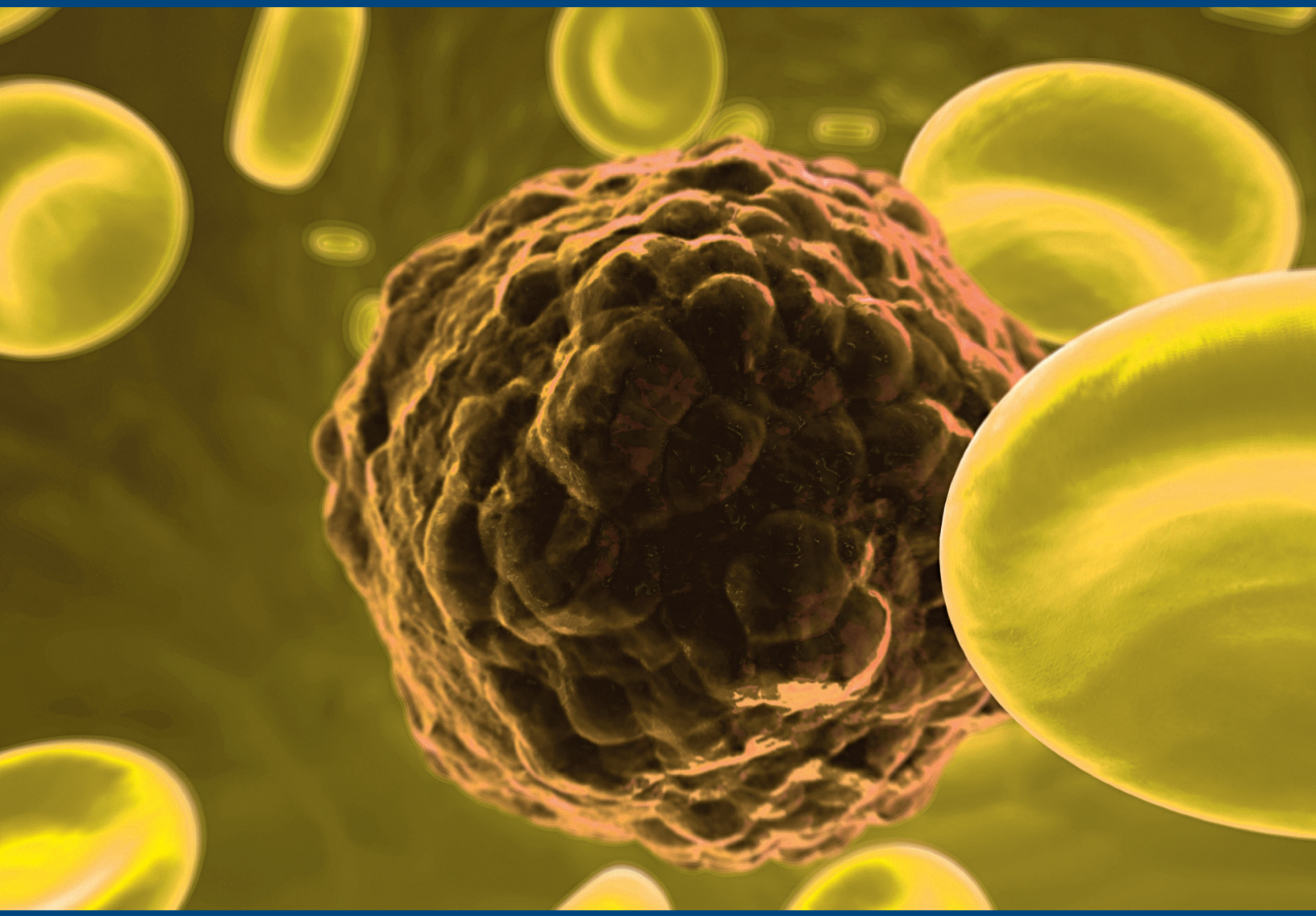
Laboratory Medicine Practice Guidelines

Use of Tumor Markers in Liver, Bladder, Cervical, and Gastric Cancers

Edited by Catharine M. Sturgeon and Eleftherios Diamandis



NATIONAL ACADEMY of CLINICAL BIOCHEMISTRY
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Presents

LABORATORY MEDICINE PRACTICE GUIDELINES

USE OF TUMOR MARKERS IN LIVER, BLADDER, CERVICAL, AND GASTRIC CANCERS

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Chapter 1

Introduction

We present here to clinical chemists, clinicians, and other practitioners of laboratory and clinical medicine the latest update of the National Academy of Clinical Biochemistry (NACB) 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) and has been included as an Appendix to this document. 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).

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 (ie, 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 (eg, heterophilic antibody interference, high-dose hooking) have previously been published (3).

Chapter **2**

Tumor Markers in Liver Cancer

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 annually. 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 B1 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, 380 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. New therapeutic antiviral strategies (eg, pegylated α-interferon combined with ribavirin or other drugs such as lamivudine) are available for treatment of hepatitis B and C (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 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 [eg, hypoglycemia (34), erythrocytosis (35), hypercalcemia (36,37)], severe watery diarrhea (37), or cutaneous features (eg, 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 (eg, 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 (eg, with 1 nodule < 5 cm in diameter or up to 3 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 (eg, 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 one 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 (ie, 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, which 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 two-site immunometric assays 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 kilo-units 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.

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

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 antigenñlabeling 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, β-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 Bñ and hepatitis Cñrelated 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 recurrenc	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

Table 1. ***(Contd.)***

Cancer Marker	Proposed Uses	Phase of Development	LOE	Reference
HCC-specific AFP band on isoelectric focusing (monosialylated AFP)	Earlier detection of HCC than "diagnostic" AFP (> 500 µg/L), positive predictive value 73% vs 42%, respectively	Not in clinical use	V	69-71
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-γGTP	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[8]	Complementary to AFP	Undergoing evaluation	V	529
α_1-Antitrypsin	Complementary to AFP	Undergoing evaluation	V	530, 531
α_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[prime]-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

α-Fucosyl-transferase	Marker of progression of HCC	Undergoing evaluation	V	541
α-L-fucosidase	Complementary to AFP	Undergoing evaluation	V	542, 543
Transforming growth factor β1	Diagnosis of small HCC tumors	Undergoing evaluation	V	544
Urinary transforming growth factor β1	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 virusñrelated 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
Plasma proteasome	Marker of malignant transformation in cirrhotic patients including those with low tumor mass	Undergoing evaluation	V	562
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

Table 1. ***(Contd.)***

Cancer Marker	Proposed Uses	Phase of Development	LOE	Reference
Genetic markers				
Plasma glutamate carboxy-peptidase, 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

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 (eg, 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 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 three 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, normal AFP-L3 and DCP concentrations 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 three 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 three markers

Table 2. Recommendations for Use of AFP in Liver Cancer by Different Expert Groups

Application	AASLD 2005 40	Asian Oncology Summit 136	BrSocGE 26 2003	EASL 131 2001	EGTM 137 1999	ESMO 2009 4	French SOR 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	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

Abbreviations: Br Soc GE, British Society of Gastroenterology; EGTM, European Group on Tumor Markers; ESMO, European Society for Medical Oncology; SOR, strength of recommendation; Japanese EBCIGI, Japanese evidence-based clinical guidelines.

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 seven 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]. Receiver operating characteristic (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 end points 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 five of 1,239 studies met all the authors' inclusion criteria (99-103). In these five 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 cut points 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, 1,069 hepatitis B virus–infected patients with proven cirrhosis had to be screened to detect 14 cases of HCC, of which only six 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 one 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 5,581 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 two 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 (eg, 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 in 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 (eg, two 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 two 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 (eg, 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 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 with 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, 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 α1-antitrypsin deficiency. Surveillance is also recommended for individuals without cirrhosis who are hepatitis B carriers or have other risk factors (eg, 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; French 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 1,000 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 10×10^6 μg/L (ie, 10 g/L), with pretreatment concentrations > 1,000 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 (eg, 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 using ultrasound 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 contrast, 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 two dynamic studies (eg, 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 (eg, 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 two imaging modalities (eg, 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 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 <zref>152,<ths>155<zrefx>. 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 two 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 one of seven 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 past decade (164). Age, degree of liver damage, AFP concentration, maximal tumor dimension, number of tumors, intrahepatic extent of tumor, extrahepatic metastasis, portal and hepatic vein invasion, surgical curability, and free surgical margins were all independent prognostic factors for HCC patients undergoing liver resection (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 three AFP groups in 1,158 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 > 1,000 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 two 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 normal 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 Strength of Recommendation (SOR) 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 post-treatment 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 (ie, monthly for 6 months, then once every 3 months up to 1 year post-transplantation, 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 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 prothrombin produced by vitamin K absence or antagonism II (PIVKA 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 mkU/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 mAkU/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 four groups according to concentrations of DCP (less than or greater than 62.5 mAkU/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 1,377 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 patients 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 three 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 (eg, 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*) codes for a member of the glypican family of glycosyl-phosphatidylinositol–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 one the 20 patients with chronic hepatitis and cirrhosis. The combined sensitivity of the two 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 two markers in a subset of early-stage HCC was essentially unchanged, and there was no correlation between sGPC-3 and AFP in the 69 patients who had HCC. The combined marker sensitivity was 72%. This preliminary study suggests that sGPC-3 may have some promise and that larger clinical trials to investigate its potential are merited.

Other Serum Markers for Liver Cancer

Many other serum markers have been reported for HCC (Table 1). Pre- and post-treatment 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 (eg, 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 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 (eg, 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.

Chapter 3

Tumor Markers in Bladder Cancer

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 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 (ie, 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, six 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, Cortlandt Manor, NY) 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 four 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 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 validated 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).

Table 3. Useful and Potentially Useful Urine Markers for Bladder Cancer

Cancer Marker	Proposed Use/Uses	Phase of Development	LOE	FDA Cleared?	Reference
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, 297, 298
FGFR3	Prognosis	In clinical trials	III	No	92-94
Microsatellite markers	Early detection	In clinical trials	III	No	299-305
HA/HAase	None at present	Not in clinical use	IV	No	307-310
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 β subunit and β core fragment 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

Nuclear Matrix Protein

The nuclear matrix protein 22 (NMP22) test (Matritech, Drummondville, Quebec, Canada) 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 mentioned 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 1,331 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, Quebec, Canada) 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 bacillus Calmette-Guerin 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 treatment 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. Over expression 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, Stockholm, Sweden) 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, Bromma, Sweden) 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 two major components, an RNA template and an enzymatic subunit.

The Telomeric Repeat Amplification Protocol (TRAP) assay (Geron, Menlo Park, CA) 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 non-neoplastic 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 one 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 two-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 one 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 three of 35 patients who had previously been treated for bladder cancer but who had negative cystoscopic evaluations (291). 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 one to four 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 two 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 two 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) β subunit and β core fragment (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 work-up 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 (eg, 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), *HCG β* 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).

Chapter **4**

Tumor Markers in Cervical Cancer

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 3,870 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 two 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 trials have indicated that HPV testing leads to earlier detection of high-grade CIN lesions or cervical cancer compared with 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 virus-like 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 longer) 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 end point 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 (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 $\leq$ 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 with 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 alone 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 versus 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 versus 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(eg, 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 (ie, 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 tumor 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 (385-415). Carcinoembryonic antigen (CEA) and CA125 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.

Table 4. Currently Available and Potentially Useful Serum Markers for Cervical Cancer

Cancer Marker	Proposed Use	Phase of Development	LOE	Reference
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
CA125	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

Table 5. NACB Recommendations for the Clinical Use of SCC in Squamous Cell Cervical Cancer

Marker	Application	NACB Recommendations (2009)	LOE	Strength of Recommendation
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

Squamous Cell Carcinoma Antigen

Biochemistry of SCC

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 two 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 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 (eg, tissue remodelling, protein processing) and pathologic processes (eg, tumor progression; 425). Structurally, SCC1 and SCC2 are almost identical, differing only in their reactive site loops. The two forms, however, may have different biological functions (423,425,426).

Reference Intervals for SCC

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 (eg, psoriasis, eczema), lung (eg, 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 (ie, > 10 µg/L) were associated with enlarged lymph nodes shown on CT scan (399). On combining SCC (cutoff value 2.5 µg/L) with CA125 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 ROC curve (395). The authors concluded that a normal pretreatment SCC concentration 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 normal preoperative SCC concentrations, 16% of IB1 and 29% of IB2/IIA patients had postoperative indications for adjuvant radiotherapy, in contrast with 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 with other reported investigations, lymph node status showed no independent prognostic value in this study (393). Another group found that SCC and CA125, 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 with node-positive patients with normal SCC concentrations (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 one 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). Normal CEA and SCC concentrations 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 work-up 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 post-treatment SCC concentrations and response to chemotherapy (408). None of the patients with a complete response had post-treatment 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 two of six 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 five (14%) of 35 patients, serum SCC elevation was the only sign of recurrent disease. Unfortunately, all of these five 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. Post-treatment 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 Post-Treatment 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 a normal SCC concentration does not exclude the presence of lymph node metastases.

Pretreatment SCC concentrations may be used to individualize treatment planning, in particular, for 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.

Chapter **5**

Tumor Markers in Gastric Cancer

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 patients 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 (eg, 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 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 (ie, 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.

Table 6. Currently Available Serum Markers for Gastric Cancer

Marker	Proposed Use	Phase of Development	LOE	Reference
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
β Subunit of HCG	Prognosis	Needs further evaluation	IV	494, 495

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 *Helicobacter pylori* infection have recently been proposed by the Practice Parameters Committee of the American College of Gastroenterology (476). Testing for *Helicobacter pylori* infection and treating as appropriate is part of the initial evaluation of patients with gastric cancer (463).

Members of families with a strong history of diffuse gastric cancer who are carriers of germline truncating E-cadherin mutations may benefit from genetic counseling, with prophylactic gastrectomy as 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, while 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, CA19-9, and CA72-4 (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).

Table 7. Reported Pretreatment Sensitivity of Serum Markers for Gastric Cancer

Marker	Cutoff Level	Early Stage	Advanced Disease	Reference
CEA	5 µg/L	< 20%	40-50	484-488, 501, 504, 505, 575
CA19.9	37 kU/L	< 20%	20-50	484-488, 501, 504, 505, 575
CA72.4	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

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 the Diagnosis and Screening of 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, CA19.9, or CA72.4 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 CA19.9 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 CA19.9 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 CA19.9 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 CA19.9 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.

REFERENCES

1. Field M, Lorh K, eds. Clinical practice guidelines: directions for a new program. Washington, DC, National Academy Press, 1990, pp 168
2. Diamandis EP, Hoffman BR, Sturgeon CM. National Academy of Clinical Biochemistry laboratory medicine practice guidelines for the use of tumor markers. Clin Chem 2008;54:1935-1939.
3. Sturgeon CM, Hoffman BR, Chan DW, Ch'ng SL, Hammond E, Hayes DF, et al. National Academy of Clinical Biochemistry laboratory medicine practice guidelines for use of tumor markers in clinical practice: Quality requirements. Clin Chem 2008;54:e1-10.
4. Jelic S. Hepatocellular carcinoma: ESMO Clinical Recommendations for diagnosis, treatment and follow-up. Ann Oncol 2009;20:iv41-45.
5. Stuart KE, Stadler ZK. Hepatic carcinoma, primary. http://emedicine.medscape.com/article/282814-overview
6. Llovet JM, Burroughs A, Bruix J. Hepatocellular carcinoma. Lancet 2003;362:1907-1917.
7. Bosch FX, Ribes J, Borràs J. Epidemiology of primary liver cancer. Semin Liver Dis 1999;19:271-285.
8. Tanaka Y, Hanada K, Mizokami M, Yeo AE, Shih JW, Gojobori T, Alter HJ. Inaugural Article: A comparison of the molecular clock of hepatitis C virus in the United States and Japan predicts that hepatocellular carcinoma incidence in the United States will increase over the next two decades. Proc Natl Acad Sci 2002;99:15584-15589.
9. El-Serag HB, Davila JA, Petersen NJ, McGlynn KA. The continuing increase in the incidence of hepatocellular carcinoma in the United States: an update. Ann Intern Med 2003;139:817-823.
10. Fattovich G, Giustina G, Degos F, Tremolada F, Diodati G, Almasio P, et al. Morbidity and mortality in compensated cirrhosis type C: A retrospective follow-up study of 384 patients. Gastroenterology 1997;112:463-472.
11. Colombo.M, Berr F, Bruix J, Hauss J, Wands J, Wittekind C. In: Berr F, Bruix J, Hauss J, Wittekind C, Wands J, eds. Risk groups and preventive strategies. Malignant liver tumors: basic concepts and clinical management (Falk Symposium). Kluwer Academic Publishers BV and Falk Foundation, 2003, pp 67-74
12. Liaw YF, Tai DI, Chu CM, Lin DY, Sheen IS, Chen TJ, Pao CC. Early detection of hepatocellular carcinoma in patients with chronic type B hepatitis. A prospective study. Gastroenterology 1986;90:263-267.
13. Sun Z, Lu P, Gail MH, Pee D, Zhang Q, Ming L, et al. Increased risk of hepatocellular carcinoma in male hepatitis B surface antigen carriers with chronic hepatitis who have detectable urinary aflatoxin metabolite M1. Hepatology 1999;30:379-383.
14. Bruno S, Silini E, Crosignani A, Borzio F, Leandro G, Bono F, et al. Hepatitis C virus genotypes and risk of hepatocellular carcinoma in cirrhosis: A prospective study. Hepatology 1997;25:754-758.
15. Bruix J, Barrera JM, Calvet X, Ercilla G, Costa J, Sanchez-Tapias JM, et al. Prevalence of antibodies to hepatitis C virus in Spanish patients with hepatocellular carcinoma and hepatic cirrhosis. Lancet 1989;2:1004-1006.
16. Colombo M, de Franchis R, Del Ninno E, Sangiovanni A, De Fazio C, Tommasini M, et al. Hepatocellular carcinoma in Italian patients with cirrhosis. N Engl J Med 1991;325:675-680.
17. Tsukuma H, Hiyama T, Tanaka S, Nakao M, Yabuuchi T, Kitamura T, et al. Risk factors for hepatocellular carcinoma among patients with chronic liver disease. N Engl J Med 1993;328:1797-1801.
18. Niederau C, Fischer R, Sonnenberg A, Stremmel W, Trampisch HJ, Strohmeyer G. Survival and causes of death in cirrhotic and in noncirrhotic patients with primary hemochromatosis. N Engl J Med 1985;313:1256-1262.
19. Zhou XD, Tang ZY, Yang BH, Lin ZY, Ma ZC, Ye SL, et al. Experience of 1000 patients who underwent hepatectomy for small hepatocellular carcinoma. Cancer 2001;91:1479-1486.
20. Fattovich G, Giustina G, Schalm SW, Hadziyannis S, Sanchez-Tapias J, Almasio P, et al. Occurrence of hepatocellular carcinoma and decompensation in western European patients with cirrhosis type B. The EUROHEP Study Group on Hepatitis B Virus and Cirrhosis. Hepatology 1995;21:77-82.
21. Beasley RP, Hwang LY, Lin CC, Chien CS. Hepatocellular carcinoma and hepatitis B virus. A prospective study of 22 707 men in Taiwan. Lancet 1981;2:1129-1133.
22. WHO. Hepatitis C: global prevalence. Wkly Epidemiol Rec 1997:341-344.
23. European Association for the Study of the Liver. EASL Clinical Practice Guidelines. Management of chronic hepatitis B. J Hepatol 2009;50:227-242.
24. Lok AS, McMahon BJ. Chronic hepatitis B. Hepatology 2007;45:507-539.
25. Ghany MG, Strader DB, Thomas DL, Seeff LB, and American Association for the Study of Liver Diseases. Diagnosis, management, and treatment of hepatitis C: An update. Hepatology 2009;49:1335-1374.
26. Ryder SD. Guidelines for the diagnosis and treatment of hepatocellular carcinoma (HCC) in adults. Gut 2003;52:iii1-8 (suppl 3).
27. Gogel BM, Goldstein RM, Kuhn JA, McCarty TM, Donahoe A, Glastad K. Diagnostic evaluation of hepatocellular carcinoma in a cirrhotic liver. Oncology (Williston Park) 2000;14:15-20.
28. Larcos G, Sorokopud H, Berry G, Farrell GC. Sonographic screening for hepatocellular carcinoma in patients with chronic hepatitis or cirrhosis: an evaluation. Am J Roentgenol 1998;171:433-5.
29. Sarasin FP, Giostra E, Hadengue A. Cost-effectiveness of screening for detection of small hepatocellular carcinoma in western patients with Child-Pugh class A cirrhosis. Am J Med 1996;101:422-434.
30. Schwartz JM, Carithers RL. Clinical features, diagnosis and screening for primary hepatocellular carcinoma. V. 17.2 May 2009. http://www.uptodateonline.com/patients/content/topic.do?topicKey=~kxb1bGB8WXPmsH
31. Kew MC, Dos Santos HA, Sherlock S. Diagnosis of primary cancer of the liver. Br Med J 1971;4:408-411.
32. A new prognostic system for hepatocellular carcinoma: a retrospective study of 435 patients: the Cancer of the Liver Italian Program (CLIP) investigators. Hepatology 1998;28:751-755.
33. Sugano S, Miyoshi K, Suzuki T, Kawafune T, Kubota M. Intrahepatic arteriovenous shunting due to hepatocellular carcinoma and cirrhosis, and its change by transcatheter arterial embolization. Am J Gastroenterol 1994;89:184-188.

