-L3) is known to be a marker of poor prognosis in patients with hepatocellular carcinoma (HCC). However, it has been difficult to measure AFP-L3 under low AFP ( $\leq$  20 ng/mL). The aim of this study was to elucidate the role of AFP-L3 in HCC patients with low AFP conditions. Methods: One hundred and ninety six consecutive newly developed HCC patients with low AFP ( $\leq$  20 ng/mL) were examined for serum AFP-L3 expression by a newly-developed micro-total analysis system that could stably measure AFP-L3 in low AFP circumstances, and its clinical importance was analyzed. Results: Positivity of AFP-L3 in HCC patients was 13.3% at a cut-off level of 10%. Five-year survivals of HCC patients with AFP-L3 (< 10%) and AFP-L3 ( $\geq$  10%) were 69.4% and 41.1%, respectively ( $P = 0.001$ ). Among 18 clinical parameters, low alanine aminotransferase, large tumor size, presence of portal vein tumor thrombus, high AFP and high des-gamma carboxy prothrombin were observed in the high AFP-L3 ( $\geq$  10%) group. Multivariate analysis revealed that high aspartate aminotransferase (AST) (risk ratio [RR]= 3.24, 95% confidence interval [CI] =1.27-8.26), the presence of ascites (RR = 3.44, 95% CI = 1.22-9.34), multiple tumor number (RR= 3.06, 95% CI = 1.33-7.17), and high AFP-L3 (RR = 8.36, 95% CI= 2.79-25.5) were risk factors for survival. High AFP-L3 was also a risk factor for survival in HCC patients who received radiofrequency ablation ( $P = 0.048$ ). Conclusions: AFP-L3 is a strong prognostic factor for survival even in HCC patients with low AFP ( $\leq$  20 ng/mL).

**(5) Clinical utility of highly sensitive Lens culinaris agglutinin-reactive alpha-fetoprotein in hepatocellular carcinoma patients with alpha-fetoprotein <20 ng/mL. Toyoda H, et al. Cancer Sci. 2011;102:1025-31.**

The *Lens culinaris* agglutinin-reactive fraction of alpha-fetoprotein (AFP-L3) has been used as a diagnostic and prognostic marker of hepatocellular carcinoma (HCC). The analytical sensitivity of a conventional method for AFP-L3% is not sufficient in patients with a low AFP level. This study was performed to determine the clinical utility of a newly developed highly sensitive AFP-L3% (hs-AFP-L3%) assay in patients with an AFP level <20 ng/mL. In the cohort study, serum samples obtained from 270 patients with newly diagnosed HCC before treatment and 396 patients with chronic liver disease at Ogaki Municipal Hospital, in both of which the AFP level was <20 ng/mL, were measured for conventional AFP-L3% (c-AFP-L3%), hs-AFP-L3% and des-gamma-carboxy prothrombin (DCP). Diagnostic sensitivity and specificity of hs-AFP-L3% at a cut-off level of 5% were 41.5% and 85.1%, respectively, significantly increasing the sensitivity from 7.0% for c-AFP-L3%. Multivariate analysis identified hs-AFP-L3% as an independent factor associated with reduced long-term survival. The survival rate of patients with high hs-AFP-L3% ( $\geq 5\%$ ) before treatment was significantly poorer than that of patients with low hs-AFP-L3% ( $<5\%$ ) ( $P < 0.001$ ). In patients with AFP <20 ng/mL, measurements of AFP-L3% by the highly sensitive method before treatment were more useful for diagnosis and prognosis of HCC than by the conventional method.

**(6) Clinical significance of the highly sensitive fucosylated fraction of  $\alpha$ -fetoprotein in patients with chronic liver disease. Hanaoka T, et al. J Gastroenterol Hepatol. 2011;26:739-44.**

