

National Academy of Clinical Biochemistry Guidelines for the Use of Tumor Markers in Primary Liver Cancer

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Abbreviations: AFP, α -fetoprotein; AJCC, American Joint Committee for Cancer; Br Soc GE, British Society of Gastroenterology; CLIP, Cancer of the Liver Italian Program; CT = computer tomography; DCP = des- γ -carboxy-prothrombin; EASL, European Association for the Study of the Liver; EIA, enzyme immunoassay; French SOR, French group for Standards, Options, and Recommendations; EGTM, European Group on Tumor Markers; GPC-3 = glypican-3; HBV = hepatitis B virus; HCC = hepatocellular cancer; HCV = hepatitis C virus; MRI = magnetic resonance imaging; NACB = National Academy of Clinical Biochemistry; NCCN, National Comprehensive Cancer Network; PIVKA II = prothrombin produced by vitamin K absence or antagonism II; TNM = tumor – lymph node – metastasis staging system; US = ultrasound.

INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide and the third most common cause of cancer-related death (1), with 500,000 new cases diagnosed yearly. The age-adjusted worldwide incidence varies by geographic area, increasing from 5.5/100,000 of the population in the United States and Europe to 14.9/100,000 in Asia and Africa (2). The higher incidence observed in Europe during the past decade probably reflects the increasing number of cases of hepatitis C infection (3, 4) and liver cirrhosis (5), both strong predisposing factors for HCC (6). In most parts of Asia and Africa, hepatitis B virus infection is most relevant (7), with ingestion of aflatoxin B₁ from contaminated food an additional contributory factor (8). In the West and Japan hepatitis C virus infection is the main risk factor (2, 9-12), although patients with alcoholic cirrhosis or haemochromatosis are also at increased risk (13). Older patients are more likely to develop HCC (10, 11). In contrast, in developing countries HCC more frequently affects younger individuals who have chronic hepatitis B (14), carriers having twice the relative risk of developing the disease. Cirrhotic patients have a higher risk than non-cirrhotic patients with annual HCC incidences 2-6.6% (15) and 0.4% (16) respectively. Worldwide, 380 million individuals are infected with hepatitis B and 170 million with hepatitis C (17). Protective vaccination is possible for hepatitis B but not hepatitis C, although new anti-viral strategies [e.g. pegylated interferon and ribavirin] are being developed.

Diagnosis of hepatocellular carcinoma

While early detection is highly desirable, patients with early disease are often asymptomatic (18,19) and consequently HCC is frequently diagnosed late, by which time it is often untreatable (20). Suspicion of disease may first arise in patients with liver cirrhosis who develop ascites, encephalopathy or jaundice (21). Some patients initially present with upper abdominal pain, weight loss, early satiety or a palpable mass in the upper abdomen (19). Other symptoms include obstructive jaundice, diarrhoea, bone pain, dyspnoea, intraperitoneal bleeding, paraneoplastic syndromes [e.g. hypoglycemia (22), erythrocytosis (23), hypercalcemia (24,25)], severe watery diarrhoea (25), or cutaneous features (e.g. dermatomyositis) (26).

The combination of regular measurement of tumor markers and ultrasound of the liver is commonly used for surveillance of HCC. In patients with cirrhosis or chronic viral hepatitis who are being monitored in this way, a rising serum AFP level may provide the first indication of malignancy, leading to imaging of the liver. In an asymptomatic patient, a predominant solid nodule that is not consistent with haemangioma is suggestive of HCC (27), while hypervascular lesions associated with elevated AFP are almost diagnostic for malignancy.

Diagnostic imaging modalities used include ultrasound, computed tomography, magnetic resonance imaging (MRI) and angiography (1). Ultrasound is widely available, non-invasive, and commonly used in patients with HCC to assess hepatic blood supply and vascular invasion by the tumor, as well as intraoperatively to detect small tumor nodules. Computed tomography (CT) of the liver is frequently used to investigate abnormalities identified on ultrasound, but may also be used for primary screening. Magnetic resonance imaging (MRI) provides high-resolution images of the liver. Space-occupying lesions hypoperfused by portal blood are considered an early sign of HCC even in the absence of a coincident rise in circulating AFP.

Specimens for histopathology are usually obtained by biopsy under ultrasound or CT guidance. Risks of biopsy include bleeding and tumor spread along the needle track (1-2%) (28,29). The histological appearance of HCC ranges from well-differentiated to poorly differentiated lesions of large multinucleate anaplastic tumor giant cells, with frequent central necrosis and occasional bile globules and acidophilic inclusions, often dysplastic rather than carcinomatous. There is ongoing debate about the relevance of grading the dysplasia in predicting HCC.

Staging and prognosis (1,30)

Patients are rarely diagnosed with HCC at the very early stage of carcinoma *in situ* (31) when liver function is still well preserved, and five-year survival rates are 89-93% following resection and 71% following percutaneous treatment (32). Patients with early stage HCC have one tumor nodule of <5cm or 2-3 nodules each <3 cm. Prognosis depends on the number and size of the nodule(s), liver function at the time of diagnosis, and the choice of treatment (33,34). The much greater disease heterogeneity seen in intermediate and advanced HCC complicates the selection of optimal treatment, which in turn is reflected in the considerable variation in survival rates reported in randomized controlled trials [e.g. one-year, 10-72%; two-year, 8-50% (35)]. It has been suggested that considering AFP and alkaline phosphatase, Child-Pugh score and the absence or presence of ascites could improve outcome prediction (34, 36, 37). End-stage HCC is defined by terminal cancer-related symptoms with <6 months' life expectancy and no survival benefit from treatment.