34. Tietge UJ, Schöfl C, Ocran KW, Wagner S, Böker KH, Brabant G, et al. Hepatoma with severe non-islet cell tumor hypoglycemia. Am J Gastroenterol 1998;93:997-1000.
35. Kew MC, Fisher JW. Serum erythropoietin concentrations in patients with hepatocellular carcinoma. Cancer 1986;58:2485-2488.
36. Knill-Jones RP, Buckle RM, Parsons V, Calne RY, Williams R. Hypercalcemia and increased parathyroid-hormone activity in a primary hepatoma. Studies before and after hepatic transplantation. N Engl J Med 1970;282:704-708.
37. Yen TC, Hwang SJ, Wang CC, Lee SD, Yeh SH. Hypercalcemia and parathyroid hormone-related protein in hepatocellular carcinoma. Liver 1993;13:311-315.
38. Gregory B, Ho VC. Cutaneous manifestations of gastrointestinal disorders. Part I. J Am Acad Dermatol 1992;26:153-166.
39. Gomaa AI, Khan SA, Leen EL, Waked I, Taylor-Robinson SD. Diagnosis of hepatocellular carcinoma. World J Gastroenterol 2009;15:1301-1314.
40. Bruix J, Sherman M. Management of hepatocellular carcinoma. Hepatology 2005;42:1208-1236.
41. Durand F, Regimbeau JM, Belghiti J, Sauvanet A, Vilgrain V, Terris B, et al. Assessment of the benefits and risks of percutaneous biopsy before surgical resection of hepatocellular carcinoma. J Hepatol 2001;35:254-258.
42. Silva MA, Hegab B, Hyde C, Guo B, Buckels JA, Mirza DF. Needle track seeding following biopsy of liver lesions in the diagnosis of hepatocellular cancer: a systematic review and meta-analysis. Gut 2008;57:1592-1596.
43. Takayama T, Makuuchi M, Hirohashi S, Sakamoto M, Yamamoto J, Shimada K, et al. Early hepatocellular carcinoma as an entity with a high rate of surgical cure. Hepatology 1998;28:1241-6.
44. Kojiro M, Tabor E. The evolution of pathologic features of hepatocellular carcinoma. In Tabor E, ed. Viruses and liver cancer: perspectives in medical virology. Amsterdam, the Netherlands, Elsevier Science, 2002, pp 113-22.
45. Llovet JM, Fuster J, Bruix J. Intention-to-treat analysis of surgical treatment for early hepatocellular carcinoma: resection versus transplantation. Hepatology 1999;30:1434-1440.
46. The Liver Cancer Study Group of Japan. Predictive factors for long term prognosis after partial hepatectomy for patients with hepatocellular carcinoma in Japan. Cancer 1994;74:2272-2280.
47. Llovet JM, Bruix J. Systematic review of randomized trials for unresectable hepatocellular carcinoma: chemoembolization improves survival. Hepatology 2003;37:429-442.
48. Lopez PM, Villanueva A, Llovet JM. Systematic review: Evidence-based management of hepatocellular carcinomaóan updated analysis of randomized controlled trials. Aliment Pharmacol Ther 2006;23:1535-1547.
49. El-Serag HB, Marrero JA, Rudolph L, Reddy KR. Diagnosis and treatment of hepatocellular carcinoma. Gastroenterology 2008;134:1752-1763.
50. Okada S. Local ablation therapy for hepatocellular carcinoma. Semin Liver Dis 1999;19:323-328.
51. Livraghi T, Giorgio A, Marin G, Salmi A, de SI, Bolondi L, et al. Hepatocellular carcinoma and cirrhosis in 746 patients: Long-term results of percutaneous ethanol injection. Radiology 1995;197:101-108.
52. Lencioni RA, Allgaier HP, Cioni D, Olschewski M, Deibert P, Crocetti L, et al. Small hepatocellular carcinoma in cirrhosis: randomized comparison of radio-frequency thermal ablation versus percutaneous ethanol injection. Radiology 2003;228:235-240.
53. Fong Y, Sun RL, Jarnagin W, Blumgart LH. An analysis of 412 cases of hepatocellular carcinoma at a Western center. Ann Surg 1999;229:790-799.
54. Cho YK, Kim JK, Kim MY, Rhim H, Han JK. Systematic review of randomized trials for hepatocellular carcinoma treated with percutaneous ablation therapies. Hepatology 2009;49:453-459.
55. Llovet JM, Bruix J. Molecular targeted therapies in hepatocellular carcinoma. Hepatology 2008;48:1312-1327.
56. Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, et al. Sorafenib in advanced hepatocellular carcinoma. N Engl J Med 2008;359:378-390.
57. Cheng AL, Kang YK, Chen Z, Tsao CJ, Qin S, Kim JS, et al. Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: A phase III randomised, double-blind, placebo-controlled trial. Lancet Oncol 2009;10:25-34.
58. Hayes DF, Bast RC, Desch CE, Fritsche H Jr, Kemeny NE, Jessup JM, et al. Tumor marker utility grading system: A framework to evaluate clinical utility of tumor markers. J Natl Cancer Inst 1996;88:1456-1466.
59. Johnson PJ, Diamandis EP, Fritsche HA, Lilja H, Chan DW, Schwartz MK. Tumor markers in primary malignanciesof the liver. In: Diamandis EP, Fritche HA, Lilja H, Chan DW, Schwartz MK, eds. Tumor markers: physiology, pathobiology, technology and clinical applications. Washington, DC, AACC Press, 2002, pp 269-279.
60. Mizejewski GJ. Alpha-fetoprotein structure and function: relevance to isoforms, epitopes, and conformational variants. Exp Biol Med (Maywood) 2001;226:377-408.
61. Christiansen M, Hogdall CK, Andersen JR, Norgaard-Pedersen B. Alpha-fetoprotein in plasma and serum of healthy adults: preanalytical, analytical and biological sources of variation and construction of age-dependent reference intervals. Scand J Clin Lab Invest 2001;61:205-215.
62. Yoshima H, Mizuochi T, Ishii M, Kobata A. Structure of the asparagine-linked sugar chains of alpha-fetoprotein purified from human ascites fluid. Cancer Res 1980;40:4276-4281.
63. Yamashita K, Taketa K, Nishi S, Fukushima K, Ohkura T. Sugar chains of human cord serum alpha-fetoprotein: characteristics of N-linked sugar chains of glycoproteins produced in human liver and hepatocellular carcinomas. Cancer Res 1993;53:2970-2975.
64. Mora J, Gascon N, Tabernero JM, Germa JR, Gonzalez F. Alpha-fetoprotein-concanavalin A binding as a marker to discriminate between germ cell tumours and liver diseases. Eur J Cancer 1995;31A:2239-2242.
65. Saitoh S, Ikeda K, Koida I, Suzuki Y, Kobayashi M, Tsubota A, et al. Diagnosis of hepatocellular carcinoma by concanavalin A affinity electrophoresis of serum alpha-fetoprotein. Cancer 1995;76:1139-1144.
66. Aoyagi Y, Suzuki Y, Igarashi K, Saitoh A, Oguro M, Yokota T, et al. The usefulness of simultaneous determinations of glucosaminylation and fucosylation indices of alpha-fetoprotein in the differential diagnosis of neoplastic diseases of the liver. Cancer 1991;67:2390-2394.
67. Taketa K, Ichikawa E, Taga H, Hirai H. Antibody-affinity blotting, a sensitive technique for the detection of a-fetoprotein separated by lectin affinity electrophoresis in agarose gels. Electrophoresis 1985;6:492-497.
68. Taketa K. Alpha-fetoprotein: Reevaluation in hepatology. Hepatology 1990;12:1420-1432.

69. Johnson PJ, Leung N, Cheng P, Welby C, Leung WT, Lau WY, et al. "Hepatoma-specific" alphafetoprotein may permit preclinical diagnosis of malignant change in patients with chronic liver disease. Br J Cancer 1997;75:236-240.
70. Fujii Y, Taketa K, Aoi T, Taga H, Hirai H. Increased serum levels of monosialo-alpha-fetoprotein in hepatocellular carcinoma and other malignancies. Tumor Biol 1993;14:319-324.
71. Poon TC, Mok TS, Chan AT, Chan CM, Leong V, Tsui SH, et al. Quantification and utility of monosialylated alpha-fetoprotein in the diagnosis of hepatocellular carcinoma with nondiagnostic serum total alpha-fetoprotein. Clin Chem 2002;48:1021-1027.
72. Shimizu K, Katoh H, Yamashita F, Tanaka M, Tanikawa K, Taketa K, et al. Comparison of carbohydrate structures of serum alpha-fetoprotein by sequential glycosidase digestion and lectin affinity electrophoresis. Clin Chim Acta 1996;254:23-40.
73. Johnson PJ, Poon TC, Hjelm NM, Ho CS, Ho SK, Welby C, et al. Glycan composition of serum alpha-fetoprotein in patients with hepatocellular carcinoma and non-seminomatous germ cell tumour. Br J Cancer 1999;81:1188-1195.
74. Taketa K, Endo Y, Sekiya C, Tanikawa K, Koji T, Taga H, et al. A collaborative study for the evaluation of lectin-reactive alpha-fetoproteins in early detection of hepatocellular carcinoma. Cancer Res 1993;53:5419-5423.
75. Sato Y, Nakata K, Kato Y, Shima M, Ishii N, Koji T, et al. Early recognition of hepatocellular carcinoma based on altered profiles of alpha-fetoprotein. N Engl J Med 1993;328:1802-1806.
76. Taketa K, Sekiya C, Namiki M, Akamatsu K, Ohta Y, Endo Y, Kosaka K. Lectin-reactive profiles of alpha-fetoprotein characterizing hepatocellular carcinoma and related conditions. Gastroenterology 1990;99:508-518.
77. Yamashita F, Tanaka M, Satomura S, Tanikawa K. Prognostic significance of Lens culinaris agglutinin A-reactive alpha-fetoprotein in small hepatocellular carcinomas. Gastroenterology 1996;111:996-1001.
78. Wang SS, Lu RH, Lee FY, Chao Y, Huang YS, Chen CC, Lee SD. Utility of lentil lectin affinity of alpha-fetoprotein in the diagnosis of hepatocellular carcinoma. J Hepatol 1996;25:166-171.
79. Kumada T, Nakano S, Takeda I, Kiriyama S, Sone Y, Hayashi K, et al. Clinical utility of Lens culinaris agglutinin-reactive alpha-fetoprotein in small hepatocellular carcinoma: Special reference to imaging diagnosis. J Hepatol 1999;30:125-130.
80. Oka H, Saito A, Ito K, Kumada T, Satomura S, Kasugai H, et al. Multicenter prospective analysis of newly diagnosed hepatocellular carcinoma with respect to the percentage of Lens culinaris agglutinin-reactive alpha-fetoprotein. J Gastroenterol Hepatol 2001;16:1378-1383.
81. Song BC, Suh DJ, Yang SH, Lee HC, Chung YH, Sung KB, Lee YS. Lens culinaris agglutinin-reactive alpha-fetoprotein as a prognostic marker in patients with hepatocellular carcinoma undergoing transcatheter arterial chemoembolization. J Clin Gastroenterol 2002;35:398-402.
82. Leerapun A, Suravarapu SV, Bida JP, Clark RJ, Sanders EL, Mettler TA, et al. The utility of Lens culinaris agglutinin-reactive alpha-fetoprotein in the diagnosis of hepatocellular carcinoma: evaluation in a United States referral population. Clin Gastroenterol Hepatol 2007;5:394-402.
83. Sterling RK, Jeffers L, Gordon F, Venook AP, Reddy KR, Satomura S, et al. Utility of Lens culinaris agglutinin-reactive fraction of alpha-fetoprotein and des-gamma-carboxy prothrombin, alone or in combination, as biomarkers for hepatocellular carcinoma. Clin Gastroenterol Hepatol 2009;7:104-113.
84. Carr BI, Kanke F, Wise M, Satomura S. Clinical evaluation of lens culinaris agglutinin-reactive alpha-fetoprotein and des-gamma-carboxy prothrombin in histologically proven hepatocellular carcinoma in the United States. Dig Dis Sci 2007;52:776-782.
85. Tateishi R, Shiina S, Yoshida H, Teratani T, Obi S, Yamashiki N, et al. Prediction of recurrence of hepatocellular carcinoma after curative ablation using three tumor markers. Hepatology 2006;44:1518-1527.
86. Marrero JA, Feng Z, Wang Y, Nguyen MH, Befeler AS, Roberts LR, et al. Alpha-fetoprotein, des-gamma carboxyprothrombin, and lectin-bound alpha-fetoprotein in early hepatocellular carcinoma. Gastroenterology 2009;137:110-118.
87. Llovet JM, Di Bisceglie AM, Bruix J, Kramer BS, Lencioni R, Zhu AX, et al. Design and endpoints of clinical trials in hepatocellular carcinoma. J Natl Cancer Inst 2008;100:698-711.
88. Johnson JR, Williams G, Pazdur R. End points and United States Food and Drug Administration approval of oncology drugs. J Clin Oncol 2003;21:1404-1411.
89. Collier J, Sherman M. Screening for hepatocellular carcinoma. Hepatology 1998;27:273-278.
90. Chen DS, Sung JL, Sheu JC, Lai MY, How SW, Hsu HC, et al. Serum alpha-fetoprotein in the early stage of human hepatocellular carcinoma. Gastroenterology 1984;86:1404-1409.
91. Kondo F, Wada K, Nagato Y, Nakajima T, Kondo Y, Hirooka N, et al. Biopsy diagnosis of well-differentiated hepatocellular carcinoma based on new morphologic criteria. Hepatology 1989;9:751-755.
92. Forner A, Vilana R, Ayuso C, Bianchi L, Sole M, Ayuso JR, et al. Diagnosis of hepatic nodules 20 mm or smaller in cirrhosis: prospective validation of the noninvasive diagnostic criteria for hepatocellular carcinoma. Hepatology 2008;47:97-104.
93. Yoshino M. Growth kinetics of hepatocellular carcinoma. Jpn J Clin Oncol 1983;13:45-52.
94. Barbara L, Benzi G, Gaiani S, Fusconi F, Zironi G, Siringo S, et al. Natural history of small untreated hepatocellular carcinoma in cirrhosis: a multivariate analysis of prognostic factors of tumor growth rate and patient survival. Hepatology 1992;16:132-137.
95. Okuda K. Early recognition of hepatocellular carcinoma. Hepatology 1986;6:729-738.
96. Forner A, Reig M, Bruix J. Alpha-fetoprotein for hepatocellular carcinoma diagnosis: the demise of a brilliant star. Gastroenterology 2009;137:26-29.
97. Lok AS, Sterling RK, Everhart JE, Wright EC, Hoefs JC, Di Bisceglie AM, et al. Des-gamma-carboxy Prothrombin and Alpha fetoprotein as Biomarkers for the Early Detection of Hepatocellular Carcinoma. Gastroenterology 2009; 138:493-502.
98. Gupta S, Bent S, Kohlwes J. Test characteristics of alpha-fetoprotein for detecting hepatocellular carcinoma in patients with hepatitis C: A systematic review and critical analysis. Ann Intern Med 2003;139:46-50.
99. Trevisani F, D'Intino PE, Morselli-Labate AM, Mazzella G, Accogli E, Caraceni P, et al. Serum alpha-fetoprotein for diagnosis of hepatocellular carcinoma in patients with chronic liver disease: influence of HBsAg and anti-HCV status. J Hepatol 2001;34:570-575.
100. Tong MJ, Blatt LM, Kao VW. Surveillance for hepatocellular carcinoma in patients with chronic viral hepatitis in the United States of America. J Gastroenterol Hepatol 2001;16:553-559.
101. Cedrone A, Covino M, Caturelli E, Pompili M, Lorenzelli G, Villani MR, et al. Utility of alpha-fetoprotein (AFP) in the screening of patients with virus-related chronic liver disease: Does different viral etiology influence AFP levels in HCC? A study in 350 Western patients. Hepatogastroenterology 2000;47:1654-1658.

