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Special Report

# National Academy of Clinical Biochemistry Laboratory Medicine Practice Guidelines for Use of Tumor Markers in Liver, Bladder, Cervical, and Gastric Cancers

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**BACKGROUND:** Updated National Academy of Clinical Biochemistry Laboratory Medicine Practice Guidelines for the use of tumor markers in the clinic have been developed.

**METHODS:** Published reports relevant to use of tumor markers for 4 cancer sites—liver, bladder, cervical, and gastric—were critically reviewed.

**RESULTS:** $\alpha$-Fetoprotein (AFP) may be used in conjunction with abdominal ultrasound for early detection of hepatocellular carcinoma (HCC) in patients with chronic hepatitis or cirrhosis associated with hepatitis B or C virus infection. AFP concentrations $>200$ $\mu$g/L in cirrhotic patients with typical hypervascular lesions $>2$ cm in size are consistent with HCC. After a diagnosis of HCC, posttreatment monitoring with AFP is recommended as an adjunct to imaging, especially in the absence of measurable disease.

Although several urine markers have been proposed for bladder cancer, none at present can replace routine cystoscopy and cytology in the management of patients with this malignancy. Some may, however, be used as complementary adjuncts to direct more effective use of clinical procedures.

Although carcinoembryonic antigen and CA 19-9 have been proposed for use gastric cancer and squamous cell carcinoma antigen for use in cervical cancer, none of these markers can currently be recommended for routine clinical use.

**CONCLUSIONS:** Implementation of these recommendations should encourage optimal use of tumor markers for patients with liver, bladder, cervical, or gastric cancers.



We present here to clinical chemists, clinicians, and other practitioners of laboratory and clinical medicine the latest update of the National Academy of Clinical

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Biochemistry (NACB)[17] Laboratory Medicine Practice Guidelines for the use of tumor markers in liver, bladder, cervical, and gastric cancers. These guidelines are intended to encourage more appropriate use of tumor marker tests by primary care physicians, hospital physicians, and surgeons, specialist oncologists, and other health professionals.

Clinical practice guidelines are systematically developed statements intended to assist practitioners and patients in making decisions about appropriate health care for specific clinical circumstances *(1)*. An explanation of the methods used when developing these guidelines has previously been published *(2)*. As might be expected, many of the NACB recommendations are similar to those made by other groups, as is made clear from the tabular comparisons presented for each malignancy *(2)*. The disciplines of all authors and statements of conflicts of interest, declared according to NACB requirements, are provided as required by *Clinical Chemistry*. All comments received about these guidelines, together with responses to these comments, are also recorded in the Comments Received Table in the Data Supplement that accompanies the online version of this report at http://www.clinchem.org/content/vol56/issue6.

To prepare these guidelines, the literature relevant to the use of tumor markers was reviewed. Particular attention was given to reviews, including the few relevant systematic reviews, and to guidelines issued by expert panels. If possible, the consensus recommendations of the NACB panels reported here were based on available evidence, i.e., were evidence based. NACB recommendations relating to general quality requirements for tumor marker measurements, including tabulation of important causes of false-positive tumor marker results that must also be taken into account (e.g., heterophilic antibody interference, high-dose hooking) have previously been published *(3)*.

[17] Nonstandard abbreviations: NACB, National Academy of Clinical Biochemistry; HCC, hepatocellular carcinoma; AFP, α-fetoprotein; CT, computed tomography; AASLD, American Association for the Study of Liver Diseases; BCLC, Barcelona Clinic liver cancer classification; LOE, level of evidence; IS, International Standard; LCA, *Lens culinaris* agglutinin; AFP-L3, AFP from HCC; DCP, des-γ-carboxy-prothrombin; AU, arbitrary units; EASL, European Association for the Study of the Liver; NCCN, National Comprehensive Cancer Network; LOE, level of evidence; SOR, Strength of Recommendation; RFA, radiofrequency ablation; GPC-3, glypican-3; sGPC-3, GPC-3 soluble serological marker; RT, reverse transcription; FDA, US Food and Drug Administration; CFH, complement factor H; NMP22, nuclear matrix protein 22; CK, cytokeratin; TPA, tissue polypeptide antigen; TPS, tissue polypeptide specific antigen; UBC, urinary bladder cancer; TRAP, Telomeric Repeat Amplification Protocol; hTR, human telomerase RNA; hTERT, human telomerase reverse transcriptase; BLCA, bladder cancer protein; HA, hyaluronic acid; HAase, hyaluronidase; HCG, human chorionic gonadotropin; PMF1, polyamine-modulated factor 1; CIN, cervical intraepithelial neoplasia; HPV, human papilloma virus; VLP, viruslike particles; FIGO, International Federation of Gynecology and Obstetrics; SCC, squamous cell carcinoma antigen; CEA, carcinoembryonic antigen.

## Tumor Markers in Liver Cancer[18,19]

### BACKGROUND

Hepatocellular carcinoma (HCC) is the fifth most common cancer in men and the eighth most common cancer in women worldwide *(4, 5)*. It is also the third most common cause of cancer-related death *(6)*, 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 US and Europe to 14.9/100 000 in Asia and Africa *(7)*. The higher incidence observed in Europe during the past decade probably reflects the increasing number of cases of hepatitis C infection *(8, 9)* and liver cirrhosis *(10)*, both strong predisposing factors for HCC *(11)*.

In most parts of Asia and Africa, hepatitis B virus infection is most relevant *(12)*, with ingestion of aflatoxin $B_1$ from contaminated food an additional contributory factor *(13)*. In the West and Japan, hepatitis C virus infection is the main risk factor *(7, 14–17)*, although patients with alcoholic cirrhosis or hemochromatosis are also at increased risk *(18)*. In these parts of the world, older patients are more likely than young patients to develop HCC *(15, 16)*. In contrast, in developing countries HCC more frequently affects younger individuals who have chronic hepatitis B *(19)*, with carriers having twice the relative risk of developing the disease. Cirrhotic patients have a higher risk than noncirrhotic patients, with annual HCC incidences of 2%–6.6% *(20)* and 0.4% *(21)*, respectively. Worldwide, 350 million individuals are infected with hepatitis B and 170 million with hepatitis C *(22)*. Protective vaccination is possible for hepatitis B but not hepatitis C. Antiviral strategies (e.g., pegylated α-interferon combined with ribavirin for hepatitis C or drugs such as lamivudine, adefovir, entecavir, and tenofovir for hepatitis B) are widely available *(23–25)*.

The rationale behind screening for HCC by regular liver ultrasound and tumor marker measurement in high-risk but asymptomatic groups is that screening facilitates early identification of tumors when they are still potentially curable. In patients with cirrhosis or chronic viral hepatitis monitored in this way, an increasing serum α-fetoprotein (AFP) concentration may provide the first indication of malignancy, prompting additional imaging of the liver and additional investigations *(26)*. In an asymptomatic patient, a predominant solid nodule that is not consistent with

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[19] All comments received about the NACB Recommendations for Liver Cancer are included in the online Data Supplement. Professor John Iredale was an invited Expert Reviewer.

hemangioma is suggestive of HCC *(27)*, whereas hypervascular lesions associated with elevated AFP (>400 μg/L) are almost diagnostic for malignancy. Ideally, randomized, controlled trials should be carried out to demonstrate the efficacy of screening in terms of decreased disease-related mortality and improved survival and cost-effectiveness *(28)*. It is unlikely that such trials will be undertaken, because it is already generally accepted that where surveillance has been systematically implemented, it is beneficial for selected cirrhotic patients *(29)*. In developed countries, about 30%–40% of patients with HCC are now diagnosed sufficiently early for curative treatments.

Because many patients with early disease are asymptomatic *(30, 31)*, HCC is frequently diagnosed late, by which time it is often untreatable *(32)*. Suspicion of disease may first arise in patients with liver cirrhosis who develop ascites, encephalopathy, or jaundice *(33)*. Some patients initially present with upper abdominal pain, weight loss, early satiety, or a palpable mass in the upper abdomen *(31)*. Other symptoms include obstructive jaundice, diarrhea, bone pain, dyspnea, intraperitoneal bleeding, paraneoplastic syndromes [e.g., hypoglycemia *(34)*, erythrocytosis *(35)*, hypercalcemia *(36, 37)*], severe watery diarrhea *(37)*, or cutaneous features (e.g. dermatomyositis) *(38)*.

Diagnostic imaging modalities include ultrasound, computed tomography (CT), and MRI *(6, 39)*. Ultrasound is widely available, noninvasive, 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. Although CT of the liver is sometimes used to investigate abnormalities identified on ultrasound, it is rarely used for primary screening. American Association for the Study of Liver Diseases (AASLD) guidelines specifically state that there are no data to support surveillance with CT scanning *(40)*. MRI provides high-resolution images of the liver.

Specimens for histopathology are usually obtained by biopsy under ultrasound or CT guidance. Risks of biopsy include tumor spread along the needle track (1%–2.7% overall) *(41, 42)*. The histological appearance of HCC ranges from well-differentiated to poorly differentiated lesions of large multinucleate anaplastic tumor giant cells, with frequent central necrosis. There is ongoing debate about the relevance of grading the dysplasia in predicting HCC.

Except in Japan, patients are rarely diagnosed with HCC at the very early stage of carcinoma in situ malignancy *(43)*, when 5-year survival rates are 89%–93% after resection and 71% after percutaneous treatment *(44)*. Patients with early stage HCC have 1 tumor nodule of <5 cm 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 *(45, 46)*. The much greater disease heterogeneity seen in more advanced disease 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., 1-year, 10%–72%, 2-year, 8%–50% *(47)*].

Curative treatments are offered to 30%–40% of HCC patients in referral centers in Western countries and to 60%–90% of patients in Japan *(6)*. Hepatic resection is the treatment of choice in noncirrhotic 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 1 nodule <5 cm in diameter or 3–5 nodules <3 cm each. Modern management of HCC has recently been reviewed *(40, 48, 49)*.

Potential 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 *(50)*. Percutaneous ethanol injection has been associated with few adverse events, response rates of up to 90%–100% and 5-year survival rates as high as 50% *(51)* in selected patient groups. Radiofrequency ablation or ethanol injection are very successful for patients with 1 tumor <3 cm. Radiofrequency ablation is also effective, with comparable objective responses, fewer sessions needed *(52)* and better 5-year survival rates for patients with larger tumors *(53, 54)*.

Palliative treatments in advanced disease include arterial chemoembolization, with survival advantages in well-selected candidates *(47)*. Embolization agents such as gelfoam 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. On the basis of improved understanding and detection of aberrant activation of several signaling cascades involved in liver cell transformation, molecular targeted therapies for HCC are being developed *(55)*. In multicenter phase III placebo-controlled trials 1 of these new drugs, the multikinase inhibitor Sorafenib, has been shown to be modestly effective in the treatment of advanced stage HCC [Barcelona Clinic liver cancer classification (BCLC) stages B and C] *(55–57)*.

It is clear from the above discussion that early detection of HCC, preferably when still asymptomatic, is desirable for a favorable outcome. The aim of this report is to present new NACB Guidelines for the use of

serum and tissue tumor markers in the early detection of HCC and its management. To prepare these guidelines, the literature relevant to the use of tumor markers in HCC was reviewed. Particular attention was given to reviews, including systematic reviews, prospective randomized trials that included the use of markers, and guidelines issued by expert panels. When possible, the consensus recommendations of the NACB Panel were based on available evidence, i.e., were evidence based. A summary of guidelines on these topics published by other expert panels is also presented.

### CURRENTLY AVAILABLE MARKERS FOR HCC

The most widely investigated tissue-based and serum-based tumor markers for HCC are listed in Table 1, together with the phase of development of each marker and the level of evidence (LOE) for its clinical use *(58)* (level 1, evidence from a single, high-powered, prospective, controlled study that is specifically designed to test the marker, or evidence from a metaanalysis, pooled analysis, or overview of level II or III studies; level II, evidence from a study in which marker data are determined in relationship to a prospective therapeutic trial that is performed to test therapeutic hypothesis but not specifically designed to test marker utility; level III, evidence from large prospective studies; level IV; evidence from small retrospective studies; level V, evidence from small pilot studies). Of the markers listed, only AFP is widely used in clinical practice.

### TUMOR MARKERS IN LIVER CANCER: NACB RECOMMENDATIONS

A summary of recommendations from representative guidelines published on the use of AFP in HCC is presented in Table 2. Table 2 also summarizes the current NACB guidelines for the use of markers in this malignancy. Below, we present a more detailed discussion of some of the markers listed in Tables 1 and 2.

### α-FETOPROTEIN

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 *(59, 60)*]. Normally produced during gestation by the fetal liver and yolk sac, AFP is highly elevated in the circulation of newborns with concentrations decreasing during the next 12 months to 10–20 μg/L.

*Analytical considerations: assay methods, standardization, and reference values.* AFP is currently measured by 2-site immunometric assays by using monoclonal and/or polyclonal antibodies, with results similar to those of the RIAs that preceded them. Most commercial assays are calibrated against WHO International Standard (IS) 72/225. Clinical results are reported in mass units (μg/L) or in kiloUnits per liter of IS 72/225, for which 1 IU of AFP corresponds to 1.21 ng. The upper reference limit used by most treatment centers is 10–15 μg/L (8.3–12.4 kU/L). AFP concentrations reportedly increase with age, the upper reference limit increasing from 11.3 μg/L in persons <40 years old to 15.2 μg/L in those >40 years old *(61)*. Ideally, reference values should be established for each assay, because there is some between-method variation in results.

*Analytical considerations: AFP carbohydrate microheterogeneity.* AFP is a glycoprotein and contains 4% carbohydrate as a single biantennary chain that is *N*-linked to asparagine-232 of the protein backbone *(62, 63)*. The microheterogeneity of this carbohydrate chain has been investigated extensively by use of both lectin affinity electrophoresis *(64–68)* and isoelectric focusing *(69–73)*. Distinct glycoform patterns characteristic of malignant or benign tissue have been 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 *(74–76)*. AFP from HCC patient sera, for example, binds more strongly to concanavalin A than does AFP from nonseminomatous 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 nonseminomatous germ cell tumors (AFP-L2). Assay kits are now available commercially that specifically measure the AFP-L3 and AFP-P4 glycoforms *(74, 76)*.

Numerous reported studies from Japan and other Asian countries have demonstrated 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 unfavorable outcome *(77–81)*. In a study comparing measurement of AFP-L3 and AFP in a US referral population (166 patients with HCC, 77 with chronic liver disease, and 29 with benign liver mass), AFP-L3 concentrations were found to be relevant only at AFP concentrations between 10 and 200 μg/L *(82)*. Within this range, AFP-L3 exhibited sensitivity of 71% and specificity of 63% at a cutoff of 10%. At a cutoff of >35% sensitivity decreased to 33% but specificity increased to 100%, enabling reliable diagnosis of an additional 10% of HCC cases that would not have been diagnosed using AFP alone at a cutoff of 200 μg/L.

In a multicenter prospective 2-year longitudinal North American study, serum AFP was compared with

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

| Cancer marker | Proposed uses | Phase of development | LOE | Reference |
|---|---|---|---|---|
| Tissue markers | | | | |
| *GPC3* | Differentiating HCC from other hepatic disorders at the tissue level. | Undergoing evaluation. | V | *(196, 197)* |
| *GPC3* + heat shock protein 70 + glutamine synthetase | Raised levels of 2 of the 3 markers indicate a need for biopsy (accuracy 78% at 100% specificity). | Undergoing evaluation. | | *(511)* |
| Telomerase | Independent prediction of recurrence after HCC resection. | Undergoing evaluation. | V | *(512–515)* |
| Proliferating cell nuclear antigen–labeling index | Prediction of recurrence and survival in small HCC. | Undergoing evaluation. | V | *(516)* |
| Ki-67 | Assessment of prognosis after resection of HCC. | Undergoing evaluation. | V | *(517)* |
| MIB-1, E-cadherin, $\beta$-catenin | Prognostic marker for recurrence when selecting HCC patients for orthotopic liver transplantation. | Undergoing evaluation. | V | *(518)* |
| Serum markers | | | | |
| AFP | Screening patients at high risk for HCC, especially those with hepatitis B– and hepatitis C–related liver cirrhosis. | In clinical use, but value not validated in a high-level evidence study. | III | *(89, 90, 99–104)* |
| | In conjunction with ultrasound, diagnosis of HCC in patients at high risk of disease. | In clinical use, but value not validated in a high-level evidence study. | III | *(30, 106–115, 118–120)* |
| | Assessing prognosis preoperatively. | Value not validated in a high-level evidence study. | III | *(32, 154, 166, 170, 179, 519)* |
| | Monitoring HCC patients, in conjunction with ultrasound, to detect early recurrence. | In clinical use, but value not validated in a high-level evidence study. | III | *(89, 90, 99–103, 179)* |
| | Monitoring patients with no evidence of disease after resection or transplantation. | In clinical use, but value not validated in a high-level evidence study. | IV | *(98, 99, 101, 103, 168)* |
| | Monitoring therapy in advanced disease. | In clinical use, but value not validated in a high-level evidence study. | IV | *(172, 174–178)* |
| AFP–concanavalin 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 | *(64–66)* |
| AFP–LCA binding | Differentiating malignant (high) from nonmalignant (low) origin of elevated AFP, independent of location (fucosylation index). | Not in general clinical use, but effective for AFP source origin on suspicion of malignant vs benign liver disease. | V | *(66, 520)* |
| HCC-specific AFP band on isoelectric focusing (monosialylated AFP) | Earlier detection of HCC than "diagnostic" AFP (>500 $\mu$g/L), positive predictive value 73% vs 42%, respectively. | Not in clinical use. | V | *(69–71)* |

