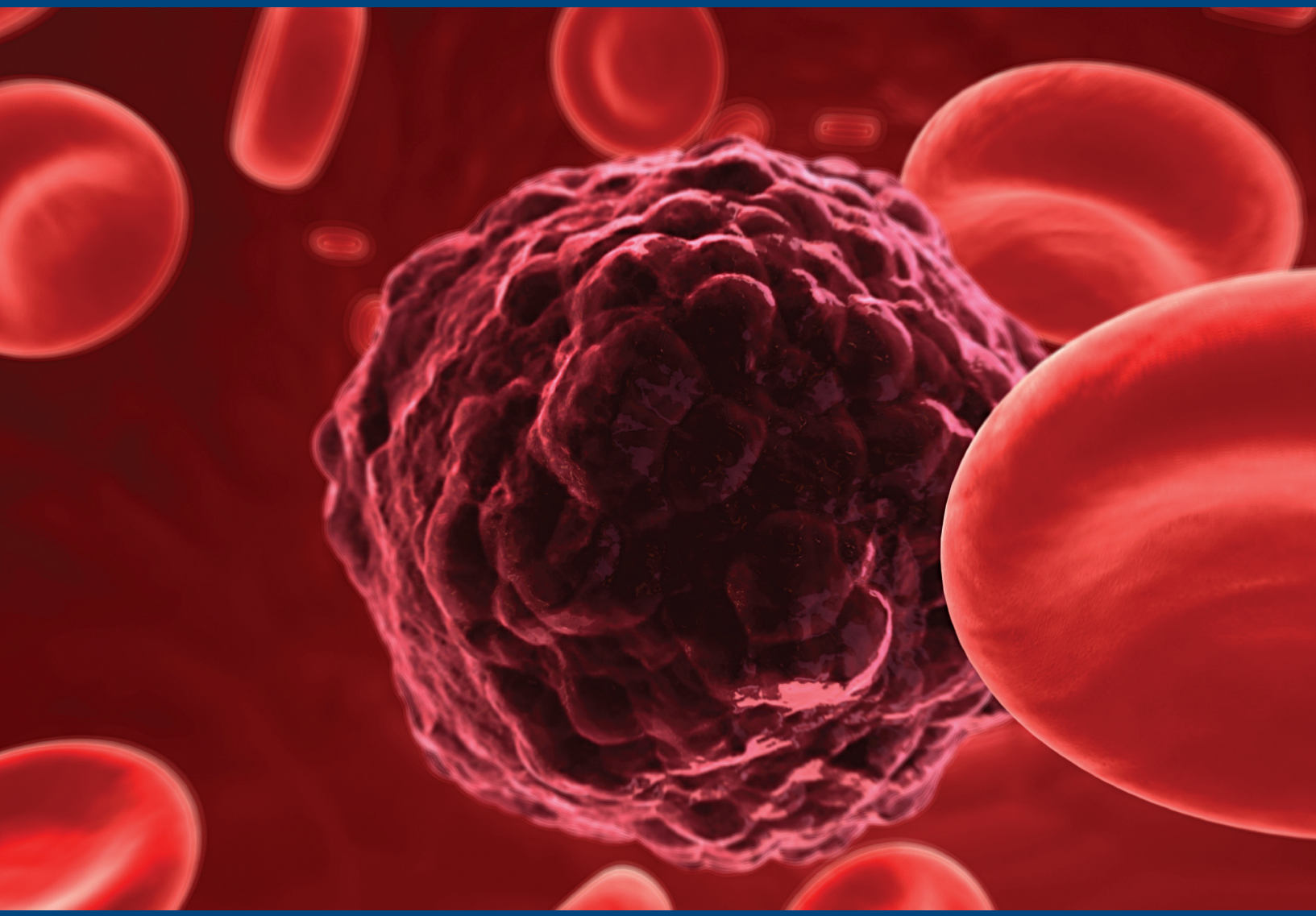
Laboratory Medicine Practice Guidelines

Use of Tumor Markers in Testicular, Prostate, Colorectal, Breast, and Ovarian Cancers

Edited by Catharine M. Sturgeon and Eleftherios Diamandis



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Chapter 1

Introduction

We present 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 (LPMG) for the use of tumor markers in testicular, prostate, colorectal, breast, and ovarian 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 to assist practitioners and patients to make decisions about appropriate health care for specific clinical circumstances (1). An explanation of the methodology used when developing these Guidelines appears in the introduction of the *Tumor Markers: Quality Requirements* LMPG, which can be found on the NACB web site (2) and 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). In order 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. Where possible, these consensus recommendations of the NACB panels were evidence based. The *Tumor Markers: Quality Requirements* LMPG presents NACB recommendations relating to general quality requirements for tumor measurements, and includes tabulation of important causes of false positive tumor marker results (eg, due to heterophilic antibody interference, "high dose hooking") which must also be taken into account (3).

Chapter **2**

Tumor Markers in Testicular Cancers

Ulf-Håkan Stenman, Rolf Lamerz, and Leendert H. Looijenga

BACKGROUND

Approximately 95% of all malignant testicular tumors are of germ cell origin, most of the rest being lymphomas, Leydig or Sertoli cell tumors and mesotheliomas. Germ cell tumors of adolescents and adults are classified into two main types, seminomas and nonseminomatous germ cell cancers of the testis (NSGCT). Testicular cancers represent about 1% of all malignancies in males, but they are the most common tumors in men age 15 to 35 years. They represent a significant cause of death in this age group in spite of the fact that presently more than 90% of the cases are cured (4). Germ cell tumors may also originate in extragonadal sites (eg, the sacrococcygeal region, mediastinum, and pineal gland (5)). Those of the sacrum are predominantly found in young males. Based on the histology, age of the patient at diagnosis, clinical behavior, and chromosomal constitution, these tumors can be subdivided into three distinct entities with different clinical and biological characteristics (6-9): teratomas and yolk sac tumors of newborns and infants; seminomas and nonseminomas of adolescents and young adults; and spermatocytic seminoma of the elderly. Seminomas and nonseminomas in adolescence and adulthood were the focus of attention when developing these recommendations.

The incidence of testicular cancers varies considerably in different countries. In the United States, approximately 7,200 new cases are diagnosed each year (4) and the age-adjusted incidence is 5.2/100,000. The incidence is about 4-fold higher in white than in black men. In Europe, the age-adjusted incidence is lowest in Lithuania (0.9/100,000), intermediate in Finland (2.5/100,000), and highest in Denmark (9.2/100 000) (10). The incidence in various European countries has increased by 2% to 5% per year. In the United States, the incidence increased by 52% from the mid-1970s to the mid-1990s (11). The cause of germ cell tumors is unknown, but familial clustering has been observed and cryptorchidism and Klinefelter's syndrome are predisposing factors (4). At presentation, most patients have diffuse testicular swelling, hardness, and pain. At an early stage, a painless testicular mass is a pathognomonic finding but a testicular mass is most often caused by infectious epididymitis or orchitis. The diagnosis can usually be confirmed by ultrasonography. If testicular cancer is suspected, the serum concentrations of α-fetoprotein (AFP), human chorionic gonadotropin (hCG), and lactate dehydrogenase (LDH) should be determined before therapy. As a rule, orchiectomy is performed prior to any further treatment, but may be delayed until after chemotherapy in individuals with life-threatening metastatic disease. After orchiectomy, additional therapy depends on the type and stage of the disease. Surveillance is increasingly used for seminoma patients with stage I disease, but radiation to the retroperitoneal and ipsilateral pelvic lymph nodes, which is standard treatment for stage IIa and IIb disease, is also used, as is short (single) course carboplatin (12). About 4% to 10% of patients relapse with more than 90% of these cured by chemotherapy. About 15% to 20% of stage I seminoma under surveillance relapse and need to be treated with chemotherapy. Patients with stage I nonseminomatous tumors are treated by orchiectomy. After orchiectomy, surveillance and nerve-sparing retroperitoneal lymph-node dissection are accepted treatment options. About 20% of patients under surveillance will have a relapse and require chemotherapy. Patients with stage II nonseminomatous tumors are treated with either chemotherapy or retroperitoneal lymph node dissection. Testicular cancer patients with advanced disease are treated with chemotherapy (4).

Serum tumor markers have an important role in the management of patients with testicular cancer, contributing to diagnosis, staging and risk assessment, evaluation of response to therapy and early detection of relapse. Increasing marker concentrations alone are sufficient to initiate treatment. AFP, hCG, and LDH are established serum markers. Most cases of nonseminomatous germ cell tumors (NSGCT) have elevated serum levels of one or more of these markers while LDH, and hCG are useful in seminomas. Other markers have been evaluated but provide limited additional clinical information.

To prepare these guidelines, we reviewed the literature relevant to the use of tumor markers for testicular cancer. Particular attention was given to reviews, prospective randomised trials that included the use of markers, and guidelines issued by expert panels. Only one relevant systematic review was identified (109). Where possible, the consensus recommendations of the NACB panel were evidence based.

CURRENTLY AVAILABLE MARKERS FOR TESTICULAR CANCER

Table 1 lists the most widely investigated tissue-based and serum-based tumor markers for testicular cancer. Also listed is the phase of development of each marker as well as the level of evidence (LOE) for its clinical use.

Table 1. Currently Available Serum and Tissue Markers for Testicular Tumors

Marker	Proposed Use	Phase of Development	Level of Evidence	Reference
Established serum markers				
AFP	Diagnosis Prognosis/staging Monitoring/surveillance	Generally available	II I II	(4, 65, 73, 89)
hCG	Diagnosis Prognosis/staging Monitoring/surveillance	Generally available	II I II	(4, 89, 103)
LDH	Prognosis/staging	Generally available	I	(63, 109)
Potentially useful experimental serum markers				
hCGb	Diagnosis Monitoring	Experimental	IV	(96, 103)
LD-1	Diagnosis Risk stratification	Experimental	IV	(109)
PLAP	Diagnosis	Experimental	IV	(111, 112)
NSE	Diagnosis	Experimental	IV	(116, 117)
Established tissue markers				
PLAP	Histological typing ITGCNU	Antibodies for immunohistochemistry generally available	II	(24)
c-KIT, stem cell factor rec.	Typing of seminoma and ITGCNU	Antibodies for immunohistochemistry available	II	(28)
CD30	Embryonal carcinoma	Antibodies for immunohistochemistry generally available	IV	(60, 519)
AFP	Typing of yolk sac tumors and embryonal carcinoma	Antibodies for immunohistochemistry generally available	II	(24)
hCG	Typing of seminoma and choriocarcinoma	Antibodies for immunohistochemistry generally available	II	(24)
Amplification of 12p	Diagnosis of extragonadal tumors	Limited availability	II	(107, 108)
Vascular invasion	Risk stratification	Limited availability	II	(54)
OCT3/4, POUF1	Risk stratification	Experimental	IV	(58)

Abbreviations: AFP, α-fetoprotein; hCG, human chorionic gonadotropin; hCGβ, free β subunit of human chorionic gonadotropin; LDH, lactic dehydrogenase; NSE, neuron specific enolase; NSGCT, nonseminomatous germ cell tumors; PLAP, placental (germ cell) alkaline phosphatase.