The TNM system (38) and the Okuda classification (39) are most frequently used for staging. Prognostic classifications from Japan (40), France (36), Italy (37,41), Spain (42,43) and China (44) have also been published (see also 45,46), but none has received universal acceptance. Most of these systems include as major prognostic factors severity of the underlying liver disease, tumor size, tumor extension into adjacent structures, and presence of metastases (37,39).

Two staging systems include AFP. A French system scores the Karnofsky-index, ultrasonographic portal vein obstruction, and serum bilirubin, alkaline phosphatase and AFP (cut-off 35 µg/L) (36). Based on the score, patients are classified as being at low (A), moderate (B) or high risk (C) for death with one-year survival rates of 72%, 34% and 7% respectively. A second classification, proposed by the Cancer of the Liver Italian Program (CLIP) (41), includes as variables Child-Pugh stage, morphology, portal vein thrombosis and serum AFP (cut-off 400 µg/L). With a simple scoring system patients are assigned to one of seven categories (0-6) with validated median survival rates of 35.7, 22.1, 8.5, 6.9 and 3.2 (for combined groups 7-6) months (last combined group 4-6) (37). Both classifications incorporate AFP as an indicator of tumor spread and burden, cellular differentiation and aggressive potential.

Additional prognostic factors

An Italian study of prognostic factors in 176 patients with HCC demonstrated that low albumin, high bilirubin, elevated AFP, portal vein thrombosis and an untreatable lesion were independent risk factors for worse survival (47). Survival depended most strongly on the degree of functional liver impairment, presence of HBV infection, type of diagnosis and aggressiveness of the tumor. A more recent nationwide Japanese survey of prognostic factors influencing survival after liver resection in HCC patients demonstrated improvement in outcomes and operative mortality rates over the last decade (48). Age, degree of liver damage, AFP level, maximal tumor dimension, number of tumors, intrahepatic extent of tumor, extrahepatic metastasis, portal and hepatic vein invasion, surgical curability and free surgical margins were all independent prognostic factors for HCC patients undergoing liver resection (48).

Primary treatment

Curative treatments are offered to 30-40% of HCC patients in referral centers in Western countries and to 60-90% of patients in Japan (1). Hepatic resection is the treatment of choice in non-cirrhotic patients, with 5-year survivals of 70% achievable in carefully selected patients. Similarly high survival rates can be achieved by transplantation in appropriately selected cirrhotic patients, e.g. with one nodule <5cm in diameter or up to three nodules <3cm each.

Post-treatment monitoring

According to National Comprehensive Cancer Network (NCCN) practice guidelines for hepatobiliary cancer (49), post-treatment follow-up of HCC patients consists of imaging every 3 to 6 months for 2 years and then annually, and AFP determinations, if initially elevated,

every 3 months for 2 years, and then every 6 months. In case of disease progression, recommendations on initial investigation should be consulted. According to the French Standard, Options and Recommendations (SOR) guidelines (50), there is no consensus about patterns or modalities of follow-up other than clinical examination. Surveillance plans may incorporate ultrasound, AFP measurement, abdominal CT scan, chest X-ray and/or MRI imaging, with optimal choice and timing of these dependent on treatment options.

Role of adjuvant therapy

Adjuvant treatments include percutaneous ablation, chemoembolization and chemotherapy. Percutaneous treatments provide the best treatment options for early unresectable HCC, destruction of neoplastic cells being achieved by chemical (alcohol, acetic acid) or physical (radiofrequency, microwave, laser, cryoablation) treatments (51). Percutaneous ethanol injection appears to be the most effective technique with few adverse events, response rates of up to 90-100% and 5-year survival rates as high as 50% (52) in selected patient groups. This technique represents the gold standard for patients with one tumor <3cm. Radiofrequency ablation is also effective, with comparable objective responses, fewer sessions needed (53) and better 5-year survival rates for larger tumors (54).

Palliative treatments in advanced disease include arterial chemoembolization and tamoxifen, with survival advantages demonstrated for the former (but not the latter) in well-selected candidates (35). Embolization agents such as gelatine administered with selective chemotherapy agents (e.g. doxorubicin, mitomycin or cisplatin) mixed with lipiodol (chemoembolization) can delay tumor progression and vascular invasion in 15-55% of patients.

It is clear from the above discussion that early detection of HCC, preferably when still asymptomatic, is desirable for a favourable outcome. The aim of this article is to present new NACB Guidelines for the use of serum tumor markers in HCC, both in early detection and in monitoring following treatment. A summary of guidelines published by other Expert Panels on these topics is also presented.

CURRENTLY AVAILABLE MARKERS FOR HEPATOCELLULAR CARCINOMA

Table 1 lists the most widely investigated tissue-based and serum-based tumor markers for hepatocellular carcinoma. Also listed is the phase of development of each marker as well as the level of evidence (LOE) for its clinical use. The level of evidence grading system used is based on that previously described by Hayes et al. [*See Section 1*].

