

This is a selection of clinical papers that highlight the clinical use of AFP-L3% in HCC management (January 2009). The summary is directly from the abstract of each paper. Two separate annotated bibliographies for DCP and three HCC marker combination (AFP, AFP-L3, DCP) assay for HCC management are also available.

### Diagnostic usefulness of AFP-L3 for hepatocellular carcinoma

- (1) Clinical utility of AFP-L3% measurement in North American patients with HCV-related cirrhosis. Sterling RK et al., Am J Gastroenterol. 2007 Oct;102(10):2196-205

#### Summary

The aim of this prospective study was to compare the clinical utility of AFP-L3% with that of total AFP in North American patients. Patients with chronic hepatitis (CH) C virus-related cirrhosis from 7 clinical sites were prospectively followed every 3-6 months for 2 yr. Of the 372 patients evaluated, 40 had hepatitis C virus-related HCC at entry and 332 entered the prospective trial. Of the latter, 34 developed HCC and 298 remained free of HCC. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for AFP were 60.8%, 71.1%, 34.4%, and 88.0% with a cutoff of 20 ng/mL and 21.6%, 98.7%, 80.0%, and 83.5% with a cutoff of 200 ng/mL, compared to 36.5%, 91.6%, 51.9%, and 85.3% for AFP-L3% with a cutoff of 10%. In those with an elevated AFP (20-200 ng/mL), AFP-L3% had a specificity of 86.6% and an NPV of 80.7%. Multivariate analysis identified AFP, AFP-L3%, and age as independent predictors of HCC. Elevated AFP-L3% was associated with a lower cumulative HCC-free rate at 2 yr (58.9%) than was AFP (82.0%,  $P=0.01$ ). The incidence of HCC was significantly higher in patients with elevated AFP-L3% than in those with elevated AFP. The high specificity of AFP-L3% persisted among patients with elevated AFP (20-200 ng/mL) and suggests that AFP-L3% has clinical utility in HCV patients with AFP of 20-200 ng/mL.

- (2) The utility of *Lens culinaris* agglutinin-reactive  $\alpha$ -fetoprotein in the diagnosis of hepatocellular carcinoma: Evaluation in a United States referral population. Leerapun A et al., Clin Gastroenterol Hepatol. 2007 Mar;5(3): 394-402.

#### Summary

This retrospective study evaluated for the first time the utility of AFP-L3% for diagnosis of HCC in a referral population of the USA. 272 patients were included: 166 with HCC and 106 with benign liver disease. AFP-L3% was measured in all cases of previously determined AFP between 10 and 200 ng/mL. AFP-L3% greater than 10% had a sensitivity of 71% and a specificity of 63% for diagnosis of HCC. An AFP-L3% greater than 35% had a reduced sensitivity of 33%, but an increased specificity of 100%. The high specificity of the AFP-L3% cut-off of 35% allowed the confident diagnosis of an additional 10% of HCCs not diagnosed using an AFP cut-off of 200 ng/mL. After adjustment for AFP level, no association was observed between AFP-L3% and tumor size, stage, vascular invasion, grade, or survival. As conclusion AFP-L3% greater than 35% has 100% specificity for HCC in these patients. AFP-L3%, used in combination with AFP, may be a clinically useful adjunct marker for the diagnosis of HCC.

- (3) Multicenter prospective analysis of newly diagnosed hepatocellular carcinoma with respect to the percentage of *Lens culinaris* agglutinin-reactive alpha-fetoprotein. Oka H et al., J Gastroenterol Hepatol. 2001 Dec;16(12):1378-83.

#### Summary

In this collaborative study, the percentage of AFP-L3 and serum AFP concentration was evaluated with respect to background factors and tumor characteristics in a large population of newly diagnosed HCC patients. It was deemed to be appropriate to change the cut off level of AFP-L3% from the conventional 15% to 10% for the purpose of discrimination between non-neoplastic liver diseases and HCC. As a conclusion to this study, serum AFP concentrations did not reveal a malignancy of HCC. However, the AFP-L3% positive (>10%) HCC has biologically malignant characteristics, especially portal vein invasion and lower tumor

classification, and is an advanced tumor regardless of small tumor size and lower total serum AFP concentrations. As AFP-L3 shows tumor characteristics, its presence should be an important factor in the determination of therapy and prognosis of patients.

- (4) AFP-L3: A new generation of tumor marker for hepatocellular carcinoma. Li D et al., Clin Chim Acta. 2001 Nov;313(1-2):15-9.