*Continued on page e6*

**Table 1. Currently available serum and tissue markers for liver cancer.** *(Continued from page e5)*

| Cancer marker | Proposed uses | Phase of development | LOE | Reference |
|---|---|---|---|---|
| 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 | *(67, 68, 74, 75, 77–85, 165, 521)* |
| Circulating free AFP-IgM complexes | Providing information complementary to AFP. | Undergoing evaluation. | V | *(522)* |
| DCP/prothrombin produced by vitamin K absence or antagonism II | Used with AFP during and after treatment to predict adverse outcome, early recurrence, and malignant potential. False-positive results may occur in patients with severe obstructive jaundice or vitamin K action impairment (e.g., patients on warfarin or some antibiotics). Three commercial assays with differing accuracy are available. | Undergoing evaluation. | IV | *(84, 85, 173, 181–190, 192–194, 523)* |
| Soluble NH2 fragment of GPC-3, a heparan sulfate proteoglycan | Diagnosis and monitoring of HCC and cirrhosis. Enables detection of small-size HCC more sensitively than AFP. | Undergoing evaluation. | V | *(196, 199)* |
| Golgi protein 73 | Resident Golgi glycoprotein, for diagnosis of early HCC. | Undergoing evaluation. | V | *(524)* |
| Iso-$\gamma$GTP | Complementary to AFP as a diagnostic marker for HCC. | Undergoing evaluation. | V | *(525, 526)* |
| Ferritin | Monitoring HCC in patients whose tumors do not produce AFP. | No high-level evidence evaluation. | V | *(527, 528)* |
| Variant alkaline phosphatase | Complementary to AFP. | Undergoing evaluation. | V | *(529)* |
| $\alpha_1$-Antitrypsin | Complementary to AFP. | Undergoing evaluation. | V | *(530, 531)* |
| $\alpha_1$-Acid glycoprotein | Complementary to AFP. | Undergoing evaluation. | V | *(532)* |
| Osteopontin | Complementary to AFP. | Undergoing evaluation. | V | *(533)* |
| Aldolase A | Complementary to AFP. | Undergoing evaluation. | V | *(534, 535)* |
| 5′-Nucleotide phosphodiesterase | Complementary to AFP. Monitoring HCC in patients whose tumors do not produce AFP. | Undergoing evaluation. | V | *(536, 537)* |
| CK18, CK19, TPA, TPS | Complementary to AFP. | Undergoing evaluation. | V | *(538, 539)* |
| Circulating free squamous cell carcinoma antigen–IgM complexes | Complementary to AFP in diagnosis of HCC. | Undergoing evaluation. | V | *(540)* |
| $\alpha$-Fucosyl-transferase | Marker of progression of HCC. | Undergoing evaluation. | V | *(541)* |
| $\alpha$-L-fucosidase | Complementary to AFP. | Undergoing evaluation. | V | *(542, 543)* |

*Continued on page e7*

**Table 1. Currently available serum and tissue markers for liver cancer.** *(Continued from page e6)*

| Cancer marker | Proposed uses | Phase of development | LOE | Reference |
|---|---|---|---|---|
| Transforming growth factor $\beta$1 | Diagnosis of small HCC tumors. | Undergoing evaluation. | V | *(544)* |
| Urinary transforming growth factor $\beta$1 | Complementary to AFP. | Undergoing evaluation. | V | *(545)* |
| Intercellular cell adhesion molecule 1 | Predictor of prognosis of HCC. | Undergoing evaluation. | V | *(546, 547)* |
| Anti-p53 antibody | Complementary to AFP in diagnosis of HCC. | Undergoing evaluation. | V | *(548)* |
| Interleukin-8 | Predictor of prognosis of HCC. | Undergoing evaluation. | V | *(549)* |
| Interleukin-6 | Complementary to AFP in diagnosis of HCC, predictor of HCC. | Undergoing evaluation. | V | *(550, 551)* |
| Insulin-like growth factor II | Complementary to AFP. | Undergoing evaluation. | V | *(552)* |
| Telomerase or telomerase reverse transcriptase mRNA | Diagnosis of HCC and predictor of its course of HCC (also assayed in ascitic fluid). | Undergoing evaluation. | V | *(553, 554)* |
| Vascular endothelial growth factor | Prognostic marker. Predictor of poor outcome. | Undergoing evaluation. | V | *(555)* |
| Variant wild-type estrogen receptor | Predictor of unfavorable prognosis in HCC. | Undergoing evaluation. | V | *(556, 557)* |
| Vitamin B12–binding protein | Diagnosis of the AFP-negative fibrolammellar variant of HCC. | Undergoing evaluation. | V | *(558, 559)* |
| Neurotensin | Diagnosis of the AFP-negative fibrolammellar variant of HCC. | Undergoing evaluation. | V | *(560)* |
| Free nucleic acids | Early detection and monitoring of HCC. | Undergoing evaluation. | V | *(210)* |
| Circulating cell-free serum DNA | Predictive marker for distant metastasis of hepatitis C virus–related HCC. | Undergoing evaluation. | V | *(561)* |
| Epigenetic abnormalities such as p16 hypermethylation | Early detection of HCC. | Undergoing evaluation. | V | *(211)* |
| Proteomics | Early detection and monitoring of HCC. | Undergoing evaluation. | V | *(208, 209)* |
| Tumor cell markers | | | | |
| Circulating tumor cells in peripheral blood detected by RT-PCR of AFP mRNA | Assessment of prognosis pre and postoperatively. Prediction of early recurrence and distant metastases after surgery. Assist in therapeutic decisions. Clinical utility is controversial, and findings of published studies are inconsistent. | Undergoing investigation. | IV, V | *(200–204)* |

*Continued on page e8*

**Table 1. Currently available serum and tissue markers for liver cancer.** *(Continued from page e7)*

| Cancer marker | Proposed uses | Phase of development | LOE | Reference |
|---|---|---|---|---|
| Plasma proteasome | Marker of malignant transformation in cirrhotic patients including those with low tumor mass. | Undergoing evaluation. | V | *(562)* |
| Genetic markers | | | | |
| Plasma glutamate carboxypeptidase, phospholipases A2 G13 and G7 and other cDNA microarray-derived encoded proteins. | Assessment of early HCC in patients with chronic viral chronic hepatitis; assessment of metastatic potential of HCC. | Undergoing evaluation. | V | *(215, 563)* |
| Melanoma antigen gene 1, 3; synovial sarcoma on X chromosome 1, 2, 4, 5; sarcoplasmic calcium-binding protein 1; New York esophageal squamous cell carcinoma 1 | Complementary to AFP in monitoring recurrence. Candidate antigens for immunotherapy. | Undergoing evaluation. | V | *(564, 565)* |
| Circulating methylated DNA (ras association domain family 1A) | Detection and quantification of circulating methylated ras association domain family 1A useful for HCC screening, detection and prognosis. | Undergoing evaluation. | V | *(566)* |

**Table 2. Recommendations for use of AFP in liver cancer by different expert groups.**

| Application | AASLD 2005 *(40)* | Asian Oncology Summit *(136)* | BrSocGE[a] *(26)* 2003 | EASL *(131)* 2001 | EGTM *(137)* 1999 | ESMO 2009 *(4)* | French Standard, Options and Recommendations guidelines *(132)* | Japanese EBCIGI 2007/2008 *(127, 128)* | NCCN 2010 *(135)* | NACB 2010 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | LOE | SOR |
| Early detection of HCC by 6-month determination of AFP (with abdominal ultrasound) in high risk groups (i.e. patients with chronic hepatitis B or C virus or cirrhosis) | Yes (but AFP to be used only if ultrasound not available) | Yes (At 3- to 6-month intervals) | Yes | Yes | Yes | Yes (ultrasound with or without AFP) | Yes | Yes (including AFP, AFP-L3, DCP) | Yes | Yes | III | B/C |
| Indicator of increased risk of HCC when increased or increasing AFP is accompanied by negative ultrasound | None published | None published | None published | Yes | None published | None published | None published | | Yes | Yes | III | C |
| Confirmation of diagnosis of HCC | Yes (AFP >200 μg/L) | Yes (AFP >400 μg/L) | Yes | Yes (AFP >400 μg/L) | Yes | Yes (AFP >400 μg/L) | None published | Yes (AFP >200 μg/L) | Yes (AFP >400 μg/L) | Yes (AFP >200 μg/L) | III | B/C |
| Prediction of prognosis | None published | None published | None published | Yes | None published | None published | None published | None published | None published | Yes, in combination with existing factors | III | B/C |
| Posttreatment monitoring (where pretreatment AFP raised) as an adjunct to imaging | Yes | | None published | Yes | Yes | Yes | Yes | None published | Yes | Yes | IV | C |
| Monitoring after surgery, transplantation or percutaneous therapy | Yes | | None published | Yes | None published | Yes | Yes | None published | Yes, especially in absence of measurable disease | Yes, especially in absence of measurable disease | IV | C |
| Monitoring advanced disease | None published | | None published | Yes | None published | None published | Yes | None published | Yes, especially in absence of measurable disease | Yes, especially in absence of measurable disease | IV | C |

[a] Br Soc GE, British Society of Gastroenterology; EGTM, European Group on Tumor Markers; ESMO, European Society for Medical Oncology; Japanese EBCIGI, Japanese evidence-based clinical guidelines.

AFP-L3 and des-γ-carboxy-prothrombin (DCP) (an investigational tumor marker for HCC) in 372 patients with hepatitis C *(83)*, including 40 initial HCC and 34 HCC follow-up cases and 298 initially HCC-free cases *(83)*. Sensitivity, specificity, and positive/negative predictive values were, respectively, 61%, 71%, 34%, and 88% for AFP (cutoff 20 μg/L) and 22%, 99%, 80%, and 84% (cutoff 200 μg/L) compared with 37%, 92%, 52%, and 85% for AFP-L3 alone (cutoff 10%) and 39%, 90%, 48%, and 86% for DCP alone (cutoff 7.5 μg/L) *(83)*. When all 3 markers were combined, these figures increased to 77%, 59%, 32%, and 91%, respectively. In patients with raised AFP (20–200 μg/L), high specificity was found for AFP-L3 and DCP (86.6% and 90.2%, respectively). Of 29 HCC patients with AFP values <20 μg/L, 13 had increased concentrations of AFP-L3 or DCP. Compared with total AFP, AFP-L3 and DCP concentrations within reference intervals correlated more strongly with an absence of HCC, with a higher specificity and negative predictive value *(83)*.

In a prospective study comparing AFP-L3 and DCP with AFP in 99 US patients with histologically confirmed HCC, sensitivity rates were 62%, 73%, and 68%, respectively, with the highest sensitivity (86%) obtained when all 3 markers were combined *(84)*. AFP-L3 was significantly related to portal vein invasion and patient outcome, suggesting it could be a useful prognostic marker for HCC *(84)*. Use of the same 3 markers to predict HCC recurrence after curative percutaneous ablation has been investigated in 416 HCC patients, 277 of whom had recurrence during the follow-up period *(85)*. Pre- and postablation AFP >100 μg/L and AFP-L3 >15% were both significant predictors of recurrence and thus may complement imaging modalities in evaluating treatment efficacy *(85)*. A large and well-designed case-control study comparing AFP, AFP-L3, and DCP has recently been conducted in 7 academic medical centers in the US *(86)*. The study cohort included 417 patients with cirrhosis and 419 with HCC [77 with BCLC very early (BCLC 0) and 131 with early (BCLC A) stage disease]. ROC analysis revealed that AFP had higher sensitivity (67%) than DCP or AFP-L3 for patients with BCLC 0 stage disease *(86)*. Additional research is required to assess the value of AFP and related markers as surrogate endpoints for true health outcomes in clinical trials *(87, 88)*.

*AFP in screening and early detection.* Cirrhotic patients with AFP concentrations that are persistently elevated are at increased risk of developing HCC compared with those with AFP concentrations that fluctuate or remain within reference intervals (29% vs 13% vs 2.4%, respectively) *(6)*. Lower serum AFP concentrations are frequently encountered when HCC is detected during screening *(89)*, and small HCC tumors are AFP negative in up to 40% of cases *(90)*. AFP immunostaining of well-differentiated small HCCs is often negative *(91)*, rendering tissue AFP uninformative. In these instances, tumors may be detectable only by ultrasound *(92)*. Malignant lesions undetectable by imaging are likely to reach 2 cm in diameter in about 4–12 months *(93, 94)*. To detect tumors ≤2 cm in diameter, a suggested interval for surveillance in cirrhotic patients is 6 months, with the use of both serum AFP and ultrasound *(95)*. Comparison of studies is often difficult owing to differences in study design. In addition, opinions differ as to how effectively AFP measurement contributes to programs for early detection or surveillance *(96)*. Reliable markers are needed to complement ultrasound, because the interpretation of ultrasound is operator dependent and can be difficult to perform in patients who are obese or have underlying cirrhosis *(97)*.

In a systematic review of AFP test characteristics for diagnosis of HCC in HCV patients *(98)*, only 5 of 1239 studies met all the authors' inclusion criteria *(99–103)*. In these 5 studies, with the use of an AFP cutoff of 20 μg/L, sensitivity ranged from 41% to 65%, specificity from 80% to 94%, positive likelihood ratio from 3.1 to 6.8, and negative likelihood ratio from 0.4 and 0.6, additional demonstrating the limited value of AFP as a screening test. In 19 of 24 studies of patients with hepatitis C published from 1985 to 2002, AFP sensitivities and specificities for HCC were 45%–100% and 70%–95%, respectively, at cutpoints between 10 and 19 μg/L *(104)*. Ultrasound has been reported to have higher sensitivity (71%) and specificity (93%) than serum AFP, but the positive predictive value of ultrasound is low, at about 14% *(30)*. Because 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 studies suggest that 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 *(105)*.

In one study, 1069 hepatitis B virus–infected patients with proven cirrhosis had to be screened to detect 14 cases of HCC, of which only 6 were at a sufficiently early stage to be amenable to surgical cure *(106)*. 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 6-month intervals with ultrasound, AFP, and DCP. Only 1 of 14 detected HCC cases (7%) was surgically resectable at the time of diagnosis *(107)*. However, other studies have demonstrated benefit in screening chronic hepatitis B carriers for HCC. A population-based Alaskan prospective screening study of 2230 carriers with cirrhosis who were positive for hepatitis B surface antigen *(108, 109)* demonstrated that 64%–87% of detected HCCs were limited to single foci and that 43%–75% of tumors were <3 cm in size, which enabled curative surgery in 29%–66% of the detected cancers *(12, 110, 111)*. In another study, tumor size was significantly reduced and survival improved (35% vs 10% at 30 months) when HCC was detected by screening *(112)*.

There is some evidence that screening high-risk populations for HCC can be cost-effective in high-prevalence regions such as Hong Kong *(113)* and that screening imparts a survival advantage, as demonstrated in an asymptomatic Asian Hawaiian population with chronic hepatitis B or C and cirrhosis *(114)* and also in an Italian study of cirrhotic patients with screen-detected HCC *(115)*. These conclusions are supported by results of a randomized, controlled trial of screening of 18 816 patients age 35–59 years recruited in urban Shanghai between 1993 and 1995 who had hepatitis B infection or a history of chronic hepatitis *(116)*. Biannual screening with AFP and ultrasound reduced HCC mortality by 37%. Although results of a screening study of 5581 hepatitis B carriers between 1989 and 1995 in Qidong county demonstrated that screening with AFP resulted in earlier diagnosis of liver cancer, the gain in lead time did not result in any overall reduction in mortality *(117)*. It seems likely that this finding reflects differences in therapy in the 2 studies, 75% of patients with subclinical HCC identified in the Shanghai study having received radical treatment compared with only 25% in the Qidong study *(116)*.

A national survey of practice in the US *(118)* has documented that a majority of institutions routinely screen patients with cirrhosis for HCC, especially those with high-risk etiologies. 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 carriers positive for hepatitis B surface antigen who have additional risk factors (e.g. active chronic hepatitis, cirrhosis) and in patients with cirrhosis of any etiology *(119)*. 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 *(120)*. Biannual 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 *(120)*. Results of a later systematic review and economic analysis indicated that AFP measured biannually and ultrasound performed every 6 months provide the most effective surveillance strategy in high-risk patients *(121)*. Because of high costs, however, the authors questioned whether ultrasound should be routinely offered to those with serum AFP <20 μg/L, in view of the cost-benefit ratio, which depends on the etiology of cirrhosis.

These conclusions are generally supported by results of a recent modeling study in which effectiveness and cost-effectiveness of surveillance for HCC were evaluated in separate and mixed cohorts of individuals with cirrhosis due to alcoholic liver disease, hepatitis B, or hepatitis C *(122)*. Algorithms including the use of AFP and/or ultrasound at 6- and 12-month intervals were compared. In the mixed cohort, the model found that AFP and ultrasound performed every 6 months to be most effective, tripling the number of patients with operable tumors at diagnosis and almost halving the number of deaths from HCC compared with no surveillance. Based on this report, the most cost-effective strategy would involve triage with 6-month AFP measurements. It was concluded that in the UK National Health Service, surveillance of individuals with cirrhosis at high risk for HCC should be considered to be both effective and cost-effective *(122)*.

Given the widespread use of AFP measurements and liver ultrasound to screen prospectively for the onset of HCC in cirrhotic patients, particularly those who are suitable candidates for curative therapy *(109, 123, 124)*, there is an urgent need to establish and validate optimal follow-up protocols when suspicious nodules are detected *(10, 125, 126)*.