102. Nguyen MH, Garcia RT, Simpson PW, Wright TL, Keeffe EB. Racial differences in effectiveness of alpha-fetoprotein for diagnosis of hepatocellular carcinoma in hepatitis C virus cirrhosis. Hepatology 2002;36:410-417.
103. Peng YC, Chan CS, Chen GH. The effectiveness of serum alpha-fetoprotein level in anti-HCV positive patients for screening hepatocellular carcinoma. Hepatogastroenterology 1999;46:3208-3211.
104. Gebo KA, Chander G, Jenckes MW, Ghanem KG, Herlong HF, Torbenson MS, et al. Screening tests for hepatocellular carcinoma in patients with chronic hepatitis C: A systematic review. Hepatology 2002;36:S84-S92.
105. Takano S, Yokosuka O, Imazeki F, Tagawa M, Omata M. Incidence of hepatocellular carcinoma in chronic hepatitis B and C: a prospective study of 251 patients. Hepatology 1995;21:650-655.
106. Sherman M, Peltekian KM, Lee C. Screening for hepatocellular carcinoma in chronic carriers of hepatitis B virus: Incidence and prevalence of hepatocellular carcinoma in a North American urban population. Hepatology 1995;22:432-438.
107. Pateron D, Ganne N, Trinchet JC, Aurousseau MH, Mal F, Meicler C, et al. Prospective study of screening for hepatocellular carcinoma in Caucasian patients with cirrhosis. J Hepatol 1994;20:65-71.
108. McMahon BJ, Alberts SR, Wainwright RB, Bulkow L, Lanier AP. Hepatitis B-related sequelae: prospective study in 1400 hepatitis B surface antigen-positive Alaska native carriers. Arch Intern Med 1990;150:1051-1054.
109. McMahon BJ, Bulkow L, Harpster A, Snowball M, Lanier A, Sacco F, et al. Screening for hepatocellular carcinoma in Alaska natives infected with chronic hepatitis B: A 16-year population-based study. Hepatology 2000;32:842-846.
110. Tanaka S, Kitamura T, Nakanishi K, Okuda S, Yamazaki H, Hiyama T, Fujimoto I. Effectiveness of periodic checkup by ultrasonography for the early diagnosis of hepatocellular carcinoma. Cancer 1990;66:2210-2214.
111. Solmi L, Primerano AM, Gandolfi L. Ultrasound follow-up of patients at risk for hepatocellular carcinoma: results of a prospective study on 360 cases. Am J Gastroenterol 1996;91:1189-1194.
112. Yuen MF, Cheng CC, Lauder IJ, Lam SK, Ooi CG, Lai CL. Early detection of hepatocellular carcinoma increases the chance of treatment: Hong Kong experience. Hepatology 2000;31:330-335.
113. Yuen MF, Lai CL. Screening for hepatocellular carcinoma: survival benefit and cost-effectiveness. Ann Oncol 2003;14:1463-1467.
114. Wong LL, Limm WM, Severino R, Wong LM. Improved survival with screening for hepatocellular carcinoma. Liver Transpl 2000;6:320-325.
115. Sangiovanni A, Del NE, Fasani P, De FC, Ronchi G, Romeo R, et al. Increased survival of cirrhotic patients with a hepatocellular carcinoma detected during surveillance. Gastroenterology 2004;126:1005-1014.
116. Zhang BH, Yang BH, Tang ZY. Randomized controlled trial of screening for hepatocellular carcinoma. J Cancer Res Clin Oncol 2004;130:417-422.
117. Chen JG, Parkin DM, Chen QG, Lu JH, Shen QJ, Zhang BC, Zhu YR. Screening for liver cancer: results of a randomised controlled trial in Qidong, China. J Med Screen 2003;10:204-209.
118. Chalasani N, Said A, Ness R, Hoen H, Lumeng L. Screening for hepatocellular carcinoma in patients with cirrhosis in the United States: results of a national survey. Am J Gastroenterol 1999;94:2224-2229.
119. Nguyen MH, Keeffe EB. Screening for hepatocellular carcinoma. J Clin Gastroenterol 2002;35:S86-S91.
120. Lin OS, Keeffe EB, Sanders GD, Owens DK. Cost-effectiveness of screening for hepatocellular carcinoma in patients with cirrhosis due to chronic hepatitis C. Aliment Pharmacol Ther 2004;19:1159-1172.
121. Thompson Coon J, Rogers G, Hewson P, Wright D, Anderson R, Cramp M, et al. Surveillance of cirrhosis for hepatocellular carcinoma: systematic review and economic analysis. Health Technol Assess 2007;11:1-206.
122. Thompson Coon J, Rogers G, Hewson P, Wright D, Anderson R, Jackson S, et al. Surveillance of cirrhosis for hepatocellular carcinoma: a cost-utility analysis. Br J Cancer 2008;98:1166-1175.
123. Oka H, Tamori A, Kuroki T, Kobayashi K, Yamamoto S. Prospective study of alpha-fetoprotein in cirrhotic patients monitored for development of hepatocellular carcinoma. Hepatology 1994;19:61-66.
124. Colombo M. Screening for cancer in viral hepatitis. Clin Liver Dis 2001;5:109-122.
125. Bonis PA, Tong MJ, Blatt LM, Conrad A, Griffith JL. A predictive model for the development of hepatocellular carcinoma, liver failure, or liver transplantation for patients presenting to clinic with chronic hepatitis C. Am J Gastroenterol 1999;94:1605-1612.
126. Velazquez RF, Rodriguez M, Navascues CA, Linares A, Perez R, Sotorrios NG, et al. Prospective analysis of risk factors for hepatocellular carcinoma in patients with liver cirrhosis. Hepatology 2003;37:520-527.
127. Kudo M, Okanoue T. Management of hepatocellular carcinoma in Japan: Consensus-based clinical practice manual proposed by the Japan Society of Hepatology. Oncology 2007;72:2-15 (suppl 1).
128. Makuuchi M, Kokudo N, Arii S, Futagawa S, Kaneko S, Kawasaki S, et al. Development of evidence-based clinical guidelines for the diagnosis and treatment of hepatocellular carcinoma in Japan. Hepatol Res 2008;38:37-51.
129. Arase Y, Ikeda K, Suzuki F, Suzuki Y, Kobayashi M, Akuta N, et al. Prolonged-interferon therapy reduces hepatocarcinogenesis in aged-patients with chronic hepatitis C. J Med Virol 2007;79:1095-1102.
130. Murashima S, Tanaka M, Haramaki M, Yutani S, Nakashima Y, Harada K, et al. A decrease in AFP level related to administration of interferon in patients with chronic hepatitis C and a high level of AFP. Dig Dis Sci 2006;51:808-812.
131. Bruix J, Sherman M, Llovet JM, Beaugrand M, Lencioni R, Burroughs AK, et al. Clinical management of hepatocellular carcinoma. Conclusions of the Barcelona-2000 EASL conference. European Association for the Study of the Liver. J Hepatol 2001;35:421-430.
132. Giovannini M, Elias D, Monges G, Raoul JL, Rougier P. Hepatocellular carcinoma. Br J Cancer 2001;84:74-77 (suppl 2).
133. Bialecki ES, Ezenekwe AM, Brunt EM, Collins BT, Ponder TB, Bieneman BK, Di Bisceglie AM. Comparison of liver biopsy and noninvasive methods for diagnosis of hepatocellular carcinoma. Clin Gastroenterol Hepatol 2006;4:361-368.
134. Sturgeon CM, Lai LC, Duffy MJ. Serum tumour markers: How to order and interpret them. BMJ 2009;339:b3527.
135. National Comprehensive Cancer Network (NCCN) clinical practice guidelines in oncology, hepatobiliary cancers V.I. 2010. http://www.nccn.org/
136. Poon D, Anderson BO, Chen LT, Tanaka K, Lau WY, Van Cutsem E, et al. Management of hepatocellular carcinoma in

Asia: consensus statement from the Asian Oncology Summit 2009. Lancet Oncol 2009;10:1111-1118.
137. European Group on Tumor Markers (EGTM): Consensus recommendations. Anticancer Res 1999;19:2785-2820.
138. Sturgeon CM, Duffy MJ, Stenman UH, Lilja H, Brunner N, Chan DW, et al. National Academy of Clinical Biochemistry laboratory medicine practice guidelines for use of tumor markers in testicular, prostate, colorectal, breast, and ovarian cancers. Clin Chem 2008;54:e11-79.
139. McIntire KR, Waldmann TA, Moertel CG, Go VL. Serum alpha-fetoprotein in patients with neoplasms of the gastrointestinal tract. Cancer Res 1975;35:991-996.
140. Sawabu N, Hattori N, Okuda K, Ishak KG. Serological tumor markers in hepatocellular carcinoma. In: Okuda K, Ishak KG, eds. Neoplasms of the liver. Tokyo, Japan, Springer-Verlag; 1987, pp 227-238.
141. Wu JT. Serum alpha-fetoprotein and its lectin reactivity in liver diseases: A review. Ann Clin Lab Sci 1990;20:98-105.
142. Daniele B, Bencivenga A, Megna AS, Tinessa V. Alpha-fetoprotein and ultrasonography screening for hepatocellular carcinoma. Gastroenterology 2004;127:S108-S112.
143. Talwalkar JA, Gores GJ. Diagnosis and staging of hepatocellular carcinoma. Gastroenterology 2004;127:S126-S132.
144. Fujiyama S, Izuno K, Yamasaki K, Sato T, Taketa K. Determination of optimum cutoff levels of plasma des-gamma-carboxy prothrombin and serum alpha-fetoprotein for the diagnosis of hepatocellular carcinoma using receiver operating characteristic curves. Tumour Biol 1992;13:316-323.
145. Liver cancer study group of Japan. The 15th report on nationwide follow-up studies of primary liver cancer. Acta Hep Jap 2003;44:157-175.
146. Lee HS, Chung YH, Kim CY. Specificity of serum a-fetoprotein in $HBsAg^+$ and HBxAg- patients in the diagnosis of hepatocellular carcinoma. Hepatology 1991;14:68-72.
147. Namieno T, Kawata A, Sato N, Kondo Y, Uchino J. Age-related, different clinicopathologic features of hepatocellular carcinoma patients. Ann Surg 1995;221:308-314.
148. Taketa K. Alpha-fetoprotein. J Med Tech 1989;33:1380-1384.
149. The Liver Study Group of Japan: Primary cancer in Japan. Sixth Report. Cancer 1987;60:1400-1411.
150. Matsui H, Rimal N, Kamakura K, Uesugi S, Yamamoto H, Ikeda S, Taketa K. Serum alpha-fetoprotein levels in healthy Japanese adults. Acta Med Okayama 1998;52:149-154.
151. Greene FL, Page DL, Fleming ID, et al. AJCC (American Joint Committee on Cancer) Cancer Staging Manual. Vol. 6. New York, NY, Springer-Velag; 2002, pp 131-138.
152. Okuda K, Ohtsuki T, Obata H, Tomimatsu M, Okazaki N, Hasegawa H, et al. Natural history of hepatocellular carcinoma and prognosis in relation to treatment. Study of 850 patients. Cancer 1985;56:918-928.
153. Kudo M, Chung H, Haji S, Osaki Y, Oka H, Seki T, et al. Validation of a new prognostic staging system for hepatocellular carcinoma. The JIS score compared with the CLIP score. Hepatology 2004;40:1396-1405.
154. Chevret S, Trinchet JC, Mathieu D, Rached AA, Beaugrand M, Chastang C. A new prognostic classification for predicting survival in patients with hepatocellular carcinoma. Groupe d'Etude et de Traitement du Carcinome Hepatocellulaire. J Hepatol 1999;31:133-141.
155. Prospective validation of the CLIP score: A new prognostic system for patients with cirrhosis and hepatocellular carcinoma. The Cancer of the Liver Italian Program (CLIP) Investigators. Hepatology 2000;31:840-845.
156. Llovet JM, Bru C, Bruix J. Prognosis of hepatocellular carcinoma. The BCLC staging classification. Semin Liver Dis 1999;19:329-338.
157. Bruix J, Llovet JM. Prognostic prediction and treatment strategy in hepatocellular carcinoma. Hepatology 2002;35:519-524.
158. Leung TW, Tang AM, Zee B, Lau WY, Lai PB, Leung KL, et al. Construction of the Chinese University Prognostic Index for hepatocellular carcinoma and comparison with the TNM staging system, the Okuda staging system, and the Cancer of the Liver Italian Program staging system. A study based on 926 patients. Cancer 2002;94:1760-1769.
159. Llovet JM, Beaugrand M. Hepatocellular carcinoma. Present status and future prospects. J Hepatol 2003;38:S136-S49 (suppl 1).
160. Henderson JM, Sherman M, Tavill A, Abecassis M, Chejfec G, Gramlich R. AHPBA/AJCC consensus conference on staging of hepatocellular carcinoma: consensus statement HPB (Oxford) 2003;5:243-250.
161. Marrero JA, Fontana RJ, Barrat A, Askari F, Conjeevaram HS, Su GL, Lok AS. Prognosis of hepatocellular carcinoma: comparison of 7 staging systems in an American cohort. Hepatology 2005;41:707-16.
162. Cho CS, Gonen M, Shia J, Kattan MW, Klimstra DS, Jarnagin WR, et al. A novel prognostic nomogram is more accurate than conventional staging systems for predicting survival after resection of hepatocellular carcinoma. J Am Coll Surg 2008;206:281-291.
163. Lerose R, Molinari R, Rocchi E, Manenti F, Villa E. Prognostic features and survival of hepatocellular carcinoma in Italy: impact of stage of disease. Eur J Cancer 2001;37:239-245.
164. Ikai I, Arii S, Kojiro M, Ichida T, Makuuchi M, Matsuyama Y, et al. Reevaluation of prognostic factors for survival after liver resection in patients with hepatocellular carcinoma in a Japanese nationwide survey. Cancer 2004;101:796-802.
165. Shiraki K, Takase K, Tameda Y, Hamada M, Kosaka Y, Nakano T. A clinical study of lectin-reactive alpha-fetoprotein as an early indicator of hepatocellular carcinoma in the follow-up of cirrhotic patients. Hepatology 1995;22:802-807.
166. Tangkijvanich P, Anukulkarnkusol N, Suwangool P, Lertmaharit S, Hanvivatvong O, Kullavanijaya P, Poovorawan Y. Clinical characteristics and prognosis of hepatocellular carcinoma: analysis based on serum alpha-fetoprotein levels. J Clin Gastroenterol 2000;31:302-308.
167. Farinati F, Marino D, De Giorgio M, Baldan A, Cantarini M, Cursaro C, et al. Diagnostic and prognostic role of alpha-fetoprotein in hepatocellular carcinoma: both or neither? Am J Gastroenterol 2006;101:524-532.
168. Andorno E, Salizzoni M, Schieroni R, De HB. Role of serum alpha-fetoprotein in pre- and post-orthotopic liver transplantation (OLT) for malignant disease. J Nucl Med Allied Sci 1989;33:132-134.
169. Ebara M, Ohto M, Shinagawa T, Sugiura N, Kimura K, Matsutani S, et al. Natural history of minute hepatocellular carcinoma smaller than three centimeters complicating cirrhosis. A study in 22 patients. Gastroenterology 1986;90:289-298.
170. Matsumoto Y, Suzuki T, Asada I, Ozawa K, Tobe T, Honjo I. Clinical classification of hepatoma in Japan according to serial changes in serum alpha-fetoprotein levels. Cancer 1982;49:354-360.
171. Scottish Hepatopancreatobiliary Managed Clinical Network: Guidelines for the management of hepatocelluar carcinoma. www.scan.scot.nhs.uk

172. Johnson PJ, Williams R. Serum alpha-fetoprotein estimations and doubling time in hepatocellular carcinoma. Influence of therapy and possible value in early detection. J Natl Cancer Inst 1980;64:1329-1332.
173. Toyoda H, Kumada T, Kaneoka Y, Osaki Y, Kimura T, Arimoto A, et al. Prognostic value of pretreatment levels of tumor markers for hepatocellular carcinoma on survival after curative treatment of patients with HCC. J Hepatol 2008;49:223-232.
174. Urabe T, Hayashi S, Terasaki S, Terada M, Matsushita E, Kaneko S, et al. An assessment of therapeutic effect of hepatocellular carcinoma by the serial changes in serum AFP value [Japanese]. Nippon Shokakibyo Gakkai Zasshi 1990;87:100-108.
175. McIntire KR, Vogel CL, Primack A, Waldmann TA, Kyalwazi SK. Effect of surgical and chemotherapeutic treatment on alpha-fetoprotein levels in patients with hepatocellular carcinoma. Cancer 1976;37:677-683.
176. Matsumoto Y, Suzuki T, Ono H, Nakase A, Honjo I. Response of alpha-fetoprotein to chemotherapy in patients with hepatomas. Cancer 1974;34:1602-1606.
177. Leung TW, Patt YZ, Lau WY, Ho SK, Yu SC, Chan AT, et al. Complete pathological remission is possible with systemic combination chemotherapy for inoperable hepatocellular carcinoma. Clin Cancer Res 1999;5:1676-1681.
178. Nauta RJ, Heres EK, Thomas DS, Harter KW, Rodgers JE, Holt RW, et al. Intraoperative single-dose radiotherapy. Observations on staging and interstitial treatment of unresectable liver metastases. Arch Surg 1987;122:1392-1395.
179. Chan SL, Mo FK, Johnson PJ, Hui EP, Ma BB, Ho WM, et al. New utility of an old marker: serial alpha-fetoprotein measurement in predicting radiologic response and survival of patients with hepatocellular carcinoma undergoing systemic chemotherapy. J Clin Oncol 2009;27:446-452.
180. Vora SR, Zheng H, Stadler ZK, Fuchs CS, Zhu AX. Serum alpha-fetoprotein response as a surrogate for clinical outcome in patients receiving systemic therapy for advanced hepatocellular carcinoma. Oncologist 2009;14:717-725.
181. Liebman HA, Furie BC, Tong MJ, Blanchard RA, Lo KJ, Lee SD, et al. Des-gamma-carboxy (abnormal) prothrombin as a serum marker of primary hepatocellular carcinoma. N Engl J Med 1984;310:1427-1431.
182. Weitz IC, Liebman HA. Des-gamma-carboxy (abnormal) prothrombin and hepatocellular carcinoma. A critical review. Hepatology 1993;18:990-997.
183. Mita Y, Aoyagi Y, Yanagi M, Suda T, Suzuki Y, Asakura H. The usefulness of determining des-gamma-carboxy prothrombin by sensitive enzyme immunoassay in the early diagnosis of patients with hepatocellular carcinoma. Cancer 1998;82:1643-1648.
184. Okuda H, Nakanishi T, Takatsu K, Saito A, Hayashi N, Watanabe K, et al. Measurement of serum levels of des-gamma-carboxy prothrombin in patients with hepatocellular carcinoma by a revised enzyme immunoassay kit with increased sensitivity. Cancer 1999;85:812-818.
185. Tanaka Y, Kashiwagi T, Tsutsumi H, Nagasawa M, Toyama T, Ozaki S, et al. Sensitive measurement of serum abnormal prothrombin (PIVKA-II) as a marker of hepatocellular carcinoma. Hepatogastroenterology 1999;46:2464-2468.
186. Okuda H, Nakanishi T, Takatsu K, Saito A, Hayashi N, Takasaki K, et al. Serum levels of des-gamma-carboxy prothrombin measured using the revised enzyme immunoassay kit with increased sensitivity in relation to clinicopathologic features of solitary hepatocellular carcinoma. Cancer 2000;88:544-549.
187. Marrero JA, Su GL, Wei W, Emick D, Conjeevaram HS, Fontana RJ, Lok AS. Des-gamma carboxyprothrombin can differentiate hepatocellular carcinoma from nonmalignant chronic liver disease in American patients. Hepatology 2003;37:1114-1121.
188. Koike Y, Shiratori Y, Sato S, Obi S, Teratani T, Imamura M, et al. Des-gamma-carboxy prothrombin as a useful predisposing factor for the development of portal venous invasion in patients with hepatocellular carcinoma. A prospective analysis of 227 patients. Cancer 2001;91:561-569.
189. Imamura H, Matsuyama Y, Miyagawa Y, Ishida K, Shimada R, Miyagawa S, et al. Prognostic significance of anatomical resection and des-gamma-carboxy prothrombin in patients with hepatocellular carcinoma. Br J Surg 1999;86:1032-1038.
190. Hamamura K, Shiratori Y, Shiina S, Imamura M, Obi S, Sato S, et al. Unique clinical characteristics of patients with hepatocellular carcinoma who present with high plasma des-gamma-carboxy prothrombin and low serum alpha-fetoprotein. Cancer 2000;88:1557-1564.
191. Nakamura S, Nouso K, Sakaguchi K, Ito YM, Ohashi Y, Kobayashi Y, et al. Sensitivity and specificity of des-gamma-carboxy prothrombin for diagnosis of patients with hepatocellular carcinomas varies according to tumor size. Am J Gastroenterol 2006;101:2038-2043.
192. Kobayashi M, Ikeda K, Kawamura Y, Yatsuji H, Hosaka T, Sezaki H, et al. High serum des-gamma-carboxy prothrombin level predicts poor prognosis after radiofrequency ablation of hepatocellular carcinoma. Cancer 2009;115:571-580.
193. Durazo FA, Blatt LM, Corey WG, Lin JH, Han S, Saab S, et al. Des-gamma-carboxyprothrombin, alpha-fetoprotein and AFP-L3 in patients with chronic hepatitis, cirrhosis and hepatocellular carcinoma. J Gastroenterol Hepatol 2008;23:1541-1548.
194. Fujiyama S, Tanaka M, Maeda S, Ashihara H, Hirata R, Tomita K. Tumor markers in early diagnosis, follow-up and management of patients with hepatocellular carcinoma. Oncology 2002;62:57-63 (suppl 1).
195. Hsu HC, Cheng W, Lai PL. Cloning and expression of a developmentally regulated transcript MXR7 in hepatocellular carcinoma: biological significance and temporospatial distribution. Cancer Res 1997;57:5179-5184.
196. Capurro M, Wanless IR, Sherman M, Deboer G, Shi W, Miyoshi E, Filmus J. Glypican-3. A novel serum and histochemical marker for hepatocellular carcinoma. Gastroenterology 2003;125:89-97.
197. Sung YK, Hwang SY, Park MK, Farooq M, Han IS, Bae HI, et al. Glypican-3 is overexpressed in human hepatocellular carcinoma. Cancer Sci 2003;94:259-262.
198. Capurro M, Filmus J. Glypican-3 as a serum marker for hepatocellular carcinoma. Cancer Res 2005;65:372; author reply 372-373.
199. Hippo Y, Watanabe K, Watanabe A, Midorikawa Y, Yamamoto S, Ihara S, et al. Identification of soluble NH2-terminal fragment of glypican-3 as a serological marker for early-stage hepatocellular carcinoma. Cancer Res 2004;64:2418-2423.
200. Matsumura M, Shiratori Y, Niwa Y, Tanaka T, Ogura K, Okudaira T, et al. Presence of alpha-fetoprotein mRNA in blood correlates with outcome in patients with hepatocellular carcinoma. J Hepatol 1999;31:332-339.
201. Ijichi M, Takayama T, Matsumura M, Shiratori Y, Omata M, Makuuchi M. alpha-fetoprotein mRNA in the circulation as a predictor of postsurgical recurrence of hepatocellular carcinoma. A prospective study. Hepatology 2002;35:853-860.