NOTE. LOE, level of evidence (120), level evidence from a single, high-powered, prospective, controlled study that is specifically designed to test the marker, or evidence from a meta-analysis, 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 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.

Table 2. Recommendations for Use of Tumor Markers in Testicular Cancer by Different Expert Groups

	EAU 2001 (14)	EGTM 1999 (13)	ESMO 2007 (17) and 2008 (21)	NACB 2002 (15)	NCCN 2007 (18)	NACB 2008	
						Recommendation	Strength of Recommendation*
AFP and hCG for							
Screening	No	No	No	No	No	No	A
Diagnosis/case-finding	Yes	Yes	Yes	Yes	Yes	Yes	B
Staging/prognosis	Yes	Yes	Yes	Yes	Yes	Yes	A
Detecting recurrence	Yes	Yes	Yes	Yes	Yes	Yes	A
Monitoring therapy	Yes	Yes	Yes	Yes	Yes	Yes	A
AFP for							
Differential diagnosis of NSGCT	Yes	Yes	Yes	Yes	Yes	Yes	A
LDH for							
Diagnosis/case-finding	Yes	Yes	Yes	Yes	Yes	Yes	B
Staging/prognosis	Yes	Yes	Yes	Yes	Yes	Yes	A
Detecting recurrence	Yes	Yes	Yes	Yes	Yes	Yes	B
Monitoring therapy	Yes	Yes	Yes	Yes	Yes	Yes	B

Abbreviations: AFP, α-fetoprotein; hCG, human chorionic gonadotropin; LDH, lactic dehydrogenase; EGTM, European Group on Tumour Markers; EAU, European Association of Urologists; NACB, National Academy of Clinical Biochemistry

*Strength of recommendation (520): 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 of 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.]

TUMOR MARKERS IN TESTICULAR CANCER: NACB RECOMMENDATIONS

Table 2 presents a summary of recommendations from representative guidelines published on the use of tumor markers in testicular cancer. This Table also summarizes the NACB guidelines for the use of markers in this malignancy. A number of groups have made detailed recommendations regarding the management of testicular cancer (13-21), with some of those relating to tumor marker use summarized in Table 3. Table 4 summarizes the prognostic significance of serum tumor markers in metastatic testicular cancer, according to the consensus statement of the International Germ Cell Consensus Classification (IGCCC), which remains the cornerstone for diagnosis and treatment of testicular germ cell tumors. Below, we briefly review the histological types of testicular cancer and present a more detailed discussion on the markers listed in these Tables.

HISTOLOGICAL TYPES OF TESTICULAR CANCER

In the most recent WHO-Mostofi classification (8, 22), testicular cancers are subdivided into two major types, seminomas and NSGCT, which differ with respect to both marker expression and treatment. The incidence of seminoma peaks in the fourth decade of life and that of NSGCT in the third. Seminomas can be either pure seminomas or the rare spermatocytic seminomas that occur in older age groups. Most NSGCTs are a mixture of histological types (ie, embryonal carcinomas, choriocarcinomas, teratomas, and yolk sac tumors). About 10% to 20% of the nonseminomas also contain a seminoma component. These are classified as combined tumors according to the British classification (23), but as nonseminomas according to the WHO classification system (22). Teratomas are further subdivided as mature or immature. Somatic cancers of various types occasionally develop from a teratoma and are classified as non-germ cell malignancies. Metastases may contain any component occurring in the primary tumor and occasionally components not detected in the primary tumor (22). Fewer than 10% of NSGCT contain a single tissue type and all histological types of tissue should be described (24).

The precursor lesion of testicular seminomas and nonseminomas is carcinoma in situ (CIS) (25), also referred to as intratubular germ cell neoplasia unclassified (ITGCNU) and testicular intratubular neoplasia (TIN). CIS cells are found within the spermatogonial niche of the seminiferous tubule in the adult testis in close proximity to the Sertoli cells, the nursing cells of spermatogenesis (26). The CIS cells can be detected in the adjacent parenchyma of most invasive tumors, and are more frequently associated with NSGCTs than with seminomas (27). ITGCNU is considered to represent the pre-malignant counterpart of an embryonic germ cell, most likely a primordial germ cell or gonocyte. This theory is supported by multiple findings, including epidemiology, morphology, immunohistochemistry, and molecular characterization (28, 29).

Recent data indicate that infertile men with bilateral microlithiasis have an increased risk (up to 20%) of developing testicular seminomas and NSGCTs (30). Surgical biopsy to assess the presence of ITGCNU (31) is indicated in this condition.

TISSUE MARKERS FOR TESTICULAR CANCER

Genetic Aberrations

A gain of 12p is observed in germ cell tumors both of testicular and extragonadal origin. This indicates that gain of 12p sequences may be of crucial importance for the development of this cancer and, indeed, this finding is used to diagnose germ cell tumors at extragonadal sites (32). However, the expression

Table 3. Recommended Frequency of Tumor Marker Measurements in the Follow-Up of Testicular Cancer Patients (16)

	Frequency of Tumor Marker Measurements (No. of times per year)					
	Year 1	Year 2	Year 3	Year 4	Year 5	Years 6-10
Stage 1 seminoma after radiotherapy	4	3	3	2	2	
Stage I seminoma surveillance after chemotherapy	6	4	3	2	2	1
Stage I NSGCT surveillance	6[a]	4[b]	2	2	2	[c]
Stage I NSGCT after RPLND or adjuvant chemotherapy	6	3	2	2	2	[c]
Stage IIa-IIb seminoma after radiotherapy	6	4	3	2	2	1
Stage IIa-IIB NSGCT after RPLND and chemotherapy or primary chemotherapy	4	2	2	2	2	1
Seminoma and NSGCT of advanced stage	12	6	4	3	2	1

Abbreviations: NSGCT, nonseminomatous germ cell tumors; RPLND, retroperitoneal lymph node dissection.

[a] Measurements every two months recommended; measurements every month for the first six months advisable.

[b] Measurements every three months recommended; measurements every two months advisable.

[c] Measurement once a year advisable.

Table 4. Classification of Metastatic Germ Cell Tumors Into Various Risk Groups According to the International Germ Cell Consensus Classification (66)*

Good Prognosis **Nonseminoma**	**Seminoma**
Testis/retroperitoneal primary **And** No non-pulmonary visceral metastases **And** Good markers - all of: AFP $<$ 1000 μg/L and hCG $<$ 5000 U/L (1000 μg/L) and LDH $<$ 1.5 x N (upper limit of normal)	Any primary site **and** No non-pulmonary visceral metastases **and** Normal AFP, any hCG, any LDH
56% of non-seminomas **5 year PFS 89%** **5 year Survival 92%**	**90% of seminomas** **5 year PFS 82%** **5 year Survival 86%**
Intermediate Prognosis **Nonseminoma**	**Seminoma**
Testis/retroperitoneal primary **And** No non-pulmonary visceral metastases **And** Intermediate markers - any of: AFP $\geq$ 1000 and $\leq$ 10,000 μg/L or hCG $\geq$ 5000 U/L and $\leq$ 50,000 U/L or LDH $\geq$ 1.5 x N and $\leq$ 10 x N	Any primary site **and** No non-pulmonary visceral metastases **and** Normal AFP, any hCG, any LDH
28% of non-seminomas **5 year PFS 75%** **5 year Survival 80%**	**10% of seminomas** **5 year PFS 67%** **5 year Survival 72%**
Poor Prognosis **Non-seminoma**	**Seminoma**
Mediastinal primary **Or** Non-pulmonary visceral metastases **Or** Poor markers - any of: AFP $>$ 10,000 μg/L or hCG $>$ 50,000 U/L (10000 μg/L or LDH $>$ 10 x N	No patients classified as poor prognosis
16% of non-seminomas **5 year PFS 41%** **5 year Survival 48%**	

*Adapted from Reference **(66)** and reproduced with permission.

Abbreviations: AFP, α-fetoprotein; hCG, human chorionic gonadotropin; hCGβ; LDH, lactic dehydrogenase; N, upper limit of normal; PFS, progression free survival.

level of 12p sequences does not correlate with stage of the disease and treatment sensitivity/resistance (33-35). The crucial determinant of response to cisplatin-based compounds appears to occur downstream of DNA binding in the intrinsic or extrinsic pathways of apoptosis or DNA repair (36-38).

While the majority of germ cell tumors show an intact DNA mismatch repair pathway, a defect leading to microsatellite instability has been observed in tumors refractory to cisplatin (39-41). Other potentially relevant findings in the context of treatment sensitivity and resistance relate to a possible defect in caspase 9 function (42). All these factors might be important and it is unlikely that a single factor determines treatment sensitivity or resistance. This is illustrated by the finding that mature teratomas are resistant to various DNA-damaging

treatment protocols (38), possibly due to epigenetic changes occurring during somatic differentiation.

The majority of invasive seminomas and nonseminomas contain additional copies of the X chromosome (43). This is interesting, as during normal (female) development, X-inactivation can occur in these tumors, in which *XIST* is the regulatory gene (6). Detection of unmethylated *XIST* DNA in plasma has been suggested to be useful for molecular diagnosis and the monitoring of testicular GCT patients (44). This observation merits further investigation.

A number of studies have linked the development of germ cell tumors to a deregulated G_1/S checkpoint, possibly related to the lack of a functional retinoblastoma (*RB*) gene cell cycle regulator (45) and consequently no upregulation of p21 after induction of DNA damage. Cells without p21 show reduced cisplatin-induced DNA damage repair capacity and increased sensitivity to cisplatin (46). The treatment-resistant mature teratomas show, in contrast to other invasive components, positive staining for multiple proteins potentially related to treatment resistance. In addition, they are positive for the RB gene and p21 allowing them to go into G_1/S cycle arrest (47, 48). This might explain the observation that residual mature teratoma is found in about 30% to 40% of remnants of initial metastases after chemotherapy. A predictive model for the histology of a residual retroperitoneal mass, based on primary tumor histology, prechemotherapy markers, mass size, and size reduction under chemotherapy, has been developed (49). Absence of teratoma elements or viable cancer cells in the primary tumor has been identified as the most powerful predictor for benign residual tissue (50). However, caution is warranted because small teratoma areas may be missed in the primary tumor, and absence of teratoma elements does not exclude occurrence of malignant cells in residual masses. These findings may again be related to the origin of these tumors (51) because *RB* expression is not found in human fetal gonocytes and ITGCNU (52, 53).