Recently published Japanese evidence-based clinical guidelines for diagnosis and treatment of HCC differentiate the risk of HCC in patients with cirrhosis as being super high (hepatitis B/C–related cirrhosis) or high (chronic hepatitis B/C or liver cirrhosis with a cause other than hepatitis B/C) *(127, 128)*. For the super high-risk group, ultrasound examination and measurements of AFP, DCP, and AFP-L3 are recommended at intervals of 3–4 months, with a dynamic CT or MRI scan every 6–12 months. For the high-risk group, ultrasound and tumor-marker measurements are recommended every 6 months. Addition of DCP or AFP-L3 is considered necessary because these are diagnostic markers whereas AFP is a marker of risk *(129, 130)*. Detection of a nodular lesion by ultrasound and/or a continuous rise in AFP (>200 μg/L), DCP [in

arbitrary units (AU) with 1 AU = 1 μg prothrombin] (>40 mAU/mL), or AFP-L3 (>15%) requires further evaluation by dynamic CT or MRI *(127, 128)*.

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 at 6 months, that fine-needle biopsy and histology be added to investigate nodules of 1–2 cm (false-positive rate 30%–40%), and that additional noninvasive diagnostic criteria (e.g., 2 imaging techniques) be employed for tumors >2 cm *(131)*. French recommendations published in 2001 *(132)* state that the diagnosis of HCC should be based on histopathological examination of 1 or more liver samples obtained by open surgery, laparoscopy, or ultrasound/CT-guided biopsy (standard) with the option of fine-needle aspiration for cytology if liver biopsy is impossible.

In a recent US retrospective study in which patients with hepatic lesions suspicious for HCC underwent both fine-needle aspiration and core biopsy, results were correlated with those from commonly used noninvasive methods *(133)*. Patients with positive biopsy results had significantly higher serum AFP concentrations than those with negative biopsy results, although the 2 groups were otherwise similar. Biopsy results had greater sensitivity, specificity, and predictive value compared with noninvasive diagnostic criteria. The authors recommended an increased role for image-guided biopsy of suspicion lesions >1 cm in size to allow adequate treatment planning, and commented that the risks of biopsy appear small and the potential benefits significant *(133)*.

It is of course essential to be aware of the caveats of use of AFP, including the benign and malignant diseases that may cause raised serum AFP and the fact that a value within reference intervals never necessarily excludes malignancy *(99, 134)*. An elevated AFP detected by a single measurement may be transient (e.g., arising from an inflammatory flare of underlying chronic viral hepatitis), whereas elevated but stable concentrations decrease the likelihood that HCC is the causal agent. Sequential measurements of serum AFP may therefore provide useful information, but this is still under investigation and not yet fully validated for routine clinical practice. A steadily rising pattern of elevated AFP should always be rigorously investigated by using ultrasound and other imaging techniques, which if initially negative should be repeated to identify any possible occult hepatic malignancy *(131)*.

In 2003 the British Society of Gastroenterology presented guidelines on the use of serial tumor marker measurements to screen for HCC *(26)*. The expert group concluded that in high-risk groups, screening by abdominal ultrasound and AFP compared to no surveillance detected HCC of smaller size. Such detection enables a greater proportion of curative therapies, with earlier detection leading to improved long-term survival and/or cost savings. It was suggested that surveillance for HCC should be restricted to males and females with cirrhosis due to hepatitis B or C virus or genetic hemochromatosis and to males with cirrhosis due to primary biliary cirrhosis and alcoholic cirrhosis (if abstinent or likely to comply with treatment). The likelihood of HCC arising in cirrhosis of other etiology was considered to be low. Surveillance using AFP and abdominal ultrasound was recommended at 6-month intervals, with appropriate equipment and skilled operators essential for the ultrasound component. Patients should be counseled on the implications of early diagnosis and its lack of proven benefit *(26)*.

These recommendations are in accord with National Comprehensive Cancer Network (NCCN) guidelines *(135)*, which recommend surveillance using both AFP and ultrasound in patients at risk for HCC *(135)*. Those considered as being at risk include patients with cirrhosis associated with hepatitis B or alcohol, genetic hemochromatosis, autoimmune hepatitis, nonalcoholic steatohepatitis, primary biliary cirrhosis, or $\alpha_1$-antitrypsin deficiency. Surveillance is also recommended for individuals without cirrhosis who are hepatitis B carriers or have other risk factors (e.g., active viral replication, high hepatitis B virus DNA concentrations, family history of HCC, Asian males >40 years old, females >50 years old, Africans <20 years old). The NCCN recommends additional imaging if serum AFP is rising or after identification of a liver mass nodule on ultrasound *(135)*. The 2009 consensus statement of the Asian Oncology Summit also recommends liver ultrasound and measurement of AFP concentrations every 3–6 months in all patients with liver cirrhosis, regardless of etiology, with the caveat that such surveillance is best established in hepatitis B virus–related liver cirrhosis, for which the LOE is relatively high *(136)*. The AASLD currently recommends use of AFP for surveillance but only when ultrasound is not available *(40)*. This organization also states that HCC screening should be "offered in the setting of a program or a process in which screening tests and recall procedures have been standardized and in which quality control procedures are in place" *(40)*.

In accord with these and other recommendations *(26, 131, 132, 135, 137)* (Table 2), the NACB supports the use of determinations of AFP every 6-months and abdominal ultrasound to screen prospectively for the onset of HCC in high-risk patients, especially those with liver cirrhosis related to hepatitis B or C virus.

**NACB LIVER CANCER PANEL RECOMMENDATION 1: AFP IN SCREENING PATIENTS AT HIGH RISK FOR HCC**

AFP should be measured and abdominal ultrasound performed at 6-month intervals in patients at high risk of HCC, especially in those with liver cirrhosis related to hepatitis B and hepatitis C virus. AFP concentrations that are >20 μg/L and increasing should prompt further investigation even if ultrasound is negative [LOE, III/IV; Strength of Recommendation (SOR), C].

*AFP in diagnosis.* Elevated serum AFP concentrations are not specific for HCC because increased concentrations also occur in normal pregnancy, in certain benign liver diseases, and in some malignancies. Non-HCC malignancies that may give rise to high AFP concentrations include nonseminomatous germ cell tumors, for which AFP is an important tumor marker with well-established clinical use *(138)*. AFP may also be raised in stomach cancer, biliary tract cancer, and pancreatic cancers *(139)*. Elevated AFP concentrations exceeding 1000 μg/L are, however, rare in these malignancies, occurring in <1% of cases.

Approximately 20%–40% of adult patients with hepatitis or liver cirrhosis have raised AFP concentrations (>10 μg/L) *(140)*. In these patients, an AFP concentration between 400 and 500 μg/L was initially generally accepted as the optimal decision point to differentiate HCC from chronic liver disease *(26, 136, 141–143)*. However, a Japanese study advocated an optimal cutoff of 150 μg/L based on ROC analysis (sensitivity 54%, specificity 95.9%, comparing results for patients with HCC and benign chronic liver disease) *(144)*. Using the same ROC technique, an Italian group demonstrated the same specificity of 99.4% with cutoffs of 200 and 400 μg/L, but with higher sensitivity at the lower cutoff *(99)*. The 2001 EASL guidelines state that AFP >400 μg/L together with detection of a suspicious liver node on imaging is diagnostic of HCC *(131)*. This guideline is in accord with recommendations of the Asian Oncology Summit panel, which concluded that a characteristic image on dynamic CT or dynamic MRI, regardless of tumor size, will suffice for diagnosis of HCC, and obviate the need for biopsy, with AFP >400 μg/L diagnostic in patients with liver cirrhosis or chronic hepatitis *(136)*. This group also recommended that needle biopsy be avoided when curative surgery is possible. Both the AASLD *(40)* and Japanese expert panels *(131)* state that in patients with a suspicious liver node on imaging, AFP concentrations >200 μg/L are also suspicious and should be investigated. After exclusion of hepatic inflammation, a sustained rise in AFP is suggestive of HCC and should prompt further liver imaging studies, whereas stable or decreasing results make it less likely.

Circulating AFP concentrations in patients presenting with HCC range from within the reference interval to as high as $10 \times 10^6$ μg/L (i.e. 10 g/L), with pretreatment concentrations >1000 μg/L in approximately 40% of patients *(145)*. AFP has been reported to be higher in patients with HCC arising from chronic viral conditions compared to those with alcoholic liver disease *(146)* and in younger *(147)* and male *(147)* 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% *(148)*. According to some Japanese investigators *(149)*, any circulating AFP value >10 μg/L in patients with chronic liver disease should be regarded as suspicious of HCC and prompt further investigation, e.g., using AFP-L3 (LCA) or AFP-P4 (E-PHA) lectin tests and imaging. These investigators advocate a lower decision point of 10 μg/L rather than 20 μg/L to take into account the improvements in imaging that have led to more HCC being detected when AFP is <20 μg/L. In Japan, for example, the percentage of HCC patients with AFP concentrations <20 μg/L at presentation increased from 3.6% in 1978 to 38.1% in 2000. From 2001 to 2003, after a change in AFP cutoff to <15 μg/L, 36.4% of HCC patients had increased AFP concentrations *(127)*. Introduction of a lower cutoff was supported by a previous report that healthy Japanese individuals do not have AFP concentrations >10 μg/L *(150)*, but this finding may apply only to the population studied.

The Japanese guidelines state that HCC can be diagnosed by imaging (dynamic CT/MRI/contrast-enhanced ultrasound) or other techniques (hypervascularity in the arterial phase and wash-out in the portal venous phase) *(127, 128)*. Continuous increases in AFP (>200 μg/L) and/or DCP (>40 mAU/mL) and/or AFP-L3 (>15%) are highly suggestive of typical HCC, even in the absence of ultrasound evidence of an apparent liver nodule *(127)* and should prompt the use of dynamic CT or MRI *(128)*.

According to recent guidelines from the AASLD, surveillance/screening in patients at risk for HCC should be performed by ultrasound scanning at intervals of 6–12 months and AFP alone not be used unless ultrasound is not available *(40)*, whereas the NCCN guidelines recommend periodic screening with ultrasound and AFP every 6–12 months *(135)*. On ultrasound detection of a nodule <1 cm, the AASLD panel recommends follow-up by ultrasound at intervals of 3–6 months, reverting to routine surveillance if there is no growth after a period of up to 2 years *(40)*. In con-

trast, the NCCN guidelines recommend imaging control by CT/MRI/ultrasound every 3–4 months for nodules <1 cm, reverting to routine surveillance if the nodule does not increase in size for 18 months *(135)*. Nodules of 1–2 cm that are detected by ultrasound in cirrhotic liver should be investigated by 2 dynamic studies (e.g., CT, MRI) and treated as HCC if their appearance is consistent with this diagnosis, but if not characteristic, the lesion should be biopsied.

For a nodule >2 cm at initial diagnosis with typical HCC features (e.g., classic arterial enhancement on triphasic CT or MRI) or cases in which AFP is >200 μg/L, results can be considered diagnostic of HCC, and biopsy unnecessary, but if the lesion is not characteristic, or the liver is noncirrhotic, biopsy is recommended. For small lesions that are negative on biopsy, ultrasound or CT follow-up at 3- to 6-month intervals is recommended, with repeat biopsy if the lesion enlarges but remains atypical. 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.

The use of AFP as an adjunct in the diagnosis of HCC is recommended by EASL*(131)*, the British Society of Gastroenterology *(26)*, the European Group on Tumor Markers *(137)* and the NCCN *(135)*. These recommendations are supported by the NACB Panel, which also stresses the importance of serial AFP measurements together with consideration of sustained increases in AFP even at low concentrations (Table 2).

**NACB LIVER CANCER PANEL RECOMMENDATION 2: AFP IN THE EARLY DETECTION OF HCC IN PATIENTS AT HIGH RISK**

In patients at risk for HCC, sustained increases in serum AFP may be used in conjunction with ultrasound to aid early detection of HCC and guide further management. Ultrasound detected nodules <1 cm should be monitored at 3-month intervals with ultrasound. Nodules of 1–2 cm in cirrhotic liver should be investigated by 2 imaging modalities (e.g., CT and MRI). If the appearance of the nodules is consistent with HCC, they should be treated as such, with biopsy required if not. If lesions are >2 cm in size, AFP is >200 μg/L, and the ultrasound appearance is typical of HCC, results may be considered diagnostic of HCC and biopsy is not necessary (LOE, III; SOR, B).

*AFP in prognosis.* The TNM (tumor-node-metastasis) system *(151)* and the Okuda classification *(152)* are the most frequently used staging systems for HCC. Prognostic classifications from Japan *(153)*, France *(154)*, Italy *(32, 155)*, Spain *(156, 157)*, and China *(158)* have also been published [see also *(159, 160)*]. Of these, the Spanish BCLC staging system showed the best prognostic stratification *(161)* and was also adopted in the AASLD guidelines *(40)*. 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 *(152, 155)*. According to AASLD guidelines *(40)*, for optimal assessment of the prognosis of HCC patients, the staging system should include tumor stage, liver function, and physical status and consider life expectancy, all of which are included in the Spanish BCLC system.

The Chinese staging system (AFP cutoff 500 μg/L) *(158)* and 2 European staging systems include AFP. The French system includes the Karnofsky index, ultrasonographic portal vein obstruction, and serum bilirubin, alkaline phosphatase, and AFP (cutoff 35 μg/L) *(154)*. Based on the score, patients are classified as being at low, moderate, or high risk for death, with 1-year survival rates of 72%, 34%, and 7%, respectively. Another classification, proposed by the Cancer of the Liver Italian Program *(155)*, includes Child-Pugh stage, morphology, portal vein thrombosis, and serum AFP (cutoff 400 μg/L). By use of a simple scoring system, patients are assigned to 1 of 7 categories with validated median survival rates *(155)*. Both classifications incorporate AFP as an indicator of tumor spread and burden, cellular differentiation, and aggressive potential. With the aim of improving available systems for postoperative risk classification, a nomogram based on clinicopathological variables including serum AFP, patient age, tumor size and margin status, postoperative blood loss, presence of satellite lesions, and vascular invasion has recently been developed *(162)*. The nomogram reportedly enables accurate prediction of postoperative survival and risk stratification in patients undergoing liver resection for HCC and is currently undergoing evaluation *(162)*.

It has been suggested that considering AFP and alkaline phosphatase, Child-Pugh score, and the absence or presence of ascites could improve outcome prediction *(46, 154, 155)*. An Italian study of prognostic factors in 176 patients with HCC demonstrated that low albumin (<33 μg/L), high bilirubin (>22.5 μmol/L), elevated AFP (>32.5 kU/L), portal vein thrombosis, and an untreatable lesion were independent risk factors for worse survival *(163)*. Survival depended most strongly on the degree of functional liver impairment, presence of hepatitis B virus 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 *(164)*. Age, degree of liver damage, AFP concentration, max-

imal 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 *(164)*.

Large studies using multivariate analyses confirm that raised AFP concentrations predict poor prognosis compared with AFP-negative cases in HCC *(32, 154, 165)*. In a retrospective study of 309 HCC patients stratified according to pretreatment AFP concentrations (<20, 20–399 or ≥400 μg/L), patients with higher AFP concentrations tended to have larger tumors, but there was no correlation with Okuda stage, degree of tumor differentiation, or extrahepatic metastasis *(166)*. In contrast, a more recent large Italian multicenter survey that used the same 3 AFP groups in 1158 HCC patients *(167)* revealed a low sensitivity (54%) for AFP in diagnosis of HCC, but confirmed its prognostic value by demonstrating its significant correlation with tumor size, lesion focality, TNM and Okuda stage, Edmonson score, and survival ($p < 0.0001$) in treated as well as in untreated patients.

According to other authors *(168, 169)*, AFP, as well as tumor size, 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) *(170)*. AFP concentrations >1000 μg/L predict a relatively worse prognosis, even after attempted curative resection *(70)*. Serum AFP concentrations <12 000 μg/L are required to meet UK criteria for liver transplantation *(171)*.

AFP doubling time has also been reported to be an important prognostic factor *(172)*. Persistence of a positive AFP-L3 fraction after intervention also has been reported to indicate residual or recurrent disease *(77)*. The NACB supports the prognostic use of pretreatment serum AFP concentration in combination with other prognostic factors (Table 2).

**NACB LIVER CANCER PANEL RECOMMENDATION 3: AFP FOR DETERMINING PROGNOSIS**

In combination with other prognostic factors, AFP concentrations may provide prognostic information in untreated HCC patients and in those undergoing liver resection, with high concentrations indicating poor prognosis (LOE, IV; SOR, C).

*AFP in monitoring patients after treatment.* For patients with increased AFP concentrations before therapy, monitoring treatment of HCC by use of serial AFP determinations is a well-accepted procedure. After complete removal of the tumor, AFP concentrations typically decrease, with a half-life of 3.5–4 days. Incomplete resection yields a longer half-life, which is associated with poorer survival *(166, 172)*, whereas failure of the AFP to normalize implies residual malignancy or severe liver damage. [Determination of the AFP-L3 fraction can help to differentiate these 2 conditions *(81, 142, 173)*.] However, normalization of AFP does not necessarily indicate complete clearance of the disease. Recurrence after transplantation may occur, even when AFP is stable and within reference limits *(168, 172, 174)*, presumably reflecting the presence of micrometastases too small to produce measurable serum concentrations.

Changes in AFP concentrations also reflect tumor response after chemotherapy, with longer survival in patients showing a significantly prolonged decrease in AFP than in those with slowly increasing concentrations *(175, 176)*. In patients receiving new and effective combined systemic therapies *(177)*, 75% have shown dramatic decreases in serum AFP, with concentrations normalizing completely in some patients. Progressive disease was found in patients with continued AFP increase and doubling times between 6.5 and 112 days (mean 41 days), again correlating with survival *(172)*. Similar results were observed after 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 *(178)*. Discrepancies between tumor marker and imaging results may be due to residual fibrosis and other factors that can complicate interpretation of CT scans *(178)*.