202. Lemoine A, Le BT, Salvucci M, Azoulay D, Pham P, Raccuia J, et al. Prospective evaluation of circulating hepatocytes by alpha-fetoprotein mRNA in humans during liver surgery. Ann Surg 1997;226:43-50.
203. Witzigmann H, Geissler F, Benedix F, Thiery J, Uhlmann D, Tannapfel A, et al. Prospective evaluation of circulating hepatocytes by alpha-fetoprotein messenger RNA in patients with hepatocellular carcinoma. Surgery 2002;131:34-43.
204. Iavarone M, Lampertico P, Ronchi G, Del NE, Zanella A, Colombo M. A prospective study of blood alpha-fetoprotein messenger RNA as a predictor of hepatocellular carcinoma in patients with cirrhosis. J Viral Hepat 2003;10:423-426.
205. Katoh H, Ojima H, Kokubu A, Saito S, Kondo T, Kosuge T, et al. Genetically distinct and clinically relevant classification of hepatocellular carcinoma: putative therapeutic targets. Gastroenterology 2007;133:1475-1486.
206. Kittaka N, Takemasa I, Takeda Y, Marubashi S, Nagano H, Umeshita K, et al. Molecular mapping of human hepatocellular carcinoma provides deeper biological insight from genomic data. Eur J Cancer 2008;44:885-897.
207. Mann CD, Neal CP, Garcea G, Manson MM, Dennison AR, Berry DP. Prognostic molecular markers in hepatocellular carcinoma: a systematic review. Eur J Cancer 2007;43:979-992.
208. Sun S, Lee NP, Poon RT, Fan ST, He QY, Lau GK, Luk JM. Oncoproteomics of hepatocellular carcinoma: from cancer markers' discovery to functional pathways. Liver Int 2007;27:1021-1038.
209. Zinkin NT, Grall F, Bhaskar K, Otu HH, Spentzos D, Kalmowitz B, et al. Serum proteomics and biomarkers in hepatocellular carcinoma and chronic liver disease. Clin Cancer Res 2008;14:470-477.
210. Lo YM. Circulating nucleic acids in plasma and serum: an overview. Ann N Y Acad Sci 2001;945:1-7.
211. Wong IH, Lo YM, Zhang J, Liew CT, Ng MH, Wong N, et al. Detection of aberrant p16 methylation in the plasma and serum of liver cancer patients. Cancer Res 1999;59:71-73.
212. Lee HS, Kim BH, Cho NY, Yoo EJ, Choi M, Shin SH, et al. Prognostic implications of and relationship between CpG island hypermethylation and repetitive DNA hypomethylation in hepatocellular carcinoma. Clin Cancer Res 2009;15:812-820.
213. Ladeiro Y, Couchy G, Balabaud C, Bioulac-Sage P, Pelletier L, Rebouissou S, Zucman-Rossi J. MicroRNA profiling in hepatocellular tumors is associated with clinical features and oncogene/tumor suppressor gene mutations. Hepatology 2008;47:1955-1963.
214. Mishra L, Banker T, Murray J, Byers S, Thenappan A, He AR, et al. Liver stem cells and hepatocellular carcinoma. Hepatology 2009;49:318-329.
215. Smith MW, Yue ZN, Geiss GK, Sadovnikova NY, Carter VS, Boix L, et al. Identification of novel tumor markers in hepatitis C virus-associated hepatocellular carcinoma. Cancer Res 2003;63:859-864.
216. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Thun MJ. Cancer Statistics, 2009. CA Cancer J Clin 2009;59:225-249.
217. Vineis P, Esteve J, Hartge P, Hoover R, Silverman DT, Terracini B. Effects of timing and type of tobacco in cigarette-induced bladder cancer. Cancer Res 1988;48:3849-3852.
218. Lamm DL, Torti FM. Bladder cancer, 1996. CA Cancer J Clin 1996;46:93-112.
219. Bryan RT, Wallace DM. 'Superficial' bladder cancer - time to uncouple pT1 tumours from pTa tumours. BJU Int 2002;90:846-852.
220. Sauter G, Algaba F, Amin M. Tumours of the urinary system: noninvasive urothelial neoplasias. Lyon, France, IARCC Press, 2004, pp 29-34.
221. Busch C, Algaba F. The WHO/ISUP 1998 and WHO 1999 systems for malignancy grading of bladder cancer. Scientific foundation and translation to one another and previous systems. Virchows Arch 2002;441:105-108.
222. Agarwal PK, Black PC, Kamat AM. Considerations on the use of diagnostic markers in management of patients with bladder cancer. World J Urol 2008;26:39-44.
223. Theodorescu D, Wittke S, Ross MM, Walden M, Conaway M, Just I, et al. Discovery and validation of new protein biomarkers for urothelial cancer: a prospective analysis. Lancet Oncol 2006;7:230-240.
224. Sanchez-Carbayo M, Cordon-Cardo C. Molecular alterations associated with bladder cancer progression. Semin Oncol 2007;34:75-84.
225. Ecke TH. Focus on urinary bladder cancer markers: a review. Minerva Urol Nefrol 2008;60:237-246.
226. Cordon-Cardo C, Cote RJ, Sauter G. Genetic and molecular markers of urothelial premalignancy and malignancy. Scand J Urol Nephrol 2000;82-93 (suppl).
227. Wolff EM, Liang G, Jones PA. Mechanisms of disease. Genetic and epigenetic alterations that drive bladder cancer. Nat Clin Pract Urol 2005;2:502-510.
228. Vrooman OP, Witjes JA. Urinary markers in bladder cancer. Eur Urol 2008;53:909-916.
229. Droller MJ. Bladder cancer. State-of-the-art care. CA Cancer J Clin 1998;48:269-284.
230. Parmar MK, Freedman LS, Hargreave TB, Tolley DA. Prognostic factors for recurrence and followup policies in the treatment of superficial bladder cancer. Report from the British Medical Research Council Subgroup on Superficial Bladder Cancer (Urological Cancer Working Party). J Urol 1989;142:284-288.
231. Atkins D, Best D, Briss PA, Eccles M, Falck-Ytter Y, Flottorp S, et al. Grading quality of evidence and strength of recommendations. BMJ 2004;328:1490.
232. National Comprehensive Cancer Network (NCCN). Clinical Practice Guidelines in Oncology: bladder cancer. V. 1.2010. http://www.nccn.org/professionals/physician_gls/PDF/bladder.pdf
233. Zipfel PF, Skerka C. Complement factor H and related proteins. An expanding family of complement-regulatory proteins? Immunol Today 1994;15:121-126.
234. Gutierrez Banos JL, Martin Garcia B, Hernandez Rodriguez R, Portillo Martin JA, Correas Gomez MA, del Valle Schaan JI, et al. Usefulness of BTA Stat test (Bard) in the diagnosis of bladder cancer. Preliminary results and comparison with cytology and cystoscopy. Arch Esp Urol 1998;51:778-782.
235. Sharma S, Zippe CD, Pandrangi L, Nelson D, Agarwal A. Exclusion criteria enhance the specificity and positive predictive value of NMP22 and BTA stat. J Urol 1999;162:53-57.
236. Takashi M, Schenck U, Kissel K, Leyh H, Treiber U. Use of diagnostic categories in urinary cytology in comparison with the bladder tumour antigen (BTA) test in bladder cancer patients. Int Urol Nephrol 1999;31:189-196.
237. Landman J, Chang Y, Kavaler E, Droller MJ, Liu BC. Sensitivity and specificity of NMP-22, telomerase, and BTA in the detection of human bladder cancer. Urology 1998;52:398-402.
238. Wiener HG, Mian C, Haitel A, Pycha A, Schatzl G, Marberger M. Can urine bound diagnostic tests replace cystoscopy in the management of bladder cancer? J Urol 1998;159:1876-1880.
239. Leyh H, Marberger M, Conort P, Sternberg C, Pansadoro V, Pagano F, et al. Comparison of the BTA stat test with voided urine cytology and bladder wash cytology in the diagnosis and monitoring of bladder cancer. Eur Urol 1999;35:52-56.

240. Leyh H, Mazeman E. Bard BTA test compared with voided urine cytology in the diagnosis of recurrent bladder cancer. Eur Urol 1997;32:425-428.
241. Sarosdy MF, Hudson MA, Ellis WJ, Soloway MS, DeVere White R, Sheinfeld J, et al. Improved detection of recurrent bladder cancer using the Bard BTA stat Test. Urology 1997;50:349-353.
242. Thomas L, Leyh H, Marberger M, Bombardieri E, Bassi P, Pagano F, et al. Multicenter trial of the quantitative BTA TRAK assay in the detection of bladder cancer. Clin Chem 1999;45:472-477.
243. Mattioli S, Seregni E, Caperna L, Botti C, Savelli G, Bombardieri E. BTA-TRAK combined with urinary cytology is a reliable urinary indicator of recurrent transitional cell carcinoma (TCC) of the bladder. Int J Biol Markers 2000;15:219-225.
244. Herman MP, Svatek RS, Lotan Y, Karakiewizc PI, Shariat SF. Urine-based biomarkers for the early detection and surveillance of non-muscle invasive bladder cancer. Minerva Urol Nefrol 2008;60:217-235.
245. Soloway MS, Briggman V, Carpinito GA, Chodak GW, Church PA, Lamm DL, et al. Use of a new tumor marker, urinary NMP22, in the detection of occult or rapidly recurring transitional cell carcinoma of the urinary tract following surgical treatment. J Urol 1996;156:363-367.
246. Miyanaga N, Akaza H, Ishikawa S, Ohtani M, Noguchi R, Kawai K, et al. Clinical evaluation of nuclear matrix protein 22 (NMP22) in urine as a novel marker for urothelial cancer. Eur Urol 1997;31:163-168.
247. Miyanaga N, Akaza H, Tsukamoto S, Shimazui T, Ohtani M, Ishikawa S, et al. Usefulness of urinary NMP22 to detect tumor recurrence of superficial bladder cancer after transurethral resection. Int J Clin Oncol 2003;8:369-373.
248. Stampfer DS, Carpinito GA, Rodriguez-Villanueva J, Willsey LW, Dinney CP, Grossman HB, et al. Evaluation of NMP22 in the detection of transitional cell carcinoma of the bladder. J Urol 1998;159:394-398.
249. Lahme S, Bichler KH, Feil G, Zumbragel A, Gotz T. Comparison of cytology and nuclear matrix protein 22 (NMP 22) for the detection and follow-up of bladder-cancer. Adv Exp Med Biol 2003;539:111-119.
250. Ponsky LE, Sharma S, Pandrangi L, Kedia S, Nelson D, Agarwal A, Zippe CD. Screening and monitoring for bladder cancer: refining the use of NMP22. J Urol 2001;166:75-78.
251. Tomera KM. NMP22 BladderChek Test: point-of-care technology with life- and money-saving potential. Expert Rev Mol Diagn 2004;4:783-794.
252. Yokoyama T, Sekigawa R, Hayashi T, Horita S, Kanamuro T, Nonami Y, et al. The clinical efficacy of Bladder Chek NMP22 in urothelial cancer. Rinsho Byori 2004;52:199-203.
253. Atsu N, Ekici S, Oge OO, Ergen A, Hascelik G, Ozen H. False-positive results of the NMP22 test due to hematuria. J Urol 2002;167:555-558.
254. Grossman HB, Messing E, Soloway M, Tomera K, Katz G, Berger Y, Shen Y. Detection of bladder cancer using a point-of-care proteomic assay. JAMA 2005;293:810-816.
255. Lokeshwar VB, Habuchi T, Grossman HB, Murphy WM, Hautmann SH, Hemstreet GP 3rd, et al. Bladder tumor markers beyond cytology: International Consensus Panel on bladder tumor markers. Urology 2005;66:35-63.
256. Grossman HB, Soloway M, Messing E, Katz G, Stein B, Kassabian V, Shen Y. Surveillance for recurrent bladder cancer using a point-of-care proteomic assay. JAMA 2006;295: 299-305.
257. Mian C, Pycha A, Wiener H, Haitel A, Lodde M, Marberger M. Immunocyt: a new tool for detecting transitional cell cancer of the urinary tract. J Urol 1999;161:1486-1489.
258. Messing EM, Teot L, Korman H, Underhill E, Barker E, Stork B, et al. Performance of urine test in patients monitored for recurrence of bladder cancer: a multicenter study in the United States. J Urol 2005;174:1238-1241.
259. Toma MI, Friedrich MG, Hautmann SH, Jakel KT, Erbersdobler A, Hellstern A, Huland H. Comparison of the ImmunoCyt test and urinary cytology with other urine tests in the detection and surveillance of bladder cancer. World J Urol 2004;22:145-149.
260. Feil G, Zumbragel A, Paulgen-Nelde HJ, Hennenlotter J, Maurer S, Krause S, et al. Accuracy of the ImmunoCyt assay in the diagnosis of transitional cell carcinoma of the urinary bladder. Anticancer Res 2003;23:963-967.
261. Lodde M, Mian C, Negri G, Berner L, Maffei N, Lusuardi L, et al. Role of uCyt+ in the detection and surveillance of urothelial carcinoma. Urology 2003;61:243-247.
262. Halling KC, King W, Sokolova IA, Meyer RG, Burkhardt HM, Halling AC, et al. A comparison of cytology and fluorescence in situ hybridization for the detection of urothelial carcinoma. J Urol 2000;164:1768-1775.
263. Sarosdy MF, Schellhammer P, Bokinsky G, Kahn P, Chao R, Yore L, et al. Clinical evaluation of a multi-target fluorescent in situ hybridization assay for detection of bladder cancer. J Urol 2002;168:1950-1954.
264. Friedrich MG, Toma MI, Hellstern A, Pantel K, Weisenberger DJ, Noldus J, Huland H. Comparison of multitarget fluorescence in situ hybridization in urine with other noninvasive tests for detecting bladder cancer. BJU Int 2003;92:911-914.
265. Bollmann D, Bollmann M, Bankfalvi A, Heller H, Bollmann R, Pajor G, Hildenbrand R. Quantitative molecular grading of bladder tumours: a tool for objective assessment of the biological potential of urothelial neoplasias. Oncol Rep 2009;21: 39-47.
266. Kipp BR, Karnes RJ, Brankley SM, Harwood AR, Pankratz VS, Sebo TJ, et al. Monitoring intravesical therapy for superficial bladder cancer using fluorescence in situ hybridization. J Urol 2005;173:401-404.
267. Gudjonsson S, Isfoss BL, Hansson K, Domanski AM, Warenholt J, Soller W, et al. The value of the UroVysion assay for surveillance of non-muscle-invasive bladder cancer. Eur Urol 2008;54:402-408.
268. Skacel M, Fahmy M, Brainard JA, Pettay JD, Biscotti CV, Liou LS, et al. Multitarget fluorescence in situ hybridization assay detects transitional cell carcinoma in the majority of patients with bladder cancer and atypical or negative urine cytology. J Urol 2003;169:2101-2105.
269. Pycha A, Lodde M, Comploj E, Negri G, Egarter-Vigl E, Vittadello F, et al. Intermediate-risk urothelial carcinoma: an unresolved problem? Urology 2004;63:472-475.
270. Mengual L, Marin-Aguilera M, Ribal MJ, Burset M, Villavicencio H, Oliver A, Alcaraz A. Clinical utility of fluorescent in situ hybridization for the surveillance of bladder cancer patients treated with bacillus Calmette-Guerin therapy. Eur Urol 2007;52:752-759.
271. Whitson J, Berry A, Carroll P, Konety B. A multicolour fluorescence in situ hybridization test predicts recurrence in patients with high-risk superficial bladder tumours undergoing intravesical therapy. BJU Int 2009;104:336-339.