Vascular Invasion

Particular attention must be paid to the presence or absence of vascular invasion as a predictor of metastatic spread and occult metastases (54). Distinguishing venous from lymphatic invasion does not add information as to the risk of occult metastasis. Besides vascular invasion, high proliferative activity (assessed with the monoclonal antibody MIB-1), and to a lesser extent the presence of embryonal carcinoma in the primary tumor and a high pathologic stage, have been reported to be predictors of systemic spread in clinical stage I NSGCT (for review, see (55)). However, the predictive value of this model is limited, as the group defined as high risk in fact has a 50% risk of occult metastasis, and the low-risk group a 16% risk. Prospective assessment of risk factors for relapse in clinical stage I NSGCT also showed that vascular invasion was the strongest predictive factor (56). With the addition of two other risk parameters (MIB-1 score $>$ 70% and embryonal carcinoma $\geq$ 50%), the positive predictive value increased to 63.6%. Thus, even with an optimal combination of prognostic factors and reference pathology, more than one third of patients predicted to have pathologic stage II or a relapse during follow-up will not have metastatic disease and will be overtreated with adjuvant therapy. In contrast, patients at low risk can be predicted with better accuracy (86.5%), suggesting that surveillance may be an option for highly compliant patients. Recently, cluster analysis has been used to identify prognostic subgroups in patients with embryonal carcinoma (57).

SERUM MARKERS FOR TESTICULAR CANCER

Marker Expression and Tumor Type

Certain markers have been found to be informative for the classification of seminomas and NSGCTs. Placental/germ cell alkaline phosphatase (PLAP) is detected in most seminomas and embryonal carcinomas, in 50% of yolk sac tumors and choriocarcinomas, but only rarely in teratomas. Human chorionic gonadotrophin (hCG) is expressed by syncytiotrophoblasts, choriocarcinoma, and approximately 30% of seminomas. Of the other tissue markers, the stem cell factor receptor (c-KIT) has been used mainly to detect ITGCNU and seminoma, CD30 to detect embryonal carcinoma, and α-fetoprotein (AFP) to detect yolk sac tumors and a 10% to 20% subset of embryonal carcinomas and teratomas. Recently, a potentially valuable marker OCT3/4, also known as POU5F1, has been identified (58-61).

Although a large number of serum markers have been studied, only hCG, AFP, and lactate dehydrogenase (LDH) have thus far been shown to have independent diagnostic and prognostic value (Tables 2 and 3). The clinical value of other markers remains to be established. Table 5 summarizes analytical limitations of the assays available for some of the most important established and experimental tumor markers. The implications of these limitations for tumor marker use in routine clinical practice are discussed in greater detail later.

CLINICAL APPLICATIONS OF SERUM TUMOR MARKERS IN TESTICULAR CANCER

Diagnosis

Patients with a testicular germ cell tumor may present with a painless testicular mass, while others also have symptoms caused by metastatic disease. The clinical workup comprises physical examination, ultrasound of the testis, and computerized tomography (CT) scan of the pelvis, abdomen, and chest (62). Determination of hCG, AFP and LDH in serum before therapy is mandatory in all patients. The marker concentration in serum is dependent on histological type and tumor load (ie, stage). In a recent large collaborative study 64% of the tumors were NSGCT and 36% seminomas (63). Of the latter, 77% presented with stage I disease (ie, tumor localized to the testis), and 21% had elevated serum levels of hCG. Of those with NSGCT 52% had stage I disease and 79% had elevated marker

Table 5. Analytical Requirements and Potential Interfering Factors for Established And Experimental Serum Markers For Germ Cell Tumors

Established Markers			
Marker	**Sample type**	**Analytical requirements**	**Confounding factors**
Experimental Markers			
AFP	Serum or plasma	Detection limit <1 μg/L	Hepatitis Heterophilic antibodies Drug-induced hepatic damage Hepatocellular cancer
hCG	Serum or plasma Urine to confirm false results	Detection limit <2 U/L Cross-reaction with LH $<2\%$ Equimolar recognition of hCGβ (or use of separate assay for hCGβ)	Chemotherapy-induced elevation of hCG to >10 U/L Heterophilic antibodies Nontrophoblastic cancers producing hCGβ
LDH	Serum	Reference values are method-dependent Clinical decision limits based on upper reference limit	Elevated values also caused by – Hemolysis – Liver disease – Muscle disease – Myocardial infarction
Experimental Markers			
hCGβ	Serum or plasma	Detection limit 0.5 pmol/L	Nontrophoblastic cancers
LD-1	Serum	Reference values method-dependent	Hemolysis, muscle disease, heart disease
PLAP	Serum	Reference values method-dependent	Smokers may have 10-fold increased values
NSE	Serum	Reference values method-dependent	Hemolysis causes falsely elevated values

Abbreviations: AFP, α-fetoprotein; hCG, human chorionic gonadotropin; hCGβ, free β-subunit of human chorionic gonadotropin; hCGα, free α-subunit of human chorionic gonadotropin; LDH, lactic dehydrogenase; NSE, neuron specific enolase; PLAP, placental (germ cell) alkaline phosphatase.

levels (both hCG and AFP elevated in 44%, only AFP in 26% and only hCG in 9% (63). In seminoma patients hCG concentrations are usually below 300 U/L. Levels >1000 U/L are mostly associated with NSGCT. Levels >10000 U/L are mainly seen in patients with pure choriocarcinoma but occasionally may occur in seminoma. LDH is elevated in 40-60% of patients with seminoma or NSGCT (64). The classification of a tumor is based on histological examination, but if serum AFP is elevated, a tumor classified as a seminoma is reclassified as NSGCT and treated accordingly (4).

NACB Testicular Cancer Panel Recommendation 1: Tumor Markers in the Diagnosis of Testicular Cancer

When testicular cancer is suspected, pretreatment determination of hCG, AFP, and LDH is mandatory [LOE, II; SOR, B].

Staging, Risk Stratification, and Selection of Therapy

Elevated serum concentrations of AFP, hCG, and LDH are associated with adverse prognosis (65, 66). A high serum hCG concentration is a strong prognostic factor, and the risk of recurrence increases with increasing concentration (67). The International Germ Cell Cancer Collaborative Group (IGCCCG) has incorporated serum concentrations of hCG, AFP, and LDH in a scheme for classification of metastatic germ cell tumors (Table 4). Tumors are classified as having good, intermediate or poor prognosis based on marker levels, primary site of the tumor, and presence or absence of non-pulmonary visceral metastases (66).

The selection of treatment is based on tumor type and prognostic group. Stage I seminomas may be treated by orchiectomy alone, which leads to cure in 80% to 85% of patients. Orchiectomy in combination with radiotherapy of the abdominal lymph nodes leads to cure in 97% to 99% of patients, and this approach is routinely used in many centers. Without radiotherapy 15% to 20% of patients relapse, but most of these are cured by second-line therapy. Therefore, surveillance at increased frequency is an alternative to radiotherapy.

When treated by orchiectomy only, stage I NSGCT patients have a 30% risk of relapse. The risk is higher (50%) if perivascular infiltration is present than if it is absent (risk 15% to 20%). The relapse risk if very low if retroperitoneal lymph node dissection (RPLND) is performed in connection with primary therapy. This procedure is associated with morbidity and therefore surveillance is used as an alternative to RPLND. Chemotherapy is another alternative to RPLND, but residual retroperitoneal tumors consisting of teratomas, which need to be treated by surgery, are often observed. If serum marker levels do not normalize or increase after RPLND, positive retroperitoneal lymph nodes or systemic disease requiring chemotherapy are most likely present (68, 69).

Further Risk Stratification

Embryonal carcinoma is the most common cell type in NSGCT. It is totipotential and tumors with pure embryonal carcinoma are associated with early metastatic disease. There is therefore a need to estimate prognosis of tumors containing this cell type more accurately. Cluster analysis of the serum markers AFP and hCG in combination with the tissue markers p53, Ki67, and apoptosis index suggest that a pattern with high Ki67, low apoptosis, and low p53 is associated with better survival than other patterns. Classification with this algorithm has been reported to be independent of the IGCCCG classification (67). If these results can be confirmed, this could provide a tool for more precise tailoring of therapy.

NACB Testicular Cancer Panel Recommendation 2: Tumor Markers in the Clinical Staging Of Testicular Cancer

Measurement of hCG, AFP, and LDH is mandatory for staging and risk stratification according to the International Germ Cell Consensus Classification (Table 4) [LOE, I; SOR, A].

Monitoring Response to Therapy

If AFP or hCG in serum is elevated before therapy, the rate of marker decline reflects the response to therapy. Persistent marker elevation after chemotherapy indicates residual disease and the need for further therapy (70, 71). Chemotherapy may induce a transient increase or surge in marker concentrations during the first week of treatment (72).

In the absence of residual disease after orchidectomy, the half-life of hCG is approximately 1.5 days and that of AFP is 5 days (73, 74). During chemotherapy, half-lives $>$ 3.5 days for hCG or $>$ 7 days for AFP predict recurrence and adverse prognosis (75). Marker half-life is calculated from the slope of the logarithm of the marker concentration versus time. It is preferable to use marker concentrations from several time points and to calculate the half-life from the slope of the regression line (64). The half-life should be determined after the initial marker surge during two cycles of chemotherapy between days 7 and 56. A slow rate of marker decline is of potential use in poor-risk patients and may imply a need for more aggressive therapy (75).

NACB Testicular Cancer Panel Recommendation 3: Tumor Markers in Monitoring Response to Treatment in Patients With Testicular Cancer

If raised prior to therapy, serum markers (AFP, hCG, and/or LDH) should be monitored weekly until concentrations are within the reference interval. Wherever possible, the marker half-life should be determined. Marker levels exceeding the upper reference limit after therapy suggest residual disease, which should be confirmed or excluded by other methods [LOE, II; SOR, A].

Surveillance

After successful primary therapy, all patients are monitored with physical examination, tumor marker determinations, and CT scan. With such surveillance, relapse is in most cases detected before clinical symptoms appear. Most relapses occur within the first year and relapses after 2 years are rare but some cases may relapse even after 10 years. The surveillance is tailored to take into account tumor type, stage, treatment, and likelihood of relapse (Table 3). Patients with low-risk disease treated with surgery alone are monitored most frequently (eg, every 1-2 weeks during the first 6 months). Some centers recommend weekly monitoring in order to detect a relapse before tumor grows to a size associated with adverse prognosis, as estimated by serum concentrations of AFP $>$ 500 kU/L and of hCG $>$ 1,000 U/L (76). In all patients monitoring is continued for 5 years (16).

NACB Testicular Cancer Panel Recommendation 4: Tumor Markers in Surveillance of Patients With Testicular Cancer

Serial monitoring with AFP, hCG, and LDH is recommended even when these are not raised prior to therapy as marker expression can change during therapy. Frequency of measurement depends on the stage and pathology of disease but should be according to agreed protocols (eg, as in Table 3). Because baseline levels are individual, increases are more important than absolute concentrations. A single increasing value must be confirmed with a second sample and the possibility of transient elevation due to non-specific interference (eg, iatrogenic hypogonadism) should be actively considered [LOE, II; SOR, A].

Analytical Considerations

Tumor marker measurements are mandatory in the treatment of testicular cancer patients. It is therefore appropriate to review analytical requirements for these important tests in more detail.