A recent phase III randomized trial of systemic chemotherapy in HCC patients evaluated clinical and radiological outcome and included prospectively collected serial AFP measurements *(179)*. In 117 patients with initially elevated serum AFP (cutoff 20 μg/L) and an AFP response (≥20% decrease) after the second cycle of chemotherapy, 47 had improved survival compared with 70 AFP nonresponders (13.5 vs 5.6 months; $P < 0.0001$). AFP concentrations were strongly associated with radiological response ($P < 0.0001$) and also with survival (multivariate analysis: hazard ratio 0.413, $P < 0.0001$). It was therefore concluded that in HCC patients undergoing systemic chemotherapy, serial AFP determinations may be useful both for prognosis and for monitoring treatment response, as well as providing a surrogate marker for the evaluation of new therapeutic agents *(179)*. Similarly, authors of a recent study from Massachusetts General Hospital Cancer Center and Harvard Medical School concluded that serum AFP change during treatment may serve as a useful surrogate marker for clinical outcome in patients with advanced HCC receiving systemic therapy *(180)*.

According to the French Standard, Options and Recommendations guidelines *(132)*, there is no consensus about patterns or modalities of follow-up other than clinical examination and surveillance plans that may incorporate ultrasound, AFP measurement, abdominal CT scan, chest x-ray, and/or MRI, with optimal choice and timing of these dependent on treatment options. The NCCN is more specific, recommending posttreatment follow-up of HCC patients that includes imaging every 3 to 6 months for 2 years and then annually, with AFP (if initially elevated) measured every 3 months for 2 years, and then every 6 months *(135)*. Similarly, ESMO recommends that patients undergoing curative resection should be followed up with liver imaging and AFP measurement for 2 years at 3- to 6-month intervals, and then annually, because curative therapy can be offered to a minority of patients after relapse *(4)*. After liver transplantation, follow-up should be more frequent, i.e., monthly for 6 months, then once every 3 months up to 1 year posttransplantation, then twice a year up to 2 years, and annually thereafter *(4)*.

In accord with other expert groups *(131, 132, 135)*, the NACB recommends serial determinations of serum AFP (if elevated before treatment) to monitor efficacy of treatment, course of disease, and recurrence, and supports the frequency of measurement recommended by the NCCN *(135)*.

**NACB LIVER CANCER PANEL RECOMMENDATION 4: AFP IN MONITORING TREATMENT**

Measurement of AFP at follow-up visits is recommended to monitor disease status after liver resection or liver transplantation for detection of recurrence or after ablative therapies and application of palliative treatment. Although monitoring intervals are as yet undefined, current practice suggests following patients every 3 months for 2 years and then every 6 months (LOE, IV; SOR, C).

*Tumor markers other than AFP.* Des-γ-carboxy-prothrombin. DCP, also known as PIVKA II (prothrombin produced by vitamin K absence or antagonism II), is an abnormal prothrombin devoid of coagulation activity and is potentially a marker for HCC. Mainly developed and investigated in Japan, DCP was first described in the US in 1984 *(181)* and critically reviewed there in 1993 *(182)*. 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 AU/L.

A number of published investigations have reported DCP sensitivities for the diagnosis of HCC ranging from 54% to 70% at a decision point of 40 AU/L, 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% *(183–186)*. DCP, AFP, and combined DCP/AFP sensitivities for solitary HCC (<2 cm) were 30%–53%, 13%, and 57%, respectively, and for larger tumors (>3 cm) were 78%–81%, 49%–69%, and 84%–94%, respectively, *(183, 184, 186)*. 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) *(186)*. Both DCP and AFP concentrations correlated with tumor size and grading, but not significantly with each other.

A cross-sectional case control study that compared serum AFP and DCP in a US population has confirmed the apparent superiority of DCP as a tumor marker for HCC *(187)*. The study included 48 healthy adults, 51 patients with chronic hepatitis (mostly hepatitis C), 53 individuals with compensated cirrhosis, and 55 people with proven HCC. With the use of ROC analysis, DCP was found to perform better than AFP in differentiating HCC from cirrhosis (sensitivity 90% vs 77%, specificity 91% vs 71%, positive predictive value 85% vs 81%, negative predictive value 90% vs 74%, area under the ROC curve 0.921 vs 0.815). There was no improvement over DCP alone when the 2 markers were combined.

DCP has also been reported to have prognostic significance. In a study of HCC patients treated by percutaneous ethanol injection 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 *(188)*, whereas ROC analysis results suggested it was an effective predictor of HCC recurrence after resection *(189)*. In another study 237 HCC patients were categorized into 4 groups according to concentrations of DCP (less than or greater than 62.5 AU/L) and AFP (less than or greater than 100 μg/L) *(190)*. The 22 patients with low AFP and high DCP were predominantly male and had large lesions but few nodules. Outcome was particularly poor in patients who had high concentrations of both DCP and AFP *(190)*. According to a more recent report comparing serum AFP and DCP determinations in 1377 HCC and 355 chronic liver disease patients, the utility of DCP was lower in smaller tumors (<3 cm diameter) than in larger ones (>5 cm diameter) *(191)*.

A retrospective analysis of 199 HCC patients with early stage HCC in Child Pugh A cirrhotic patients treated by resection or radiofrequency ablation (RFA) showed similar 3- and 5-year survival rates (90%/79% vs 87%/75%) *(192)*. One- and 3-year tumor recurrence-free survival rates were higher in the pa-

tients treated by resection (83%/51% vs 83%/42% for RFA; $P = 0.011$) *(192)*. With multivariate analysis, prothrombin time ≥80% was found to be an independent prognostic factor for the resected group whereas platelet count ≥100 000 and DCP concentration <100 AU/L were prognostic for the RFA group. At DCP concentrations ≥100 AU/L the treatment procedure became a significant prognostic factor for survival. These results suggest that a high DCP concentration reflects biological aggressiveness and that surgical resection rather than RFA treatment is advantageous in these patients. The prognostic value of pretreatment concentrations of AFP (cutoff 400 μg/L), AFP-L3 (cutoff 15%), and DCP (cutoff 100 AU/L) has been investigated in HCC patients after curative treatment by hepatectomy (n = 345) and compared to locoregional thermal ablation (n = 456) *(173)*. Multivariate analysis results in hepatectomy patients indicated that no tumor marker was associated with decreased survival. In patients who had undergone locoregional thermal ablation, elevation of AFP-L3 ($P = 0.0171$) or DCP ($P = 0.0004$) was significantly associated with decreased survival and DCP was also associated with increased rate of recurrence ($P < 0.0001$).

An investigation of AFP, AFP-L3, and DCP in 240 patients with hepatitis B or C (144 HCC, 47 chronic hepatitis, and 49 cirrhotic cases) at optimal cutoffs according to ROC analysis (DCP, 84 AU/L; AFP, 25 μg/L; AFP-L3, 10%) yielded sensitivity, specificity, and positive predictive value rates of 87%, 85%, and 86.8% for DCP; 69%, 87%, and 69.8% for AFP; and 56%, 90%, and 56.1% for AFP-L3 *(193)*. DCP concentrations were below cutoff in all non-HCC cases but increased in all HCC cases including those with single lesions. DCP correlated with tumor size, high AFP concentrations with diffuse type HCC, and all 3 markers with metastatic HCC. The authors recommended routine use of DCP for HCC detection.

False-positive elevated DCP concentrations are found in patients with severe obstructive jaundice due to intrahepatic cholestasis or in conditions in which the action of vitamin K is impaired (e.g., in individuals with longstanding vitamin K deficiency and those who have ingested warfarin and some wide-spectrum antibiotics) *(194)*. Despite these limitations, DCP is a promising emerging marker with considerable potential.

Glypican-3. Glypican-3 (GPC-3), initially termed MXR7 *(195)*, is another promising new tissue and serum marker for HCC. The gene glypican 3 (*GPC3*)[20] codes for a member of the glypican family of glycosylphosphatidylinositol–anchored cell-surface heparan sulfate proteoglycans *(196)*. GPC-3 was first detected via its mRNA, which was increased in 75% of tissue samples from patients with primary and recurrent HCC but in only 3.2% of samples from normal liver tissue *(195)*. These data were later confirmed immunohistochemically *(196, 197)*. Elevated GPC-3 mRNA concentrations were also found in the serum of HCC patients *(195)*. Sensitivity exceeded that of AFP (88% vs 55%) for the entire group of HCC patients tested as well as for those with smaller HCC tumors <3 cm (77% vs 43%). In a later study of 34 HCC patients *(196)*, sensitivity was somewhat lower (53%) and similar to that of AFP (54%). However, specificity was excellent, with no significant elevations in healthy sample donors or patients with acute hepatitis, and in only 1 of the 20 patients with chronic hepatitis and cirrhosis. The combined sensitivity of the 2 markers was 82%. Neither marker correlated with the other.

Although another group has demonstrated the presence of the C-terminus in serum *(198)*, a recent report on the GPC protein suggests that the only fragment present in the circulation is the amino terminal, which constitutes the GPC-3 soluble serological marker (sGPC-3) *(199)*. With the use of an ELISA with highly specific monoclonal antibodies to analyze 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 (cutoff 2 μg/L) comparable to those of AFP [55%/90% (cutoff 20 μg/L]. The sensitivity of the 2 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 for HCC (Table 1). Pre- and posttreatment detection of circulating HCC cells by reverse transcription (RT)-PCR of AFP mRNA has been suggested by some groups to be useful in predicting HCC recurrence and poor outcome *(200, 201)*, although other investigators have questioned its value *(202–204)*. Other techniques under investigation include genetic profiling, transcriptomics *(205–207)*, proteome analysis *(208, 209)*, and determination of free nucleic acids *(210)* and epigenetic abnormalities (e.g., p16 hypermethylation) in serum or plasma *(211)*. Also being explored are the prognostic implications of CpG-island hypermethylation and DNA hypomethylation *(212)*, microRNA profiling *(213)* and exploration of liver cancer stem cells *(214)*. Fifty upregulated HCC marker genes, which are potential tumor marker

[20] Genes: *GPC3*, glypican 3; *FGFR3*, fibroblast growth factor receptor 3; *CGB*, chorionic gonadotropin, beta polypeptide (*hCGβ*); *PMF1*, polyamine-modulated factor 1; *TP53*, tumor protein p53.

candidates, have been identified in hepatitis C virus–associated HCC by use of cDNA microarray analysis of surgical liver samples from patients infected with hepatitis C virus *(215)*.

The NACB panel does not recommend the use of any HCC-related biomarkers except AFP for the routine surveillance of patients with or at risk of HCC. The NACB does, however, support further evaluation of the clinical utility of potential markers for which there is increasing published evidence (e.g., AFP-L3, DCP, and GPC-3) in suitably designed prospective randomized clinical studies.

**NACB LIVER CANCER PANEL RECOMMENDATION 5: TUMOR MARKERS OTHER THAN AFP**

AFP is currently the only marker that can be recommended for clinical use in liver malignancies. New liver cancer markers offer promise but their contribution to the current standard of care is unknown and further investigations in properly designed clinical trials are needed (LOE, not applicable; SOR, C).

**KEY POINTS: TUMOR MARKERS IN HCC**

HCC 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 of these diseases is instituted early, the risk of 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 lesions. The clinical utility of AFP measurement, together with ultrasound and other more sensitive imaging techniques, is already well established for this application, whereas other tumor markers require further investigation. Future developments in molecular genetics and proteomic analysis may lead to earlier diagnosis and more effective treatment of HCC patients.

## Tumor Markers in Bladder Cancer[21,22]

**BACKGROUND**

Each year in the US, nearly 71 000 new cases of bladder cancer are diagnosed and approximately 14 000 people die from this disease *(216)*. The prevalence of bladder cancer in the US is estimated at almost 500 000 cases. Almost twice as many cases of bladder cancer occur in men than in women, with cigarette smoking the leading cause *(217)*. Other risk factors include exposure to industrial carcinogens and chronic infection with *Schistosomiasis haematobium*.

The most common symptom of bladder cancer is intermittent hematuria (80%–85% of patients). Other urinary tract symptoms include increased frequency, urgency, and dysuria (15%–20% of patients). The diagnosis is usually established by cystoscopic evaluation, prompted by hematuria or urinary tract symptoms, and biopsy. In some cases, urine cytology is positive for tumor cells. Bladder cancer is staged according to the degree of tumor invasion into the bladder wall *(218)*. Carcinoma in situ (stage Tis) and stages Ta and T1 are grouped as nonmuscle invasive bladder cancers because they are restricted to the inner epithelial lining of the bladder and do not involve the muscle wall. Of the nonmuscle invasive tumors, stage Ta tumors are confined to the mucosa, whereas stage T1 tumors invade the lamina propria. T1 tumors are regarded as being more aggressive than Ta tumors *(219)*. Muscle invasive tumors (stages T2, T3, and T4) extend into the muscle (stage T2), the perivesical fat layer beyond the muscle (stage T3), and adjacent organs (T4). Metastatic tumors involve lymph nodes (N1–3) or distant organs (M1).

The most common cell type of bladder cancer is transitional cell carcinoma, although adenocarcinomas, squamous cell carcinomas, and sarcomas also occur. The cellular morphology of nonmuscle invasive bladder tumors is graded according to the degree of cellular differentiation. The grading consists of well-differentiated (grade 1), moderately differentiated (grade 2), and poorly differentiated (grade 3) tumors. Grading of cell morphology is important for establishing prognosis, because grade 3 tumors are the most aggressive and the most likely to become invasive. Use of the WHO classification from 2004 is widely advocated, because it facilitates uniform diagnosis of tumors *(220)*. A modified grading system (WHO International Society of Urological Pathology 1998), which is increasingly being used *(221)*, eliminates the numerical grades and categorizes most bladder cancers as either low grade or high grade.

The heterogeneity of urological tumors—in terms of both histological origin and clinical behavior *(222)*—means that clinical parameters such as tumor grade and stage are not sufficiently accurate to predict biological behavior or to guide treatment reliably, especially in high-risk cases *(223–225)*. New markers to aid diagnosis, assess prognosis, identify optimal treatment, and monitor progression of urological cancers are urgently required.

Bladder cancer may be regarded as a genetic disease caused by the multistep accumulation of genetic and epigenetic factors *(226–228)*. Nonmuscle invasive bladder tumors are generally treated by transurethral

[21] NACB Bladder Cancer Sub-Committee Members: Herbert A. Fritsche (Chair), Thorsten H. Ecke, H. Barton Grossman, Seth P. Lerner, Ihor Sawczuk.

[22] All comments received about the NACB Recommendations for Bladder Cancer are included in the online Data Supplement.

resection of the bladder with or without intravesical treatments with bacille Calmette-Guérin immunotherapy or intravesical chemotherapy. Muscle invasive tumors are usually treated by cystectomy, or with bladder-sparing therapies that consist of chemotherapy and radiation. Patients who have metastatic disease require systemic chemotherapy with multiple anticancer agents *(229)*. A thorough understanding of cancer progression pathways facilitates development of drug therapies against specific tumor targets *(225)*.

The majority of bladder cancer patients are diagnosed with nonmuscle invasive tumors. Even though these tumors can be completely resected, there is a high risk of recurrence; 50%–70% of these patients will develop tumor recurrence within 5 years. With intensive medical surveillance, the 5-year survival rates for these patients range from 95% to 75% for Ta and T1 tumors, respectively. However, almost 25% of patients with Ta and T1 noninvasive tumors will eventually develop invasive disease. The 5-year survival rate decreases with tumor invasiveness and the presence of metastasis. Patients with stage T2 tumors have a 5-year survival rate of 60%, but only 35% of patients with stage T3 tumors and 10% of patients with stage T4 metastatic tumors survive 5 years *(218)*.

Lifelong surveillance is therefore required for bladder cancer patients who are initially diagnosed with nonmuscle invasive disease. Current patient-monitoring protocols generally consist of regularly scheduled cystoscopic evaluations, usually together with urine cytology, performed every 3 months during the first 2 years of follow-up, twice a year during years 3 and 4, and annually thereafter, until disease recurrence is documented *(230)*.

Urine tumor markers have been proposed for use as diagnostic aids in patients who present with hematuria, as prognostic indicators of disease recurrence and survival, and as early detectors of recurrent disease in monitored patients. Potential applications of urine tumor marker tests in patient surveillance include serial tests for earlier detection of recurrent disease, adjuncts to urine cytology to improve the detection of disease recurrence, less expensive and more objective alternatives to urine cytology, and indicators to direct the frequency of cystoscopy evaluation in the follow-up of patients with bladder cancer.

To prepare these guidelines, we reviewed the literature relevant to the use of tumor markers in bladder cancer. Particular attention was given to reviews, including systematic reviews, prospective randomized trials that included the use of markers, and guidelines issued by expert panels. Where possible, the consensus recommendations of the NACB panel were based on available evidence, i.e., were evidence based.

#### CURRENTLY AVAILABLE TUMOR MARKERS FOR BLADDER CANCER

Currently available bladder cancer tumor markers and some of those in development are listed in Table 3, with an assessment of each marker and the LOE for its clinical use. The LOE grading system *(58)* and SOR *(231)* have been applied as previously described *(2)* [SOR *(231)*, A = high (further research is very unlikely to change the panel's confidence in the estimate of effect); B = moderate (further research is likely to have an important impact on the panel's confidence in the estimate of effect and is likely to change the estimate); C = low (further research is very likely to have an important effect on the panel's confidence in the estimate of effect and is likely to change the estimate); D = very low (any estimate of effect is very uncertain)]. As indicated in Table 3, 6 tumor marker tests, all of which are measured in urine, have been cleared by the US Food and Drug Administration (FDA) for use in routine patient care.