TUMOR MARKERS IN LIVER CANCER: NACB RECOMMENDATIONS

Table 2 summarizes the current National Academy of Clinical Biochemistry (NACB) guidelines for the use of α -fetoprotein (AFP) in this malignancy. Below, we present a more detailed discussion of the markers listed in Tables 1 and 2.

α -Fetoprotein (AFP)

AFP is a 70 kD glycoprotein consisting of 591 amino acids and 4% carbohydrate residues, encoded by a gene on chromosome 4q11-q13 (for reviews see 161,162). Normally produced during gestation by the fetal liver and yolk sac, AFP is highly elevated in the circulation of newborns with levels declining over the next 12 months to 10-20 μ g/L.

Assay methods, standardization and reference values. AFP is currently measured by two-site immunometric assays using monoclonal and/or polyclonal antibodies, with results similar to those of the radioimmunoassays that preceded them. Most commercial assays are calibrated against WHO International Standard (IS) 72/225. Clinical results are reported in mass units (μ g/L) or in kU/L of IS 72/225, where one International Unit (IU) of AFP corresponds to 1.21 nanograms. The upper reference limit used by most centers is 10-15 μ g/L (8.3–12.4 kU/L). AFP concentrations reportedly increase with age, the upper reference limit increasing from 9.3 kU/L in subjects <40 years old to 12.6 in those >40 (163). Ideally reference values should be established for each assay, as there is some between-method variation in results.

AFP carbohydrate-microheterogeneity. AFP is a glycoprotein, containing 4% carbohydrate as a single bi-antennary chain that is N-linked to asparagine-232 of the protein backbone (164,165). The microheterogeneity of this carbohydrate chain has been investigated extensively by both lectin affinity electrophoresis (84-86, 93,94) and isoelectric focusing (88-90,166,167). Distinct glycoform patterns characteristic of malignant or benign tissue are found, raising the possibility of improving AFP specificity for HCC by measurement of an HCC-specific glycoform.

AFP glycoforms can be differentiated on the basis of their lectin-binding affinity (91,92,168). AFP from HCC patient sera, for example, binds more strongly to concanavalin A than does AFP from non-seminomatous germ cell tumors, and both bind more strongly to *Lens culinaris* lectin (LCA) than does AFP from patients with benign liver disease. The affinity for LCA is slightly higher for AFP from HCC (AFP-L3) than that from non-seminomatous germ cell tumors (AFP-L2). Assay kits are now available commercially that specifically measure the AFP-L3 and AFP-P4 glycoforms (91,168).

Numerous publications from Japan and other Asian countries demonstrate that an increase in the AFP-L3 fraction of serum AFP correlates more strongly than conventional serum AFP with adverse histological characteristics of HCC (e.g. greater portal vein invasion, more advanced tumor irrespective of size) and predicts unfavourable outcome (97-101).

AFP in diagnosis. 20-40% of adult patients with hepatitis or liver cirrhosis have raised AFP levels (>10 µg/L) (169). AFP levels between 10 and 1,000 µg/L may be associated with benign conditions. An AFP concentration between 400 and 500 µg/L is now generally accepted as the optimal decision point to discriminate HCC from chronic liver disease. In general, after exclusion of hepatic inflammation, a sustained rise in AFP is suggestive of HCC, while stable or decreasing results make it less likely.

Circulating AFP levels range from within the reference interval to as high as 10×10^6 µg/L (i.e. 10 g/L) in patients presenting with HCC, with pre-treatment levels >1,000 µg/L in approximately 40% (170). AFP has been reported to be higher in HCC arising from chronic viral conditions as compared to alcoholic liver disease (175), and in younger (174) and male (174) patients. In one cohort study of 239 patients with chronic hepatitis, 277 with cirrhosis, and 95 with HCC, AFP gave sensitivities for HCC of 79% and 52.6% at decision points of 20 µg/L and 200 µg/L respectively, with corresponding specificities of 78% and 99.6% (173). According to some Japanese investigators (171), any circulating AFP value >10 µg/L in patients with chronic liver disease should be regarded as suspicious of HCC and indicates the need for further follow up using AFP-L3 (LCA) or AFP-P4 (E-PHA) lectin tests and imaging. These investigators advocate a decision point of 10 µg/L instead of 20 µg/L to take into account improvements in imaging that have resulted in a greater proportion of HCC being detected when AFP is <20 µg/L. [In Japan, for example, the percentage of HCC patients with AFP levels <20 µg/L at presentation increased from 3.6 to 29% from 1978 to 1986 (169)].

Serum AFP may also be raised in malignancies other than HCC. Such elevations are frequently associated with non-seminomatous germ cell tumours, for which AFP is an important tumor marker with well-established clinical use. Elevations also occur in stomach (172), biliary tract and pancreatic cancers, but levels exceed 1,000 µg/L in <1% of cases.

The use of AFP as adjunct in the diagnosis of HCC is recommended by the European Association for the Study of the Liver (EASL) (158), the British Society of Gastroenterology (159), the European Group on Tumor Markers (EGTM) (160) and the NCCN (49). These recommendations are also supported by the NACB Panel, which stresses the importance of serial AFP measurements together with consideration of sustained increases in AFP even at low concentrations (Table 2).