272. Southgate J, Harnden P, Trejdosiewicz LK. Cytokeratin expression patterns in normal and malignant urothelium: a review of the biological and diagnostic implications. Histol Histopathol 1999;14:657-664.
273. Nisman B, Barak V, Shapiro A, Golijanin D, Peretz T, Pode D. Evaluation of urine CYFRA 21-1 for the detection of primary and recurrent bladder carcinoma. Cancer 2002;94:2914-2922.
274. Pariente JL, Bordenave L, Jacob F, Gobinet A, Leger F, Ferriere JM, Le Guillou M. Analytical and prospective evaluation of urinary cytokeratin 19 fragment in bladder cancer. J Urol 2000;163:1116-1119.
275. Siracusano S, Niccolini B, Knez R, Tiberio A, Benedetti E, Bonin S, et al. The simultaneous use of telomerase, cytokeratin 20 and CD4 for bladder cancer detection in urine. Eur Urol 2005;47:327-333.
276. Sanchez-Carbayo M, Herrero E, Megias J, Mira A, Soria F. Comparative sensitivity of urinary CYFRA 21-1, urinary bladder cancer antigen, tissue polypeptide antigen, tissue polypeptide antigen and NMP22 to detect bladder cancer. J Urol 1999;162:1951-1956.
277. Sanchez-Carbayo M, Urrutia M, Silva JM, Romani R, Garcia J, Alferez F, et al. Urinary tissue polypeptide-specific antigen for the diagnosis of bladder cancer. Urology 2000;55:526-532.
278. Mian C, Lodde M, Haitel A, Vigl EE, Marberger M, Pycha A. Comparison of the monoclonal UBC-ELISA test and the NMP22 ELISA test for the detection of urothelial cell carcinoma of the bladder. Urology 2000;55:223-226.
279. Kim NW, Piatyszek MA, Prowse KR, Harley CB, West MD, Ho PL, et al. Specific association of human telomerase activity with immortal cells and cancer. Science 1994;266:2011-2015.
280. Yoshida K, Sugino T, Tahara H, Woodman A, Bolodeoku J, Nargund V, et al. Telomerase activity in bladder carcinoma and its implication for noninvasive diagnosis by detection of exfoliated cancer cells in urine. Cancer 1997;79:362-369.
281. Muller M, Krause H, Heicappell R, Tischendorf J, Shay JW, Miller K. Comparison of human telomerase RNA and telomerase activity in urine for diagnosis of bladder cancer. Clin Cancer Res 1998;4:1949-1954.
282. de Kok JB, Ruers TJ, van Muijen GN, van Bokhoven A, Willems HL, Swinkels DW. Real-time quantification of human telomerase reverse transcriptase mRNA in tumors and healthy tissues. Clin Chem 2000;46:313-318.
283. Sanchini MA, Bravaccini S, Medri L, Gunelli R, Nanni O, Monti F, et al. Urine telomerase: an important marker in the diagnosis of bladder cancer. Neoplasia 2004;6:234-239.
284. Saad A, Hanbury DC, McNicholas TA, Boustead GB, Morgan S, Woodman AC. A study comparing various noninvasive methods of detecting bladder cancer in urine. BJU Int 2002;89:369-373.
285. Lee MY, Tsou MH, Cheng MH, Chang DS, Yang AL, Ko JS. Clinical application of NMP22 and urinary cytology in patients with hematuria or a history of urothelial carcinoma. World J Urol 2000;18:401-405.
286. Konety BR, Nguyen TS, Dhir R, Day RS, Becich MJ, Stadler WM, Getzenberg RH. Detection of bladder cancer using a novel nuclear matrix protein, BLCA-4. Clin Cancer Res 2000;6:2618-2625.
287. Van Le TS, Myers J, Konety BR, Barder T, Getzenberg RH. Functional characterization of the bladder cancer marker, BLCA-4. Clin Cancer Res 2004;10:1384-1391.
288. Konety BR, Nguyen TS, Brenes G, Sholder A, Lewis N, Bastacky S, et al. Clinical usefulness of the novel marker BLCA-4 for the detection of bladder cancer. J Urol 2000;164:634-639.
289. Ambrosini G, Adida C, Altieri DC. A novel anti-apoptosis gene, survivin, expressed in cancer and lymphoma. Nat Med 1997;3:917-921.
290. Dabrowski A, Filip A, Zgodzinski W, Dabrowska M, Polanska D, Wojcik M, et al. Assessment of prognostic significance of cytoplasmic survivin expression in advanced oesophageal cancer. Folia Histochem Cytobiol 2004;42:169-172.
291. Smith SD, Wheeler MA, Plescia J, Colberg JW, Weiss RM, Altieri DC. Urine detection of survivin and diagnosis of bladder cancer. Jama 2001;285:324-328.
292. Moussa O, Abol-Enein H, Bissada NK, Keane T, Ghoneim MA, Watson DK. Evaluation of survivin reverse transcriptase-polymerase chain reaction for noninvasive detection of bladder cancer. J Urol 2006;175:2312-2316.
293. Lehner R, Lucia MS, Jarboe EA, Orlicky D, Shroyer AL, McGregor JA, Shroyer KR. Immunohistochemical localization of the IAP protein survivin in bladder mucosa and transitional cell carcinoma. Appl Immunohistochem Mol Morphol 2002;10:134-138.
294. Pina-Cabral L, Santos L, Mesquita B, Amaro T, Magalhaes S, Criado B. Detection of survivin mRNA in urine of patients with superficial urothelial cell carcinomas. Clin Transl Oncol 2007;9:731-736.
295. Kenney DM, Geschwindt RD, Kary MR, Linic JM, Sardesai NY, Li ZQ. Detection of newly diagnosed bladder cancer, bladder cancer recurrence and bladder cancer in patients with hematuria using quantitative rt-PCR of urinary survivin. Tumour Biol 2007;28:57-62.
296. Shariat SF, Casella R, Khoddami SM, Hernandez G, Sulser T, Gasser TC, Lerner SP. Urine detection of survivin is a sensitive marker for the noninvasive diagnosis of bladder cancer. J Urol 2004;171:626-630.
297. Schultz IJ, Kiemeney LA, Karthaus HF, Witjes JA, Willems JL, Swinkels DW, et al. Survivin mRNA copy number in bladder washings predicts tumor recurrence in patients with superficial urothelial cell carcinomas. Clin Chem 2004;50:1425-1428.
298. Schultz IJ, Wester K, Straatman H, Kiemeney LA, Babjuk M, Mares J, et al. Gene expression analysis for the prediction of recurrence in patients with primary Ta urothelial cell carcinoma. Eur Urol 2007;51:416-422; discussion 422-423.
299. Simoneau M, Aboulkassim TO, LaRue H, Rousseau F, Fradet Y. Four tumor suppressor loci on chromosome 9q in bladder cancer: evidence for two novel candidate regions at 9q22.3 and 9q31. Oncogene 1999;18:157-163.
300. Czerniak B, Chaturvedi V, Li L, Hodges S, Johnston D, Roy JY, et al. Superimposed histologic and genetic mapping of chromosome 9 in progression of human urinary bladder neoplasia: implications for a genetic model of multistep urothelial carcinogenesis and early detection of urinary bladder cancer. Oncogene 1999;18:1185-1196.
301. Mao L, Schoenberg MP, Scicchitano M, Erozan YS, Merlo A, Schwab D, Sidransky D. Molecular detection of primary bladder cancer by microsatellite analysis. Science 1996;271:659-662.
302. Steiner G, Schoenberg MP, Linn JF, Mao L, Sidransky D. Detection of bladder cancer recurrence by microsatellite analysis of urine. Nat Med 1997;3:621-624.
303. von Knobloch R, Brandt H, Hofmann R. Molecular serological diagnosis in transitional cell bladder cancer. Ann N Y Acad Sci 2004;1022:70-75.
304. Fornari D, Steven K, Hansen AB, Vibits H, Jepsen JV, Poulsen AL, et al. Microsatellite analysis of urine sediment versus urine

cytology for diagnosing transitional cell tumors of the urinary bladder. APMIS 2004;112:148-152.
305. Utting M, Werner W, Dahse R, Schubert J, Junker K. Microsatellite analysis of free tumor DNA in urine, serum, and plasma of patients: a minimally invasive method for the detection of bladder cancer. Clin Cancer Res 2002;8:35-40.
306. van der Aa MN, Zwarthoff EC, Steyerberg EW, Boogaard MW, Nijsen Y, van der Keur KA, et al. Microsatellite analysis of voided-urine samples for surveillence of low-grade non-muscle-invasive urothelial carcinoma. Feasibility and clinical utility in a prospective multicenter study (Cost-Effectiveness of Follow-Up of Urinary Bladder Cancer Trial [lsqb]CEFUB[rsqb]). Eur Urol 2008;55-659-667.
307. Lokeshwar VB, Obek C, Pham HT, Wei D, Young MJ, Duncan RC, et al. Urinary hyaluronic acid and hyaluronidase: markers for bladder cancer detection and evaluation of grade. J Urol 2000;163:348-356.
308. Pham HT, Block NL, Lokeshwar VB. Tumor-derived hyaluronidase: a diagnostic urine marker for high-grade bladder cancer. Cancer Res 1997;57:778-783.
309. Hautmann S, Toma M, Lorenzo Gomez MF, Friedrich MG, Jaekel T, Michl U, et al. Immunocyt and the HA-HAase urine tests for the detection of bladder cancer: a side-by-side comparison. Eur Urol 2004;46:466-471.
310. Schroeder GL, Lorenzo-Gomez MF, Hautmann SH, Friedrich MG, Ekici S, Huland H, Lokeshwar V. A side by side comparison of cytology and biomarkers for bladder cancer detection. J Urol 2004;172:1123-1126.
311. Cappellen D, De Oliveira C, Ricol D, de Medina S, Bourdin J, Sastre-Garau X, et al. Frequent activating mutations of FGFR3 in human bladder and cervix carcinomas. Nat Genet 1999;23:18-20.
312. Billerey C, Chopin D, Aubriot-Lorton MH, Ricol D, Gil Diez de Medina S, Van Rhijn B, et al. Frequent FGFR3 mutations in papillary non-invasive bladder (pTa) tumors. Am J Pathol 2001;158:1955-1959.
313. Powers CJ, McLeskey SW, Wellstein A. Fibroblast growth factors, their receptors and signaling. Endocr Relat Cancer 2000;7:165-197.
314. Horton WA, Lunstrum GP. Fibroblast growth factor receptor 3 mutations in achondroplasia and related forms of dwarfism. Rev Endocr Metab Disord 2002;3:381-385.
315. van Rhijn BW, Vis AN, van der Kwast TH, Kirkels WJ, Radvanyi F, Ooms EC, et al. Molecular grading of urothelial cell carcinoma with fibroblast growth factor receptor 3 and MIB-1 is superior to pathologic grade for the prediction of clinical outcome. J Clin Oncol 2003;21:1912-1921.
316. van Rhijn BW, Lurkin I, Radvanyi F, Kirkels WJ, van der Kwast TH, Zwarthoff EC. The fibroblast growth factor receptor 3 (FGFR3) mutation is a strong indicator of superficial bladder cancer with low recurrence rate. Cancer Res 2001;61:1265-1268.
317. Habuchi T, Marberger M, Droller MJ, Hemstreet GP 3rd, Grossman HB, Schalken JA, et al. Prognostic markers for bladder cancer. International Consensus Panel on bladder tumor markers. Urology 2005;66:64-74.
318. Hernandez S, Lopez-Knowles E, Lloreta J, Kogevinas M, Amoros A, Tardon A, et al. Prospective study of FGFR3 mutations as a prognostic factor in nonmuscle invasive urothelial bladder carcinomas. J Clin Oncol 2006;24:3664-3671.
319. Sawczuk IS, Pickens CL, Vasa UR, Ralph DA, Norris KA, Miller MC, et al. DD23 Biomarker. A prospective clinical assessment in routine urinary cytology specimens from patients being monitored for TCC. Urol Oncol 2002;7:185-190.
320. Gilbert SM, Veltri RW, Sawczuk A, Shabsigh A, Knowles DR, Bright S, et al. Evaluation of DD23 as a marker for detection of recurrent transitional cell carcinoma of the bladder in patients with a history of bladder cancer. Urology 2003;61:539-543.
321. Sanchez-Carbayo M, Urrutia M, Gonzalez de Buitrago JM, Navajo JA. Evaluation of two new urinary tumor markers. Bladder tumor fibronectin and cytokeratin 18 for the diagnosis of bladder cancer. Clin Cancer Res 2000;6:3585-3594.
322. Hegele A, Heidenreich A, Varga Z, von Knobloch R, Olbert P, Kropf J, Hofmann R. Cellular fibronectin in patients with transitional cell carcinoma of the bladder. Urol Res 2003;30:363-366.
323. Hotakainen K, Haglund C, Paju A, Nordling S, Alfthan H, Rintala E, Stenman UH. Chorionic gonadotropin beta-subunit and core fragment in bladder cancer: mRNA and protein expression in urine, serum and tissue. Eur Urol 2002;41:677-685.
324. Chan MW, Chan LW, Tang NL, Tong JH, Lo KW, Lee TL, et al. Hypermethylation of multiple genes in tumor tissues and voided urine in urinary bladder cancer patients. Clin Cancer Res 2002;8:464-470.
325. Friedrich MG, Weisenberger DJ, Cheng JC, Chandrasoma S, Siegmund KD, Gonzalgo ML, et al. Detection of methylated apoptosis-associated genes in urine sediments of bladder cancer patients. Clin Cancer Res 2004;10:7457-7465.
326. Dulaimi E, Uzzo RG, Greenberg RE, Al-Saleem T, Cairns P. Detection of bladder cancer in urine by a tumor suppressor gene hypermethylation panel. Clin Cancer Res 2004;10:1887-1893.
327. Vlahou A, Giannopoulos A, Gregory BW, Manousakas T, Kondylis FI, Wilson LL, et al. Protein profiling in urine for the diagnosis of bladder cancer. Clin Chem 2004;50:1438-1441.
328. Zhang YF, Wu DL, Guan M, Liu WW, Wu Z, Chen YM, et al. Tree analysis of mass spectral urine profiles discriminates transitional cell carcinoma of the bladder from noncancer patient. Clin Biochem 2004;37:772-779.
329. Varkarakis MJ, Gaeta J, Moore RH, Murphy GP. Superficial bladder tumor. Aspects of clinical progression. Urology 1974;4:414-420.
330. Grossfeld GD, Litwin MS, Wolf JS, Hricak H, Shuler CL, Agerter DC, Carroll PR. Evaluation of asymptomatic microscopic hematuria in adults: the American Urological Association best practice policy–part I: definition, detection, prevalence, and etiology. Urology 2001;57:599-603.
331. Khadra MH, Pickard RS, Charlton M, Powell PH, Neal DE. A prospective analysis of 1,930 patients with hematuria to evaluate current diagnostic practice. J Urol 2000;163:524-527.
332. Mariani J. A prospective analysis of 1930 patients with hematuria to evaluate current diagnostic practice. J Urol 2000;165:545-546.
333. Grossfeld GD, Litwin MS, Wolf JS Jr, Hricak H, Shuler CL, Agerter DC, Carroll PR. Evaluation of asymptomatic microscopic hematuria in adults: the American Urological Association best practice policy–part II: patient evaluation, cytology, voided markers, imaging, cystoscopy, nephrology evaluation, and follow-up. Urology 2001;57:604-610.
334. Zippe C, Pandrangi L, Agarwal A. NMP22 is a sensitive, cost-effective test in patients at risk for bladder cancer. J Urol 1999;161:62-65.
335. Matritech I. PMA #940035. Submitted to the Food and Drug Administration; 2000. http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfPMA/pma.cfm?id=17679

336. Stein JP, Grossfeld GD, Ginsberg DA, Esrig D, Freeman JA, Figueroa AJ, et al. Prognostic markers in bladder cancer: a contemporary review of the literature. J Urol 1998;160: 645-659.
337. Zlotta AR, Schulman CC. Biological markers in superficial bladder tumors and their prognostic significance. Urol Clin North Am 2000;27:179-89, xi-xii.
338. Grossman HB, Liebert M, Antelo M, Dinney CP, Hu SX, Palmer JL, Benedict WF. p53 and RB expression predict progression in T1 bladder cancer. Clin Cancer Res 1998;4:829-834.
339. Lazar V, Diez SG, Laurent A, Giovangrandi Y, Radvanyi F, Chopin D, et al. Expression of human chorionic gonadotropin beta subunit genes in superficial and invasive bladder carcinomas. Cancer Res 1995;55:3735-3738.
340. Gontero P, Banisadr S, Frea B, Brausi M. Metastasis markers in bladder cancer: a review of the literature and clinical considerations. Eur Urol 2004;46:296-311.
341. Esrig D, Spruck CH 3rd, Nichols PW, Chaiwun B, Steven K, Groshen S, et al. p53 nuclear protein accumulation correlates with mutations in the p53 gene, tumor grade, and stage in bladder cancer. Am J Pathol 1993;143:1389-1397.
342. Malats N, Bustos A, Nascimento CM, Fernandez F, Rivas M, Puente D, et al. P53 as a prognostic marker for bladder cancer: a meta-analysis and review. Lancet Oncol 2005;6:678-686.
343. Gontero P, Casetta G, Zitella A, Ballario R, Pacchioni D, Magnani C, et al. Evaluation of P53 protein overexpression, Ki67 proliferative activity and mitotic index as markers of tumour recurrence in superficial transitional cell carcinoma of the bladder. Eur Urol 2000;38:287-296.
344. Vatne V, Maartmann-Moe H, Hoestmark J. The prognostic value of p53 in superficially infiltrating transitional cell carcinoma. Scand J Urol Nephrol 1995;29:491-495.
345. Cordon-Cardo C. Molecular alterations associated with bladder cancer initiation and progression. Scand J Urol Nephrol Suppl 2008:154-165.
346. Ecke TH, Sachs MD, Lenk SV, Loening SA, Schlechte HH. TP53 gene mutations as an independent marker for urinary bladder cancer progression. Int J Mol Med 2008;21:655-661.
347. Sachs MD, Schlechte H, Lenk VS, Brenner S, Schnorr D, Fleige B, et al. Genetic analysis of Tp53 from urine sediment as a tool for diagnosing recurrence and residual of bladder carcinoma. Eur Urol 2000;38:426-433.
348. Schlichtholz B, Presler M, Matuszewski M. Clinical implications of p53 mutation analysis in bladder cancer tissue and urine sediment by functional assay in yeast. Carcinogenesis 2004;25:2319-2323.
349. Aleman A, Cebrian V, Alvarez M, Lopez V, Orenes E, Lopez-Serra L, et al. Identification of PMF1 methylation in association with bladder cancer progression. Clin Cancer Res 2008;14:8236-8243.
350. Sanchez-Carbayo M, Socci ND, Lozano J, Saint F, Cordon-Cardo C. Defining molecular profiles of poor outcome in patients with invasive bladder cancer using oligonucleotide microarrays. J Clin Oncol 2006;24:778-789.
351. Mahnert B, Tauber S, Kriegmair M, Nagel D, Holdenrieder S, Hofmann K, et al. Measurements of complement factor H-related protein (BTA-TRAK assay) and nuclear matrix protein (NMP22 assay)–useful diagnostic tools in the diagnosis of urinary bladder cancer? Clin Chem Lab Med 2003;41:104-110.
352. Glas AS, Roos D, Deutekom M, Zwinderman AH, Bossuyt PM, Kurth KH. Tumor markers in the diagnosis of primary bladder cancer. A systematic review. J Urol 2003;169:1975-1982.
353. Malik SN, Murphy WM. Monitoring patients for bladder neoplasms: what can be expected of urinary cytology consultations in clinical practice. Urology 1999;54:62-66.
354. van der Poel HG, Debruyne FM. Can biological markers replace cystoscopy? An update. Curr Opin Urol 2001;11:503-509.
355. Sanchez-Carbayo M, Urrutia M, Gonzalez de Buitrago JM, Navajo JA. Utility of serial urinary tumor markers to individualize intervals between cystoscopies in the monitoring of patients with bladder carcinoma. Cancer 2001;92:2820-2828.
356. Lokeshwar VB, Soloway MS. Current bladder tumor tests: does their projected utility fulfill clinical necessity? J Urol 2001;165:1067-1077.
357. Raitanen MP, Kaasinen E, Lukkarinen O, Kauppinen R, Viitanen J, Liukkonen T, Tammela TL. Analysis of false-positive BTA STAT test results in patients followed up for bladder cancer. Urology 2001;57:680-684.
358. Hacker NF, Berek JS. Cervical cancer. In: Berek JS, Hacker NF, eds. Practical gynecologic oncology. Vol. 3. Philadelphia: Lippincott Williams & Wilkins; 2000:345-405.
359. Whelan SL, Parkin DM, Masuyer E, eds. Patterns of cancer on five continents. Lyon: International Agency for Research on Cancer. New York. Oxford University Press, 1990. ISBN 9283221028. IARC scientific publications no. 102.
360. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Murray T, Thun MJ. Cancer statistics, 2008. CA Cancer J Clin 2008;58:71-96.
361. Campion M. Preinvasive disease. In: Berek JS, Hacker NF, eds. Practical gynecologic oncology. Vol. 3. Philadelphia, PA, Lippincott Williams & Wilkins, 2000, pp 271-343.
362. Peto J, Gilham C, Fletcher O, Matthews FE. The cervical cancer epidemic that screening has prevented in the UK. Lancet 2004;364:249-256.
363. Sellors JW, Sankaranarayanan RE. Colposcopy and treatment of cervical intraepithelial neoplasia: a beginner's manual. Lyon, France, IARC Press; 2003.
364. Munoz N, Bosch FX, de Sanjose S, Herrero R, Castellsague X, Shah KV, et al. Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl J Med 2003;348:518-527.
365. Walboomers JM, Jacobs MV, Manos MM, Bosch FX, Kummer JA, Shah KV, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol 1999;189: 12-19.
366. Smith JS, Lindsay L, Hoots B, Keys J, Franceschi S, Winer R, Clifford GM. Human papillomavirus type distribution in invasive cervical cancer and high-grade cervical lesions: a meta-analysis update. Int J Cancer 2007;121:621-632.
367. De Vuyst H, Clifford G, Li N, Franceschi S. HPV infection in Europe. Eur J Cancer 2009;45:2632-2639.
368. Bulk S, Berkhof J, Bulkmans NW, Zielinski GD, Rozendaal L, van Kemenade FJ, et al. Preferential risk of HPV16 for squamous cell carcinoma and of HPV18 for adenocarcinoma of the cervix compared to women with normal cytology in The Netherlands. Br J Cancer 2006;94:171-175.
369. Clifford G, Franceschi S, Diaz M, Munoz N, Villa LL. Chapter 3: HPV type-distribution in women with and without cervical neoplastic diseases. Vaccine 2006;24:S326-34 (suppl 3).
370. Bosch FX, Burchell AN, Schiffman M, Giuliano AR, de Sanjose S, Bruni L, et al. Epidemiology and natural history of human papillomavirus infections and type-specific implications in cervical neoplasia. Vaccine 2008;26:K1-16 (suppl 10).
371. Mayrand MH, Duarte-Franco E, Rodrigues I, Walter SD, Hanley J, Ferenczy A, et al. Human papillomavirus DNA versus