AFP

Biochemistry and biology. AFP is a homolog of albumin and is thought to act as a carrier protein in the fetus. During pregnancy, AFP is initially produced by the yolk sac and later by the fetal liver (77). Concentrations in fetal plasma reach levels of 3 g/L in the 12 to 14 weeks of pregnancy and decrease thereafter to 10 to 200 mg/L at term (78). After birth, circulating concentrations decrease with a half life of 5 days, falling to adult levels at 8 to 10 months of age (79, 80). The high values that are normal in early childhood must be remembered when using AFP as a marker for testicular yolk sac tumors, which is the most common testicular neoplasm in infants (81, 82).

Assay methods, standardization, and reference values. AFP is quantified by two-site immunometric assays employing monoclonal antibodies or combinations of monoclonal and polyclonal antibodies. Results are generally comparable to those obtained

with the competitive radioimmunoassay (RIA) format used previously. The WHO standard 72/225, in which one International Unit (U) of AFP corresponds to 1.21 ng, is used for calibration. Laboratories report values in mass units (ng/ml or μg/L) or kU/L. Reference values should be established for each assay to reflect differences in assay bias. Most centers quote an upper reference limit for AFP in the range of 10 to 15 μg/L. Circulating concentrations increase slightly with age: in one study the upper reference limit increased from 9.3 kU/L in subjects younger than 40 years to 12.6 kU/L in those older than 40 (83).

False positive results. Rising levels of serum AFP indicate persistent germ cell tumors, even in the absence of radiographic evidence of disease, provided other possible causes can be excluded (see below) (4). Moderately elevated AFP levels may persist even after chemotherapy, particularly when persistent disease has a large cystic component, serving as a reservoir leaking AFP into the circulation (84). Elevated serum concentrations of AFP occur in most hepatocellular carcinomas and 10% to 30% of other gastrointestinal cancers, but these diseases are rare in patients with testicular cancer. Elevated AFP values may not reflect cancer, and it is therefore important to identify positive results caused by other diseases and by non-specific interference. Benign liver disease, in particular hepatitis, and liver damage induced by chemotherapy are often associated with moderately elevated serum AFP levels, and may result in misinterpretation especially if levels are rising (85, 86).

The carbohydrate composition of AFP derived from the liver and the yolk sac are different (87). Lectin binding can differentiate increased levels caused by testicular cancer and liver disease (88), but such methods are not routinely used. Patients who initially have elevated AFP levels may have normal levels during a relapse if therapy has eliminated AFP-producing elements but not all other components (89). Moderately elevated values that remain stable do not usually indicate relapse (86).

NACB Testicular Cancer Panel Recommendation 5: Analytical Requirements for Measurement of AFP

AFP methods should be calibrated against WHO Standard 72/225 and the units in which results are reported (μg/L or kU/L) clearly stated. The detection limit for AFP assays should be ≥ 1 μg/L (ie, ≤ 1.2 kU/L). Reference values should be established to reflect method bias. AFP may be raised due to benign diseases, malignancies other than testicular cancer, or nonspecific interferences and these possibilities must be considered when interpreting results [LOE, not applicable; SOR, A].

hCG and hCGβ

Biochemistry and biology. hCG is a member of the glycoprotein hormone family, which includes luteinizing hormone (LH), follicle stimulating hormone (FSH), and thyroid stimulating hormone (TSH). All four contain a common α subunit. The distinct β subunits confer biological activity and display various degrees of homology, with that between the β subunits of LH (LHβ) and hCG (hCGβ) at about 80%. hCGβ contains a 24-amino acid C-terminal extension not present in LHβ so antibodies to this part of the molecule are specific for hCG. While the subunits lack hCG activity, hCGβ has been shown to enhance the growth of tumor cells in culture by preventing apoptosis (90). hCG is expressed at very high concentrations by the placenta and trophoblastic tumors including choriocarcinoma of the testis. hCG is heavily glycosylated, hCGβ containing 6 and hCGα 2 carbohydrate chains. The glycosylation of hCG secreted by tumors is often different from that of pregnancy hCG. An antibody, B152, detects only a hyperglycosylated variant of hCG. This form predominates in early pregnancy and is possibly more cancer specific than "normal" hCG (91).

Nomenclature, assay methods, standardization, and reference values. Specific determination of hCG is based on antibodies reacting with hCGβ (92). This has caused confusion in the nomenclature of hCG assays—the expressions "β-hCG" or "hCG-beta assay" may denote assays measuring both hCG and hCGβ or only hCGβ. According to the nomenclature recommended by the International Federation of Clinical Chemistry (IFCC), hCG denotes the intact αβ heterodimer, hCGβ the free β subunit and hCGα the free α subunit (93). Assays should be defined according to what they measure (ie, hCG and hCGβ separately or hCG and hCGβ together (64, 94)).

Assays for hCG are currently calibrated against the Fourth International Standards (IS 75/589), in which concentrations are expressed in U based on bioactivity. However, it is difficult to compare concentrations of hCG with those of hCGβ and hCGα which are expressed in different arbitrary units of the relevant International Standards (IS 75/551 and IRP 75/569, respectively). Recently established WHO reference reagents have values assigned in molar concentrations, which should facilitate direct comparison of hCG and hCGβ concentrations in the future (93, 95).

As seminomas may produce solely hCGβ and not intact hCG, it is essential that both hCG and hCGβ are measured when monitoring testicular cancer (14, 96). Recommendations about antibody combinations that recognize most important forms of hCG-related isoforms and are appropriate for use in oncology have been published (94). Assays recognizing both hCG and hCGβ often utilize antibodies to epitope on the C-terminal peptides of hCGβ, but the relatively low affinities of these antibodies may limit assay sensitivity (94). Theoretically, it should be possible to improve detection of testicular cancer by using separate assays for hCG and hCGβ (64, 96) but this remains to be confirmed.

hCG is secreted at low levels by the pituitary, producing plasma levels that are measurable by sensitive methods. The serum concentrations may increase with age, particularly in women after menopause (97, 98). For most assays, the upper reference limit of hCG is stated to be 5 to 10 U/L. When determined by ultrasensitive methods, the upper limit for postmenopausal women is 5 U/L while it is 3 U/L in menstruating women The upper reference limit for men younger than 50 years is 0.7 U/L and for men older than 50 years is 2.1 U/L (98).

Cut-off values lower than the commonly used 5 to 10 U/L can be used to diagnose patients with testicular cancer. However, although most men with testicular cancer are young, their hCG levels may be increased due to testicular malfunction. Therefore, diagnosis of active disease in a patient with a history of a germ cell tumor requires sequential determinations and rising values. The detection limit of most commercial assays does not allow reliable measurement of levels below 5 U/L and the utility of ultrasensitive assays and lower cut-off values needs to be determined (64). When expressed in molar concentrations, 5 U/L of hCG corresponds to 15 pmol/L. The upper reference limit for hCGβ is 2 pmol/L and is independent of age and sex (98).

Specificity and confounding factors. It is important to note that chemotherapy often causes gonadal suppression that increases the hCG levels. Such hypogonadism can also be spontaneous. This can be confirmed by measurement of serum LH and FSH and, when necessary, suppression with testosterone replacement (99). Therefore, levels increasing from below 2 up to 5 to 8 U/L during chemotherapy are often iatrogenic and do not necessarily indicate relapse. Moderately elevated levels of hCG may be of pituitary origin, especially if accompanying serum levels of LH and FSH exceed 30 to 50 U/L and are attributed to interrupted feedback inhibition from the gonads. This can be confirmed by short-term testosterone treatment, which suppresses pituitary secretion of hCG (100, 101).

Nontrophoblastic tumors may in extremely rare cases produce hCG, whereas hCGβ is often expressed at moderate levels by a large variety of tumors, including ovarian, gastrointestinal, bladder, lung, and head and neck cancers (101). Some patients with such tumors will have elevated hCG levels when measurement is carried out by an assay recognizing both hCG and hCGβ.

Falsely elevated results for serum hCG can be caused by heterophilic antibodies. This has only been reported in women (102) but there is no reason why it should not also occur in men. False-positive results can be identified by analysis of hCG in urine or by repeating the assay after adding a blocking agent (eg, non-immune mouse IgG) to the sample to block the interference (64, 102).

Apparently false-negative results will be obtained with assays measuring only hCG if the tumor produces hCGβ but not hCG. While more common in seminoma (103) it may also occur in NSGCT patients (104).

NACB Testicular Cancer Panel Recommendation 6: Analytical Requirements for Measurement of hCG

It is essential that both hCG and its free β subunit (hCGβ) be measured when using hCG to monitor testicular cancer patients, either using a method recognizing a broad spectrum of hCG-related isoforms or separate specific assays. hCG and hCGβ should be recognized on an equimolar basis with a detection limit of ≥ 1 U/L. IFCC hCG nomenclature should be used to describe the method used. The possibility of interferences (eg, from heterophilic antibodies) and transient increases (eg, due to chemotherapy) must be considered when interpreting hCG results [LOE, not applicable; SOR, A].

LDH

Biochemistry and biology. LDH in the circulation exists as a tetramer that may contain various combinations of two subunits, LDH-A and LDH-B. The various subunits can combine in five isoenzymes, LDH-1 [consisting of four B subunits (B_4)], LDH-2 (B_3A_1), LDH-3 (B_2A_2), LDH-4 (B_1A_3) and LDH-5 (A_4). The gene encoding LDH-A is located on chromosome 11 while the gene for LDH-B is located on the short arm of chromosome 12 (ie, *12p*) (105). Interestingly, all invasive seminomas and NSGCTs show additional copies of this chromosomal arm (106), suggesting that it may play a role in disease progression. No gain of 12p is detected in ITGCNU (107, 108). A correlation between copy number of 12p, tumor invasiveness, and the serum level of LDH-1 has been reported, but thus far the relevant *12p* genes have not been identified (109). While theoretically interesting, these findings need to be confirmed.

Specificity and confounding factors. Serum concentrations of LDH are measured enzymatically and the values are method dependent. The degree of elevation is therefore most conveniently expressed relative to the upper reference limit. LDH-1 can be determined by zymography or by immunoprecipitation of the other isoenzymes and determination of residual catalytic activity. LDH is expressed in many tissues and elevated levels may be caused by a wide variety of diseases. Despite its lack of specificity, LDH is a useful marker, especially for staging of seminoma and NSGCT (108). Hemolysis may cause falsely elevated values and should be avoided.

NACB Testicular Cancer Panel Recommendation 7: Analytical Requirements for Measurement of LDH

As LDH is measured enzymatically and the values are method dependent, the degree of elevation should be expressed relative to the appropriate upper reference limit. Care must be taken to avoid hemolysis, which may cause falsely elevated values [LOE, not applicable; SOR, A].