AFP in screening. The rationale behind screening for HCC in high-risk but asymptomatic groups is to identify early curable tumors. Ideally, randomized controlled trials should be carried out to demonstrate the efficacy of screening, in terms of decreased disease-related mortality, improved survival and cost-effectiveness (176). However it is unlikely that such trials will ever be undertaken, as it is already generally accepted that surveillance is beneficial for selected cirrhotic patients (177). In developed countries about 30-40% of patients with HCC are now diagnosed at a sufficiently early stage for the malignancy to be amenable to curative treatments.

Cirrhotic patients with persistently elevated AFP are at increased risk of developing HCC compared to those with fluctuating or normal AFP levels (29% versus 13% versus 2.4%, respectively) (1). Lower serum AFP levels are frequently encountered when HCC is detected during screening (66) with small HCC tumors being AFP-negative in up to 40% of cases (67). AFP immunostaining of well differentiated small HCCs is often negative (178), rendering AFP uninformative. In these instances, the tumor can only be detected by ultrasound.

Lesions undetectable by imaging are likely to reach 2 cm in diameter in about 4-12 months (179,180), so in order to detect tumors below 2 cm the suggested interval for surveillance in cirrhotic patients is 6 months, using both serum AFP and ultrasound (181). Differences in study design mean that it is often difficult to compare different studies. In a systematic review of AFP test characteristics for diagnosis of HCC in HCV patients (73), only 5 of 1239 studies met all the authors' inclusion criteria (68-72). In these five studies, at a serum AFP cut-off value of 20 µg/L, sensitivity ranged between 41 and 65%, specificity between 80 and 94%, positive likelihood ratio between 3.1 and 6.8 and negative likelihood ratio between 0.4 and 0.6, underscoring its limited value as screening test. In patients with hepatitis C (47), 19 of 24 studies published from 1985-2002 reported AFP sensitivities and specificities for HCC of 45%-100% and 70%-95% respectively at cut-points between 10 and 19 µg/L. Ultrasound has been reported to have higher sensitivity (71%) and specificity (93%) than serum AFP, but its positive predictive value is still low at about 14% (18). As the success of ultrasound detection is critically dependent on the skill of the ultrasonographer, investigation of patients with increases in serum AFP or suspicious screen-detected nodules is best performed in specialist referral centers.

The incidence of HCC in patients with chronic hepatitis is lower than in patients with cirrhosis, which may decrease the benefit of screening in the former. Japanese experiences suggests differences in the natural history of hepatitis B and C mean that hepatitis B patients are more likely to develop HCC even when young and asymptomatic or following seroconversion.

In one study, 1069 HBV patients with proven cirrhosis had to be screened in order to detect 14 cases of HCC, of which only 6 were at a sufficiently early stage to be amenable to surgical cure (182). The frequency of detection of curable malignancy was even lower in a study of 118 French patients with Child-Pugh A or B cirrhosis who were screened at six month intervals with ultrasound, AFP and des- γ -carboxy-prothrombin (an investigational tumor marker for HCC). Only 1 of 14 detected HCC cases (7%) was surgically resectable at the time of diagnosis (184). However, other studies have demonstrated benefit in screening chronic hepatitis B carriers for HCC. A population-based Alaskan prospective screening study of 2230 hepatitis B surface antigen positive carriers with cirrhosis (183,186) found that 64-87% of detected HCCs were limited to single foci and that in 43-75% of cases tumors were <3 cm in size enabling curative surgery in 29-66% of the detected cancers (7,187,188). In another study, tumor size was significantly reduced and survival improved (35% versus 10% at 30 months) when HCC was detected by screening (185).

There is additional evidence that screening high risk populations for HCC can be cost effective in high prevalence countries such as Hong Kong (190) and that screening imparts a survival advantage, as demonstrated in an asymptomatic Asian Hawaiian population with chronic hepatitis B or C and cirrhosis (189) and also in an Italian study of cirrhotic patients with screen-detected HCC (191). A national survey of practice in the United States (192) has documented that a majority of institutions routinely screen patients with cirrhosis for HCC, especially in high-risk aetiologies. Systematic screening with twice yearly AFP and liver ultrasound is considered by many to offer the best hope for early diagnosis of HCC in healthy hepatitis B surface antigen positive carriers with additional risk factors (e.g. active chronic hepatitis, cirrhosis) and in patients with cirrhosis of any aetiology (193). Markov analysis has clearly demonstrated that in US patients with cirrhosis arising from chronic hepatitis C, screening for HCC is as cost-effective as other accepted screening protocols (194). Bi-annual AFP and annual ultrasound gave the greatest gain in terms of quality-adjusted life-years, while still maintaining a cost-effectiveness ratio of <\$50,000/quality-adjusted life-year. The authors suggested that biannual AFP with annual CT screening might even be cost-effective (194).