Papanicolaou screening tests for cervical cancer. N Engl J Med 2007;357:1579-1588.
372. Dunne EF, Datta SD, L EM. A review of prophylactic human papillomavirus vaccines: recommendations and monitoring in the US. Cancer 2008;113:2995-3003.
373. Heideman DA, Snijders PJ, Berkhof J, Verheijen RH, Helmerhorst TJ, Meijer CJ. Vaccination against HPV: indications for women and the impact on the cervical screening programme. BJOG 2008;115:938-946.
374. Kesic V. Management of cervical cancer. Eur J Surg Oncol 2006;32:832-837.
375. Peters WA III, Liu PY, Barrett RJ, Stock RJ, Monk BJ, Berek JS, et al. Concurrent chemotherapy and pelvic radiation therapy compared with pelvic radiation therapy alone as adjuvant therapy after radical surgery in high-risk early-stage cancer of the cervix. J Clin Oncol 2000;18:1606-1613.
376. Monk BJ, Wang J, Im S, Stock RJ, Peters WA 3rd, Liu PY, et al. Rethinking the use of radiation and chemotherapy after radical hysterectomy: a clinical-pathologic analysis of a Gynecologic Oncology Group/Southwest Oncology Group/Radiation Therapy Oncology Group trial. Gynecol Oncol 2005;96:721-728.
377. Rotman M, Sedlis A, Piedmonte MR, Bundy B, Lentz SS, Muderspach LI, Zaino RJ. A phase III randomized trial of postoperative pelvic irradiation in stage IB cervical carcinoma with poor prognostic features: follow-up of a gynecologic oncology group study. Int J Radiat Oncol Biol Phys 2006;65:169-176.
378. Green J, Kirwan J, Tierney J, Vale C, Symonds P, Fresco L, et al. Concomitant chemotherapy and radiation therapy for cancer of the uterine cervix. Cochrane Database Syst Rev 2005:CD002225.
379. Lukka H, Hirte H, Fyles A, Thomas G, Elit L, Johnston M, et al. Concurrent cisplatin-based chemotherapy plus radiotherapy for cervical cancer–a meta-analysis. Clin Oncol (R Coll Radiol) 2002;14:203-212.
380. Neoadjuvant Chemotherapy for Locally Advanced Cervical Cancer Meta-analysis Collaboration. Neoadjuvant chemotherapy for locally advanced cervical cancer. A systematic review and meta-analysis of individual patient data from 21 randomised trials. Eur J Cancer 2003;39:2470-2486.
381. Hirte HW, Strychowsky JE, Oliver T, Fung-Kee-Fung M, Elit L, Oza AM. Chemotherapy for recurrent, metastatic, or persistent cervical cancer: a systematic review. Int J Gynecol Cancer 2007;17:1194-1204.
382. Fuller AF Jr, Elliott N, Kosloff C, Hoskins WJ, Lewis JL Jr. Determinants of increased risk for recurrence in patients undergoing radical hysterectomy for stage IB and IIA carcinoma of the cervix. Gynecol Oncol 1989;33:34-39.
383. Hale RJ, Wilcox FL, Buckley CH, Tindall VR, Ryder WDJ, Logue JP. Prognostic factors in uterine cervical carcinoma: a clinicopathological analysis. Int J Gynecol Cancer 1991;1:19-23.
384. Kamura T, Tsukamoto N, Tsuruchi N, Saito T, Matsuyama T, Akazawa K, Nakano H. Multivariate analysis of the histopathologic prognostic factors of cervical cancer in patients undergoing radical hysterectomy. Cancer 1992;69:181-186.
385. Avall-Lundqvist EH, Sjovall K, Nilsson BR, Eneroth PH. Prognostic significance of pretreatment serum levels of squamous cell carcinoma antigen and CA 125 in cervical carcinoma. Eur J Cancer 1992;28A:1695-1702.
386. Bolli JA, Doering DL, Bosscher JR, Day TG Jr, Rao CV, Owens K, et al. Squamous cell carcinoma antigen: clinical utility in squamous cell carcinoma of the uterine cervix. Gynecol Oncol 1994;55:169-173.
387. Bonfrer JM, Gaarenstroom KN, Korse CM, Van Bunningen BN, Kenemans P. Cyfra 21-1 in monitoring cervical cancer: a comparison with tissue polypeptide antigen and squamous cell carcinoma antigen. Anticancer Res 1997;17:2329-2334.
388. Brioschi PA, Bischof P, Delafosse C, Krauer F. Squamous-cell carcinoma antigen (SCC-A) values related to clinical outcome of pre-invasive and invasive cervical carcinoma. Int J Cancer 1991;47:376-379.
389. Chou CY, Wang ST, Kuo HC, Tzeng CC, Yao BL. Serum level of squamous cell carcinoma antigen and tumor size are useful to identify preoperatively patients at high risk of cervical cancer. Cancer 1994;74:2497-2501.
390. Crombach G, Scharl A, Vierbuchen M, Wurz H, Bolte A. Detection of squamous cell carcinoma antigen in normal squamous epithelia and in squamous cell carcinomas of the uterine cervix. Cancer 1989;63:1337-1342.
391. Crombach G, Wurz H, Herrmann F, Kreienberg R, Mobus V, Schmidt-Rhode P, et al. The importance of the SCC antigen in the diagnosis and follow-up of cervix carcinoma. A cooperative study of the Gynecologic Tumor Marker Group (GTMG). Dtsch Med Wochenschr 1989;114:700-705.
392. Duk JM, de Bruijn HW, Groenier KH, Hollema H, ten Hoor KA, Krans M, Aalders JG. Cancer of the uterine cervix. Sensitivity and specificity of serum squamous cell carcinoma antigen determinations. Gynecol Oncol 1990;39:186-194.
393. Duk JM, Groenier KH, de Bruijn HW, Hollema H, ten Hoor KA, van der Zee AG, Aalders JG. Pretreatment serum squamous cell carcinoma antigen. A newly identified prognostic factor in early-stage cervical carcinoma. J Clin Oncol 1996;14:111-118.
394. Gaarenstroom KN, Bonfrer JM, Kenter GG, Korse CM, Hart AA, Trimbos JB, Helmerhorst TJ. Clinical value of pretreatment serum Cyfra 21-1, tissue polypeptide antigen, and squamous cell carcinoma antigen levels in patients with cervical cancer. Cancer 1995;76:807-813.
395. Gaarenstroom KN, Kenter GG, Bonfrer JM, Korse CM, van dV, Fleuren GJ, Trimbos JB. Can initial serum cyfra 21-1, SCC antigen, and TPA levels in squamous cell cervical cancer predict lymph node metastases or prognosis? Gynecol Oncol 2000;77:164-170.
396. Gitsch G, Kainz C, Joura E, Frohlich B, Bieglmayr C, Tatra G. Squamous cell carcinoma antigen, tumor associated trypsin inhibitor and tissue polypeptide specific antigen in follow up of stage III cervical cancer. Anticancer Res 1992;12:1247-1249.
397. Gocze PM, Vahrson HW, Freeman DA. Serum levels of squamous cell carcinoma antigen and ovarian carcinoma antigen (CA 125) in patients with benign and malignant diseases of the uterine cervix. Oncology 1994;51:430-434.
398. Holloway RW, To A, Moradi M, Boots L, Watson N, Shingleton HM. Monitoring the course of cervical carcinoma with the squamous cell carcinoma serum radioimmunoassay. Obstet Gynecol 1989;74:944-949.
399. Hong JH, Tsai CS, Chang JT, Wang CC, Lai CH, Lee SP, et al. The prognostic significance of pre- and posttreatment SCC levels in patients with squamous cell carcinoma of the cervix treated by radiotherapy. Int J Radiat Oncol Biol Phys 1998;41:823-830.
400. Kato H, Tamai K, Morioka H, Nagai M, Nagaya T, Torigoe T. Tumor-antigen TA-4 in the detection of recurrence in cervical squamous cell carcinoma. Cancer 1984;54:1544-1546.
401. Lozza L, Merola M, Fontanelli R, Stefanon B, Seregni E, Bombardieri E, De PG. Cancer of the uterine cervix: clinical value of squamous cell carcinoma antigen (SCC) measurements. Anticancer Res 1997;17:525-529.

402. Maiman M, Feuer G, Fruchter RG, Shaw N, Boyce J. Value of squamous cell carcinoma antigen levels in invasive cervical carcinoma. Gynecol Oncol 1989;34:312-316.
403. Neunteufel W, Tatra G, Bieglmayer C. Serum squamous cell carcinoma antigen levels in women with neoplasms of the lower genital tract and in healthy controls. Arch Gynecol Obstet 1989;246:243-250.
404. Neunteufel W, Tatra G, Bieglmayer C. Squamous cell carcinoma (SCC) antigen in patients with invasive cervical carcinoma during primary irradiation. Gynecol Obstet Invest 1990;29:154-157.
405. Ngan HY, Chan SY, Wong LC, Choy DT, Ma HK. Serum squamous cell carcinoma antigen in the monitoring of radiotherapy treatment response in carcinoma of the cervix. Gynecol Oncol 1990;37:260-263.
406. Ngan HY, Cheng GT, Yeung WS, Wong LC, Ma HK. The prognostic value of TPA and SCC in squamous cell carcinoma of the cervix. Gynecol Oncol 1994;52:63-68.
407. Pectasides D, Economides N, Bourazanis J, Pozadzizou P, Gogou L, Koutsiouba P, Athanassiou A. Squamous cell carcinoma antigen, tumor-associated trypsin inhibitor, and carcinoembryonic antigen for monitoring cervical cancer. Am J Clin Oncol 1994;17:307-312.
408. Scambia G, Benedetti PP, Foti E, Amoroso M, Salerno G, Ferrandina G, et al. Squamous cell carcinoma antigen: prognostic significance and role in the monitoring of neoadjuvant chemotherapy response in cervical cancer. J Clin Oncol 1994;12:2309-2316.
409. Schmidt-Rhode P, Schulz KD, Sturm G, Hafner H, Prinz H, Kunzig HJ. Squamous cell carcinoma antigen for monitoring cervical cancer. Int J Biol Markers 1988;3:87-94.
410. Takeshima N, Hirai Y, Katase K, Yano K, Yamauchi K, Hasumi K. The value of squamous cell carcinoma antigen as a predictor of nodal metastasis in cervical cancer. Gynecol Oncol 1998;68:263-266.
411. Tsai SC, Kao CH, Wang SJ. Study of a new tumor marker, CYFRA 21-1, in squamous cell carcinoma of the cervix, and comparison with squamous cell carcinoma antigen. Neoplasma 1996;43:27-29.
412. Yazigi R, Munoz AK, Richardson B, Risser R. Correlation of squamous cell carcinoma antigen levels and treatment response in cervical cancer. Gynecol Oncol 1991;41:135-138.
413. Yoon SM, Shin KH, Kim JY, Seo SS, Park SY, Kang S, Cho KH. The clinical values of squamous cell carcinoma antigen and carcinoembryonic antigen in patients with cervical cancer treated with concurrent chemoradiotherapy. Int J Gynecol Cancer 2007;17:872-878.
414. Gadducci A, Tana R, Cosio S, Genazzani AR. The serum assay of tumour markers in the prognostic evaluation, treatment monitoring and follow-up of patients with cervical cancer: a review of the literature. Crit Rev Oncol Hematol 2008;66:10-20.
415. Borras G, Molina R, Xercavins J, Ballesta A, Iglesias J. Tumor antigens CA 19.9, CA 125, and CEA in carcinoma of the uterine cervix. Gynecol Oncol 1995;57:205-211.
416. Crombach G, Scharl A, Wurz H. CA 125 in normal tissues and carcinomas of the uterine cervix, endometrium and Fallopian tube, II: immunoradiometric determination in secretions, tissue extracts and serum. Arch Gynecol Obstet 1989;244:113-122.
417. Duk JM, de Bruijn HW, Groenier KH, Fleuren GJ, Aalders JG. Adenocarcinoma of the uterine cervix. Prognostic significance of pretreatment serum CA 125, squamous cell carcinoma antigen, and carcinoembryonic antigen levels in relation to clinical and histopathologic tumor characteristics. Cancer 1990;65:1830-1837.
418. Leminen A. Tumor markers CA 125, carcinoembryonic antigen and tumor-associated trypsin inhibitor in patients with cervical adenocarcinoma. Gynecol Oncol 1990;39:358-363.
419. Ngan HY, Cheung AN, Lauder IJ, Cheng DK, Wong LC, Ma HK. Tumour markers and their prognostic value in adenocarcinoma of the cervix. Tumour Biol 1998;19:439-444.
420. Bonfrer JMG, Duffy MJ, Radtke M, Segurado O, Torre GC, Van Dalen A, et al. Tumour markers in gynaecological cancers: EGTM recommendations. Anticancer Res 1999;19:2807-2810.
421. Kato H, Torigoe T. Radioimmunoassay for tumor antigen of human cervical squamous cell carcinoma. Cancer 1977;40:1621-1628.
422. Suminami Y, Kishi F, Sekiguchi K, Kato H. Squamous cell carcinoma antigen is a new member of the serine protease inhibitors. Biochem Biophys Res Commun 1991;181:51-58.
423. Schneider SS, Schick C, Fish KE, Miller E, Pena JC, Treter SD, et al. A serine proteinase inhibitor locus at 18q21.3 contains a tandem duplication of the human squamous cell carcinoma antigen gene. Proc Natl Acad Sci U S A 1995;92:3147-3151.
424. Kato H, Suehiro Y, Morioka H, Torigoe T, Myoga A, Sekiguchi K, Ikeda I. Heterogeneous distribution of acidic TA-4 in cervical squamous cell carcinoma: immunohistochemical demonstration with monoclonal antibodies. Jpn J Cancer Res 1987;78:1246-1250.
425. Silverman GA, Bartuski AJ, Cataltepe S, Gornstein ER, Kamachi Y, Schick C, Uemura Y. SCCA1 and SCCA2 are proteinase inhibitors that map to the serpin cluster at 18q21.3. Tumour Biol 1998;19:480-487.
426. Schick C, Kamachi Y, Bartuski AJ, Cataltepe S, Schechter NM, Pemberton PA, Silverman GA. Squamous cell carcinoma antigen 2 is a novel serpin that inhibits the chymotrypsin-like proteinases cathepsin G and mast cell chymase. J Biol Chem 1997;272:1849-1855.
427. Molina R, Filella X, Torres MD, Ballesta AM, Mengual P, Cases A, Balaque A. SCC antigen measured in malignant and nonmalignant diseases. Clin Chem 1990;36:251-254.
428. Montag TW. Tumor markers in gynecologic oncology. Obstet Gynecol Surv 1990;45:94-105.
429. Farghaly SA. Tumor markers in gynecologic cancer. Gynecol Obstet Invest 1992;34:65-72.
430. Bae SN, Namkoong SE, Jung JK, Kim CJ, Park JS, Kim JW, et al. Prognostic significance of pretreatment squamous cell carcinoma antigen and carcinoembryonic antigen in squamous cell carcinoma of the uterine cervix. Gynecol Oncol 1997;64:418-424.
431. Bolger BS, Dabbas M, Lopes A, Monaghan JM. Prognostic value of preoperative squamous cell carcinoma antigen level in patients surgically treated for cervical carcinoma. Gynecol Oncol 1997;65:309-313.
432. Lin H, ChangChien CC, Huang EY, Tseng CW, Eng HL, Huang CC. The role of pretreatment squamous cell carcinoma antigen in predicting nodal metastasis in early stage cervical cancer. Acta Obstet Gynecol Scand 2000;79:140-144.
433. Massuger LF, Koper NP, Thomas CM, Dom KE, Schijf CP. Improvement of clinical staging in cervical cancer with serum squamous cell carcinoma antigen and CA 125 determinations. Gynecol Oncol 1997;64:473-476.
434. Patsner B, Orr JW Jr, Allmen T. Does preoperative serum squamous cell carcinoma antigen level predict occult extracervical disease in patients with stage Ib invasive squamous cell carcinoma of the cervix? Obstet Gynecol 1989;74:786-788.
435. Reesink-Peters N, van der Velden J, Ten Hoor KA, Boezen HM, de Vries EG, Schilthuis MS, et al. Preoperative serum squamous cell carcinoma antigen levels in clinical decision making