Placental Alkaline Phosphatase

Biochemistry and biology. A tumor-associated isoenzyme of alkaline phosphatase was first described in a patient with lung cancer and later detected in serum of patients with other cancers and identified as placental alkaline phosphatase (PLAP) (110). In fact, two genes encode the proteins detected as PLAP activity (ie, PLAP and germ cell [GCAP] enzymes). Both genes map to chromosome 2 and the proteins cannot be distinguished from each other using routine enzymatic or immunohistochemical methods (111). PLAP is elevated most frequently in patients with seminoma (60% to 70%) (112, 113), and less frequently in those with other germ cell tumors, including ITGCNU (24). An enzymatic method can be used to detect ITGCNU cells in frozen tissue sections (114).

Assay methods, standardization, and reference values. PLAP has usually been determined by zymography but it can be also be measured by immunoassay or enzymatically after immunocapture (113). The result should be compared with locally

determined reference values. Because of homology with other alkaline phosphatase isoenzymes, antibody selection is critical. However, the antibodies available so far cannot distinguish between the PLAP and GCAP isozymes. Therefore, PLAP denotes both of these isozymes.

Specificity and confounding factors. Serum concentrations of PLAP are increased up to 10-fold in smokers and its measurement is therefore of little value in this group (113). This and the paucity of commercial assays limit its clinical application and serum assays for PLAP are not routinely included in the diagnostic work up of testicular cancer patients.

Other Markers

Although pregnancy-specific beta-1 glycoprotein (or SP1) and hCG are both expressed in trophoblastic cells, hCG is the superior marker (115). Consequently, SP1 is not routinely measured. Neuron-specific enolase (NSE) is elevated in about 30% to 50% of patients with seminomas and less often in NSGCT patients (16, 116, 117), but in spite of these promising results the use of NSE is limited.

KEY POINTS: TUMOR MARKERS IN TESTICULAR CANCER

Tumor markers are of central importance in the diagnosis, staging, risk assessment and monitoring of patients with testicular cancer. Several serum markers have been described but only AFP, hCG, and LDH have been thoroughly validated and shown to have independent prognostic value. Several tissue markers may prove to be clinically important in the diagnosis and classification of testicular germ cell tumors. Germ cell tumors also display typical chromosomal abnormalities and amplification of 12p is sufficiently characteristic to be useful in the clinic to identify extratesticular germ cell tumors. Developments in DNA-based diagnostics have revealed a number of changes that may in the future enable more accurate stratification of prognosis.

Chapter **3**

Tumor Markers in Prostate Cancer

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BACKGROUND

Prostate cancer is the most common cancer in men in the United States. In 2007, 218,890 new cases and 27,050 deaths were predicted. While prostate cancer is unequivocally lethal in some patients, most men die with, rather than of, their cancer (118). Autopsy data suggest that 42% of men older than 50 years have cancerous foci in their prostates but only approximately 16% of men will be diagnosed as having prostate cancer during their lifetime and only one fourth of these will die from it. Many more men die with, rather than of, prostate cancer. (119). Current incidence rates of clinical disease are 15-fold higher in the United States than in Japan despite similar frequencies of histological cancer. Hence, the far greater prevalence of histological than symptomatic cancer has been cited to support a conservative, non-interventionist approach to this disease. However, once prostate cancer reaches advanced stages either locally or systemically with bone metastases, or becomes refractory to hormone therapy, there is little if any therapeutic means for cure.

The optimal treatment of patients with prostate cancer requires the use of the tumor marker prostate-specific antigen (PSA) in all instances and disease states. The use of PSA-related isoforms is appropriate in certain specific circumstances. Herein we present new NACB guidelines on the use these and other serum-based tumor markers in prostate cancer. A summary of relevant guidelines published by other expert panels on this topic is also provided.

In order to prepare these guidelines, the literature relevant to the use of tumor markers in prostate 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 PROSTATE CANCER

Commercially available PSA markers approved by the US Food and Drug Administration (FDA) for use in the treatment of patients with prostate cancer are listed in Table 6, together with the phase of development for each marker as well as the level of evidence (LOE) for their clinical use (120).

TUMOR MARKERS IN PROSTATE CANCER: NACB RECOMMENDATIONS

Table 7 summarizes the NACB guidelines for the use of PSA markers in prostate cancer together with recommendations from other representative guidelines published on the use of tumor markers in prostate cancer, including recently published recommendations issued by the United Kingdom National Institute for Health and Clinical Excellence (NICE) which has undertaken a systematic review of best available evidence (121). While other markers have been investigated (Table 8), based on currently available evidence, only the use of PSA and its isoforms can be recommended in prostate cancer. Below we present a more detailed discussion of the use of these measurements.

PSA MARKERS IN PATIENT TREATMENT

PSA Markers in the Screening and Early Detection of Prostate Cancer

The widespread measurement of serum PSA is largely responsible for the increased incidence of prostate cancer in the US during the past two decades. As epidemiological data demonstrate both a marked increase in the number of men diagnosed with prostate cancer and a profound migration toward earlier stage disease at the time of diagnosis (122), there is strong evidence in support of the growing concern that such "stage migration" causes overdiagnosis and overtreatment of men with indolent cancer, a condition that may pose little threat to the life or health of the patient (123). Screening with PSA has also been questioned due to poor specificity when serum concentrations are modestly elevated (124). While there is extensive evidence to show that elevations of PSA in serum are exclusively associated with disease conditions in the prostate, they are not cancer specific, occurring also in other conditions, such as benign prostatic hyperplasia and prostatitis. This well-documented lack of specificity of the conventional PSA test even prompted researchers to question whether any association exists between serum PSA levels and prostate cancer (125). In contrast, reports from many other investigators have shown that there is very strong evidence of a very significant association between serum PSA levels and presence or

Table 6. NACB Recommendations for the Clinical Use of PSA Serum Markers in the Management of Prostate Cancer

Marker	Application	NACB Recommendations (2008)	LOE*	Strength of Recommendation**	Reference
PSA	Screening	No	III	B	(136,138,521,522)
	Early detection (with DRE)	Yes	III	B	(136, 183, 521, 522)
	Early Detection: Age-specific reference ranges	No	Expert opinion	B	(146)
	Staging/prognosis	Yes	III	B	(193, 201, 205, 206, 523-526)
	Surveillance/monitoring	Yes	III	B	(527, 528)
% fPSA	Differentiation of prostate cancer from benign prostatic disease when total PSA is between 2-10 μg/L	Yes	III	B	(160, 529)

Abbreviations: NACB, National Academy of Clinical Biochemistry; PSA, prostate-specific antigen; LOE, level of evidence; DRE, digital rectal examination; %fPSA, percent free prostate-specific antigen.

*LOE (120): level 1, evidence from a single, high-powered, prospective, controlled study that is specifically designed to test the marker, or evidence from a meta-analysis, 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 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.

**Strength of recommendation (520): 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 of 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.].

outcome of prostate cancer (126-130). Also, the lack in specificity of the PSA test is less critical in monitoring patients with a prostate cancer diagnosis for whom PSA is the most important marker in evaluating response to therapeutic interventions and in detecting tumor relapse. Although potentially valuable as part of multivariate panels to identify aggressive cancers and/or cancer recurrence, measurement of prostatic acid phosphatase alone does not provide any clinically useful information additional to PSA measurement (131, 132), and therefore is not recommended by the NACB.

NACB Prostate Cancer Panel Recommendation 1: Choice of Tumor Marker for Treatment of Patients With Prostate Cancer

PSA is currently the most useful serum tumor marker in treatment of prostate cancer patients and is required in all states of the disease [LOE, III; SOR, A].

Population-based median levels are lower than 0.6 μg/L for men ≥ 50 years, the vast majority of whom have yet to develop any signs or symptoms of prostate cancer or benign enlargement of the gland (130, 133, 134). The 80th centile is close to 1 μg/L and the 90th centile is about 1.25 μg/L (130). An upper limit of normal according to the 95th percentile for men ≥ 50 years has never been implemented in clinical practice, but would correspond to a PSA level of about 1.5 μg/L. A modest increase in PSA levels in older men reflects a higher frequency of benign prostate conditions at higher age. Population-based demographics of PSA levels for 50 to 70-year-old men show that 8% to 9% of these men have PSA levels ≥ 4.0 μg/L, while 11% to 12% have PSA levels ≥ 3.0 μg/L, and as many as 20% of all men have serum PSA levels ≥ 2.0 μg/L (135).

In men who present with modestly elevated levels of PSA in serum (ie, 4 to 10 μg/L), there is extensive evidence showing that histopathologic examination of tissue harvested by systematic prostate biopsies confirms presence of prostate cancer in 25% to 35% of these men (136, 137). When serum PSA levels rise above 10 μg/L, the cancer-specificity of the test is 40% to 50% or higher. Current recommendations in the United States suggest that most men older than 50 years should have annual prostate cancer screening with PSA and digital rectal examination (DRE), and that men should be advised to have biopsies when the DRE is abnormal or when the PSA level in serum is ≥ 4.0 μg/L (138). The NICE guidelines conclude that the serum PSA level alone is a poor predictor of the presence of prostate cancer and should not automatically lead to a prostate biopsy, particularly as many cancers diagnosed on this basis alone will be of low risk, causing little or no impact on life expectancy (121, 139).

These recommendations all have some limitations, as has recently been discussed (140). The PSA cut-off of ≥ 4.0 μg/L represents a clinical decision limit that was introduced on the basis of a single report evaluating the optimal combination of sensitivity and specificity of the PSA test in a study cohort, and the distribution of values observed in this original study may no longer apply (141). It is debatable whether a PSA cut-point lower than 4 μg/L should be recommended. Also debatable is whether decisions to recommend prostate biopsy should be based solely on a single PSA cut-point value (eg, ≥ 4 μg/L).

Table 7. Recommendations by Different Expert Groups for Use of PSA, Complexed PSA, and Percent Free: Total PSA As Tumor Markers for Prostate Cancer

Marker	Application	ACS (138)	ACP (530)	ASTRO (527)	AUA (528)	EAU (531)	EGTM (148)	ESMO (532)	NACB/ EGTM 2002 (15)	NCCN (533)	USPSTF (534)	NICE 2008 (121, 139)	NACB 2008*
PSA	Screening (with DRE)	**Yes**	**No**[1]	None published	**Yes**	**Yes**	**No**[1]	**No**[2]	**Yes (NACB)**	**Yes**	Insufficient evidence available for men <75 years of age. Screening for men 75 years or older not recommended (535)	Insufficient evidence available	No at present
	Early detection: Age-specific reference ranges	None published	None published	None published	None published	None published	**No**	None published	**Yes (NACB)**	None published	None published	None published	**No**
	Early detection: PSA velocity	None published	None published	None published	None published	None published	None published	None published	None published	**Yes**	None published	**Yes**	**Yes**
	Staging/Prognosis	None published	None published	None published	**Yes**	**Yes**[3]	None published	**Yes**	None published	**Yes**[3]	None published	**Yes**	**Yes**[3]
	Follow-up negative biopsy (with DRE)	None published	None published	None published	None published	None published	None published	None published	None published	**Yes**	None published	**Yes**	**Yes**
	Surveillance/ monitoring	None published	None published	**Yes**[4]	**Yes**	**Yes**	**Yes**	**Yes**	None published	**Yes**	None published	**Yes**	**Yes**
% fPSA5	Differentiation of prostate cancer and benign prostatic disease when total PSA is between 2-10 μg/L	None published	None published	None published	None published	None published	Yes	None published	Yes	Yes	None published	None published	Yes
	Follow-up negative biopsy (with DRE) or patients with increased biopsy risk	None published	None published	None published	None published	None published	None published	None published	None published	**Yes**	None published	None published	**Yes**

*For Strengths of Recommendation, see Table 6.