#### URINE TUMOR MARKERS IN BLADDER CANCER: NACB RECOMMENDATIONS

At this time, and in accord with NCCN practice guidelines for bladder cancer *(232)*, no tumor marker tests can be recommended for use in the routine diagnosis and clinical management of bladder cancer. This includes tests for making a differential diagnosis, assessing prognosis, staging the disease, and monitoring patients for the early detection of recurrent disease. There are no prospective clinical trial data that establish the utility of any of the FDA-cleared markers or the proposed markers for increasing survival time, decreasing the cost of treatment, or improving the quality of life of bladder cancer patients. In the following report, we describe the FDA-cleared markers and the variety of newly proposed markers.

#### FDA-CLEARED MARKERS FOR BLADDER CANCER

*BTA-Stat and Trak tests for complement factor H and related proteins.* The BTA-Stat test (Polymedco) detects complement factor H (CFH) and CFH-related proteins in urine *(233)*. Factor H, a 155-kDa protein, has a central role in regulating the alternate pathway of complement activation to prevent complement-mediated damage to healthy cells. At least 4 other factor H–related proteins have been identified as products of a cluster of genes on chromosome 1 called the regulators of complement activation locus, and although some of these proteins possess complement regulatory activity, others do not *(233)*.

The BTA-Stat test provides semiquantitative detection of CFH and the CFH-related protein antigens by use of a double monoclonal antibody, immunochromatographic point-of-care device. For both noninvasive (Tis, Ta, T1) and invasive (T2–T4) tumors, the

**Table 3. Useful and potentially useful urine markers for bladder cancer.**

| Cancer marker | Proposed use/uses | Phase of development | LOE | FDA cleared? | References |
|---|---|---|---|---|---|
| BTA Stat | An aid in the early diagnosis and monitoring for recurrence of disease. | In clinical use | III | Yes | *(233, 241–243)* |
| BTA Trak | An aid in the early diagnosis and monitoring for recurrence of disease. | In clinical use | III | Yes | *(233, 241–243)* |
| NMP22 | An aid in the early diagnosis and monitoring for recurrence of disease. | In clinical use | III | Yes | *(245–254)* |
| Bladder Chek | An aid in the early diagnosis and monitoring for recurrence of disease. | In clinical use | III | Yes | *(245–254)* |
| ImmunoCyt | An aid in the early diagnosis and monitoring for recurrence of disease. | In clinical use | III | Yes | *(257–261)* |
| UroVysion | An aid in the early diagnosis and monitoring for recurrence of disease. | In clinical use | III | Yes | *(262–264)* |
| CK8, 18, 19 | None at present. | Not in clinical use | IV | No | *(272, 273, 276–279)* |
| Telomerase: TRAP, hTERT, hTR | None at present. | Not in clinical use | IV | No | *(279–284)* |
| BLCA-4 | Early detection. | In clinical trials | IV | No | *(286–288)* |
| Survivin protein and mRNA | Prognosis. | In clinical trials | III | No | *(289, 291, 296–298)* |
| Microsatellite markers | Early detection. | In clinical trials | III | No | *(299–305)* |
| HA/HAase | None at present. | Not in clinical use | IV | No | *(307–310)* |
| *FGFR3* | Prognosis. | In clinical trials | III | No | *(311–318)* |
| DD23 monoclonal antibody | None at present. | Not in clinical use | IV | No | *(319, 320)* |
| Fibronectin | None at present. | Not in clinical use | IV | No | *(321, 322)* |
| HCG $\beta$-subunit and $\beta$-core protein and mRNA | None at present. | Not in clinical use | IV | No | *(323)* |
| DNA promoter regions of hypermethylated tumor suppressor and apoptosis genes | None at present. | In research | IV | No | *(324–326)* |
| Proteomic profiles (mass spectrometry) | None at present. | In research | V | No | *(327, 328)* |

BTA-Stat test is variously reported to have sensitivities within the range 50%–83% *(234–238)* and specificities within the range 60%–92% *(236, 239, 240)*. False-positive test results are reported to occur in some patients after trauma and in patients with infection of the bladder or urinary tract, nephritis, urinary calculi, or benign prostatic hyperplasia *(241)*.

The BTA-Trak test is a quantitative enzyme immunoassay version of the BTA-Stat test. The manufacturer reports sensitivities of 67% (Tis), 59% (Ta), 92% (T1), and 89% (T2–T4) for the stages of bladder cancer indicated. Specificities of 60% are observed in benign renal disease, urinary tract infections and sexually transmitted diseases, and rise to 80%–90% in various other genitourinary diseases.

Both tests have sensitivities comparable to that of cytology for high-grade tumors and better than cytology for low-grade tumors. However, because of their high false-positive rate, these tests are not sufficiently accurate to be used for screening or early detection of bladder tumors. The NACB panel therefore does not recommend the BTA-Stat or Trak tests for use in screening or diagnosis.

**NACB BLADDER CANCER PANEL RECOMMENDATION 1: BTA TESTS FOR SCREENING AND DIAGNOSIS OF BLADDER CANCER**

The BTA-Stat and Trak tests are not recommended for screening or diagnosis of bladder tumors (LOE, III; SOR, B).

The BTA tests are FDA cleared only for use in combination with cystoscopy for monitoring of bladder cancer. Although confirmatory reports have vali-

dated the high sensitivity of the BTA-Trak test in patients with recurrent disease *(242, 243)*, the test has not been generally accepted for patient surveillance because of its high false-positive rate *(243)*. The NACB panel does not recommend the use of either the BTA-Stat or -Trak test alone for monitoring patients with a diagnosis of bladder cancer, but in accord with the FDA, recognizes that when these tests are used in combination with cystoscopy they may be helpful in selected high-risk patients *(243, 244)*.

**NACB BLADDER CANCER PANEL RECOMMENDATION 2: BTA TESTS FOR MONITORING PATIENTS WITH BLADDER CANCER**

The BTA-Stat and -Trak tests are not recommended for monitoring patients after treatment for bladder cancer (LOE, III; SOR, B). In selected patients and when used in combination with cystoscopy, their measurement may provide additional information, but there is no evidence that this improves outcome (LOE, III; SOR, B).

*Nuclear matrix protein.* The nuclear matrix protein 22 (NMP22) test (Matritech) is a double monoclonal antibody test designed to measure quantitatively the nuclear mitotic apparatus protein. This component of the nuclear matrix is overexpressed by bladder cancer and is released into the urine in increased quantity. NMP22 is not stable in urine, and the use of a protein preservative is recommended *(228)*. Clinical trial data showed that the NMP22 test, when performed 6–40 days postsurgery, correctly predicted the presence of recurrent disease at the first cystoscopic follow-up visit in 71% of patients (24 of 34) with positive NMP22 results *(245)*. In patients with negative NMP22 test values, 86% (61 of 71) had no clinical evidence of disease at the first follow-up cystoscopy. Miyanaga et al. *(246)* reported similar results for the NMP22 test but with a 35% false-positive rate. In that study and a follow-up report *(247)*, NMP22 clearly performed better than voided urine cytology in detecting bladder cancer. Similar results were also reported by Stampfer et al. in a multicenter study involving 171 patients with 274 cystoscopies *(248)* and by other investigators *(249, 250)*.

A point-of-care version of the NMP22 test called the Bladder Chek NMP22 test is available *(251)*. One published report has considered the false-positive effect of red blood cells on this test *(252)*, whereas another recent report suggested that the presence of white blood cells was responsible for false-positive NMP22 results *(253)*. In a recent comparison of Bladder Chek with cytology in which 1331 patients with hematuria were tested, the Bladder Chek test had a sensitivity of 55.7%, whereas cytology detected 15.8% of the cancers. The specificity of Bladder Chek was 85.7% compared with 99.2% specificity for urine cytology *(254)*. The high false-positive rate of NMP22-based tests has limited their general acceptance for routine use in patient care.

Reported values for sensitivity of the NMP22 ELISA test range from 47% to 100% *(255)*. Other studies have shown that NMP22 performs less well in surveillance compared with primary detection of bladder cancer, although NMP22 has a better sensitivity for surveillance than cytology *(256)*. A combination of NMP22 and cystoscopy was reportedly more sensitive than cystoscopy alone in detecting recurrences *(222)*. NMP22, however, was evaluated as an adjunct to cystoscopy or cytology alone *(256)*. In conclusion, the NMP22 test is easy to perform with better sensitivity than cytology and reasonable specificity and is also sensitive in low-grade tumors *(247, 249, 250)*. Although the false-positive rate is high, NMP22 may be superior to cytology in sensitivity, and by careful patient selection NMP22 specificity could be improved.

The FDA has cleared the NMP22 test for use as an aid in the diagnosis of patients at risk of or with symptoms of bladder cancer *(255)*.

**NACB BLADDER CANCER PANEL RECOMMENDATION 3: NMP22 AND BLADDER CHEK NMP22 TESTS FOR EARLY DETECTION OF BLADDER CANCER AND SURVEILLANCE MONITORING OF PATIENTS WITH BLADDER CANCER**

The NMP22 and Bladder Chek NMP22 tests are not recommended for primary detection of bladder cancer or for routine monitoring of patients after treatment for bladder cancer (LOE, III; SOR, B). In selected patients and when used in combination with cystoscopy, NMP22 measurement by use of these tests may provide additional information but there is no evidence that performing these measurements leads to improved outcome (LOE, III; SOR, B).

*ImmunoCyt test.* The ImmunoCyt test (Diagno-Cure) detects bladder cancer–associated markers present on exfoliated cells using a cocktail of fluorescent antibodies (19A211, M344, and LDQ10) *(257)*. The monoclonal antibody 19A211 detects high molecular weight carcinoembryonic antigen, whereas M344 and LDQ10 detect a cancer-related mucin. According to one recent report, the test has a sensitivity of 81% and specificity of 75% in detecting bladder cancer *(258)*. The ImmunoCyt test was evaluated in several earlier investigations *(259, 260)* with similar findings *(259, 260)*. When used with cytology, the ImmunoCyt test appears to improve the detection of low-grade tumors *(261)*.

*UroVysion test.* Multitarget FISH detects cancer cells based on the aneuploidy of selected chromosomes. The

UroVysion test (Vysis) employs centromere probes specific to chromosomes 3, 7, and 17 and a locus-specific probe for 9p21 to detect aneuploidy associated with bladder cancer *(262)*. A multisite study of the UroVysion test demonstrated 71% sensitivity and 94.5% specificity for bladder cancer, which is much better than that of the BTA Stat test *(263)*. A similar finding was reported by Friedrich et al. in a comparison of UroVysion with BTA Stat and NMP22 *(264)*.

In other studies, the sensitivity of the UroVysion test is between 69% and 87% *(255, 265–267)*. The test has excellent sensitivity to detect carcinoma in situ and high-grade/high-stage tumors (range 83% to 100%). Indeed FISH analysis may be useful in predicting occult disease in those patients with no cystoscopic evidence of tumor, thereby resolving cases with ambiguous cytology, and in monitoring response to therapy. A study demonstrated that 89% of patients with a negative bladder biopsy results and atypical cytology in the setting of a positive FISH developed biopsy-proven transitional cell carcinoma within 12 months *(268)*. Results of recent studies suggest that different markers in the UroVysion test may have different significance when used to predict the biologic behavior of bladder cancer *(269)*. Several studies have shown that UroVysion may also be useful for monitoring patients after bacille Calmette-Guérin treatment *(270, 271)*.

Thus the UroVysion test appears to be a promising test for detection of high-grade bladder cancer, as well as having the potential to predict bladder cancer recurrence and progression within 6–12 months. At present, FISH testing should be reserved for selected clinical situations in which it may provide more information than cytology. The high cost and complexity of the test, which requires highly trained personnel and sophisticated equipment, have slowed its adoption in routine practice. Other limitations include the requirement for intact urothelial cells and lack of consensus about what constitutes a positive result *(228)*.

**NACB BLADDER CANCER PANEL RECOMMENDATION 4: IMMUNOCYT AND UROVYSION TESTS FOR EARLY DETECTION OF BLADDER CANCER AND SURVEILLANCE MONITORING OF PATIENTS WITH BLADDER CANCER**

The ImmunoCyt and UroVysion tests are not recommended for primary detection of bladder cancer or for routine monitoring patients after treat-ment for bladder cancer (LOE, III; SOR, B). In selected patients and when used in combination with cystoscopy, ImmunoCyt and UroVysion tests may provide additional information but there is no evidence that this improves outcome (LOE, III; SOR, B).

**PROPOSED BIOMARKERS NOT CLEARED BY THE FDA**

*Cytokeratins.* Cytokeratins (CK) are intermediate filament proteins characteristic of epithelial cells. Overexpression of certain cytokeratins occurs in transitional cell carcinoma of the bladder *(272)*. Recent studies using an ELISA method to measure cytokeratin-19 fragment (CYFRA 21-1) demonstrated 75% to 97% sensitivity and approximately 70% specificity *(255)*. A specific assay for urinary CK19 (CYFRA 21-1) has also been shown to have high sensitivity and specificity for bladder cancer *(273)*. However, the performance of this marker in early stage bladder cancer is disappointing, perhaps reflecting the fact that CYFRA 21-1 concentrations are influenced by benign urological diseases and intravesical instillations *(274)*. CK20 concentrations have been measured in exfoliated cells using both RT-PCR and immunocytochemical techniques *(255, 275)*. The sensitivity of CK20 detected by either method varies between 78% and 87%, with specificity between 55% and 80% *(255, 275)*.

The tissue polypeptide antigen (TPA) test (Sangtec Medical) employs polyclonal antisera for detection of CK8, 18, and 19. Although the overall sensitivity is reported to be 80%, a false-positive rate of 30%–40% has limited TPA use in routine patient care *(276)*. Subsequently, a tissue polypeptide-specific (TPS) test (IDL Biotech) was developed, which employs monoclonal antibodies against CK8 and 18 *(277)*. Another version, called the urinary bladder cancer (UBC) test (IDL), also detects CK8 and 18. A preliminary report suggests a sensitivity of 65% and specificity of 92% for this test *(276, 278)*. In one method comparison study, the UBC test outperformed the BTA Stat and NMP22 tests, showing higher sensitivity and specificity for bladder cancer *(279)*. In general, however, the relatively low specificity of cytokeratin markers, particularly in relation to patients with benign inflammatory conditions, limits their clinical applicability.

*Telomerase.* Telomeres are regions located at the end of human chromosomes and are composed of many identical short repetitive sequences of TTAGGG. Their function is to stabilize and protect chromosomes *(279, 280)*. With each cell cycle, the ends of the telomeres shorten, until a critical length is reached after which cell division leads to breakdown of the telomere. Telomerase is a ribonucleoprotein enzyme that adds telomere repeats to maintain telomere length. Telomerase is inactivated in normal human epithelial tissue, but is reactivated in neoplasia *(279)*. Telomerase has 2 major components, an RNA template and an enzymatic subunit.

The Telomeric Repeat Amplification Protocol (TRAP) assay (Geron) measures the enzymatic activity

of telomerase. Telomeric repeats are synthesized in vitro and amplified by PCR, and the products are visualized by various methods *(279)*. In a tissue study of bladder tumors, 86% of samples (48 of 56) were shown to be telomerase positive, but no activity was detected in nonneoplastic bladder tissue. The same study evaluated exfoliated cells in 109 urine samples from urological patients, 26 of whom had bladder cancer. The authors reported 62% sensitivity and 96% specificity for telomerase activity in exfoliated urothelial cells *(280)*. Advances in the measurement of telomerase include RT-PCR assays for the human telomerase RNA (hTR) and mRNA for human telomerase reverse transcriptase (hTERT). These assays have demonstrated a sensitivity of 83% for hTR and 80% for hTERT *(281, 282)*. Sanchini et al. compared the TRAP and hTERT assays and confirmed the high sensitivity of both assays for telomerase, but suggested that the hTERT assay may be subject to a high false-positive rate in patients with inflammation of the urinary tract *(283)*. Saad et al. reported that the combined use of the TRAP assay with NMP22 gave sensitivity and specificity comparable to that of voided urine cytology *(284)*. However, many bladder cancer patients have other comorbidities, limiting the clinical applicability of telomerase assays. In 1 study, the sensitivity was as low as 7% because of the inactivation of telomerase enzyme in urine *(285)*. In conclusion, telomerase assays are not useful in their current form for detection and monitoring of bladder cancer.

*BLCA-4.* A bladder cancer–specific nuclear matrix protein (BLCA-4) has been described *(286, 287)*. The BLCA proteins were identified on 2-dimensional gels and sequenced; antibodies were subsequently raised to synthetic peptides corresponding to those sequences. Preliminary immunoassay data showed the BLCA-4 protein to be present in the urine of 53 of 54 bladder cancer patients (4 stage Tis, 25 stage Ta–T1, 13 stage T2–T3, and 6 stage T4). BLCA-4 urine concentrations in all 51 healthy controls were below the upper limit of the reference interval. However, 38 of 202 patients with spinal cord injury had elevated values. Superficial tumor was subsequently found in only 1 of these 38 patients *(288)*. Because spinal cord injury patients are at high risk for developing bladder cancer, these patients will require additional follow-up to assess the diagnostic role of BLCA-4. Clinical studies are under way to confirm the encouraging preliminary data on the utility of BLCA-4 in bladder cancer.

*Survivin.* The protein survivin is an inhibitor of apoptosis that is associated with the mitotic spindle *(289)* and is expressed in most common cancers *(290)*, with expression low in normal adult tissues but high in cancer tissues and transformed cell lines *(291)*. Survivin expression can be detected in all bladder cancer tissues, but not in normal urothelium specimens *(292, 293)*. The expression patterns of survivin in patients with bladder cancer can be examined in urine, as can the diagnostic potential of RT-PCR detection of survivin mRNA *(294, 295)*. Smith et al. have developed a polyclonal semiquantitative immunoassay to assess the role of survivin as a urine marker for bladder cancer *(291)*. The protein was detected in all 46 new and recurrent cases of bladder cancer, but in none of 17 healthy individuals. Survivin was present in 3 of 35 patients who had previously been treated for bladder cancer but who had negative cystoscopic evaluations. More recently, Shariat et al. reported sensitivity and specificity and positive and negative predictive values for the survivin protein of 64%, 93%, 92%, and 67%, respectively, in precystoscopy urine samples *(296)*. In this study, urine survivin outperformed the NMP22 test in detecting bladder cancer. The detection of mRNA survivin transcripts in exfoliated cells and bladder washings rather than the survivin protein may further improve the detection of bladder cancer *(297)*.