Background and Aim: The purpose of the present study was to investigate the clinical significance of the highly sensitive fucosylated fraction of  $\alpha$ -fetoprotein (hs-AFP-L3) in patients with chronic liver disease (CLD) and low serum  $\alpha$ -fetoprotein (AFP) concentration. Methods: A total of 241 patients being treated at our institute with CLD and low serum AFP concentration (3-10 ng/mL) were investigated retrospectively. We measured total AFP and the percentage of AFP-L3 using a  $\mu$ TAS Wako i30 device. The possible presence of hepatocellular carcinoma (HCC) was thoroughly investigated by various examinations carried out from 1 month before to 1 month after measurements. In addition, hs-AFP-L3 elevated and non-elevated groups, divided by the cut-off value based on a receiver-operator characteristic (ROC) curve, were followed for possible future development of HCC. Results: hs-AFP-L3 was above the detectable range in 60 patients (24.9%). Among those AFP-L3 positive cases, 20 (33.3%) were found to be HCC prevalent, whereas HCC was found in just 16 patients (8.8%) with undetectable hs-AFP-L3 levels. We determined the cut-off value of hs-AFP-L3%, which shows the proportion of AFP L3 in total AFP, to be 5.75%. During the follow-up period, HCC was newly detected in six patients (22.2%) in the hs-AFP-L3% elevated group and in 10 (5.6%) in the non-elevated group. Analysis using the Kaplan-Meier method showed the HCC-free rate of the hs-AFP-L3% elevated group was significantly lower than that of the non-elevated group ( $P=0.0038$ ). Independent predicting variants were female sex ( $P=0.0024$ ) and hs-AFP-L3% elevation ( $P=0.0036$ ). Conclusion: Our results suggest hs-AFP-L3 level is a useful tumor marker for HCC in patients with CLD and low serum AFP concentration.

**(7) Clinical advantage of highly sensitive on-chip immunoassay for fucosylated fraction of alpha-fetoprotein in patients with hepatocellular carcinoma. Tamura Y, et al. Dig Dis Sci. 2010;55:3576-83.**

Background: Alpha-fetoprotein (AFP) has been widely used as a diagnostic master for hepatocellular carcinoma (HCC), and the fucosylated fraction of AFP (AFP-L3) has been reported to be a specific marker for HCC. However, AFP-L3 has not always been reliable in cases with low serum AFP concentrations. Recently, a novel automated immunoassay for AFP-L3, the micro-total analysis system ( $\mu$ -TAS), has been developed. Aim: The aim of this study is to evaluate the clinical usefulness of  $\mu$ -TAS AFP-L3. Methods: Serum AFP-L3 was measured in 295 patients with HCC and in 350 with benign liver diseases. The diagnostic accuracy of  $\mu$ -TAS AFP-L3 was compared with that of the conventional assay (liquid-phase binding assay; LiBASys). The relationship between  $\mu$ -TAS AFP-L3 and clinical features was investigated. Results: When the cutoff value was set at 7%, the sensitivity, specificity, accuracy, positive predictive value, and negative predictive value of  $\mu$ -TAS AFP-L3 were 60.0%, 90.3%, 76.4%, 83.9%, and 72.8%, respectively. Its sensitivity was particularly good (41.1%) in HCC subgroups with lower AFP concentrations (<20 ng/ml). The positivity rates for  $\mu$ -TAS AFP-L3 were higher at each tumor stage than those of LiBASys AFP-L3 ( $\mu$ -TAS/LiBASys: stage I, 44.2%/16.3%; stage II, 52.9%/37.5%; stage III, 66.4%/44.5%; stage IV, 82.8%/65.5%). Conclusions:  $\mu$ -TAS AFP-L3 is more sensitive for discriminating HCC than the conventional LiBASys AFP-L3, particularly in subgroups with lower AFP concentrations and early-stage HCC.

**(8) Automated immunoassay system for AFP-L3% using on-chip electrokinetic reaction and separation by affinity electrophoresis. Kagebayashi C, et al. Anal Biochem. 2009;388:306-11.**

Implementation of the on-chip immunoassay for alpha-fetoprotein (AFP)-L3% was achieved using a fully automated microfluidic instrument platform that will prepare the chip and run the assay with a total assay time of less than 10min. Reagent/sample mixing, concentration, and reaction in microfluidic channels occur by the electrokinetic analyte transport assay (EATA) technique, enabling the integration of all assay steps on-chip. The determination of AFP-L3%, a biomarker for hepatocellular carcinoma, was achieved by the presence of *Lens culinaris* agglutinin in the separation channel, causing separation of the fucosylated isoform, AFP-L3, from the nonfucosylated AFP-L1 by lectin affinity electrophoresis. Laser-induced-fluorescence (LIF) detection was used to quantitate the labeled immunocomplexes. The limit of detection (LOD) was 0.1 ng/ml AFP, and assay precision of less than 2% coefficient of variation (CV) was obtained for quantitation from 24 to 922ng/ml total AFP in spiked serum samples. Assay precision of less than 3% CV was obtained for AFP-L3% measurements from 8.5 to 81%. Furthermore, good correlation of test results for 68 patient serum samples with a commercially available reference method (LiBASys assay for AFP-L3%) was obtained, with  $r(2)=0.981$  and slope=1.03.