Given the widespread use of AFP measurements and liver ultrasound to screen prospectively screen for the onset of HCC in cirrhotic patients, particularly those who would be suitable candidates for curative therapy (186,195,196), there is a practical urgency to establish and validate optimal follow up protocols when suspicious nodules are detected (5,197,198). The European Association for the Study of the Liver (EASL) has recommended that nodules <1 cm in diameter be followed up with repeat ultrasound and AFP in six months, that fine needle biopsy and histology be added to investigate nodules of 1-2 cm (false-positive rate 30-40%), and that additional non-invasive diagnostic criteria (e.g. two imaging

techniques) be employed for tumors >2cm (158). French recommendations published in 2001 (50) state the diagnosis of HCC should be based on histopathological examination of one or more liver samples obtained by open surgery, laparoscopy or US-/CT-guided biopsy (standard) with the option of fine-needle aspiration for cytology if liver biopsy is impossible. Sequential measurements of serum AFP may provide useful information, but this is still under investigation and not yet fully validated for routine clinical practice. An elevated AFP detected by a single measurement may be transient [e.g. arising from an inflammatory flare of underlying chronic viral hepatitis], while elevated but stable levels decrease the likelihood that HCC is the causal agent. A steadily rising pattern of elevated AFP should always be rigorously investigated using ultrasound and other imaging techniques, which should be repeated if initially negative to identify any occult hepatic malignancy (158).

The British Society of Gastroenterology (159) presented guidelines on the use of serial tumor marker measurements to screen for HCC in 2003. The following statements were graded with a Level of Evidence (LOE) of IIa:

- Screening by abdominal ultrasound and AFP as compared to no surveillance detects HCC of smaller size.
- Screening by abdominal ultrasound and AFP enables a greater proportion of curative therapies, and earlier detection of HCC leads to improved long-term survival or cost savings.

Based on Grade III evidence, the guidelines proposed that surveillance for HCC be restricted to males and females with cirrhosis due to HBV, HCV, or genetic haemochromatosis and to males with cirrhosis due to primary biliary cirrhosis and alcoholic cirrhosis, if abstinent. The likelihood of HCC arising in cirrhosis of other aetiology was considered to be low (LOE IIb). Surveillance using AFP and abdominal ultrasound was recommended at six month intervals (LOE III), with the ultrasound component noted to require appropriate equipment and skilled operators, and it was recommended that patients should be counselled on the implications of early diagnosis and its lack of proven benefit was also recognised (LOE IIb).

In accord with recommendations from other Expert Groups (49, 50, 158-160) (Table 2), the NACB supports the use of six-monthly determinations of AFP and abdominal ultrasound to screen prospectively for the onset of HCC in high-risk patients, especially those with HBV- and HCV-related liver cirrhosis.

AFP in prognosis. An increased AFP level in HCC is an adverse prognostic factor (199), generally reflecting a more aggressive tumor (200). Large multivariate analyses confirm that raised AFP levels predict poor prognosis when compared with AFP-negative cases in HCC (20, 36, 96). A retrospective study of 309 HCC patients stratified according to pre-treatment

AFP levels (<20, 20-399 or ≥400 µg/L) found that patients with higher AFP levels tended to have larger tumors, but there was no correlation with Okuda stage, degree of tumor differentiation or extra-hepatic metastasis (82). Together with tumor size and extent (74,201), AFP seems to be an independent predictor of survival. Survival of patients with serum AFP >10,000 µg/L at diagnosis was significantly shorter than in those with AFP <200 µg/L (median survival time 7.6 vs 33.9 months, respectively) (83). AFP levels >1000 µg/L predict a relatively worse prognosis in patients even after attempted curative resection (89).

AFP doubling time has also been reported to be an important prognostic factor (75). Persistence of a positive AFP-L3 fraction following intervention also has been reported to indicate residual or recurrent disease (97). In agreement with EASL (158), the NACB supports the prognostic use of pre-treatment serum AFP concentration in combination with other prognostic factors (Table 2).

AFP in monitoring. Using serial AFP determinations to monitor treatment of HCC is well accepted in patients with increased AFP levels prior to therapy. Following complete removal of the tumor, AFP levels typically decrease with a half-life of 3.5-4 days. Incomplete resection yields a longer half-life associated with poorer survival (75,82), while failure of the AFP to normalize implies residual malignancy or severe liver damage. [Determination of the AFP-L3 fraction can help to differentiate these two conditions.] However, normalization of AFP does not necessarily indicate complete clearance of the disease. Recurrence following transplantation may occur, even when AFP is stable and within normal limits (74-76), presumably reflecting the presence of micrometastases too small to produce measurable serum levels.

Changes in AFP levels also reflect tumor response following chemotherapy, with patients showing a significantly prolonged fall in AFP surviving longer than those with slowly rising levels (77,78). 75% of patients receiving new and effective combined systemic therapies (79) have shown dramatic decreases in serum AFP, with levels normalizing completely in some patients. Progressive disease was found in patients with continued AFP rise and doubling times between 6.5 and 112 days (mean 41 days), again correlating with survival (75). This is also the case following radiotherapy for primary and secondary liver tumors. Decreases in tumor markers reflected tumor regression more consistently than later changes in tumor size and volume as determined by CT (80). Discrepancies between tumor marker and imaging results may be due to residual fibrosis and other factors that can complicate interpretation of CT scans (80).

In accord with other Expert Groups (49, 50, 158) (Table 2), the NACB recommends serial determinations of serum AFP (if elevated prior to treatment) to monitor efficacy of treatment, course of disease and recurrence.