In one study, survivin mRNA detection in urine sediment by use of RT-PCR showed high sensitivity (94%) and specificity (95%) for bladder cancer and may prove useful for the routine screening and monitoring of patients *(292)*. Similarly, Schultz et al. identified survivin as the most promising candidate to distinguish between patients with primary Ta urothelial cell carcinoma and a long (71.4%) or short (69.6%) recurrence-free interval *(298)*. In the future, survivin mRNA expression analysis may help the urologist to individualize patient treatment and prevent unnecessary cystoscopy in a subgroup of patients with bladder cancer.

*Microsatellite detection.* Repetitive sequences of DNA, each containing 1 to 4 bp, are present throughout the genome and may undergo mutational changes associated with neoplasia, thereby serving as genetic cancer markers. The most common genetic change seen in bladder cancer is loss of heterozygosity in chromosome 9. From 60% to 70% of bladder neoplasms show loss of heterozygosity in either the long or the short arm of chromosome 9, which indicates that loss of suppressor genes may be the early initiating event in bladder carcinogenesis *(299, 300)*.

Using 20 microsatellite DNA markers, Mao et al. *(301)* detected 95% of patients with bladder cancer. Steiner et al. *(302)* tested 2 microsatellite markers in serial urine samples from 21 patients who had been treated for bladder cancer. Recurrent lesions were detected in 10 of 11 patients independently verified to have recurrent disease. Results of several other studies

*(303–305)* that used different panels of DNA markers suggest that it may be possible to identify a small set of microsatellite markers that reflect key DNA alterations specific and sensitive for bladder cancer. All of these reports suggest that microsatellite analysis of exfoliated cells is potentially useful to detect bladder cancer.

A prospective multicenter validation study for detection of incident bladder cancer and prediction of recurrence initiated by investigators at Johns Hopkins University and supported by the National Cancer Institute Early Detection Research Network has been completed and results are pending. A similar study conducted in the Netherlands for detection and follow-up of low-grade disease, which evaluated the value of microsatellite polymorphisms for bladder cancer detection, demonstrated sensitivity of 58% and specificity of 73% for detection of recurrence *(306)*. A persistently positive test was associated with an 83% probability of recurrence at 2 years.

*Hyaluronic acid and hyaluronidase.* Hyaluronic acid (HA), the glycosaminoglycan ligand for CD44, can promote tumor cell adhesion, migration and angiogenesis. Hyaluronidase (HAase) degrades HA into angiogenically active fragments. Lokeshwar et al. *(307)* have demonstrated that the HA test has a sensitivity of 83% and specificity of 90% for detecting bladder cancer. In addition, they found that HAase was elevated 5-fold to 8-fold in the urine of patients with grade 2 and 3 tumors compared to healthy individuals. Urinary HAase measurement has demonstrated a sensitivity of 100% and a specificity of 89% for detection of these high-grade bladder tumors in 139 patients *(308)*. Hautmann and coworkers have used these analytes together in a combined HA-HAase test *(309)*. In 2 method comparison studies, the HA-HAase test outperformed the ImmunoCyt test *(309)* and BTA-Stat and UBC tests *(310)* in the detection of bladder cancer.

*Fibroblast growth factor receptor 3.* An important recent advance in knowledge of the molecular pathogenesis of bladder cancer has been the identification of activating fibroblast growth factor receptor 3 (*FGFR3*) mutations *(311, 312)*. *FGFR3* regulates cell growth, differentiation, and angiogenesis *(313)*. The *FGFR3* mutations identified in bladder cancer are identical to those present in autosomal dominant human skeletal disorders *(314)*. *FGFR3* mutations, which occur predominantly in noninvasive papillary low-grade bladder tumor tissue, have been proposed to be associated with a favorable prognosis, and mutations are associated with improved survival of patients with Ta and T1 tumors *(315)*.

*FGFR3* mutations characterize the papillary low-grade pathway of bladder carcinoma and the mutation frequency decreases steadily among noninvasive tumors as stage and grade increase. The presence of *FGFR3* mutations might be a prognostic variable *(316)*. However, no large study to date has shown whether *FGFR3* mutation has significant prognostic independence *(317)*. *FGFR3* mutation detection may in the future provide a useful tool in the standard management of patients with low-grade papillary bladder tumors *(228, 316, 318)*. The NACB panel recommends that this should be studied further in prospective clinical trials.

*Other proposed markers.* DD23 monoclonal antibody recognizes a 185-kDa antigen expressed by bladder cancer cells and has been proposed as an adjunct to cytology for the detection of bladder cancer *(319, 320)*. Urine fibronectin *(321, 322)* and human chorionic gonadotropin (HCG) $\beta$-subunit and $\beta$-core (protein and mRNA transcript) may also be markers for transitional cell carcinoma of the bladder *(323)*. Detection of hypermethylation of promoter regions of tumor suppressor genes and apoptosis genes also appears to have potential diagnostic value for bladder cancer *(324–326)*. Recently, the use of urine proteomic profiles has been suggested as a diagnostic approach for bladder cancer *(327, 328)*.

*Role of urine markers in early detection of bladder cancer.* Almost all cases of bladder cancer are found during the workup of patients who present with hematuria *(329)*, but most cases of hematuria are not caused by bladder cancer. Urologic disease is detected in 50% of patients who present with hematuria (in whom benign prostatic hypertrophy is the most common abnormality), and bladder cancer is detected in 10% of patient with gross hematuria and 2%–3% of patients with microhematuria *(330–332)*. The workup of patients with hematuria is costly and may require cytology, cystoscopy, intravenous urography, or CT *(333)*. Thus, tumor markers could be useful in identifying the patients in this high-risk group, which requires more intensive clinical workup for bladder cancer. Zippe et al. reported on the value of the urine NMP22 test in the evaluation of 330 patients with hematuria *(334)*. The NMP22 test, used with a cutoff value of 10.0 U/mL, detected all 18 cases of bladder cancer with 45 false-positive cases (sensitivity, 100%; specificity, 85%). In this study, 267 unnecessary cystoscopies could have been avoided if cystoscopy had been directed by the NMP22 test. In a clinical trial submitted to the FDA (as premarket approval data), NMP22 test results were elevated in 69.6% of 56 bladder cancer cases that were detected in the high-risk group. In this report, the specificity was 67.7% *(335)*. The NMP22 test has been cleared by the FDA for use as an aid to diagnose bladder

cancer in individuals with risk factors or who have symptoms of bladder cancer. It is highly likely that other urine markers (e.g., BTA-Stat, UroVysion, and ImmunoCyt) may also have value for cancer detection in subjects who present with hematuria. The high false-positive rate is the major criticism of the urine-based tests when they are used to assess patients who present with hematuria or are used in patient surveillance. The low false-negative rate of these tests is their strength, leading to a high negative predictive value that effectively rules out disease in a significant proportion of patients, thereby eliminating unnecessary clinical workups for bladder cancer. The high false-positive rate of urine biomarkers has limited their role as an adjunct to cystoscopy and cytology for the detection of recurrent disease. More importantly, there are no evidence-based data to demonstrate that urine biomarker–based surveillance leads to improved patient survival outcome, improved quality of life, or reduced cost of care.

*Role of tissue markers for prognosis.* Considerable research continues to be directed toward the identification of markers that predict the aggressive potential of noninvasive bladder tumors. Such information may lead to more effective surveillance protocols and permit more aggressive treatment of those patients with tumors most likely to progress to invasive or metastatic disease *(336)*. Stein et al. have performed an exhaustive review of a variety of biological markers reported to have prognostic value *(336)*. More recently, p53 and other cell cycle control genes *(337, 338)*, chorionic gonadotropin, beta polypeptide (*CGB*; *hCGβ*) gene transcripts *(339)*, and various cell matrix and adhesion proteins and differentially expressed genes (early vs late stage tumors) have all been reported to have prognostic value *(340)*. However, at the present time, none of these markers have yet been validated for use in routine patient care.

Although many studies have demonstrated that the prevalence of p53 alterations in bladder cancer increases with stage and grade *(341, 342)*, there is no definitive evidence that p53 overexpression is an independent prognostic factor *(342)*. Some results, however, suggest that tumor protein p53 (*TP53*) genetic mutations may be independent prognostic factors for poor progression-free survival in noninvasive bladder cancer *(343–345)*. Furthermore, mutations at certain sites of the *TP53* gene, particularly at exon 8, may be responsible for worse prognosis because these sites involve the biological function of p53 *(346)*. Mutations in defined structural and functional domains of p53 may therefore serve as useful molecular biological markers for determining prognosis and treatment strategies in patients with noninvasive transitional cell carcinomas. This finding is potentially even more significant, because *TP53* mutations can be analyzed in urine cells by noninvasive methods *(347, 348)*. As newer and faster techniques for genetic analysis become available, such testing may become routine in the future.

Hypermethylation of the polyamine-modulated factor 1 (*PMF1*) gene has also been shown to be a strong indicator of tumor progression for bladder cancer patients *(349)*. In addition, the loss of PMF1 protein expression has been reported to stratify bladder tumors histopathologically and predict clinical outcome *(349, 350)*.

*Role of urine markers for patient surveillance.* Many reported studies have established the value of urine tumor marker tests in the early detection of recurrent bladder tumors, but as yet these urine tests cannot replace routine cystoscopy and cytology in the management of bladder cancer patients. Instead, these markers may be used as complementary adjuncts that direct more effective use of clinical procedures, thus potentially reducing the cost of patient surveillance. Patients with superficial lesions of low-grade (Ta, grade 1 and II) are at lower risk for recurrence than patients with Ta grade III and T1 tumors, and these lower-risk patients may need less intensive follow-up *(248)*.

The urine markers used in patient surveillance have on occasion been criticized for their low sensitivity in detecting disease *(351, 352)*, but in most studies they have significantly improved the detection of bladder cancer when used in conjunction with cytology and cystoscopy. Because of its low sensitivity, voided urine cytology has limitations in detecting carcinoma in situ (Tis) and low-grade bladder tumors *(353)*. It appears that urine markers can assist in the early detection of recurrence in patients with carcinoma in situ and low-grade superficial tumors *(354)*.

#### KEY POINTS: TUMOR MARKERS IN BLADDER CANCER

The availability of many new markers for bladder cancer raises the possibility of improving the rate of cancer detection by combined use of selected markers, measured either simultaneously or sequentially *(355)*. The objective of such panel testing should be to improve both the sensitivity and the specificity for bladder cancer detection. Prospective clinical trials are undoubtedly necessary to prove the clinical value of such panels before they can be implemented in routine patient care *(356)*. It should also be noted that the stability of these tumor marker analytes must be better defined to minimize false-negative test results. Improved definition of the disease conditions that can produce false-positive test results for urine based markers could

lead to more effective use of these tests for cancer detection *(357)*.

### Tumor Markers in Cervical Cancer[23,24]

BACKGROUND

Cancer of the uterine cervix is the major cause of death from gynecologic cancer worldwide. Reported incidence rates in developing countries are much higher than those in developed countries, ranging from 83.2 per 100 000 women in Recife, Brazil, to 3 per 100 000 for non-Jews in Israel *(358, 359)*. In 2008, cervical cancer was diagnosed in an estimated 11 070 women within the US, with 3870 estimated deaths *(360)*. The mean age for cervical cancer is 51 years *(358)*. Cervical cancer progresses slowly from preinvasive cervical intraepithelial neoplasia (CIN) or adenocarcinoma in situ to squamous cell carcinoma or adenocarcinoma, respectively. Screening asymptomatic women with regular Papanicolau smears allows diagnosis of treatable preinvasive lesions *(361)*. However, in developed countries, most cases of cervical cancer occur in women who have not had regular Papanicolau smear screening. In developing countries, screening facilities are not readily available and most women present with advanced stage disease that may have already spread into the bladder, rectum, pelvic nerves, or bone *(358)*.

Abnormal vaginal bleeding, including postcoital, intermenstrual, and postmenopausal bleeding, is the most common symptom of cervical cancer. In women who are not sexually active, however, cervical cancer is often asymptomatic until relatively advanced *(358)*. Large tumors may present with vaginal discharge. In advanced cases, pelvic pain, pressure symptoms pertaining to the bowel or bladder, and occasionally vaginal loss of urine or feces may occur *(358)*.

Cervical cytology screening is the current method for early detection of premalignant cervical lesions and cancer. It has been shown to reduce both the incidence and mortality of this malignancy in Western countries *(361, 362)*. Screening techniques include conventional Papanicolau smears or liquid-based cytology, and national screening programs have been established in a number of countries. Women with abnormal cytology are referred for colposcopy and directed biopsy for histological diagnosis *(361)*. Premalignant cervical lesions can be treated by loop electrosurgical excision, cold-knife conization, cryosurgery, $CO_2$ laser, or hysterectomy *(361, 363)*.

It is generally accepted that specific high-risk human papilloma virus (HPV) types are causally involved in the pathogenesis of cervical cancer. The HPV types HPV-16, HPV-18, HPV-31, HPV-33, HPV-35, HPV-39, HPV-45, HPV-51, HPV-52, HPV-56, HPV-58, HPV-59, HPV-68, HPV-73, and HPV-82 are considered oncogenic HPV types *(364)*. Oncogenic types can cause cervical cancers and other anogenital cancers. Nononcogenic types HPV-6 and HPV-11 can cause benign or low-grade cervical cell changes, genital warts, and recurrent respiratory papillomatosis *(364)*. It has been demonstrated that 99% of cervical cancers worldwide are associated with high-risk HPV *(364–366)*. Most cervical cancers (70%) are caused by 2 high-risk HPV types, HPV-16 and HPV-18 *(364, 366, 367)*. Persistent infection with high-risk HPV has been recognized as necessary for the development of cervical cancer and its precursor lesions *(368–370)*. It has been suggested that HPV testing can improve the efficacy of cervical cancer screening. Recent follow-up data on longitudinal population-based randomized controlled trails have indicated that HPV testing leads to earlier detection of high-grade CIN lesions or cervical cancer compared to cytological screening *(371)*.

Because persistent infection with high-risk HPV is the most important risk-factor for the development of cervical cancer precursor lesions and cervical cancer, primary prevention of (pre)malignant cervical disease is feasible. The currently available prophylactic HPV vaccines are based on viruslike particles (VLPs) and are composed of HPV L1 proteins *(372, 373)*. Three prophylactic HPV-VLP vaccines have been clinically evaluated to date, including a monovalent HPV16 L1 VLP vaccine, a bivalent HPV16/18 L1 VLP vaccine, and a quadrivalent HPV6/11/16/18 L1 VLP vaccine *(373)*. Efficacy data of the bivalent and quadrivalent vaccines demonstrate protection against persistent HPV-16 and/or HPV-18 infections (lasting 6 months or more) for more than 90% of those vaccinated for up to at least 5 years after vaccination *(372, 373)*. The efficacy against high-grade CIN and adenocarcinoma in situ is documented as an intermediate endpoint because these lesions are the obligate precursors to invasive cancer. Estimation of the efficacy against cervical cancer will require long-term follow-up in clinical trials *(372, 373)*. It is expected that the maximum effect of current HPV vaccines in the long term (15–20 years) will be a reduction of 75%–80% of cervical cancers *(372, 373)*.

Approximately 85% of cervical cancers are of the squamous cell type. Other histological types less frequently found include adenocarcinoma (approximately 10%–15%) and adenosquamous carcinoma

[23] NACB Cervical Cancer Sub-Committee Members: Katja N Gaarenstroom (Chair), Johannes Bonfrer.

[24] All comments received about the NACB Recommendations for Cervical Cancer are included in the online Data Supplement. Professor Heather Cubie and Professor Hextan Ngan were invited Expert Reviewers.

(approximately 3%). Treatment planning of patients with cervical cancer is primarily determined by the clinical stage of disease, usually according to the International Federation of Gynecology and Obstetrics (FIGO) staging criteria *(358)*.

Early stage cervical cancer (stage IB1, IIA, tumor ≤4 cm diameter) is primarily treated with either radical hysterectomy and pelvic lymphadenectomy or radiotherapy, which are equally effective *(358, 374)*. However, with radical surgery, ovarian function can be preserved and vaginal stenosis secondary to radiation avoided, which is of great advantage for younger patients *(374)*. Therefore, most patients with early stage cancer will be treated by radical hysterectomy and pelvic lymphadenectomy. For cases in which preservation of fertility is desired, radical vaginal trachelectomy and laparoscopic pelvic lymphadenectomy or abdominal trachelectomy and pelvic lymphadenectomy may be an option in patients with small tumors (<2 cm in diameter) *(374)*. If there are pelvic lymph node metastases, parametrial involvement, or positive surgical margins, adjuvant radiation therapy to the pelvis is given to increase local control *(374)*. In these cases, it has been reported that concomitant chemoradiation with platinum-based chemotherapy significantly improved disease-free survival and survival compared to radiotherapy alone *(375, 376)*. For lymph node–negative patients with unfavorable prognostic factors such as large tumor volume, deep stromal invasion, or lymphovascular invasion, adjuvant radiation therapy reduces the risk of recurrence and prolongs progression-free survival *(374, 377)*.