Abbreviations: ACS, American Cancer Society; ACP, American College of Physicians; ASTRO, American Society for Therapeutic Radiology and Oncology; AUA, American Urological Association; EGTM, DRE, digital rectal examination; European Group on Tumor Markers; ESMO, European Society for Medical Oncology; Ins, Insufficient evidence for recommendation; NACB, National Academy of Clinical Biochemistry; NCCN, National Comprehensive Cancer Network; NICE, National Institute for Health and Clinical Excellence; PCa, Prostate cancer; USPSTF, U.S. Preventive Services Task Force.

[1] Not routinely, individual decision; [2] Except in men with urinary symptoms; [3] As part of nomograms with DRE and biopsy Gleason grade (Partin Tables); [4]Following radiation therapy; [5] In men with a total PSA of 4-10 μg/L and a negative DRE.

Table 8. Biomarkers Currently Being Explored for Prostate Cancer

	Proposed Use or Uses and Comments	Phase of Development	LOE	Reference
Circulating biomarkers				
PSA sub-fractions: complexed PSA, free PSA, proPSA, intact PSA, benign PSA	Absolute concentrations in serum and percentage relative to total PSA may help discriminate between malignancy and benign conditions.	Undergoing evaluation. [Clinical assays in development]	IV, V	(536-538)
Human kallikrein 2 (hK2)	Shares 80% amino acid sequence with PSA and is produced in prostatic epithelium at concentrations 50-100 times less than PSA. Generally elevated in prostate cancer vs BPH, and is more sensitive than PSA at detecting extracapsular extension.	Undergoing evaluation.	IV, V	(538, 539)
Insulin-like growth factor (IGF-1), insulin-like growth factor binding protein (IGFBP-3)	High serum IGF-1 concentrations associated with increased risk for prostate cancer. IGFBP-3 can be detected in tissue with ProstaScint; serum concentrations elevated in prostate cancer; discriminates between cancer and BPH or no disease; also being investigated as a therapeutic target.	Undergoing evaluation.	IV, V	(540, 541)
Molecular urine markers				
PCA3	Prostate-specific gene highly expressed in prostate cancer compared to other genitourinary tissues and non-neoplastic prostatic tissues. Urine assays measure PCA3 mRNA following an attentive DRE; the mRNA is non-coding, no protein products are made.	Undergoing evaluation. [Next generation ASR PCA3 test]	IV, V	(542, 543)
Alpha-methylacyl-CoA racemase (AMACR)	Mitochondrial and peroxisomal enzyme involved in oxidation; over-expressed in prostate cancer; detected in tissue by IHC, and in conjunction with loss of basal cell markers (e.g. basal cytokeratins, p63) can help establish diagnosis of cancer on prostate needle biopsy. Assays to detect a humoral response may supplement PSA screening in identifying significant tumors.	Undergoing evaluation (urine and tissue).	IV, V	(544-548)
Glutathione S-transferase-pi (GSTPi)	Protects cells from oxidative damage; reduced expression in prostate cancer due to hypermethylation of its promoter region; distinguishes between BPH and cancer; methylation status of GSTPi gene promoter quantified in prostatic tissue, cells derived from serum, urine and seminal plasma by PCR.	Undergoing evaluation in a clinical trial	IV, V	(549, 550)
Methylation panel	Hypermethylation of a panel of markers in combination with histology may aid in prostate cancer diagnosis; aberrant methylation profiles in prostate tissue samples correlated with clinicopathological features of poor prognosis.	Undergoing evaluation. [ASR in development].	IV, V	(551, 552)
Telomerase activity	Telomerase activity is detectable in the vast majority of prostate cancers but not in benign prostate tissues. Improved methods of telomerase detection may make this marker useful for early detection of prostate cancer in tissue samples or in urine.	Undergoing evaluation.	IV, V	(553, 554)

Cell/Gene tests				
Circulating prostate cells RT-PCR gene targets PSA, hK2, and PMSA mRNAs	Measurements of the frequency in the shedding of circulating prostate/tumor cells in blood–using RT-PCR assays for PSA-, hK2- and/or PSMA-mRNAs–as a means to define invasive and/or systemic disease stage.	Undergoing evaluation in a clinical trial	IV, V	(536, 555)
PTEN	A lipid phosphatase that functions as a tumor suppressor by inhibiting the phosphatidylinositol 3-kinase/protein kinase B (P13K/Akt) signaling pathway. Gene somatically deleted or mutated in some prostate cancers. Protein can be detected by IHC and decreased levels are associated with higher grade and stage.	Undergoing evaluation	IV, V	(556, 557)
CDKN1B (P27)	Cyclin-dependent kinase inhibitor. Protein decreased in prostate tumor cells and levels correlated with worse outcome.	Undergoing evaluation	IV, V	(558, 559)
Ki-67	Marker of cellular proliferation. Fractions of cells staining positive by IHC associated with worse outcome.	Undergoing evaluation	IV, V	(560)
Chromosome 8p22 loss and 8q24 (C-MYC) gain	Bq24 over-representation, especially in combination with loss of 8q22 using a FISH assay, is associated with prostate cancer progression in men with stage pT2N0M0, pT3N0M0 and pT23N1-3M0 prostate cancers.	Undergoing evaluation	IV, V	(561)
Prostate stem cell antigen (PSCA)	Cell surface protein found primarily in the prostate; increased expression in many higher-grade prostate cancers and most metastatic lesions; correlated with late-stage disease; detection in prostatic tissue via FISH, PCR, IHC.	Undergoing evaluation	IV, V	(562)

NOTE. Table based on Table 3 of the Prostate Cancer Foundation *Report to the Nation on Prostate Cancer* (563)].

Abbreviations: BPH, benign prostatic hyperplasia; FISH, fluorescence in situ hybridization; IHC, immunohistochemistry; LOE, levels of evidence, as defined in Table 6; PCR, polymerase chain reaction.

Lower PSA cut-offs increase the cancer detection rate at the expense of increasing the number of men advised to undergo biopsy. However, it has also been clearly demonstrated that 20% or more of all men who have PSA levels from 2.0 (or 3.0) up to 4.0 μg/L are found to have prostate cancer at biopsy (142, 143). This was confirmed in a recent study, where as many as 15.2% of all 2,950 biopsied men with PSA values < 4.0 μg/L were diagnosed with prostate cancer by biopsy. This study showed that the prevalence of prostate cancer in 62 to 91-year-old men increased from 6.6% in men with PSA between 0 to 0.5 μg/L, 10% between 0.6 to 1.0 μg/L, 17% between 1 to 2 μg/L, up to 23.9% between 2.1 to 3.0 μg/L, and 26.9% between PSA values of 3.1 to 4.0 μg/L (128). Also, the prevalence of high-grade prostate cancer increased with increasing PSA values. Hence, the positive predictive value of the PSA test in terms of biopsy-proven (histological) prostate cancer is similar for men with a PSA value between 2 to 4 μg/L and those with a PSA value between 4 to 10 μg/L (136, 144).

NACB Prostate Cancer Panel Recommendation 2: Clinical Decision Limits

Given the controversy regarding the use of PSA to detect very small tumors, reported benefits arising from lowering the clinical decision limit for biopsy lower than 4 μg/L are too uncertain to mandate any general recommendation. Cut-points lower than the commonly used 4 μg/L limit will increase sensitivity with a concomitant decrease in specificity unless other adjunctive tests or measures are employed to increase specificity. Conversely, use of clinical decision limits for PSA higher than 4.0 μg/L decreases the sensitivity, which results in the missed diagnoses of clinically significant tumors in men who might potentially benefit from early treatment [LOE, not applicable; SOR, B].

The across-the-board recommendation of annual PSA testing for men older than 50 years (138) is overly simplistic, and fails to alter testing frequency based on the individualized risk imparted by previously determined PSA levels. For example, a 55-year-old male with a baseline PSA of 0.4 μg/L is much less likely to develop prostate cancer in the future than a similarly aged man with a baseline PSA of 3.3 μg/L. Stenman et al (126) used frozen serum samples and information from a Health Examination Survey in Finland, and Gann et al (145) used information from the Physicians' Health Study to examine the ability of PSA to identify men who subsequently were or were not clinically diagnosed with prostate cancer. Gann et al's data suggest that men with PSA levels between 2.0 and 3.0 μg/L have 5.5-fold higher relative risk for diagnosis of prostate cancer than men with PSA levels lower than 1.0 μg/L. In the former group, serum PSA levels reached 2 to 3 μg/L on average more than 5 years before the cancer was detected by DRE. Recently, Lilja et al (130) demonstrated a very strong association between PSA levels in blood collected more than 20 years prior to prostate cancer diagnosis and the likelihood of that diagnosis in a large representative population of Swedish men age 44 to 50 years who had not previously been exposed to PSA testing. These data and those reported from others (129) suggest that risk stratification at early middle-age may be important to consider in refining current imperfect early cancer detection strategies. Several additional issues particularly relevant to screening programs are discussed below.

Age-specific reference intervals for PSA. Since serum PSA levels gradually increase with age in men older than 40 years, age-specific reference ranges have been proposed with the expectation that their implementation would increase cancer detection rates in younger men by lowering the cut-point, and would increase specificity in older men by raising the cut-point (146). Although there is no consensus, many experts—including a majority of opinion of the National Comprehensive Cancer Network (NCCN)—favor the use of clinical decision limits lower than 4.0 μg/L for serum PSA in younger men. The NACB, however, is not yet convinced of the net benefit in doing this in the absence of additional test(s) that could significantly increase diagnostic specificity (ie, reduce unnecessary biopsies). At the same time the NACB advises caution in increasing the decision limit higher than 4.0 μg/L, since this could result in failure to diagnose clinically significant tumors in men who might potentially benefit from early treatment (147). Hence, contrary to previously issued recommendations (148), the NACB does not endorse the use of age-specific reference ranges.

NACB Prostate Cancer Panel Recommendation 3: Age-Specific Reference Ranges for PSA

Age-specific reference ranges should not be used for PSA [LOE, expert opinion; SOR B].