Tumor markers other than AFP

Des-γ-carboxy-prothrombin. Des-γ-carboxy-prothrombin (DCP) [also known as “Prothrombin produced by vitamin K absence or antagonism II” (PIVKA II), an abnormal prothrombin devoid of coagulation activity] is potentially a promising marker for HCC. Mainly developed and investigated in Japan, it was first described in the USA in 1984 (103) and critically reviewed there in 1993 (104). A single commercially available EIA kit from Japan has dominated the market for DCP testing. The sensitivity of this method has been markedly improved since 1996 and is currently 10 mAU/ml (AU, arbitrary unit; 1 AU=1 μg prothrombin). A number of published investigations have reported DCP sensitivities for HCC ranging from 54% to 70% at a decision point of 40 mAU/ml, with corresponding specificities in cirrhotic patients between 87% and 95%. AFP tested concurrently in the same patients has shown, at a decision point of 20 μg/L, 47%-72% sensitivity and 72%-86% specificity. Combined DCP/AFP sensitivity was about 80% (105-108). DCP, AFP, and combined DCP/AFP sensitivities for solitary HCC [<2cm] were 30%-53%, 13% and 57% respectively and for larger tumors [>3 cm] were 78%-81%, 49%-69% and 84%-94% respectively (105,106,108). The sensitivity of both markers was better for moderately to poorly differentiated tumors (DCP, 68%; AFP, 61%; DCP/AFP, 85%; n=41) than for well-differentiated tumors (DCP, 13%; AFP, 33%; DCP/AFP, 40%; n=15) (108). DCP and AFP both correlated with tumor size and grading, but not significantly with each other.

An American cross-sectional case control study comparing serum AFP and DCP has confirmed the apparent superiority of DCP as a tumor marker for HCC (112). The study included 48 American healthy adults, 51 patients with chronic hepatitis (mostly hepatitis C), 53 individuals with compensated cirrhosis and 55 people with proven HCC and used ROC-analyzed optimal cut-offs of 11 μg/L for AFP and 125 mAU/ml for DCP. DCP performed better than AFP in differentiating HCC from cirrhosis [sensitivity 90% cf 77%; specificity 91% cf 71%; PPV 85% cf 81%; NPV=90% cf 74%; AUC 0.921 cf 0.815]. There was no improvement over DCP alone when the two markers were combined.

DCP has also been reported to have prognostic significance. In a study of HCC patients treated by percutaneous ethanol injection (PEI) or microwave coagulation therapy, multivariate analysis showed that after histological grade and tumor differentiation, DCP was the strongest predisposing factor for later development of portal venous invasion (110) while ROC analysis suggested it was an effective predictor of HCC recurrence following resection (113). Another report (109) has suggested that high DCP (>62.5 mAU/ml) with low AFP (<100 μg/L) correlate with large HCC and few tumor foci. Outcome is particularly poor in patients who have high levels of both DCP and AFP (109).

False-positive elevated DCP levels are found in patients with severe obstructive jaundice due to intrahepatic cholestasis or in conditions when the action of Vitamin K is

impaired [e.g. long-standing vitamin K deficiency or the ingestion of warfarin and some wide-spectrum antibiotics] (111). Despite these limitations, DCP is a promising emerging marker of considerable potential.

Glypican-3 Glypican-3 (GPC-3) is another promising new tissue and serum marker for HCC. The gene GPC3 codes for a member of the glypican family of glycosyl-phosphatidylinositol-anchored cell-surface heparan sulfate proteoglycans (55). GPC-3 was first detected *via* its mRNA, which was increased in 75% tissue specimens from primary and recurrent HCC but in only 3.2% of specimens from normal liver. These data were later confirmed immunohistochemically (70,72). Elevated GPC-3 mRNA levels were also found in the serum of HCC patients (56). Sensitivity exceeded that of AFP (88% versus 55%) for the entire group of HCC patients tested as well as for those with smaller HCCs <3 cm (77% versus 43%). In a later study of 34 HCC patients (55), sensitivity was somewhat lower (53%) and similar to that of AFP (54%). However specificity was excellent with no significant elevations in healthy donors or patients with acute hepatitis, and in only one of the twenty patients with chronic hepatitis and cirrhosis. The combined sensitivity of the two markers was 82%. Neither marker correlated with the other.

Recent work on the GPC protein (114) has shown that only the amino-terminal fragment is present in the circulation, constituting the soluble serological marker (sGPC-3). Using an ELISA with highly specific monoclonal antibodies to analyse sera from 69 HCC patients, 38 liver cirrhosis patients and 96 healthy adults, ROC analysis yielded sensitivity/specificity rates of 51%/90% for sGPC-3 (cut-off 2 µg/L) comparable to those of AFP [55%/90% (cut-off 20 µg/L)]. The sensitivity of the two markers in a subset of early stage HCC was essentially unchanged, and there was no correlation between sGPC-3 and AFP in the 69 patients who had HCC. The combined marker sensitivity was 72%. This preliminary study suggests that sGPC-3 may have some promise and that larger clinical trials to investigate its potential are merited.