for patients with early-stage cervical cancer. J Clin Oncol 2005;23:1455-1462.
436. Strauss HG, Laban C, Lautenschlager C, Buchmann J, Schneider I, Koelbl H. SCC antigen in the serum as an independent prognostic factor in operable squamous cell carcinoma of the cervix. Eur J Cancer 2002;38:1987-1991.
437. Ogino I, Nakayama H, Okamoto N, Kitamura T, Inoue T. The role of pretreatment squamous cell carcinoma antigen level in locally advanced squamous cell carcinoma of the uterine cervix treated by radiotherapy. Int J Gynecol Cancer 2006;16:1094-1100.
438. Lin H, ChangChien CC, Huang EY, Eng HL, Huang CC. The role of radical surgery followed by adjuvant therapy for high-risk early-stage cervical carcinoma patients with pelvic lymph node metastasis. Eur J Obstet Gynecol Reprod Biol 2000;93:85-90.
439. Duyn A, Van Eijkeren M, Kenter G, Zwinderman K, Ansink A. Recurrent cervical cancer: detection and prognosis. Acta Obstet Gynecol Scand 2002;81:759-763.
440. Zola P, Fuso L, Mazzola S, Piovano E, Perotto S, Gadducci A, et al. Could follow-up different modalities play a role in asymptomatic cervical cancer relapses diagnosis? An Italian multicenter retrospective analysis. Gynecol Oncol 2007;107:S150-S154.
441. Kew FM, Roberts AP, Cruickshank DJ. The role of routine follow-up after gynecological malignancy. Int J Gynecol Cancer 2005;15:413-419.
442. Chan YM, Ng TY, Ngan HY, Wong LC. Monitoring of serum squamous cell carcinoma antigen levels in invasive cervical cancer: is it cost-effective? Gynecol Oncol 2002;84:7-11.
443. Chang TC, Law KS, Hong JH, Lai CH, Ng KK, Hsueh S, et al. Positron emission tomography for unexplained elevation of serum squamous cell carcinoma antigen levels during follow-up for patients with cervical malignancies: a phase II study. Cancer 2004;101:164-171.
444. Crew KD, Neugut AI. Epidemiology of gastric cancer. World J Gastroenterol 2006;12:354-362.
445. An JY, Ha TK, Noh JH, Sohn TS, Kim S. Proposal to subclassify stage IV gastric cancer into IVA, IVB, and IVM. Arch Surg 2009;144:38-45; discussion 45.
446. Enzinger PC, Benedetti JK, Meyerhardt JA, McCoy S, Hundahl SA, Macdonald JS, Fuchs CS. Impact of hospital volume on recurrence and survival after surgery for gastric cancer. Ann Surg 2007;245:426-434.
447. Gospodarowicz M, Mackillop W, O'Sullivan B, Sobin L, Henson D, Hutter RV, Wittekind C. Prognostic factors in clinical decision making: the future. Cancer 2001;91:1688-1695.
448. Wittekind C, Compton CC, Greene FL, Sobin LH. TNM residual tumor classification revisited. Cancer 2002;94:2511-2516.
449. Siewert JR, Bottcher K, Stein HJ, Roder JD. Relevant prognostic factors in gastric cancer. Ten-year results of the German Gastric Cancer Study. Ann Surg 1998;228:449-461.
450. Kim JP, Lee JH, Kim SJ, Yu HJ, Yang HK. Clinicopathologic characteristics and prognostic factors in 10 783 patients with gastric cancer. Gastric Cancer 1998;1:125-133.
451. Gospodarowicz MK, Henson DE, Hutter RVP, O'Sullivan B, Sobin LH, Wittekind C. Prognostic factors in cancer. New York, NY, Wiley-Liss, 2001.
452. Lauren P. The two histological main types of gastric carcinoma: diffuse and so-called intestinal-type carcinoma. An attempt at a histo-clinical classification. Acta Pathol Microbiol Scand 1965;64:31-49.
453. Cuschieri A, Weeden S, Fielding J, Bancewicz J, Craven J, Joypaul V, et al. Patient survival after D1 and D2 resections for gastric cancer: long-term results of the MRC randomized surgical trial. Surgical Co-operative Group. Br J Cancer 1999;79:1522-1530.
454. Hartgrink HH, van de Velde CJ, Putter H, Bonenkamp JJ, Klein Kranenbarg E, Songun I, et al. Extended lymph node dissection for gastric cancer: who may benefit? Final results of the randomized Dutch gastric cancer group trial. J Clin Oncol 2004;22:2069-2077.
455. Bonenkamp JJ, Hermans J, Sasako M, van dV, Welvaart K, Songun I, et al. Extended lymph-node dissection for gastric cancer. N Engl J Med 1999;340:908-914.
456. Scottish Intercollegiate Guidelines Network (SIGN): SIGN 87. Management of oesophageal and gastric cancer. www.sign.ac.uk
457. Moertel CG, Childs DS Jr, Reitemeier RJ, Colby MY Jr., Holbrook MA. Combined 5-fluorouracil and supervoltage radiation therapy of locally unresectable gastrointestinal cancer. Lancet 1969;2:865-867.
458. Leong T. Evolving role of chemoradiation in the adjuvant treatment of gastric cancer. Expert Rev Anticancer Ther 2004;4:585-594.
459. The Gastrointestinal Tumor Study Group. The concept of locally advanced gastric cancer. Effect of treatment on outcome. Cancer 1990;66:2324-2330.
460. Macdonald JS, Smalley SR, Benedetti J, Hundahl SA, Estes NC, Stemmermann GN, et al. Chemoradiotherapy after surgery compared with surgery alone for adenocarcinoma of the stomach or gastroesophageal junction. N Engl J Med 2001;345:725-730.
461. Macdonald JS, Smalley SR, Benedetti J, Estes N, Haller DG, Ajani JAea. Post-operative combined radiation and chemotherapy improves disease-free survival (DFS) and overall survival (OS) in resected adenocarcinoma of the stomach and gastresophageal junction. Update of the results of Intergroup Study INT-0116 (SWOG 9008). ASCO Gastrointestinal Cancers Symposium, 2004 (abstract 6).
462. Chua YJ, Cunningham D. The UK NCRI MAGIC trial of perioperative chemotherapy in resectable gastric cancer: implications for clinical practice. Ann Surg Oncol 2007;14:2687-2690.
463. National Comprehensive Cancer Network (NCCN). Clinical Practice Guidelines in Oncology. Gastric Cancer. V. 2.2009. http://www.nccn.org/professionals/physician_gls/PDF/gastric.pdf
464. Macdonald JS. Adjuvant therapy for gastric cancer. Semin Oncol 2003;30:19-25.
465. Hamilton JP, Meltzer SJ. A review of the genomics of gastric cancer. Clin Gastroenterol Hepatol 2006;4:416-425.
466. Ohtsu A. Chemotherapy for metastatic gastric cancer: past, present, and future. J Gastroenterol 2008;43:256-264.
467. Allum WH, Griffin SM, Watson A, Colin-Jones D. Guidelines for the management of oesophageal and gastric cancer. Gut 2002;50:v1-23 (suppl 5).
468. Jackson C, Cunningham D, Oliveira J, On behalf of the EGWG. Gastric cancer: ESMO Clinical Recommendations for diagnosis, treatment and follow-up. Ann Oncol 2009;20:iv34-36.
469. Ychou M, Bouche O, Marchal F, Merrouche Y, Gory-Delabaere G. Recommendations for clinical practice: management of stomach adenocarcinoma. Bull Cancer 2006;93:192-196.
470. Nishida T, Hirota S, Yanagisawa A, Sugino Y, Minami M, Yamamura Y, et al. Clinical practice guidelines for gastrointestinal stromal tumor (GIST) in Japan: English version. Int J Clin Oncol 2008;13:416-430.

471. Leung WK, Wu MS, Kakugawa Y, Kim JJ, Yeoh KG, Goh KL, et al. Screening for gastric cancer in Asia: current evidence and practice. Lancet Oncol 2008;9:279-287.
472. Hamashima C, Shibuya D, Yamazaki H, Inoue K, Fukao A, Saito H, Sobue T. The Japanese guidelines for gastric cancer screening. Jpn J Clin Oncol 2008;38:259-267.
473. Dinis-Ribeiro M, Yamaki G, Miki K, Costa-Pereira A, Matsukawa M, Kurihara M. Meta-analysis on the validity of pepsinogen test for gastric carcinoma, dysplasia or chronic atrophic gastritis screening. J Med Screen 2004;11:141-147.
474. Marshall BJ, Windsor HM. The relation of Helicobacter pylori to gastric adenocarcinoma and lymphoma: pathophysiology, epidemiology, screening, clinical presentation, treatment, and prevention. Med Clin North Am 2005;89:313-44,viii.
475. de Vries AC, van Grieken NC, Looman CW, Casparie MK, de Vries E, Meijer GA, Kuipers EJ. Gastric cancer risk in patients with premalignant gastric lesions. A nationwide cohort study in the Netherlands. Gastroenterology 2008;134:945-952.
476. Chey WD, Wong BC. American College of Gastroenterology guideline on the management of Helicobacter pylori infection. Am J Gastroenterol 2007;102:1808-1825.
477. Norton JA, Ham CM, Van Dam J, Jeffrey RB, Longacre TA, Huntsman DG, et al. CDH1 truncating mutations in the E-cadherin gene: an indication for total gastrectomy to treat hereditary diffuse gastric cancer. Ann Surg 2007;245:873-879.
478. Fitzgerald RC, Caldas C. E-cadherin mutations and hereditary gastric cancer: prevention by resection? Dig Dis 2002;20:23-31.
479. Talley NJ, Silverstein MD, Agreus L, Nyren O, Sonnenberg A, Holtmann G. AGA technical review: evaluation of dyspepsia. American Gastroenterological Association. Gastroenterology 1998;114:582-595.
480. Hundahl SA, Peeters KC, Kranenbarg EK, Hartgrink H, van de Velde CJ. Improved regional control and survival with "low Maruyama Index" surgery in gastric cancer: autopsy findings from the Dutch D1-D2 Trial. Gastric Cancer 2007;10:84-86.
481. Gold P, Freedman SO. Demonstration of tumor-specific antigens in human colon carcinomata by immunological tolerance and absorption techniques. J Exp Med 1965;121:439-462.
482. Ritts RE Jr, Del Villano BC, Go VL, Herberman RB, Klug TL, Zurawski VR Jr. Initial clinical evaluation of an immunoradiometric assay for CA 19-9 using the NCI serum bank. Int J Cancer 1984;33:339-345.
483. Johnson VG, Schlom J, Paterson AJ, Bennett J, Magnani JL, Colcher D. Analysis of a human tumor-associated glycoprotein (TAG-72) identified by monoclonal antibody B72.3. Cancer Res 1986;46:850-857.
484. Gaspar MJ, Arribas I, Coca MC, ez-Alonso M. Prognostic value of carcinoembryonic antigen, CA 19-9 and CA 72-4 in gastric carcinoma. Tumour Biol 2001;22:318-322.
485. Lai IR, Lee WJ, Huang MT, Lin HH. Comparison of serum CA72-4, CEA, TPA, CA19-9 and CA125 levels in gastric cancer patients and correlation with recurrence. Hepatogastroenterology 2002;49:1157-1160.
486. Nakane Y, Okamura S, Akehira K, Boku T, Okusa T, Tanaka K, Hioki K. Correlation of preoperative carcinoembryonic antigen levels and prognosis of gastric cancer patients. Cancer 1994;73:2703-2708.
487. Kodera Y, Yamamura Y, Torii A, Uesaka K, Hirai T, Yasui K, et al. The prognostic value of preoperative serum levels of CEA and CA19-9 in patients with gastric cancer. Am J Gastroenterol 1996;91:49-53.
488. Ishigami S, Natsugoe S, Hokita S, Che X, Tokuda K, Nakajo A, et al. Clinical importance of preoperative carcinoembryonic antigen and carbohydrate antigen 19-9 levels in gastric cancer. J Clin Gastroenterol 2001;32:41-44.
489. Wobbes T, Thomas CM, Segers MF, Nagengast FM. Evaluation of seven tumor markers (CA 50, CA 19-9, CA 19-9 TruQuant, CA 72-4, CA 195, carcinoembryonic antigen, and tissue polypeptide antigen) in the pretreatment sera of patients with gastric carcinoma. Cancer 1992;69:2036-2041.
490. Webb A, Scott-Mackie P, Cunningham D, Norman A, Andreyev J, O'Brien M, Bensted J. The prognostic value of serum and immunohistochemical tumour markers in advanced gastric cancer. Eur J Cancer 1996;32A:63-68.
491. Hsieh MC, Wu CW, Tsay SH, Lui WY, P'Eng FK. Pre-operative serum levels of tissue polypeptide antigen in patients with gastric cancer. J Gastroenterol Hepatol 1995;10:60-65.
492. Nakata B, Chung YS, Kato Y, Ogawa M, Ogawa Y, Inui A, et al. Clinical significance of serum CYFRA 21-1 in gastric cancer. Br J Cancer 1996;73:1529-1532.
493. Kornek G, Schenk T, Raderer M, Djavarnmad M, Scheithauer W. Tissue polypeptide-specific antigen (TPS) in monitoring palliative treatment response of patients with gastrointestinal tumours. Br J Cancer 1995;71:182-185.
494. Louhimo J, Kokkola A, Alfthan H, Stenman UH, Haglund C. Preoperative hCGbeta and CA 72-4 are prognostic factors in gastric cancer. Int J Cancer 2004;111:929-933.
495. Marcillac I, Troalen F, Bidart JM, Ghillani P, Ribrag V, Escudier B, et al. Free human chorionic gonadotropin beta subunit in gonadal and nongonadal neoplasms. Cancer Res 1992;52: 3901-3907.
496. Tocchi A, Costa G, Lepre L, Liotta G, Mazzoni G, Cianetti A, Vannini P. The role of serum and gastric juice levels of carcinoembryonic antigen, CA19.9 and CA72.4 in patients with gastric cancer. J Cancer Res Clin Oncol 1998;124:450-455.
497. Asao T, Fukuda T, Yazawa S, Nagamachi Y. Carcinoembryonic antigen levels in peritoneal washings can predict peritoneal recurrence after curative resection of gastric cancer. Cancer 1991;68:44-47.
498. Nishiyama M, Takashima I, Tanaka T, Yoshida K, Toge T, Nagata N, et al. Carcinoembryonic antigen levels in the peritoneal cavity: useful guide to peritoneal recurrence and prognosis for gastric cancer. World J Surg 1995;19:133-137.
499. Wang JY, Lin SR, Lu CY, Chen CC, Wu DC, Chai CY, et al. Gastric cancer cell detection in peritoneal lavage: RT-PCR for carcinoembryonic antigen transcripts versus the combined cytology with peritoneal carcinoembryonic antigen levels. Cancer Lett 2005;223:129-135.
500. Seo JH, Choi CW, Kim BS, Shin SW, Kim YH, Kim JS, et al. Follow-up study of peripheral blood carcinoembryonic antigen mRNA using reverse transcription-polymerase chain reaction as an early marker of clinical recurrence in patients with curatively resected gastric cancer. Am J Clin Oncol 2005;28:24-29.
501. Marrelli D, Roviello F, De SA, Farnetani M, Garosi L, Messano A, Pinto E. Prognostic significance of CEA, CA 19-9 and CA 72-4 preoperative serum levels in gastric carcinoma. Oncology 1999;57:55-62.
502. Guadagni F, Roselli M, Amato T, Cosimelli M, Perri P, Casale V, et al. CA 72-4 measurement of tumor-associated glycoprotein 72 (TAG-72) as a serum marker in the management of gastric carcinoma. Cancer Res 1992;52:1222-1227.

503. Gonzalez Vitores AM, Duro GE, Fraile BB, Carrasco MA. Prognostic value of the glycoprotein TAG-72 in patients with gastric cancer. Int J Biol Markers 2001;16:121-125.
504. Safi F, Kuhns V, Beger HG. Comparison of CA 72-4, CA 19-9 and CEA in the diagnosis and monitoring of gastric cancer. Int J Biol Markers 1995;10:100-106.
505. Joypaul B, Browning M, Newman E, Byrne D, Cuschieri A. Comparison of serum CA 72-4 and CA 19-9 levels in gastric cancer patients and correlation with recurrence. Am J Surg 1995;169:595-599.
506. Takahashi Y, Takeuchi T, Sakamoto J, Touge T, Mai M, Ohkura H, et al. The usefulness of CEA and/or CA19-9 in monitoring for recurrence in gastric cancer patients. A prospective clinical study. Gastric Cancer 2003;6:142-145.
507. Yamao T, Kai S, Kazami A, Koizumi K, Handa T, Takemoto N, Maruyama M. Tumor markers CEA, CA19-9 and CA125 in monitoring of response to systemic chemotherapy in patients with advanced gastric cancer. Jpn J Clin Oncol 1999;29:550-555.
508. Pectasides D, Mylonakis A, Kostopoulou M, Papadopoulou M, Triantafillis D, Varthalitis J, et al. CEA, CA 19-9, and CA-50 in monitoring gastric carcinoma. Am J Clin Oncol 1997;20:348-353.
509. Martin EW Jr, James KK, Hurtubise PE, Catalano P, Minton JP. The use of CEA as an early indicator for gastrointestinal tumor recurrence and second-look procedures. Cancer 1977;39:440-446.
510. Nakajima T. Gastric cancer treatment guidelines in Japan. Gastric Cancer 2002;5:1-5.
511. Di Tommaso L, Destro A, Seok JY, Balladore E, Terracciano L, Sangiovanni A, et al. The application of markers (HSP70 GPC3 and GS) in liver biopsies is useful for detection of hepatocellular carcinoma. J Hepatol 2009;50:746-754.
512. Tahara H, Nakanishi T, Kitamoto M, Nakashio R, Shay JW, Tahara E, et al. Telomerase activity in human liver tissues: comparison between chronic liver disease and hepatocellular carcinomas. Cancer Res 1995;55:2734-2736.
513. Nouso K, Urabe Y, Higashi T, Nakatsukasa H, Hino N, Ashida K, et al. Telomerase as a tool for the differential diagnosis of human hepatocellular carcinoma. Cancer 1998;78:232-236.
514. Nagao K, Tomimatsu M, Endo H, Hisatomi H, Hikiji K. Telomerase reverse transcriptase mRNA expression and telomerase activity in hepatocellular carcinoma. J Gastroenterol 1999;34:83-87.
515. Kobayashi T, Kubota K, Takayama T, Makuuchi M. Telomerase activity as a predictive marker for recurrence of hepatocellular carcinoma after hepatectomy. Amer J Surg 2001;181:284-288.
516. Kitamoto M, Nakanishi T, Kira S, Kawaguchi M, Nakashio R, Suemori S, et al. The assessment of proliferating cell nuclear antigen immunohistochemical staining in small hepatocellular carcinoma and its relationship to histologic characteristics and prognosis. Cancer 1993;72:1859-1865.
517. King KL, Hwang JJ, Chau GY, Tsay SH, Chi CW, Lee TG, et al. Ki-67 expression as a prognostic marker in patients with hepatocellular carcinoma. J Gastroenterol Hepatol 1998;13:273-279.
518. Fiorentino M, Altimari A, Ravaioli M, Gruppioni E, Gabusi E, Corti B, et al. Predictive value of biological markers for hepatocellular carcinoma patients treated with orthotopic liver transplantation. Clin Cancer Res 2004;10:1789-1795.
519. Stuart KE, Anand AJ, Jenkins RL. Hepatocellular carcinoma in the United States. Prognostic features, treatment outcome, and survival. Cancer 1996;77:2217-2222.
520. Aoyagi Y, Isokawa O, Suda T, Watanabe M, Suzuki Y, Asakura H. The fucosylation index of alpha-fetoprotein as a possible prognostic indicator for patients with hepatocellular carcinoma. Cancer 1998;83:2076-2082.
521. Shimizu K, Taniichi T, Satomura S, Matsuura S, Taga H, Taketa K. Establishment of assay kits for the determination of microheterogeneities of alpha-fetoprotein using lectin-affinity electrophoresis. Clin Chim Acta 1993;214:3-12.
522. Beneduce L, Castaldi F, Marino M, Tono N, Gatta A, Pontisso P, Fassina G. Improvement of liver cancer detection with simultaneous assessment of circulating levels of free alpha-fetoprotein (AFP) and AFP-IgM complexes. Int J Biol Markers 2004;19:155-159.
523. Kaibori M, Matsui Y, Yanagida H, Yokoigawa N, Kwon AH, Kamiyama Y. Positive status of alpha-fetoprotein and des-gamma-carboxy prothrombin: important prognostic factor for recurrent hepatocellular carcinoma. World J Surg 2004;28:702-707.
524. Marrero JA, Romano PR, Nikolaeva O, Steel L, Mehta A, Fimmel CJ, et al. GP73, a resident Golgi glycoprotein, is a novel serum marker for hepatocellular carcinoma. J Hepatol 2005;43:1007-1112.
525. Kew MC, Wolf P, Whittaker D, Rowe P. Tumour-associated isoenzymes of gamma-glutamyl transferase in the serum of patients with hepatocellular carcinoma. Br J Cancer 1984;50:451-455.
526. Fiala S, Fiala AE, Dixon B. -Glutamyl transpeptidase in transplantable, chemically induced rat hepatomas and "spontaneous" mouse hepatomas. J Natl Cancer Inst 1972;48:1393-1401.
527. Melia WM, Bullock S, Johnson PJ, Williams R. Serum ferritin in hepatocellular carcinoma. A comparison with alphafetoprotein. Cancer 1983;51:2112-2115.
528. Nakano S, Kumada T, Sugiyama K, Watahiki H, Takeda I. Clinical significance of serum ferritin determination for hepatocellular carcinoma. Am J Gastroenterol 1984;79:623-627.
529. Suzuki H, Iino S, Endo Y, Torii M, Miki K. Tumor-specific alkaline phosphatase in hepatoma. Ann N Y Acad Sci 1975;259:307-320.
530. Chio LF, Oon CJ. Changes in serum alpha 1 antitrypsin, alpha1 acid glycoprotein and beta 2 glycoprotein I in patients with malignant hepatocellular carcinoma. Cancer 1979;43: 596-604.
531. Pirisi M, Fabris C, Soardo G, Toniutto P, Vitulli D, Bartoli E. Prognostic value of serum alpha-1-antitrypsin in hepatocellular carcinoma. Eur J Cancer 1996;32A:221-225.
532. Bachtiar I, Santoso JM, Atmanegara B, Gani RA, Hasan I, Lesmana LA, et al. Combination of alpha-1-acid glycoprotein and alpha-fetoprotein as an improved diagnostic tool for hepatocellular carcinoma. Clin Chim Acta 2009;399:97-101.
533. Kim J, Ki SS, Lee SD, Han CJ, Kim YC, Park SH, et al. Elevated plasma osteopontin levels in patients with hepatocellular carcinoma. Am J Gastroenterol 2006;101:2051-2059.
534. Asaka M, Nagase K, Miyazaki T, Alpert E. Aldolase A isoenzyme levels in serum and tissues of patients with liver diseases. Gastroenterology 1983;84:155-160.
535. Zong M, Wu MC, Tang ZY, Xia SS. Clinical study of aldolase A in primary liver cancer. In: In: Tang Z, Wu M, Xia S, eds. Primary liver cancer. Beijing, China, Academic Publishers, 1989, pp 269-276.
536. Lu HM, Chen C, Sze PC, Ming TH, Chiang KL, Ting CR, et al. The significance of 5[prime]-nucleotide phosphodiesterase isozymes in the diagnosis of liver carcinoma. Int J Cancer 1980;26:31-35.
537. Fujiyama S, Tsude K, Sakai M, Sato T. 5[prime]-Nucleotide phosphodiesterase isozyme-V in hepatocellular carcinoma and other liver diseases. Hepatogastroenterology 1990;37: 469-473.