Increasing PSA specificity in screening for prostate cancer. The total PSA in circulation roughly corresponds to the sum of circulating free PSA (fPSA) and PSA bound as a stable complex to alpha-1-antichymotrypsin (PSA-ACT). The free fraction constitutes from 5% up to more than 40% of the total (149). Free and bound forms may be selectively detected by commercially available assays without any significant interfering cross-reaction (150). Several composite measures have been proposed to improve the specificity of a single serum total PSA concentration for the early detection of prostate cancer. PSA density (151-153), PSA velocity (154), PSA doubling time (155, 156), and percent fPSA (%fPSA) (157-161) have all been evaluated in this context, but only %fPSA has been widely validated and implemented in clinical practice. Men with benign disease generally present with higher %fPSA than men with prostate cancer (and no benign enlargement). Unfortunately, concurrent benign prostatic enlargement and prostate cancer complicates interpretation of %fPSA data (162). Nevertheless, in a systematic review carried out in 2005 the use of %fPSA has been suggested as a means of decreasing the number of unnecessary biopsies, particularly for men with PSA levels from 4 to 10 μg/L (163). In accord with the conclusions of a recent meta-analysis (164), the current NACB panel and the European Group on Tumor Markers (EGTM) (148) both recommend the use of %fPSA as an aid in

distinguishing men with prostate cancer from men with benign disease in selected high-risk groups, (eg, when total PSA is < 10 μg/L and DRE is negative). In particular, %fPSA may be useful in identifying men who have prostate cancer despite initial negative biopsy findings. In men suspected of being at high risk of harboring malignant disease due to low %fPSA, a cancer diagnosis may become evident after a repeat biopsy. This recommendation is tempered by the need to validate the medical decision limit for each free and total PSA commercial assay combination (165).

NACB Prostate Cancer Panel Recommendation 4: Use of %fPSA in Diagnosis

The use of %fPSA is recommended as an aid in distinguishing men with prostate cancer from men with benign prostatic hypertrophy when the total PSA level in serum is within the range of 4 to 10 μg/L and DRE is negative, most frequently in men undergoing repeat biopsy, in selected high-risk groups and particularly in identifying men who have prostate cancer despite initial negative biopsy findings. The clinical decision limit must be properly validated for each combination of fpSA and total PSA assays [LOE, I; SOR A].

More than 95% of immunodetectable complexed PSA (cPSA) fraction is bound to alpha-1-antichymotrypsin with less than 5% bound to other complex ligands (eg, alpha-1-protease inhibitor (157, 166-168)). PSA bound to alpha-2-macroglobulin is not detected by current immunoassays for PSA. Levels of cPSA in blood can be determined either directly using PSA-ACT assays (157, 158, 169) which first block access to fPSA and then measure levels of cPSA (170), or indirectly by subtracting fPSA from tPSA levels (171) using two assays designed to work together and standardized appropriately. Measurement of cPSA alone provides comparable cancer detection to total PSA, but appears to give somewhat better specificity in a narrow concentration range (172). However, cPSA levels alone cannot achieve specificity similar to that of %fPSA (170).

Guidelines for the Early Detection of Prostate Cancer

The American Cancer Society (ACS) has issued guidelines related to the early detection of prostate cancer. These guidelines recommend an annual screening with DRE and serum PSA measurement beginning at the age of 50 in men at average risk with at least 10 years of life expectancy (138). Although PSA is considered the best biochemical test currently available to detect prostate cancer, a DRE should also be included whenever possible according to the ACS. Screening at earlier age (45 years or even 40 years) is warranted in men with increased risk, including those of African-American descent and those with one or more first-degree relatives with prostate cancer. Both of these groups often develop prostate cancer several years earlier than the general population and also tend to present with a more aggressive type of cancer (173).

The recommended follow-up testing of high-risk individuals initially screened at 40 years of age depends on the PSA result. Those with PSA levels < 1 μg/L would resume testing at 45 years of age, those with levels > 1 but < 2.5 μg/L would be tested annually, while those with levels ≥ 2.5 μg/L would be evaluated further and considered for biopsy (138).

These guidelines do not endorse a general recommendation for mass screening, but support the notion that individual men should be informed of the benefits and limitations of prostate cancer screening prior to making their decision, as for example is recommended in the United Kingdom through the Prostate Cancer Risk Management Programme (174) and by NICE (121, 139, 174). Much greater emphasis than previously is being placed on informed decision making by the individual. This topic has recently been the subject of a systematic review in which PSA decision aids and evaluations were identified and appraised (175). The authors concluded that PSA decision aids improve knowledge about PSA testing at least in the short-term. There are many issues to consider, including the disparity between incidence and mortality associated with prostate cancer, since many more men are diagnosed with prostate cancer than eventually die from it. However, early detection affords the opportunity to detect organ-confined disease when curative treatment is possible. Metastatic disease now constitutes only about 5% of initial diagnoses in the United States, a dramatic fall from the 50% incidence rate of the pre-PSA era (122). Nevertheless there are still many uncertainties concerning treatment of early-stage disease, including the preferred treatment for clinically localized prostate cancer.

Merits of Early Detection of Prostate Cancer

Consequently, there is still considerable debate regarding the merits of early detection of prostate cancer, and not all physician organizations advocate routine screening (176). While the American Urological Association endorses the American Cancer Society policy statement on the early detection of prostate cancer, other organizations differ over the benefit of prostate cancer screening (177, 178). Arguments against screening are based on the fact that there is no conclusive evidence from any randomized trials that early detection and treatment influence overall mortality, while the standard treatments for organ confined prostate cancer are associated with a significant frequency of side effects. Currently, the US Preventive Task Force, the American Academy of Family Physicians, the American College of Physicians, the National Cancer Institute (NCI) and the EGTM do not recommend population-based prostate cancer screening (177, 178). The over-riding concern is that current screening modalities result in overdiagnosis and overtreatment of early-stage disease that may not be clinically significant, as has recently been reviewed (179).

The NACB and the EGTM recommend that widespread implementation of screening for prostate cancer in the general population should await the final outcome of ongoing prospective randomized studies, in particular the European Randomized Screening for Prostate Cancer (ERSPC) trial (180), which are sufficiently powered to establish whether early detection and treatment decreases prostate cancer mortality. The ERSPC has been

underway for 10 years with results expected in 2010 (181). Long term multicenter trials to determine the impact of prostate cancer screening on survival are also ongoing in the United States under the aegis of the NCI and the U.S. Public Health Service (182).

With no clear-cut evidence as yet that prostate cancer screening is of net benefit, proponents of screening have pointed to the association of PSA testing with earlier cancer stage at detection and reduced mortality arising from prostate cancer. Registry data from heavily and sparsely screened male populations in Austria provide a case in point. The expected death rate from prostate cancer (183) declined much more in the Tyrol, a heavily screened section of the country, than in less intensely screened areas (184). The decrease in observed mortality was associated with a shift toward a more favorable stage at diagnosis, in particular an increase in the proportion of organ-confined disease. The inference is that early detection and availability of effective treatment resulted in a corresponding improvement in disease specific survival. A similar trend has been observed in data from the NCI's Surveillance, Epidemiology and End Results program, from a study conducted in Olmsted County, Minnesota, MN (185), and from a comparison of prostate cancer mortality in the United States and the United Kingdom between 1975 and 2004 (186).

Even though recent data suggest that the apparent stage shift to early-stage disease and subsequent treatment of localized prostate cancer detected with PSA has positively influenced mortality rates, it is still an open question whether early detection and therapeutic intervention alters the natural history of the disease, as observed benefits may be the result of selection or lead-time bias(es) (187). The stage at diagnosis may be more dependent on the biological behavior of the tumor (aggressiveness) than on delay in presentation, and early detection may not have a significant impact on mortality. An increase in the proportion of localized prostate cancers that are being treated may account for some of the change in the mortality statistics (181).

Currently there is insufficient evidence either to support or refute the routine use of mass, selective, or opportunistic PSA-based screening, and it is equally unclear whether to advise against the use of PSA-based screening, for which success in reducing prostate cancer mortality has yet to be demonstrated. Currently, no robust evidence from randomised controlled trials is available regarding the impact of screening on quality of life, the disadvantages of screening, or its economic value. Results from two ongoing large-scale multicenter randomized controlled trials that will be available in the next several years are required to make evidence-based decisions regarding prostate cancer screening (188).

NACB Prostate Cancer Panel Recommendation 5: Prostate Cancer Screening

A decision as to whether widespread implementation of PSA screening for prostate cancer in the general population can be recommended must await the outcome of ongoing prospective randomized screening studies (eg, ERSPC trial in Europe) which are due to be completed by 2010 [LOE, III; SOR A].

PSA in Patient Treatment

The optimal treatment of early-stage disease has yet to be established. Treatment options include expectant management (active surveillance or watchful waiting), radical prostatectomy, or radiation therapy (external beam radiation or brachytherapy) (139). Alternative treatment modalities (eg, cryosurgery or high intensity focused ultrasound) await evaluation of their long-term results. Patients with advanced (metastatic) disease are typically offered hormone therapy to deprive the prostate of androgen stimulation. PSA synthesis by differentiated prostate cells is greatly impaired by such treatment and the PSA levels in blood reflect tumor burden differently from before androgen deprivation. When the disease becomes refractory to either first or second line androgen deprivation, patients may be entered into chemotherapy or experimental protocols with various agents (eg, Taxotere; sanofi-aventis, Bridgewater, NJ). The assessment of PSA levels in the blood plays a cardinal role in all aspects of the management of prostate cancer from surveillance to selection of optimal treatment to estimation of prognosis to post-therapeutic monitoring. fPSA measurement has not been shown to offer any advantages over total PSA during the follow-up of prostate cancer (189).

The treatment selected after detecting prostate cancer depends critically on whether the disease is confined to the prostate. Radical prostatectomy is primarily an option for patients with organ-confined disease, although patients with extracapsular disease may also benefit from radical surgery (190). However, the extent of disease is difficult to predict accurately. PSA alone is not informative (191), but in combination with the clinical stage and Gleason score predicts reasonably well the pathological stage of localized prostate cancer. Predictive tables that incorporate these parameters have been published (192-194) and are used by physicians to estimate the probability of organ-confined disease and to determine whether radical prostatectomy is indicated. It is recommended by NICE that urological multidisciplinary teams should assign a risk category to all men with newly diagnosed localised prostate cancer, taking these parameters into account (121, 139).

Assessment of changes of PSA levels with time [PSA velocity (PSAv) or PSA doubling time (PSADT)] was first introduced in 1992 (154), with a rapid increase indicating a higher risk for subsequent development of prostate cancer. It was further suggested in several studies that a more rapid rise in PSA before treatment is correlated with aggressive disease and early recurrence after treatment. In more recent studies reported by D'Amico et al (195, 196), a PSAv of higher than 2.0 μg/L/year measured during the year before diagnosis, was shown to be significantly associated with prostate cancer–specific mortality. Recently, Carter et al reported evidence that PSAv could also be used to predict life-threatening prostate cancer up to 15 years before diagnosis (197). However, to demonstrate that PSAv has important clinical value, it must also be unequivocally shown that a multivariable model that incorporates both PSA and PSAv (eg, addition of PSAv to a model that includes tPSA, age, and date of diagnosis) is superior to the model that uses PSA alone. This level of evidence appears still to be lacking, even in the most recently reported studies on this subject.