Other serum markers for liver cancer. Many other serum markers have been reported (Table 1). Pre- and post-treatment detection of circulating HCC cells by RT-PCR of AFP mRNA has been suggested to be useful in predicting HCC recurrence and poor outcome by some groups (151,152), although others have questioned its value (149,150,153). Other techniques under investigation include proteome analysis (147,148), determination of free nucleic acids (145) and epigenetic abnormalities (e.g. p16 hypermethylation) in serum or plasma (146). Fifty up-regulated HCC marker genes, which are potential tumor marker candidates, have been identified in hepatitis C virus-associated HCC by cDNA microarray analysis of surgical liver samples from HCV-infected patients (154). A catalytic fragment of

vitronectin has recently been identified by surface-enhanced laser desorption ionization time of flight (SELDI-TOF) mass spectrometry as a promising new serum marker of HCC in patients with chronic liver disease (148).

The NACB does not recommend any HCC-related biomarkers except AFP for the routine surveillance of patients with or at risk of HCC. However it supports further evaluation of the clinical utility of potential markers (e.g. AFP-L3, DCP and glypican-3) in suitably designed prospective randomized clinical studies.

CONCLUSION

Hepatocellular carcinoma is one of the most common cancers worldwide, and is frequently preceded by chronic viral hepatitis B or C or alcoholic liver disease. If treatment is instituted early enough, the risk of developing HCC can be decreased or abolished. In patients who have already developed HCC, surgical resection or transplantation with curative intent requires early local detection of small sized malignancy. The clinical utility of AFP measurement, with other imaging techniques, is already well established for this application, while other tumour markers require further investigation (Table 1). Future developments in molecular genetics and proteomic analysis may lead to improved early detection and treatment of HCC patients.

Table 1. Currently available serum and tissue markers for liver cancer.

Cancer Marker	Proposed Use/Uses	Phase of Development	LOE¹	Ref
Tissue markers				
Glypican-3	Differentiating HCC from other hepatic disorders at the tissue level.	Undergoing evaluation	V	55-57
Telomerase	Independent prediction of recurrence following HCC resection.	Undergoing evaluation	V	58-62
Proliferating cell nuclear antigen (PCNA) labelling index	Prediction of recurrence and survival in small HCC	Undergoing evaluation	V	63
Ki-67	Assessment of prognosis following resection of HCC.	Undergoing evaluation	V	64
MIB-1, E-cadherin, β -catenin	Prognostic marker for recurrence when selecting HCC patients for orthotopic liver transplant.	Undergoing evaluation	V	65
Serum markers				
AFP	Monitoring HCC patients, in conjunction with ultrasound, in order to detect HCC early. [When specificity is adequate (>90% at AFP cut-offs between 100 and 500 μ g/L), sensitivity is poor (20-40%) and PPV 5-15%]	In clinical use, but value not validated in a high-level evidence study	IV	66-72
	Monitoring patients with no evidence of disease following resection or transplant.	In clinical use, but value not validated in a high-level evidence study	IV	68,70, 72-74
	Monitoring therapy in advanced disease	In clinical use, but value not validated in a high-level evidence study	IV	75-80
	Assessing prognosis preoperatively. [AFP >20 μ g/L predicts adverse outcome.]	In clinical use, but value not validated in a high-level evidence study	IV	20,36, 81-83
AFP-Con A-Binding	Differentiating source of elevated AFP from germ cell and metastatic liver tumors (high) from HCC (low) (glucosaminylation index)	Not in general clinical use, but effectively differentiates AFP source as HCC or GCT. Not validated in a high-level evidence study.	V	84-86
AFP-LCA-binding	Differentiating malignant (high) from non-malignant (low) origin	Not in general clinical use,	V	86,87

	of elevated AFP, independent of location (fucosylation index)	but effective for AFP source origin on suspicion of malignant versus benign liver disease		
HCC-specific AFP band on isoelectric focussing (monosialylated AFP)	Earlier detection of HCC than “diagnostic” AFP (>500 µg/L), PPV 73% vs 42%, respectively),	Not in clinical use	V	88-90
AFP lectin-affinity subgroups [LCA-reactive LCA-L3; erythroagglutinating-phytohemagglutinin-E4 reactive AFP-P4 and P5]	Prediction of more malignant stage and poor outcome. AFP-L3 is routinely used in Japan when AFP exceeds cutoff level; AFP-P4 is more sensitive, but is not used routinely.	In limited clinical use as a commercially available test in certain countries, but value not validated by a high level evidence study.	IV	91-101
Circulating free AFP-IgM complexes	Providing information complementary to AFP.	Undergoing evaluation	V	102
DCP/PIVKA II	Used with AFP during and following treatment to predict adverse outcome, early recurrence and malignant potential. CLD and warfarin therapy suppress circulating levels. Three commercial assays of differing accuracy are available.	Undergoing evaluation	IV	103-113
Soluble NH ₂ -fragment of Glypican-3, a heparan sulfate proteoglycan	Diagnosis and monitoring of HCC and patients with cirrhosis. Enables detection of small size HCC more sensitively than AFP.	Undergoing evaluation.	V	55,114
Iso-γGTP	Complementary to AFP as a diagnostic marker for HCC.	Undergoing evaluation	V	115, 116
Ferritin	Monitoring HCC in patients whose tumors do not produce AFP.	No high level evidence evaluation	V	117, 118
Variant ALP ⁸	Complementary to AFP.	Undergoing evaluation	V	119
α ₁ -Antitrypsin	Complementary to AFP.	Undergoing evaluation	V	120, 121
Aldolase A	Complementary to AFP.	Undergoing evaluation	V	122, 123
5'-nucleotide phosphodiesterase (5'-NPD)	Complementary to AFP. Monitoring HCC in patients whose tumors do not produce AFP.	Undergoing evaluation	V	124, 125
Cytokeratins [CK18,	Complementary to AFP.	Undergoing evaluation	V	126,