#### Summary

The AFP-L3 assay is a new generation of tumor marker which measures changes in the glycosylation pattern (microheterogeneity) of a protein marker in cancer. AFP-L3 is a glycoform of alpha fetoprotein (AFP) in which in additional alpha 1-6 fucose residue is attached to the reducing end of N-acetylglucosamine on AFP. AFP-L3 appears to be a highly specific marker for liver cancer. Elevation of AFP-L3 does not correlate with changes in concentration of AFP in liver cancer. It has been shown that AFP-L3% is a marker indicative of biological malignancy of liver cancer. AFP-L3 positive liver cancer has shorter doubling time with tendency of portal vein invasion and distant metastasis. Clinical utilities of AFP-L3% have been demonstrated in early recognition, differential diagnosis, following-up treatment, and predicting prognosis for liver cancer.

- (5) Clinical evaluation of lentil lectin-reactive alpha-fetoprotein-L3 in histology-proven hepatocellular carcinoma. Khien VV et al., Int J Biol Markers. 2001 Apr-Jun;16(2):105-11.

#### Summary

This is a case-control study with 65 patients with biopsy-confirmed liver cancer, and 25 cases of chronic liver disease. AFP-L3% was found to have sensitivity of 96.9%, specificity of 92.0%, and accuracy of 95.5%. The mean value of AFP-L3% was higher in moderately or poorly differentiated liver cancer than in well differentiated liver cancer.

- (6) Utility of lentil lectin affinity of alpha-fetoprotein in the diagnosis of hepatocellular carcinoma. Wang S. et al., J Hepatology 1996 Aug;25(2):166-71.

#### Summary

The proportion of  $\alpha$ -fetoprotein-L3 was significantly higher in patients with hepatocellular carcinoma than in those patients with benign chronic liver disease ( $41.0 \pm 33.6\%$  vs.  $16.4 \pm 15.3\%$ ,  $p < 0.001$ ). This difference led to a sensitivity, specificity, positive predictive value, negative predictive value and accuracy of 57, 89, 83, 67 and 73%, respectively, in detecting HCC using the proportion of  $\alpha$ -fetoprotein-L3  $> 35\%$  as a parameter. Within a 1 year period, 1500 high risk patients were collaborating, leading to 22 cases with serum total  $\alpha$ -fetoprotein  $> 20$  ng/mL. These 22 cases included 6 pregnant women. The parameter, AFP-L3  $> 35\%$  was used along with sonography to detect HCC for the remaining 16 cases. The sensitivity, specificity, positive predictive value, negative predictive value and accuracy were 75, 83, 60, 91 and 81%, respectively, by the proportion of  $\alpha$ -fetoprotein-L3  $> 35\%$ ; and 100, 92, 80, 100, and 94%, respectively, by sonography. It was concluded that AFP-L3 provides a moderately high sensitivity and high specificity in the detection of HCC for patients with high AFP levels. It may be a useful adjuvant tool of sonography and total AFP level in a mass survey of HCC for a high risk population.

**Evaluation of therapeutic effect and prognosis after treatment of HCC**

- (7) Tumor markers after radiofrequency ablation therapy for hepatocellular carcinoma. Ogawa C et al., *Hepatogastroenterology*. 2008 Jul-Aug;55(85):1454-7.

Summary

The aim of this study was to evaluate the clinical value of measurement of the AFP-L3 fraction before and after radiofrequency ablation (RFA) therapy for HCC, compared with the measurement of total AFP and des-gamma-carboxy prothrombin (DCP). One hundred and twenty-four patients with HCCs were evaluated for their complete response with a 5-mm-thick safety margin around the tumor. Three tumor markers (AFP, DCP, AFP-L3) were measured after RFA therapy, and their clinical significance was studied. Multivariate analysis revealed that of the three tumor makers only AFP-L3 showed significant differences in the survival and disease-free rates. As conclusion AFP-L3 is the most reliable tumor marker for estimating overall survival and disease-free survival in patients with HCC effectively treated by RFA in contrast to AFP and DCP.

- (8) Usefulness of measurement of *Lens culinaris* agglutinin-reactive fraction of alpha-fetoprotein as a marker of prognosis and recurrence of small hepatocellular carcinoma. Hayashi K et al., *Am J Gastroenterol*. 1999 Oct;94(10):3028-33.

Summary

In this study, AFP-L3% was measured in 60 patients with small liver cancer (less than 2 cm in maximum diameter). The study cohorts was followed after treatment in Group A with negative AFP-L3% (n=43) and Group B (n=17) with positive AFP-L3% for 5 years for recurrence and long term prognosis. It was found that patients with AFP-L3%-negative liver cancer after treatment have a longer cumulative survival rate, and less recurrence rate.

- (9) Evaluation of curability and prediction of prognosis after surgical for hepatocellular carcinoma by *Lens culinaris* agglutinin-reactive alpha-fetoprotein. Okuda K. et al., *Inter. J. Oncol.*, 1999 Feb;14(2):265-71.