Bulky stage IB2 or IIA (tumor >4 cm) cancer can be treated by radical surgery, concomitant chemoradiation, or neoadjuvant chemotherapy followed by radical surgery *(358, 374, 378–380)*. For locally advanced cervical cancer (stage IIB, III, IVA), concomitant chemoradiation, with weekly single-agent cisplatin, has been the standard treatment since 2000 *(374, 378, 379)*. A review including 24 randomized controlled trials comparing concomitant chemotherapy and radiation therapy with radiotherapy for locally advanced cervical cancer strongly suggested that chemoradiation improves overall survival and progression-free survival with absolute benefits of 10% and 13%, respectively *(378)*. Neoadjuvant chemotherapy followed by radiotherapy vs radiotherapy alone in locally advanced cervical cancer has shown disappointing results in terms of survival. However, a metaanalysis suggested that both dose intensity of cisplatin and interval duration between the chemotherapy cycles might be of critical importance, but further study is required *(380)*. A comparison of neoadjuvant chemotherapy followed by surgery vs chemoradiation is presently ongoing within the European Organisation for the Research and Treatment of Cancer Gynecologic Cancer Group (Protocol 55994), in patients with Stage IB2, Stage IIA > 4 cm, or Stage IIB cervical cancer. The role of chemotherapy in patients with recurrent or metastatic disease is merely palliative, although response rates up to 34% have been reported. Agents with the greatest activity include paclitaxel, ifosfamide, bleomycin, and topotecan *(381)*. Median survival after treatment with chemotherapy for recurrent or metastatic cervical cancer is 4 to 17 months *(381)*.

Patients with stage IB or IIA disease (early stage disease) have an overall 5-year survival rate of between 66% and 95% *(358)*. Patients with more advanced stage disease (stage IIB and higher) have a 5-year survival rate between 9% and 64% *(358)*. The FIGO staging procedure fails to detect lymph node metastases in approximately 15%–20% of patients with early stage cervical cancer *(358)*. However, the presence of lymph node metastases is the most important prognostic factor associated with recurrent disease and poor survival *(358, 374, 382–384)*. The 5-year survival rate of patients with stage IB or IIA cervical cancer declines dramatically from approximately 80%–95% in patients without lymph node metastases to approximately 50%–65% in patients with positive lymph nodes *(358)*.

Follow-up of patients after primary treatment consists of gynecological investigation. Depending on clinical symptoms and physical findings, additional cytological or histological investigations, CT scan, MRI, or ultrasound can be performed. The aim of follow-up after initial treatment is to detect recurrent disease in an early phase to improve prognosis. It has been suggested that tumor markers may be helpful in the management of patients with cervical cancer, for example in predicting prognosis, in selecting high-risk patients who need adjuvant treatment, and in monitoring after primary treatment. The aim of this report is to present guidelines on the possible clinical utility of tumor markers in cervical cancer, especially squamous cell cervical cancer.

To prepare these guidelines, the literature relevant to the use of tumor markers in cervical cancer was reviewed. Particular attention was given to reviews, including systematic reviews, prospective randomized trials that included the use of markers, and guidelines issued by expert panels. Where possible, the consensus recommendations of the NACB panel were based on available evidence, i.e., were evidence based.

### CURRENTLY AVAILABLE MARKERS FOR CERVICAL CANCER

Tumor markers that may be helpful in the management of patients with cervical cancer are listed in Table 4, together with the phase of development for each marker as well as the LOE for its clinical use. Only tu-

**Table 4. Currently available and potentially useful serum markers for cervical cancer.**

| Cancer marker | Proposed use | Phase of development | LOE | References |
|---|---|---|---|---|
| SCC | Pretreatment identification of high-risk group with lymph node metastases in squamous cell cervical cancer | Needs further evaluation for clinical usefulness | III | *(385, 391, 393, 395, 399, 408, 410, 430–434)* |
| | Pretreatment prediction of prognosis in squamous cell cervical cancer | Independent prognostic value in several studies, not validated for individualizing treatment | III | *(385, 389, 393, 399, 408)* |
| | Prediction of response to treatment in squamous cell cervical cancer | Needs further evaluation | IV | *(389, 399, 404, 405, 408, 412, 430)* |
| | Monitoring disease and detecting recurrent disease in squamous cell cervical cancer | Strong correlation with course of disease, in clinical use in some centers | III | *(386–388, 392, 396–398, 400–403, 405–407)* |
| CA 125 | Pretreatment prediction of prognosis, in particular in cervical adenocarcinoma | Needs further evaluation | III–IV | *(385, 417)* |
| | Preoperative prediction of the presence of lymph node metastases, in particular in cervical adenocarcinoma | Needs further evaluation | III–IV | *(385, 417, 433)* |
| | Monitoring disease, in particular in cervical adenocarcinoma | Needs further evaluation | IV | *(415, 416, 418, 419)* |
| CEA | Pretreatment prediction of prognosis | Results conflicting, needs further evaluation | III–IV | *(385, 407, 415, 417, 430, 567)* |
| | Preoperative prediction of the presence of lymph node metastases, in particular in cervical adenocarcinoma | Needs further evaluation | III–IV | *(385, 417, 433)* |
| | Pretreatment prediction of clinical response to neoadjuvant chemotherapy | Needs further evaluation | IV | *(430)* |
| Cytokeratins (TPA, TPS, CYFRA 21-1) | Pretreatment prediction of prognosis | Needs further evaluation, results conflicting | III–IV | *(385, 395, 406, 568, 569)* |
| | Monitoring disease after primary treatment | Needs further evaluation, results conflicting | III–IV | *(419, 567, 570–574)* |

mor markers for which possible clinical usefulness has been demonstrated in several studies are listed. For squamous cell cervical cancer, squamous cell carcinoma antigen (SCC) is the marker of choice. Serum concentrations of SCC have been found to correlate with tumor stage, tumor size, residual tumor after treatment, recurrent or progressive disease, and survival in patients with squamous cell cervical cancer *(385–414)*. Carcinoembryonic antigen (CEA) and CA 125 have demonstrated possible utility in patients with cervical adenocarcinoma *(414–419)*. These guidelines focus on the use of SCC in squamous cell cervical cancer, the most prevalent histologic type of cervical cancer.

### TUMOR MARKERS IN CERVICAL CANCER: NACB RECOMMENDATIONS

Table 5 summarizes the NACB guidelines for the use of SCC in squamous cell cervical cancer. Although other markers have been investigated (Table 4), based on currently available evidence SCC seems the most useful marker in squamous cell cervical cancer *(420)*. Detailed discussion of its use is presented here.

*SCC biochemistry.* SCC is a subfraction of TA-4, a tumor-associated antigen first described. in 1977 *(421)*. SCC belongs to the family of serine protease inhibitors *(422)*. In most studies evaluating clinical utility, total SCC has been measured.

Molecular cloning of the SCC genomic region has revealed the presence of 2 genes, SCC1 and SCC2, which are both located on chromosome 18q21.3 and arrayed in tandem. SCC1 codes for the neutral isoform of SCC and SCC2 codes for the acidic isoform *(423)*. The neutral isoform is detected in both normal epithelial cells and malignant tissues, whereas the acidic isoform is found only in tumor cells, especially those located at the periphery of the tumor. The acidic form

**Table 5. NACB Recommendations for the clinical use of SCC in squamous cell cervical cancer.**

| Marker | Application | NACB Recommendations (2009) | LOE | SOR |
|---|---|---|---|---|
| SCC | Screening and diagnosis | No | III | A |
| | Pretreatment identification of patients at high risk of having lymph node metastases | Possibly useful, further study required. | IV/V | C |
| | Predicting prognosis | Possibly useful, further study required. | III | C |
| | Monitoring disease and detecting recurrent disease | Possibly useful, further study required. | III | C |

may also be found in the sera of cancer patients with well-differentiated squamous cell carcinomas *(424)*. It has been suggested that SCC1 and SCC2 are capable of regulating proteolytic events involved in both normal (e.g., tissue remodelling, protein processing) and pathologic processes (e.g., tumor progression) *(425)*. Structurally, SCC1 and SCC2 are almost identical, differing only in their reactive site loops. The 2 forms, however, may have different biological functions *(423, 425, 426)*.

*SCC reference intervals.* In apparently healthy women, the 99th percentile of circulating SCC is found at a concentration of 1.9 μg/L. Most studies have adopted a cutoff point between 2.0 and 2.5 μg/L. SCC is not organ specific (for cervix) or malignancy specific. Elevated concentrations have been found in patients with squamous cell carcinomas of the vulva, vagina, head and neck, esophagus, and lung *(390, 427, 428)*, as well as in patients with benign diseases of the skin (e.g., psoriasis, eczema), lung (e.g., sarcoidosis), liver, and kidney. Very high values (up to 18 μg/L) have been found in patients with renal failure, lung disease, and head and neck tumors *(427)*. There is no cutoff point that is specific for cervical malignancy.

*Clinical utility of SCC in squamous cell cervical cancer: screening and diagnosis.* SCC is not sufficiently sensitive (particularly in early stage disease) or specific for cervical cancer for use in screening. Diagnosis in all cases is based on histopathological findings. Elevated concentrations of serum SCC are found at initial diagnosis in approximately 60% of patients with cervical cancer, when all stages are included *(429)*. More specifically, serum SCC is elevated in approximately 24%–53% of patients with stage IB or IIA squamous cell cervical cancer, and in approximately 75%–90% of patients with advanced stage (FIGO IIB and higher) disease *(390, 393–395, 399, 409, 413, 414)*. Pretreatment serum SSC concentrations correlate significantly with tumor stage *(388, 391–395, 398, 409, 412–414)* and tumor size *(393–395, 408, 410, 413, 414)*.

**NACB CERVICAL CANCER PANEL RECOMMENDATION 1: USE OF TUMOR MARKERS FOR SCREENING AND DIAGNOSIS OF CERVICAL CANCER**

Currently available serum tumor markers, including SCC, are not recommended for use in screening or diagnosis of cervical cancer (LOE, III; SOR, A).

*Prediction of lymph node metastases and treatment planning.* A number of studies have examined the utility of elevated pretreatment SCC as a marker for the presence of lymph node metastases *(385, 391, 393–395, 399, 408, 410, 413, 430–434)*. In patients with stage IB or IIA squamous cell cervical cancer, sensitivity of an elevated pretreatment concentration of SCC to detect lymph node metastases ranged from 60% to 87%, with specificity ranging from 41% to 91% *(385, 391, 393, 395, 408, 434)*. In a large series of 414 patients with early stage cervical cancer, elevated pretreatment SCC, large tumor size, and lymphovascular space involvement were independent risk factors for the presence of lymph node metastases *(393)*. In another study ($n = 401$), after controlling for stage, only high concentrations of SCC (i.e., >10 μg/L) were associated with enlarged lymph nodes shown on CT scan *(399)*. On combining SCC (cutoff value 2.5 μg/L) with CA 125 in 81 women with stage IB/IIA cervical cancer that included all histological types, a positive predictive value of 76% was found for detecting lymph node metastases or lymphovascular space involvement *(433)*.

Several authors have suggested using higher cutoff values for SCC to identify patients with squamous cell cervical carcinoma that has spread to lymph nodes. Sensitivity of 59% and specificity of 94% with the use of a cutoff value of 4 μg/L have been reported in 148 patients with stage IB squamous cell cervical carcinoma *(410)*. The corresponding positive and negative predictive values were 65% and 92%, respectively. Sensitivities for lymph node metastases of 58%, 45%, and 23% using cutoff values of 2, 4, and 8.6 μg/L, respectively, have been reported in a study of 171 patients

with squamous or adenosquamous cell cervical carcinoma *(431)*. The corresponding positive predictive values were 51%, 70%, and 100%. Negative predictive values varied between 84% and 89% *(431)*. About 86% of the patients in a large series of 284 patients with stage IB and IIA squamous cell cervical carcinoma with SCC concentrations below 8 μg/L showed no lymph node metastases, whereas about 65% of the patients with serum concentrations above 8 μg/L exhibited nodal metastases *(432)*.

The clinical performance of SCC over a range of decision levels has been found to be poor in identifying lymph node metastases, as reflected by the diagonal appearance of the ROC curve *(395)*. The authors concluded that a pretreatment SCC concentration within the reference interval cannot exclude the presence of lymph node metastases and extracervical spread, and hence is of limited use in treatment planning. Nevertheless, these studies confirm that a high pretreatment serum SCC concentration (>4 μg/L) significantly increases the likelihood of lymph node metastases or extracervical spread in patients with squamous cell cervical cancer *(399, 430–432)*.

It has been suggested that the pretreatment concentration of SCC can identify patients who require intensive or additional treatment and hence may be of value in treatment planning in the individual patient *(393, 399, 433)*. To prevent morbidity associated with double modality treatment, for example, surgery should be offered only when there is a low likelihood of the need for adjuvant radiotherapy. Pretreatment SCC concentration, along with tumor size, was shown to be useful in predicting recurrence and the need for postoperative adjuvant therapy in a series of 99 patients with stage IB and IIa squamous cell cervical cancer *(389)*. The value of pretreatment SCC in clinical decision-making in 337 surgically treated stage IB/IIA cervical cancer patients has also been investigated *(435)*. The frequency of postoperative adjuvant radiotherapy was related to FIGO stage, tumor size, and preoperative SCC concentrations. In patients with preoperative SCC concentrations within the reference interval, 16% of IB1 and 29% of IB2/IIA patients had postoperative indications for adjuvant radiotherapy, in contrast to 57% of IB1 and 74% of IB2/IIA patients with elevated SCC concentrations. Serum SCC was the only independent predictor for a postoperative indication for radiotherapy. The authors suggested that SCC allows a more refined preoperative estimation of the likelihood for adjuvant radiotherapy than current clinical parameters *(435)*.

It is not surprising that an elevated pretreatment SCC concentration is associated with the need for postoperative adjuvant therapy, because elevated concentrations are strongly correlated with tumor stage, tumor size, and the presence of lymph node metastases. Therefore, pretreatment SCC concentrations might be used to individualize treatment planning, in particular in patients with low-stage squamous cell cervical cancer, but no randomized trials have yet been conducted to confirm this hypothesis.

**NACB CERVICAL CANCER PANEL RECOMMENDATION 2: SERUM SCC CONCENTRATIONS IN PREDICTION OF LYMPH NODE METASTASES AND TREATMENT PLANNING**

Pretreatment SCC concentrations may provide additional information, because high SCC concentrations are associated with the presence of lymph node metastases and the need for adjuvant treatment (LOE III) and might be used to individualize treatment planning in patients with low-stage squamous cell cervical cancer, but are not recommended for routine use at this time (LOE, IV/V; SOR, C).

*Prognosis.* An elevated pretreatment SCC concentration has been found to be an independent risk factor of poor survival in several studies *(385, 393, 399, 408, 436–438)*. The pretreatment SCC concentration was the only independent risk factor of poor survival in an analysis of results for 260 patients with stage IB or IIA disease *(393)*. However, in contrast to other reported investigations, lymph node status showed no independent prognostic value in this study *(393)*. Another group found that SCC and CA 125, in addition to stage, were significantly related to survival in the multivariate analysis of 142 patients with cervical cancer ranging from stage IA through IVB *(385)*. It was concluded from a multivariate analysis of 102 women with locally advanced squamous cell cancer or adenocarcinoma of the cervix that an SCC concentration greater than 5 μg/L was an independent predictor of response to neoadjuvant chemotherapy and poor survival *(408)*. A pretreatment SCC concentration greater than 10 μg/L (but not between 2 and 10 μg/L) had a significant impact on survival in a multivariate analysis in 401 patients with stage I to IVA squamous cell cervical cancer, primarily treated with radiotherapy *(399)*. An elevated pretreatment SCC concentration >3 μg/L was an independent prognostic factor for both recurrence-free and overall survival in a series of 129 patients with squamous cell cervical cancer *(436)*. Median SCC concentration >6.0 μg/L and lymph node metastases had significant independent effects on absolute survival and disease-free survival in 352 patients with stage IIB to IVA squamous cell cervical cancer *(437)*. Finally, an elevated pretreatment SCC concentration (>5 μg/L) identified a subgroup of high-risk node-positive patients in early stage cervical cancer compared to node-positive patients with SCC concentrations within the reference interval *(438)*. Multivariate analysis showed that an elevated pretreatment SCC concentration and

S-phase fraction greater than 20%, correlated significantly with a worse disease-free survival *(438)*. However, formal trials are required to substantiate these claims and to establish that aggressive treatment triggered by elevated pretreatment SCC concentrations actually improves pelvic control and survival.

**NACB CERVICAL CANCER PANEL RECOMMENDATION 3: SERUM SCC CONCENTRATIONS IN PREDICTION OF PROGNOSIS OF CERVICAL CANCER**

An elevated pretreatment SCC concentration has been found to be an independent risk factor for poor prognosis in several studies, but the clinical usefulness in treatment planning is uncertain. SCC is thus not recommended for routinely determining prognosis in women with cervical cancer at this time (LOE, III; SOR, C).