CK19, TPA, TPS ¹]				127
Circulating free SCCA-IgM complexes	Complementary to AFP in diagnosis of HCC.	Undergoing evaluation	V	128
α-Fucosyl-transferase	Marker of progression of HCC.	Undergoing evaluation	V	129
α-L-Fucosidase	Complementary to AFP.	Undergoing evaluation	V	130, 131
TGFβ1	Diagnosis of small HCCs.	Undergoing evaluation	V	132
Urinary TGFβ1	Complementary to AFP.	Undergoing evaluation	V	133
ICAM-1	Predictor of prognosis of HCC.	Undergoing evaluation	V	134, 135
Anti-p53-Ab	Complementary to AFP in diagnosis of HCC.	Undergoing evaluation	V	136
IL-8	Predictor of prognosis of HCC.	Undergoing evaluation	V	137
IGF-II	Complementary to AFP.	Undergoing evaluation	V	138
Telomerase	Diagnosis of HCC and predictor of its course of HCC. [Also assayed in ascitic fluid.]	Undergoing evaluation	V	139
Variant wild-type ER	Predictor of unfavorable prognosis in HCC	Undergoing evaluation	V	140, 141
Vitamin B12-binding protein	Diagnosis of the AFP-negative fibrolammellar variant of HCC.	Undergoing evaluation	V	142, 143
Neurotensin	Diagnosis of the AFP-negative fibrolammellar variant of HCC.	Undergoing evaluation	V	144
Free nucl.acids	Early detection and monitoring of HCC.	Undergoing evaluation	V	145
Epigenetic Abnormalities such as p16 hypermethylation	Early detection of HCC.	Undergoing evaluation	V	146
Proteomics	Early detection and monitoring of HCC.	Undergoing evaluation	V	147, 148
Tumor cell markers				
Tumor cells in peripheral blood detected by RT-PCR of AFP mRNA	Assessment of prognosis pre- and post-operatively. Prediction of early recurrence and distant metastases following surgery. Assist in therapeutic decisions. Clinical utility is controversial, and findings of published studies are inconsistent.	Undergoing investigation	IV, V	149- 153
Genetic markers				
Plasma glutamate carboxy-peptidase (PGCP); phospholipases A2 G13 and G7 and	Assessment of early HCC in patients with chronic viral chronic hepatitis; assessment of metastatic potential of HCC.	Undergoing evaluation	V	154, 155

other cdNA microarray-derived encoded proteins.				
MAGE-1/-3, SSX-1,2,4,5, SCP-1, NY-ESO-1	Complementary to AFP in monitoring recurrence. Candidate antigens for immunotherapy.	Undergoing evaluation	V	156, 157

LOE, level of evidence; AFP, α -fetoprotein; CON A, concanavalin A; LCA, *Lens culinaris* agglutinin; DCP, des- γ -carboxy-prothrombin; PIVKA II, prothrombin produced by vitamin K absence or antagonism II; γ -GTP, γ -glutamyl-transpeptidase; ALP, alkaline phosphatase; TPA, tissue polypeptide antigen; TPS, tissue polypeptide-specific antigen; SCCA, squamous cell carcinoma antigen; TGF, transforming growth factor; ICAM, intercellular cell adhesion molecule; IL-8, interleukin-8; IGF II, insulin-like growth factor II; ER, estrogen receptor

Table 2. Recommendations for use AFP in liver cancer by different Expert Groups.

Application	EASL¹ (158) 2001	French SOR² (50) 2001	BrSocGE³ (159) 2003	EGTM⁴(160) 1999	NCCN⁵ 2004 (49)	NACB⁶ 2005
Early detection of HCC by six-monthly determination of AFP (with abdominal ultrasound) in high risk groups [i.e. patients with chronic HBV, HCV or cirrhosis].	Yes	Yes	Yes	Yes	Yes	Yes
Confirmation of diagnosis of HCC if AFP >400 or 500 µg/L.	Yes	None published	Yes	Yes	Yes	Yes
Post-treatment monitoring (where pre-treatment AFP raised) as an adjunct to imaging.	Yes	Yes	None published	Yes	Yes	Yes
Indicator of increased risk of HCC when increased or increasing AFP is accompanied by negative ultrasound.	Yes	None published	None published	None published	Yes	Yes
Prediction of prognosis	Yes	None published	None published	None published	None published	Yes , in combination with existing factors
Monitoring following surgery, transplantation or percutaneous therapy	Yes	Yes	None published	None published	Yes , especially in absence of measurable disease	Yes , especially in absence of measurable disease
Monitoring advanced disease	Yes	Yes	None published	None published	Yes , especially in absence of measurable disease	Yes , especially in absence of measurable disease

EASL, European Association for the Study of the Liver; French SOR, French group for Standards, Options, and Recommendations; Br Soc GE, British Society of Gastroenterology; EGTM, European Group on Tumor Markers; NCCN, National Comprehensive Cancer Network; NACB, National Academy of Clinical Biochemistry

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