Summary

AFP-L3% was measured pre- and post surgical treatment in 130 patients with liver cancer. Postoperative positive AFP-L3% was an indicator for poorer prognosis with significant recurrence rate. AFP-L3% was a significant independent factor for predicting survival after surgery for liver cancer.

- (10) Usefulness of *Lens culinaris* agglutinin A-reactive fraction of alpha-fetoprotein (AFP-L3) as a marker of distant metastasis from hepatocellular carcinoma. Yamashiki N. et al., *Oncology Reports*, 1999 Nov-Dec;6(6):1229-32.

Summary

In this study, AFP-L3% was measured in 170 cases with liver cancer for predicting distant metastasis at the time diagnosis was made. AFP-L3% positive cohort (n=56) was found to have more advanced cancer and poor tumor histology. Distant metastasis was diagnosed significantly more often, although there is no significant correlation of AFP-L3% levels and portal thrombus.

- (11) The fucosylation index of alpha-fetoprotein as a possible prognostic indicator for patients with hepatocellular carcinoma. Aoyagi Y. et al., Am. Cancer Soc., 1998 Nov 15;83(10):2076-82.

#### Summary

The purpose of this study was to elucidate the usefulness of measuring the fucosylation index (FI) of  $\alpha$ -fetoprotein (AFP) before the initiation of therapy as a new prognostic indicator for patients with HCC. The conclusion of this study indicates that measuring FI from sera before the initiation of treatment serves as a new prognostic factor and may improve prognostic estimates and appraisal of therapeutic outcomes for patients with HCC.

- (12) Prognostic significance of *Lens culinaris* agglutinin A-reactive alpha-fetoprotein in small hepatocellular carcinoma. Yamashita F. et al., Gastroenterology 1996 Oct;111(4):996-1001.

#### Summary

This study evaluated the prognostic value of AFP-L3% for liver cancer in 55 patients with negative AFP-L3% before treatment. AFP-L3% turned positive after therapeutic intervention in 23 patients (41.8%) in which multiple recurrence of liver cancer and portal vein tumor thrombus were observed significantly more often. Overall survival was significantly lower in patients with positive AFP-L3% cases. AFP-L3% was included as an independent prognostic factor for liver cancer.

### **Comparison of characteristics of AFP-L3 positive HCC and other findings**

- (13) A high expression of GDP-fucose transporter in hepatocellular carcinoma is a key factor for increases in fucosylation. Moriwaki K et al., Glycobiology. 2007 Dec;17(12):1311-20

#### Summary

In this study, the expression of fucosylation-related genes in HCC tissues was studied and it was found that GDP-Fuc Tr is a key factor for increases in fucosylation. A real-time reverse transcription polymerase chain reaction (RT-PCR) analysis showed significant increases in GDP-Fuc Tr and FX expression in HCC, and levels of the GMD protein were upregulated by posttranslational modification in HCC tissues. In vitro cell experiments showed that the level of GDP-Fuc Tr was the most significantly correlated with the level of cellular fucosylation and the overexpression of GDP-Fuc Tr dramatically increased fucosylation in Hep3B cells. The importance of GDP-Fuc Tr in the increase of fucosylation was also confirmed with immunohistochemical analyses. These findings suggest that the upregulation of GDP-Fuc Tr plays a pivotal role in increased fucosylation in HCC and represents an attractive target for new treatments and diagnosis for HCC.

- (14) Lectin-reactive  $\alpha$ -fetoprotein in patients with Tyrosinemia Type 1 and hepatocellular carcinoma. Baumann U., et al., J Pediatr Gastroenterol Nutr. 2006 Jul;43(1):77-82.

#### Summary

The study investigated whether the analysis of AFP-L3 could lead to earlier detection of HCC in hereditary tyrosinemia type I (HT1) compared with judgement based on total AFP alone. AFP-L3 was determined by electrophoresis using lectin-containing agarose gel. Retrospectively sequential serum samples of 12 patients with HT1 and histologically proven HCC were analysed. In 6 patients, AFP-L3 increased before the total AFP. In 3 patients, the rise in AFP-L3 was parallel to the rise of total AFP; and in 3 patients, the AFP-L3 was raised after the total AFP or did not increase at all. Lectin-affinity electrophoresis was therefore discussed to may have a role in discriminating benign liver disease from HCC in HT1.

- (15) Clinical utility of *Lens culinaris* agglutinin-reactive alpha-fetoprotein in small hepatocellular carcinoma: Special reference to imaging diagnosis. Kumada T. et al., J. Hepatol. 1999 Jan;30(1):125-30.