538. Leandro G, Zizzari S, Piccoli A, Manghisi OG. The serum tissue polypeptide antigen in the detection of hepatocellular carcinoma in cirrhotic patients. Hepatogastroenterology 1990;37:449-451.
539. Yao WJ, Wang ST, Chow NH, Chang TT, Lin PW, Tu DG. Serum tissue polypeptide specific antigen as a noninvasive prognostic indicator for early recurrence of hepatocellular carcinoma after curative resection. Cancer 2002;95:112-118.
540. Beneduce L, Castaldi F, Marion M, Quarta S, Ruvoletto M, Pontisso P, et al. Circulating squamous cell carcinoma antigen-lgM complexes as novel biomarkers for hepatocellular carcinoma. Digest Liver Dis 2004;36:A2-A3.
541. Hutchinson WL, Du MQ, Johnson PJ, Williams R. Fucosyltransferases: differential plasma and tissue alterations in hepatocellular carcinoma and cirrhosis. Hepatology 1991;13:683-688.
542. Giardina MG, Matarazzo M, Morante R, Lucariello A, Varriale A, Guardasole V, De MG. Serum alpha-L-fucosidase activity and early detection of hepatocellular carcinoma. A prospective study of patients with cirrhosis. Cancer 1998;83:2468-2474.
543. Takahashi H, Saibara T, Iwamura S, Tomita A, Maeda T, Onishi S, et al. Serum alpha-L-fucosidase activity and tumor size in hepatocellular carcinoma. Hepatology 1994;19:1414-1417.
544. Song BC, Chung YH, Kim JA, Choi WB, Suh DD, Pyo SI, et al. Transforming growth factor-beta1 as a useful serologic marker of small hepatocellular carcinoma. Cancer 2002;94:175-180.
545. Tsai JF, Jeng JE, Chuang LY, Yang ML, Ho MS, Chang WY, et al. Clinical evaluation of urinary transforming growth factor-beta1 and serum alpha-fetoprotein as tumour markers of hepatocellular carcinoma. Br J Cancer 1997;75:1460-1466.
546. Shimizu Y, Minemura M, Tsukishiro T, Kashii Y, Miyamoto M, Nishimori H, et al. Serum concentration of intercellular adhesion molecule-1 in patients with hepatocellular carcinoma is a marker of the disease progression and prognosis. Hepatology 1995;22:525-531.
547. Hamazaki K, Gochi A, Shimamura H, Kaihara A, Maruo Y, Doi Y, et al. Serum levels of circulating intercellular adhesion molecule 1 in hepatocellular carcinoma. Hepatogastroenterology 1996;43:229-234.
548. Raedle J, Oremek G, Truschnowitsch M, Lorenz M, Roth WK, Caspary WF, Zeuzem S. Clinical evaluation of autoantibodies to p53 protein in patients with chronic liver disease and hepatocellular carcinoma. Eur J Cancer 1998;34:1198-1203.
549. Ren Y, Poon RT, Tsui HT, Chen WH, Li Z, Lau C, et al. Interleukin-8 serum levels in patients with hepatocellular carcinoma: correlations with clinicopathological features and prognosis. Clin Cancer Res 2003;9:5996-6001.
550. Porta C, De Amici M, Quaglini S, Paglino C, Tagliani F, Boncimino A, et al. Circulating interleukin-6 as a tumor marker for hepatocellular carcinoma. Ann Oncol 2008;19:353-358.
551. Wong VW, Yu J, Cheng AS, Wong GL, Chan HY, Chu ES, et al. High serum interleukin-6 level predicts future hepatocellular carcinoma development in patients with chronic hepatitis B. Int J Cancer 2009;124:2766-2770.
552. Tsai JF, Jeng JE, Chuang LY, You HL, Ho MS, Lai CS, et al. Serum insulin-like growth factor-II and alpha-fetoprotein as tumor markers of hepatocellular carcinoma. Tumor Biol 2003;24:291-298.
553. Tatsuma T, Goto S, Kitano S, Lin YC, Lee CM, Chen CL. Telomerase activity in peripheral blood for diagnosis of hepatoma. J Gastroenterol Hepatol 2000;15:1064-1070.
554. Miura N, Maruyama S, Oyama K, Horie Y, Kohno M, Noma E, et al. Development of a novel assay to quantify serum human telomerase reverse transcriptase messenger RNA and its significance as a tumor marker for hepatocellular carcinoma. Oncology 2007;72:45-51 (suppl 1).
555. Poon RT, Ho JW, Tong CS, Lau C, Ng IO, Fan ST. Prognostic significance of serum vascular endothelial growth factor and endostatin in patients with hepatocellular carcinoma. Br J Surg 2004;91:1354-1360.
556. Villa E, Camellini L, Dugani A, Zucchi F, Grottola A, Merighi A, et al. Variant estrogen receptor messenger RNA species detected in human primary hepatocellular carcinoma. Cancer Res 1995;55:498-500.
557. Villa E, Colantoni A, Camma C, Grottola A, Buttafoco P, Gelmini R, et al. Estrogen receptor classification for hepatocellular carcinoma: comparison with clinical staging systems. J Clin Oncol 2003;21:441-446.
558. Kane SP, Murray-Lyon IM, Paradinas FJ, Johnson PJ, Williams R, Orr AH, Kohn J. Vitamin B12 binding protein as a tumour marker for hepatocellular carcinoma. Gut 1978;19:1105-1109.
559. Paradinas FJ, Melia WM, Wilkinson ML, Portmann B, Johnson PJ, Murray-Lyon IM, Williams R. High serum vitamin B12 binding capacity as a marker of the fibrolamellar variant of hepatocellular carcinoma. Br Med J (Clin Res Ed) 1982;285:840-842.
560. Collier NA, Weinbren K, Bloom SR, Lee YC, Hodgson HJ, Blumgart LH. Neurotensin secretion by fibrolamellar carcinoma of the liver. Lancet 1984;1:538-540.
561. Tokuhisa Y, Iizuka N, Sakaida I, Moribe T, Fujita N, Miura T, et al. Circulating cell-free DNA as a predictive marker for distant metastasis of hepatitis C virus-related hepatocellular carcinoma. Br J Cancer 2007;97:1399-1403.
562. Henry L, Lavabre-Bertrand T, Vercambre L, Ramos J, Carillo S, Guiraud I, et al. Plasma proteasome level is a reliable early marker of malignant transformation of liver cirrhosis. Gut 2009;58:833-838.
563. Iizuka N, Oka M, Yamada-Okabe H, Nishida M, Maeda Y, Mori N, et al. Oligonucleotide microarray for prediction of early intrahepatic recurrence of hepatocellular carcinoma after curative resection. Lancet 2003;361:923-929.
564. Kobayashi Y, Higashi T, Nouso K, Nakatsukasa H, Ishizaki M, Kaneyoshi T, et al. Expression of MAGE, GAGE and BAGE genes in human liver diseases: utility as molecular markers for hepatocellular carcinoma. J Hepatol 2000;32:612-617.
565. Chen CH, Chen GJ, Lee HS, Huang GT, Yang PM, Tsai LJ, et al. Expressions of cancer-testis antigens in human hepatocellular carcinomas. Cancer Lett 2001;164:189-195.
566. Chan KC, Lai PB, Mok TS, Chan HL, Ding C, Yeung SW, Lo YM. Quantitative analysis of circulating methylated DNA as a biomarker for hepatocellular carcinoma. Clin Chem 2008;54:1528-1536.
567. Gitsch G, Kainz C, Kohlberger P, Schneider B, Danihel L, Koelbl H, Breitenecker G. Immunohistochemistry in stage FIGO III cervical cancer: prognostic value of tumor associated antigens and intermediate filaments. Anticancer Res 1992;12:2017-2019.
568. Juang CM, Wang PH, Yen MS, Lai CR, Ng HT, Yuan CC. Application of tumor markers CEA, TPA, and SCC-Ag in patients with low-risk FIGO stage IB and IIA squamous cell carcinoma of the uterine cervix. Gynecol Oncol 2000;76:103-106.
569. Ngan HY, Cheung AN, Lauder IJ, Wong LC, Ma HK. Prognostic significance of serum tumour markers in carcinoma of the cervix. Eur J Gynaecol Oncol 1996;17:512-517.
570. Callet N, Cohen-Solal Le Nir CC, Berthelot E, Pichon MF. Cancer of the uterine cervix: sensitivity and specificity of serum Cyfra 21.1 determinations. Eur J Gynaecol Oncol 1998;19:50-56.

571. Inoue M, Inoue Y, Hiramatsu K, Ueda G. The clinical value of tissue polypeptide antigen in patients with gynecologic tumors. Cancer 1985;55:2618-2623.
572. Kainz C, Sliutz G, Mustafa G, Bieglmayr C, Koelbl H, Reinthaller A, Gitsch G. Cytokeratin subunit 19 measured by CYFRA 21-1 assay in follow-up of cervical cancer. Gynecol Oncol 1995;56:402-405.
573. Nasu K, Etoh Y, Yoshimatsu J, Matsu T, Narahara H, Miyakawa I. Serum levels of cytokeratin 19 fragments in cervical cancer. Gynecol Obstet Invest 1996;42:267-270.
574. Tempfer C, Hefler L, Haeusler G, Reinthaller A, Koelbl H, Zeisler H, Kainz C. Tissue polypeptide specific antigen in the follow-up of ovarian and cervical cancer patients. Int J Cancer 1998;79:241-244.
575. Reiter W, Stieber P, Reuter C, Nagel D, Cramer C, Pahl H, Fateh-Moghadam A. Prognostic value of preoperative serum levels of CEA, CA 19-9 and CA 72-4 in gastric carcinoma. Anticancer Res 1997;17:2903-2906.
576. Louhimo J, Nordling S, Alfthan H, von BK, Stenman UH, Haglund C. Specific staining of human chorionic gonadotropin beta in benign and malignant gastrointestinal tissues with monoclonal antibodies. Histopathology 2001;38:418-424.

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APPENDIX

Background to the NACB Tumor Marker Guidelines

Herein we report the updating and extension of practice guidelines first proposed in 2002 (1). Undertaken under the direction of a steering committee appointed by the NACB, the process involved consideration of 16 specific cancer sites and quality requirements for well-established tumor markers and as well as those being developed using new technologies. The draft guidelines were posted on the NACB Website in July 2005 and were presented as an EduTrak at the 2005 Joint AACC/IFCC Annual meeting in Orlando. Informed comment was also actively sought from individuals, organizations, and other interested parties.

NACB Tumor Marker Guideline Development Group

Nineteen Subcommittees developed draft guidelines. Subcommittee members included individuals with extensive expertise in the science, technology and clinical practice of tumor markers in academia, hospitals, and/or industry. In guidelines in which "expert opinion" is incorporated as part of the recommendations, bias, including conflict of interest, may intrude (2). Members of the in vitro diagnostic industry in the subcommittee membership were deliberately included so as to obtain a representative cross-section of experts and perspectives in the field. This major undertaking has involved significant input from approximately 100 scientists and clinicians from more than ten countries and diverse backgrounds.

Methodological Approach

There is extensive literature on the preparation (3,4) and evaluation (5) of practice guidelines. Many experts have emphasized the importance of a good ìevidence baseî in developing such guidelines (3,6) and the challenges of their effective implementation (6-9). Good methodology during guideline development is highly desirable, although it has recently been noted that good reporting of methodological quality does not necessarily lead to more valid recommendations or vice versa (10).

A recent assessment of nine clinical oncology practice guidelines has demonstrated significant heterogeneity in the development, structure, user and end points of these guidelines, which the authors conclude is not detrimental but rather is necessary, in order to meet divergent demands (11). No available guidelines are likely to be perfect in all situationsóall have limitations, some of which the NACB guidelines presented here undoubtedly share. However, characteristics identified as critical to the effectiveness of practice guidelines are a clear definition of purpose and intended audience, adherence to methodological standards, and systematic evaluation (audit) of their clinical impact after their introduction (11).

Here a relatively informal methodological approach was adopted and subcommittee chairs were allowed considerable latitude. While some of the diversity evident in the guidelines presented here undoubtedly reflects the predilection and idiosyncrasy of individual subcommittees, much of it arises from the different numbers of tumor markers described for each specific cancer as well as the variable maturity of clinical validation and currently available evidence for these markers. It is therefore not realistic to expect to achieve consistency of approach across the spectrum of cancers examined.

The subcommittees were, however, asked to follow a recommended structure when developing and formulating the guidelines and to consider each of the major potential clinical applications of tumor markers (screening/early detection, diagnosis, prognosis, treatment monitoring and surveillance) in order to achieve a reasonably homogeneous presentation across cancer types. Subcommittees were also strongly encouraged to undertake as thorough a review of the literature as feasible, with particular attention given to reviews (including systematic reviews), prospective randomized trials that included the use of markers and existing guidelines.

Importantly, each subcommittee was asked to compare its guidelines with those of other groups and to present these comparisons in tabular form, elaborating on any differences and also providing estimates of both the level of evidence (LOE) (7) and the strength or grade of recommendation (SOR) (12) (Table A) ascribable to each NACB recommendation. The LOE and SOR respectively reflect the strength of published evidence supporting the recommendations made and the degree of consensus within the guideline development group, while the tables relating to individual malignancies provide a convenient summary of the relevant NACB guidelines. Where consensus could not be achieved within a subcommittee, this is explained, describing the conflicting views and reasons for these.

The final result is a set of practice guidelines that follow a reasonably homogeneous style and approach. The strength and type of evidence underlying each recommendation is clearly stated, together with an estimate of the confidence with which each recommendation has been made, so the reader can readily discern which are based on incontrovertible clinical evidence and which are based on the expert consensus of committee members.

Review and Refinement of the NACB Tumor Marker Guidelines

Subcommittee chairs reviewed and responded to suggestions and corrections received after posting of the guidelines on the NACB website. These NACB guidelines will inevitably require updating, refinement, and modification in the future, as knowledge and understanding of tumor markers and their biological roles increases. As suggested in the very helpful AGREE document (5), and reflecting work in progress for a number of tumor markers, when the guidelines are next updated it may be possible to include some estimate of the cost-effectiveness of tumor marker use, to take account of patients' views, and to report on audit studies of their effectiveness. For this purpose it would be desirable to use a consultation form similar to that developed by the Scottish Intercollegiate Guideline Network (SIGN) [see eg, (13)].

Implementation of the NACB Tumor Marker Guidelines

Adoption of these guidelines is voluntary, some recommendations may not be appropriate in all settings (eg, clinical trials) and for effective implementation they may require translation and/or other modification in some settings. There is good evidence that "locally owned" guidelines are much more likely to be successfully adopted in routine clinical practice (4). In addition, carefully designed audit studies would be highly desirable before and after introduction of the guidelines (11).

These recommendations, which, to facilitate their dissemination, are being published in electronic form on the NACB web site, should encourage more optimal use of tumor marker tests by clinical and laboratory staff, thereby better informing medical decisions directed toward improved clinical outcome and/or quality of life for increasing numbers of cancer patients.

Table A. Levels of Evidence and Strengths of Recommendation Used to Grade the NACB Guidelines for Tumor Markers

Assessment	Criteria
Level of Evidence (8)	
I	Evidence from a single, high-powered, prospective, controlled study that is specifically designed to test marker, or evidence from a meta-analysis, pooled analysis or overview of level II or III studies.
II	Evidence from a study in which marker data are determined in relationship to prospective therapeutic trial that is performed to test therapeutic hypothesis but not specifically designed to test marker utility.
III	Evidence from large prospective studies.
IV	Evidence from small retrospective studies.
V	Evidence from small pilot studies.
Expert opinion *Strength of recommendation (12)*	
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.

NOTE. Adapted from Hayes et al (8) and Atkins et al (12).

APPENDIX REFERENCES

1. Fleisher M, Dnistrian A, Sturgeon C, Lamerz R, Witliff J. Practice guidelines and recommendations for use of tumor markers in the clinic. Tumor Markers: Physiology, pathobiology, technology and clinical applications, Vol. Washington: AACC Press, 2002:33-63.
2. Detsky AS. Sources of bias for authors of clinical practice guidelines. Can Med Assoc J 2006;175:1033, 1035.
3. Oosterhuis WP, Bruns DE, Watine J, Sandberg S, Horvath AR. Evidence-based guidelines in laboratory medicine: principles and methods. Clin Chem 2004;50:806-818.
4. Sturgeon C. Practice guidelines for tumor marker use in the clinic. Clin Chem 2002;48:1151-1159.
5. AGREE Collaboration. Development and validation of an international appraisal instrument for assessing the quality of clinical practice guidelines: the AGREE project. Qual Saf Health Care 2003;12:18-23.
6. Price CP, Christenson RH, eds. Evidence-based laboratory medicine: Principles, practice and outcomes. 2nd ed. Washington DC: AACC Press, 2007.
7. Hayes DF, Bast RC, Desch CE, Fritsche H, Jr., Kemeny NE, Jessup JM, et al. Tumor marker utility grading system: a framework to evaluate clinical utility of tumor markers. J Natl Cancer Inst 1996;88:1456-1466.
8. Hayes DF. Prognostic and predictive factors for breast cancer: translating technology to oncology. J Clin Oncol 2005;23:1596-1597.
9. Yamauchi H, Stearns V, Hayes DF. When is a tumor marker ready for prime time? A case study of c-erbB-2 as a predictive factor in breast cancer. J Clin Oncol 2001;19:2334-2356.
10. Watine J, Friedberg B, Nagy E, Onody R, Oosterhuis W, Bunting PS, et al. Conflict between guideline methodologic quality and recommendation validity: a potential problem for practitioners. Clin Chem 2006;52:65-72.
11. Pentheroudakis G, Stahel R, Hansen H, Pavlidis N. Heterogeneity in cancer guidelines: should we eradicate or tolerate? Ann Oncol 2008.
12. Atkins D, Best D, Briss PA, Eccles M, Falck-Ytter Y, Flottorp S, et al. Grading quality of evidence and strength of recommendations. BMJ 2004;328:1490.
13. Scottish Intercollegiate Guidelines Network (SIGN): SIGN 28. Management of adult testicular germ cell tumours. 1998. http://www.sign.ac.uk/ (Accessed 18th October 2007).