*Use of SCC in monitoring response to treatment and early detection of recurrence.* Results of several studies have indicated that serum SCC is potentially useful in monitoring the course of squamous cell cervical cancer after primary therapy *(386–388, 391, 392, 397–399, 403, 405, 407–409, 412, 428)*. Persistently elevated and/or increasing serum SCC concentrations after treatment suggest tumor persistence or progressive disease *(387, 398, 399, 408, 412–414, 428)*. In 1 study, CEA and SCC marker concentrations measured 1 month after primary treatment with chemoradiation were better than pretreatment serum concentrations in predicting clinical outcome *(413)*. CEA and SCC concentrations that have returned to reference intervals 1 month after treatment correlated with a complete remission at 3 months *(413)*. In another study, patients with residual induration and/or persistently elevated SCC concentration at 2–3 months after radiotherapy had a significantly higher incidence of treatment failure *(399)*. The authors suggested that, together with pelvic examination, SCC concentrations can indicate a need of further workup and management *(399)*. A pretreatment SCC concentration >5 μg/L was reported to be an independent predictor of response to neoadjuvant chemotherapy in a series of 102 patients with locally advanced cervical cancer *(399)*. Patients who were unresponsive to chemotherapy had significantly higher pretreatment SCC values than those who showed complete or partial response *(408)*. There was a correlation between posttreatment SCC concentrations and response to chemotherapy *(408)*. None of the patients with a complete response had posttreatment serum SCC concentrations >5 μg/L, whereas 82% of the unresponsive patients had abnormal marker values (SCC concentrations >2.5 μg/L) *(408)*. The overall correlation between the clinical course of the disease and the variation of SCC concentrations was 83% *(408)*. The authors suggested that SCC might provide useful information to improve the prognostic characterization and disease monitoring of patients with locally advanced cervical cancer undergoing neoadjuvant chemotherapy *(408)*. It has also been reported that an elevated pretreatment SCC and/or CEA concentration was useful in predicting the clinical response to neoadjuvant chemotherapy in a series of 67 patients with squamous cell cervical cancer stage IB2, IIA, or IIB *(408)*.

Serum SCC concentration has a sensitivity between 56% and 86% and specificity between 83% and 100% for detecting recurrent squamous cell cervical cancer *(386, 388, 392, 396, 398, 401, 407, 409, 412)*. With the use of SCC, a lead time of up to 14 months for detecting recurrent disease has been reported, with a mean or median between 2 and 6 months *(386, 388, 396–398, 400, 401, 403, 405, 407)*. Although SCC is suitable for monitoring the course of disease and shows a strong correlation with the clinical course, it is not yet known whether earlier detection of recurrent disease influences treatment outcome and prognosis. At most, 10% of patients with recurrent disease can be cured. Furthermore, most patients (80%) with recurrent disease have clinical symptoms *(439, 440)*. Most recurrences (about 95%) are detected by the presence of clinical symptoms or clinical examination *(439, 440)*.

The role of routine follow-up after gynecological malignancy has been reviewed *(441)*. Only 2 of 6 published reports on the role of follow-up after cervical cancer found a survival benefit. All were retrospective case series analysis. The contribution of SCC monitoring to recurrence detection and survival in the follow-up of 225 patients with early stage squamous cell cervical cancer has also been studied *(441)*. In 5 of 35 patients (14%), serum SCC elevation was the only sign of recurrent disease. Unfortunately, all these 5 patients died of disease. The authors concluded that SCC analysis resulted in earlier recurrence detection in a small proportion (14%) of the patients, but did not improve survival. Posttreatment SCC monitoring has not been found to be cost-effective in cervical cancer, because SCC monitoring does not alter clinical management and has no advantage over clinical examination in detecting local recurrence *(442)*, primarily because most recurrent disease is detected too late for curative treatment. Nevertheless, further investigation is needed to determine whether SCC monitoring is really useful or not in clinical practice. It has been reported in a small series of patients with recurrent cervical cancer that the addition of positron emission tomography to SCC monitoring significantly increased overall survival compared with a historical group of patients who had elevated SCC concentrations as a first sign of recurrent disease *(443)*.

**NACB CERVICAL CANCER PANEL RECOMMENDATION 4: SERUM SCC CONCENTRATIONS IN POSTTREATMENT MONITORING OF CERVICAL CANCER PATIENTS**

SCC monitoring after primary treatment strongly correlates with the clinical course of disease in patients with squamous cell cervical cancer but there is as yet no clear evidence that earlier detection improves outcome. Monitoring with SCC is thus not recommended for routine use at this time (LOE, III; SOR, C).

**KEY POINTS: TUMOR MARKERS IN CERVICAL CANCER**

The NACB recommendations for the use of tumor markers in cervical cancer are presented in Table 5. SCC is not suitable for screening or diagnosis of cervical cancer; serum SCC concentrations correlate with tumor stage, tumor size, residual tumor after treatment, recurrent or progressive disease, and survival. Highly elevated pretreatment SCC concentrations may indicate the presence of lymph node metastases or extracervical spread, but an SCC concentration within the reference interval does not exclude the presence of lymph node metastases.

Pretreatment SCC concentrations may be used to individualize treatment planning, in particular in patients with low-stage squamous cell cervical cancer, but no randomized trials have been conducted to confirm this hypothesis. An elevated pretreatment SCC concentration has been found to be an independent risk factor for poor survival in several studies. Whether pretreatment SCC concentrations are really useful in clinical practice remains uncertain. There is no evidence that more aggressive treatment improves pelvic control and survival in patients with elevated pretreatment SCC concentrations. SCC shows a strong correlation with the clinical course and is suitable for monitoring disease after primary treatment and may therefore be useful in the management of patients. However, there is as yet no evidence that earlier detection of recurrent disease using SCC monitoring influences treatment outcome or prognosis after primary treatment.

## Tumor Markers in Gastric Cancer[25,26]

**BACKGROUND**

Gastric cancer is a major health problem worldwide, remaining the second most common digestive tract cancer, despite decreasing incidence *(360, 444)*. Incidence is highest in those older than 60 years, and marked geographical variations have been observed. Risk factors include *Helicobacter pylori* infection, atrophic gastritis, male sex, cigarette smoking, high salt intake, and some of the genetic factors associated with a predisposition to colorectal cancer (e.g., family history of hereditary nonpolyposis colorectal cancer, familial adenomatous polyposis, and Peutz-Jeghers syndrome). Gastric cancer is frequently undiagnosed until a relatively advanced stage, when presenting symptoms may include dysphagia, recurrent vomiting, anorexia, weigh loss, and gastrointestinal blood loss. Definitive diagnosis requires gastroscopic or surgical biopsy, with histology reported by an experienced pathologist according to WHO criteria. Surgery is the only potentially curative treatment, but even when surgical resection is possible, long-term survival occurs only in a minority of patients, with overall 5-year survival of less than 30% after gastrectomy *(445, 446)*.

The most important prognostic factor influencing survival of patients with stomach cancer is the extent of disease as assessed by tumor stage *(447, 448)*. Of patients who undergo gastrectomy, 80% with stage I disease confined to the stomach are alive at 5 years, but only 7% of patients with stage IV disease which has spread to other organs reach 5-year survival. The ratio of involved and resected lymph nodes also has prognostic significance *(449)*. Patients with a proximal location of the tumor generally have a worse prognosis than those with cancer in the distal or middle section *(450)*.

The histological type of tumor is often regarded as an essential prognostic factor in gastric cancer. When diffuse lesions and the intestinal type with more nodular lesions are differentiated, it is assumed that the latter carries a better prognosis *(451, 452)*.

Only a minority of patients will be cured of gastric cancer with surgery alone. For those for whom curative resection is not possible, development of symptomatic metastatic disease from unresected microscopical tumor remnants is the main cause of death. Several prospective randomized trials have demonstrated that surgical resection of stomach, perigastric lymph nodes, and omenta (D1) yields the same survival figures as more extensive (D2) surgical procedures, including omental bursa and extensive lymph node resections, because of increased morbidity *(453–455)*.

Chemotherapy alone has not shown benefit, but postoperative treatment with a combination of chemo- and radiotherapy (chemoradiation) is advocated *(456)*. Since Moertel first reported prolonged survival in a group of patients treated with both 5-fluorouracil and radiation therapy compared with a group of patients given 5-fluorouracil alone *(457)*, several other studies have shown that concurrent chemo- and radiotherapy are superior to chemotherapy alone, although

[25] NACB Gastric Cancer Sub-Committee Members: Johannes Bonfrer (Chair), Johanna Louhimo.

[26] All comments received about the NACB Recommendations for Gastric Cancer are included in the online Data Supplement.

**Table 6. Currently available serum markers for gastric cancer.**

| Marker | Proposed use | Phase of development | Level of evidence | References |
|---|---|---|---|---|
| CEA | Prognosis, postoperative monitoring | Conflicting data; needs further trials | III, IV | *(484–488, 501, 502, 504, 506–508)* |
| CA 19-9 | Prognosis, postoperative monitoring | Conflicting data; needs further evaluation | III, IV | *(484, 485, 487, 488, 501, 502, 504, 506–508)* |
| CA 72-4 | Prognosis, postoperative monitoring | Needs further evaluation | III, IV | *(484, 485, 501–505, 507)* |
| Cytokeratins (CYFRA 21-1, TPA, TPS) | Prognosis | Needs further evaluation | IV | *(489, 492, 493)* |
| $\beta$ Subunit of HCG | Prognosis | Needs further evaluation | IV | *(494, 495)* |

combination therapy has shown more morbidity *(458, 459)*. Supported by results of an intergroup trial, chemoradiation with 5-fluorouracil/Leucoverin is currently considered to be standard treatment in the US *(460, 461)*. In most of Europe, perioperative treatment with chemotherapy has become the standard of care since results of the MAGIC (UK Medical Research Council Adjuvant Gastric Infusional Chemotherapy) trial, the first well-powered phase III trial for perioperative chemotherapy *(462)*, were reported in NCCN guidelines *(463)*. In another large trial it was observed that postoperative adjuvant chemotherapy and chemoradiotherapy gave improved disease-free survival and survival rates *(464)*. The use of cetuximab, bevacizumab, and trastuzumab in combination with chemotherapy is currently under investigation in various clinical trials but treatment with these molecular targeting agents is still experimental *(465, 466)*.

There are a number of excellent guidelines relating to the clinical management of gastric cancer *(456, 463, 467–470)*, but few make any reference to circulating tumor markers. The aim of this NACB panel was to review available evidence for use of serum tumor markers in the management of patients with gastric cancer and to present new NACB guidelines for this.

To prepare these guidelines, the literature relevant to the use of tumor markers in bladder cancer was reviewed. Particular attention was given to reviews including systematic reviews, prospective randomized trials that included the use of markers, and guidelines issued by expert panels. Where possible, the consensus recommendations of the NACB Panel were based on available evidence, i.e., were evidence based.

### CURRENTLY AVAILABLE MARKERS FOR GASTRIC CANCER

The most widely investigated serum-based tumor markers for gastric cancer are listed in Table 6. Also listed is the phase of development of each marker as well as the LOE for its clinical use.

### TUMOR MARKERS IN GASTRIC CANCER: NACB RECOMMENDATIONS

NACB recommendations for the use of tumor markers in gastric cancer are presented below, and their utility in the management of stomach cancer briefly reviewed.

### CLINICAL APPLICATION OF TUMOR MARKERS IN GASTRIC CANCER

*Screening and diagnosis.* In the Western hemisphere the low and decreasing incidence of gastric cancer together with the invasiveness of diagnostic gastroscopy and the lack of a suitable alternative test has precluded screening for gastric cancer. In certain Asian countries where the incidence of gastric cancer is high, opportunistic screening of high-risk individuals is common *(471)*. In Japan, where gastric cancer is the main cause of cancer death, nationwide screening has been carried out since 1983 on individuals ≥40 years old *(472)*. One of the few tumor markers to have undergone evaluation for screening for gastric cancer in Japan is pepsinogen. In a pooled analysis of 42 data sets involving about 300 000 individuals, sensitivity of this test for gastric cancer was 77% and specificity was 73% *(473)*.

The relationship between the presence of *Helicobacter pylori* and an increased risk (relative risk 2–5) for gastric cancer has been attributed to the resulting chronic gastritis *(474)*. Retrospective review of the histological records for 92 250 patients in the Netherlands who had premalignant gastric lesions first diagnosed between 1991 and 2004 confirmed that these patients are at considerable risk of gastric cancer and indicated a need for consensus as to best practice *(475)*. Optimal strategies for detecting and eradicating *H. pylori* infection have recently been proposed by the Practice Parameters Committee of the American College of Gastroenterology *(476)*. Testing for *H. pylori* infection and treating as appropriate is part of the initial evaluation of patients with gastric cancer *(463)*.

**Table 7. Reported pretreatment sensitivity of serum markers for gastric cancer.**

| | Cutoff level | Early stage | Advanced disease | References |
|---|---|---|---|---|
| CEA | 5 μg/L | <20% | 40–50 | *(484–488, 501, 504, 505, 575)* |
| CA 19-9 | 37 kU/L | <20% | 20–50 | *(484–488, 501, 504, 505, 575)* |
| CA 72-4 | 6 kU/L | <20% | 30–40 | *(484, 485, 489, 501, 504, 505, 575)* |
| Cytokeratins (CYFRA 21-1, TPA, TPS) | Variable | 15–25 | 30–50 | *(485, 489, 491, 492)* |
| β Subunit of HCG | 4 μg/L | 20–35 | 30–50 | *(494, 576)* |

Members of families with a strong history of diffuse gastric cancer who are carriers of germ line truncating E-cadherin mutations may benefit from genetic counseling, with prophylactic gastrectomy a possibility *(477)*. In a large Swedish study a negative result almost excluded precancerous conditions in a screening situation *(478)*.

A major problem with endoscopy is the low detection of early gastric cancer *(479)*. Similarly the low sensitivity of currently available serum tumor markers for early stage disease (<35%) (Table 7) precludes their use in screening and early diagnosis.

**NACB GASTRIC CANCER PANEL RECOMMENDATION 1: TUMOR MARKERS IN THE DIAGNOSIS AND SCREENING OF GASTRIC CANCER**

Currently available serum tumor markers are not recommended in screening or diagnosis of gastric cancer (LOE, III/IV; SOR, A).

*Prognosis.* The most important prognostic factor influencing survival of patients with gastric cancer is, as described above, the extent of disease. If a D2 resection is not performed there is a significant risk of understaging *(448, 453, 480)*.

Reports on the sensitivity of tumor markers are inevitably influenced by the accuracy of staging procedures, and use of different cutoff concentrations makes it difficult to compare results from different studies. The reported sensitivities of several markers for early and advanced disease are listed in Table 7. Univariate analysis indicates that CEA, CA 19-9, and CA 72-4 *(481–483)* have prognostic value. In multivariate analysis, however, their impact is not always independent of stage *(484–489)*. In general, increasing concentrations of tumor markers are inversely related to decreasing postoperative survival *(486, 488)*. Additional markers that have been studied in relation to prognosis include AFP *(490)*, cytokeratins (TPA, CYFRA 21-1, and TPS) *(485, 489, 491–493)*, and the free β-subunit of HCG *(494, 495)*. However, when preoperative serum concentrations of circulating tumor markers are related to recurrence, none of these markers appears to have independent prognostic value *(485, 496)*.

Peritoneal dissemination is an important cause of recurrence and death in patients with gastric cancer. Conventional cytological examination of intraoperative peritoneal lavage fluid is useful in detecting free cancer cells in the peritoneal cavity, which in turn contribute to peritoneal dissemination, but the sensitivity is low. Elevated CEA concentrations in the peritoneal lavage fluid have been shown to correlate with peritoneal recurrence and poor survival *(497, 498)*. In addition, CEA mRNA measured by RT-PCR in blood and peritoneal washings has been shown to be related to tumor burden and to predict recurrence *(499, 500)*. Intraperitoneal CEA measurement may become clinically important in the future with the development of adjuvant therapy regimens, but further confirmation is required.

**NACB GASTRIC CANCER PANEL RECOMMENDATION 2: TUMOR MARKERS IN MONITORING RESPONSE TO TREATMENT IN PATIENTS WITH GASTRIC CANCER**

Currently available serum tumor markers do not have independent prognostic value in gastric cancer and are not recommended for prognosis or prediction (LOE, III/IV; SOR, B).

*Monitoring of patients postoperatively.* In principle, postoperative follow-up of patients may be helpful for early detection of recurrence. Most studies on the use of CEA, CA 19-9, or CA 72-4 for early detection of relapse indicate a high sensitivity and a lead time of up to 10 months, especially for recurrence in the liver. However, most studies have been retrospective and clinical detection methods varied *(501–505)*, making it difficult to compare results from different studies. In a nationwide prospective study CEA and CA 19-9 detected recurrence earlier than diagnostic imaging, with an average lead time of 3 months, in some cases providing a lead time of more than 1 year *(506)*. Monitoring response to therapy is an important tool that can

spare nonresponding patients potentially serious adverse effects from chemotherapy and/or radiation therapy. Although the number of investigations is limited, results suggest that tumor markers correlate with responses as measured by conventional imaging techniques *(507, 508 )* and may be useful in the detection of recurrence.

Serum CEA and CA 19-9 measurements have been shown to be of potential value in the early detection of recurrence after surgery *(506, 509 )*, but it is not possible to determine which marker is superior for this application and there is no evidence that monitoring with either is beneficial. In accord with other investigators *(456, 510 )*, the NACB panel does not recommend regular measurement of serum tumor markers in the follow-up of patients with gastric cancer except in the context of clinical trials.

**NACB GASTRIC CANCER PANEL RECOMMENDATION 3: TUMOR MARKERS FOR MONITORING RESPONSE TO TREATMENT IN PATIENTS WITH GASTRIC CANCER**

Routine measurement of CEA or CA 19-9 is not recommended (LOE, III/IV; SOR, B).

**KEY POINTS: TUMOR MARKERS IN GASTRIC CANCER**

Most studies concerning the use of tumor markers in gastric cancer have been directed toward the prognostic power of preoperative serum concentrations. The retrospective nature of the studies, differences in study design, and inadequacy of available statistical information makes it difficult to draw any firm conclusions about the relative merits of various markers in identifying patient groups at high risk for either short disease-free survival or survival alone. Differences in surgical and diagnostic procedures also make it difficult to compare tumor marker sensitivity and specificity in relation to stage. However, no currently available marker can be recommended for use in diagnosis of gastric cancer, because specificity and sensitivity of available markers are clearly not sufficient. Results of the few reported studies of the use of CEA or CA 19-9 in follow-up of patients with this disease suggest that the measurement of these markers may be beneficial in the detection of recurrence, but this finding requires confirmation within appropriately designed clinical trials.

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