#### Summary

The objective in this study was to demonstrate the clinical utility of AFP-L3% as a useful marker to predict long term prognosis of small HCC. The study used various imaging modules such as digital subtraction angiography (DSA), Ultrasonographic angiography (USAG) and computed tomography during arterial portography (CTAP) to investigate the relationship between serum AFP-L3 and imaging modalities. AFP-L3% was measured using lectin affinity electrophoresis and 10% was used as the cutoff to indicate the presence of HCC. In conclusion, 30% of the 63 patients were AFP-L3 positive with small advanced HCC indicating that HCC patients with high malignant potential tend to be positive for AFP-L3 with small hepatocellular carcinoma of  $\leq$  2 cm in diameter.

- (16) Relationship between *Lens culinaris* agglutinin reactive alpha-fetoprotein and biological features of hepatocellular carcinoma. Kusaba T., Kurume Med. J. 1998;45(1):113-120.

#### Summary

The proportion of AFP-L3% in serum reflects some biological features of (HCC) hepatocellular carcinoma. Since AFP-L3% is sensitive and specific for localization of HCC, it is closely correlated with the clinical aggressiveness of HCC. In this study, the relationship between serum AFP-L3% in serum and pathological findings using 48 resected HCC specimens. The study used lectin-affinity blotting to measure AFP-L3 fraction and immunohistochemical staining of antigens to correlate biological features. Comparisons were made using Ki67, p53 staining and  $\alpha$ -catenin staining. The pathological examination graded samples based on histologic grade, vascular invasion and intrahepatic metastasis. The patients were divided into 3 groups based on total AFP and L3%. These comparisons along with the biological factors were examined to explain the relationship between AFP-L3% and the aggressiveness of HCC. Therefore, it can be used as an aid in the prediction of therapeutic effect and patient survival of HCC patients.

- (17) Tumor vascularity and *Lens culinaris* agglutinin reactive alpha-fetoprotein are predictors of long-term prognosis in patients with hepatocellular carcinoma after percutaneous ethanol injection therapy. Fukuda H., Kurume Med. J. 1998;45(2):187-193.

#### Summary

Using a multivariate analysis this study included Cox's proportional hazard model, tumor staining and AFP-L3% to demonstrate long term prognosis after percutaneous ethanol injection therapy (PEIT). Important factors such as histologic grade, tumor size, underlying liver disease, tumor vascularity and follow-up after PEIT are important prognostic factors for small HCC. 41 patients with HCC were studied using these factors to correlate the relationship of pretreatment clinicopathologic and biologic factors for long term prognosis. This study concluded that tumor vascularity in relation to a positive AFP-L3% were both significant prognostic factors in patients with small HCC related to survival after PEIT.

- (18) Deletion of serum lectin-reactive alpha-fetoprotein by Acyclic Retinoid: A potent biomarker in the chemoprevention of second primary hepatoma. Moriwaki H., Clin. Cancer Res. 1997 May;3(5):727-731.

#### Summary

In this evaluation 21 patients were used to evaluate serum levels of AFP-L3 and acyclic retinoid (600mg daily) over a 12 month period. AFP-L3 was measured using lectin affinity electrophoresis to identify the presence of latent hepatoma cells and acyclic retinoid to prevent second primary hepatomas. Serum AFP-L3 was performed at the beginning of the study along with a placebo group to determine if the deletion of AFP-L3 by acyclic retinoid for patients that were AFP-L3% positive. Acyclic retinoid reduced AFP-L3 levels but total AFP levels remained the same. In conclusion, patients with latent hepatoma clones producing AFP-L3 were suppressed using acyclic retinoid.

**Measurement technique of AFP-L3%**

- (19) Simultaneous determination of percentage of *Lens culinaris* agglutinin-reactive  $\alpha$ -fetoprotein and  $\alpha$ -fetoprotein concentration using the LiBASys clinical auto-analyzer. Yamagata Y. et al. 2003 Jan;327(1- 2):59-67.

Summary

LCA  $\alpha$ -fetoprotein (AFP-L3) percentage of total AFP has been used as an effective marker for earlier diagnosis, for assessment of therapeutic effects and for predicting the prognosis of HCC. The Wako LiBASys and AFP-L3% assay system to generate the data. This system correlated well to manual electrophoresis method. This method simultaneously yields both qualitative and quantitative results for AFP-L3 percentage and AFP concentration.

- (20) Automatic and simultaneous analysis of *Lens culinaris* agglutinin-reactive  $\alpha$ -fetoprotein ratio and total  $\alpha$ -fetoprotein concentration. Katoh H. et al., Analytical Chemistry 1998 May 15;70(10):2110-2114.

Summary

Wako's AFP-L3% assay was evaluated on the LiBASys instrument. The automated assay was compared to conventional manual electrophoresis methods that utilize solid-phase reactions. The data showed comparable results and the automated analysis allows for an easy to use method for simultaneous measurements of AFP and AFP-L3%.