After successful surgery, PSA should decrease to undetectable levels (198, 199). Persistently elevated PSA provides evidence of residual disease. However, the converse does not always hold, namely that undetectable PSA postoperatively indicates a surgical cure. Considerable time may elapse before residual disease becomes evident through detectable PSA. Most commonly, residual disease will declare within 3 years of surgery. Up to 20% to 30 % of the men who undergo radical prostatectomy present with residual disease during the first 10 years after surgery.

A rising PSA level after radical prostatectomy is a biochemical sign of recurrent disease that typically predates other signs of progression by many years. However, not all patients with biochemical recurrence will progress to symptoms of clinical disease and metastatic spread in their lifetimes and require treatment (200, 201). Factors reported to predict the time course to the development of metastatic disease include time to biochemical recurrence, tumor grade (Gleason score), and PSADT (156, 161). These parameters can be used to estimate the likelihood of patients remaining free of overt metastatic disease and allow physicians to stratify patients into low-risk and high-risk categories and to make better treatment decisions.

Monitoring response after initial treatment and evaluating outcome during subsequent therapy are significant clinical applications of PSA determinations. Measurement of PSA provides essential information about the efficacy of surgery or radiation therapy, helps establish the possibility of residual disease (local or distant), signals recurrent metastatic disease before it can be detected by other conventional diagnostic procedures, and provides a useful adjunct in the evaluation of therapeutic response.

PSA may provide the earliest measure of treatment efficacy or disease recurrence, and as such influence the patient's perception of well-being. For some patients, it may be most appropriate to stop measuring PSA, particularly if effective alternative treatments to counter adverse findings are not available (148).

PSA Markers in the Post-Treatment Monitoring of Prostate Cancer

After treatment, it is the panel's view that a single PSA measurement at or near the lower detection limit of the assay is not sufficient to diagnose recurrence of prostate cancer. Rising PSA levels demonstrated by repeat or serial measurements provide much more reliable evidence (121, 139, 202). After radical prostatectomy, circulating PSA declines to undetectable levels if the prostate cancer was organ-confined and all residual prostate tissue surgically excised. Sustained detection of PSA suggests either incomplete resection or metastatic deposits. If ultra-sensitive PSA assays are used in this setting, the functional detection limit of the assay should be established and should correspond to the lower reporting limit.

At present, evidence is equivocal regarding the clinical benefit of reporting biochemical recurrence of prostate cancer at PSA levels below 0.4 μg/L (200). Recently, however, salvage radiation therapy after prostatectomy has been shown to yield best results when PSA levels are still very low (≥ 0.5 μg/L) (203). The recurrence limit is less clear after radiation therapy because of the typically slower decline in circulating PSA concentration. The American Society for Therapeutic Radiation and Oncology has defined biochemical recurrence as a rise of 2 μg/L or more above the nadir PSA, after external -beam radiotherapy with or without hormone therapy (204).

Monitoring with PSA after treatment for prostate cancer is a mainstay of clinical practice, although the clinical utility of PSA is variable and depends on the disease stage of the individual patient. As has recently been observed, the lack of high quality information and paucity of clinical trials hampers development of guideline recommendations for prostate cancer, but where implemented, available guidelines are likely to improve prostate cancer outcomes while reducing unnecessary, ineffective, and costly care (140). PSA has high sensitivity for detecting recurrence after radical prostatectomy, but is less sensitive in detecting recurrence after radiation therapy. For monitoring hormone treatment, PSA provides a sensitive tool with which to verify treatment response and detect tumor growth (recurrence). However, in patients with advanced disease who recur during androgen deprivation therapy, PSA has only limited capacity to predict survival outcome.

NACB Prostate Cancer Panel Recommendation 6: Use of PSA in the Post-Treatment Monitoring of Prostate Cancer

PSA is recommended for treatment of patients with prostate cancer to monitor disease status after treatment [LOE, III; SOR, A].

Use of Nomograms Incorporating PSA to Manage Prostate Cancer

Nomograms incorporating one or more factors provide the most accurate means of individualizing therapy and predicting outcome, and reflect the most recent advances in patient treatment (205). Rather than relying on physician experience or general risk assessments of patient populations with similar characteristics, the nomograms assess treatment options or prognosis based on computerized models of Cox proportional hazards regression analysis. Predictive outcomes provided by computer models are not perfect, but nomograms can be extremely useful in assisting with treatment decisions. On occasion, it may be difficult to select the best nomogram when several competing versions apply to the same clinical decision. Kattan and colleagues (205, 206) have developed pre- and post-operative nomograms, incorporating PSA together with Gleason score and other variables, in order to predict disease recurrence after radical prostatectomy.

Pre-Analytical, Analytical, and Post-Analytical Considerations

A number of factors in the pre-analytical, analytical, and post-analytical stages can affect the clinical interpretation of PSA results and must be carefully considered. A number of these

factors were the subject of a systematic review carried out in 2001 (207).

Pre-analytical specimen processing and storage. It is desirable to collect blood prior to any manipulation of the prostate by DRE, cystoscopy, or prostate biopsy (166). If prior collection is not possible, then it is prudent to delay several days after DRE before drawing blood for PSA, although in most men DRE does not cause a clinically relevant change in circulating PSA concentration (166). After prostate biopsy or surgery, the recommended delay is several weeks to permit sufficient time for the PSA-ACT complex to be eliminated from the blood circulation, even though the kidneys rapidly clear from the blood any fPSA that was liberated from the prostate by the procedure (208, 209).

NACB Prostate Cancer Panel Recommendation 7: Pre-Analytical Requirements for PSA: Prostate Manipulation

Blood should be drawn before any manipulation of the prostate and several weeks after resolution of prostatitis [LOE, not applicable ; SOR, B].

In order to eliminate in vitro artifacts, blood should be centrifuged within 3 hours of collection to isolate the serum or plasma (210). Serum and plasma may be kept at refrigerated temperatures for up to 24 hours without loss of PSA. If analysis is delayed longer, then it is vital to store specimens frozen, preferably at or below −30ºC to avoid the eutectic point. Long-term storage at temperatures of at least −70ºC is desirable. Data show that fPSA is more susceptible to loss of immunoreactivity than cPSA (166, 211), and that for fPSA this is slower in plasma than in serum (210).

NACB Prostate Cancer Panel Recommendation 8: Pre-Analytical Requirements for PSA: Sample Handling

Samples should be centrifuged and refrigerated within 3 hours of phlebotomy; this recommendation is particularly relevant for fPSA, which is more labile than total PSA. Samples may be stored at refrigerated temperatures for up to 24 hours, but samples that will not be analyzed within 24 hours of collection should be stored frozen (at least at −20°C and preferable at −30°C or lower). For long-term storage, samples should be frozen at −70°C or lower [LOE, not applicable; SOR, B].

PSA assay standardization. Two reference standards currently are commonly used for PSA assays—those traceable to the WHO international standards and those traceable to the Hybritech standard. Most clinicians assume that all PSA assays give similar test values and that changes in these test values probably are related to pathophysiological changes in prostate glands. It is assumed PSA measurements are consistent between laboratories and between assay manufacturers, but this is not necessarily the case (212). While practice guidelines and disease management strategies vary in terms of what "number" should be used to follow up specific types of patients, these guidelines seldom contain subcategories for various analytic methods.

In practice there are considerable differences between PSA assays. Historically, the Hybritech Tandem-R PSA assay (Hybritech, Inc, San Diego, CA) was the first widely used FDA-approved commercial assay. This assay was standardized using the extinction coefficient for PSA of 1.42 mL/mg/cm reported by Graves et al in 1990 (213). The Hybritech assay was well adopted by the medical community and provided the basis for the traditional 4.0 μg/L upper reference limit (141). The second widely used commercial assay (Abbott IMx; Abbott Laboratories, Chicago, IL) was standardized to harmonize with this initial Hybritech assay and other assays also were closely aligned with these assays (214). However, in 1995, Stamey et al published an article showing that the true extinction coefficient for PSA is 1.84 ± 0.04 mL/mg/cm, based on quantitative amino acid analysis (215). It was suggested that the error in the initial gravimetric analysis was caused by the presence of bound water, salt, or carbohydrate in the lyophilized preparations. The net result of this error is that the initial Hybritech PSA values are about 20% higher than the WHO First International Standard for PSA (IRR 96/670) (216).

The First International Standards for PSA (IRR 96/670) and Free PSA (IRR 96/688) were established in 1999 using the correct extinction coefficient. The two standards contain PSA derived from seminal plasma. IRR 96/670 is a mixture of PSA and ACT in a 90:10 ratio selected to mimic circulating PSA, and IRR 96/688 contains solely free (unbound) PSA. An editorial that accompanied the standardization article entitled "WHO First International Standards for Prostate-Specific Antigen: The Beginning of the End for Assay Discrepancies" concluded that this standard would lead to greater consistency of PSA as manufacturers began to use this material to calibrate PSA assays (217). It is now recommended that PSA assays used in the United Kingdom National Health Service must be accurately calibrated against the appropriate International Standard and must be equimolar (218), with formal arrangements now in place for independent annual confirmation of satisfactory performance. While several studies suggest that between-method comparability has improved since introduction of the International Standards there are still differences in PSA assays, which may lead to clinical misinterpretation if different PSA assays are used when evaluating a particular patient (218-220).

Analytical and reporting concerns. PSA is most frequently used in conjunction with physical examination to screen for prostate cancer. A single positive PSA screen should always be verified, by repeating the PSA measurement in a specimen collected separately, before ordering confirmatory histopathological tissue examination (eg, obtained by biopsy). This may substantially reduce the number of unnecessary biopsies (221). The diagnosis of prostate cancer can only be confirmed by histopathological tissue examination.

Analytical performance should be monitored with quality control material containing PSA at concentrations near clinically relevant decision points. Information on assay characteristics and utility, including the lowest reportable concentration of the assay [often defined as the PSA concentration below

which the analytical coefficient of variation [CV] exceeds 20%] and assay CVs at concentrations corresponding to relevant clinical decision points, should be available to clinicians through laboratory test information sources.

NACB Prostate Cancer Panel Recommendation 9: Analytical Requirements for PSA: Quality Control

The lowest reportable concentration should be determined by the laboratory and reported to physicians. Quality control at these concentrations should be in place [LOE, not applicable; SOR, A].

Biological variability. To interpret PSA data from any individual or serially collected specimens, PSA variability in the blood should also be taken into account (207, 222). The EGTM recently reviewed publications concerning the variability of PSA and reported that a fair estimate of the biological variation of PSA is 20% in men older than 50 years within the PSA concentration range of 0.1 to 20 μg/L (223). In healthy men with PSA concentrations lower than 2 μg/L, biological variation was lower than 14%, while a change of 30% between successive PSA measurements was suggested to be clinically significant (224). In monitoring men with prostate cancer, a critical difference of 50% to 60% has been suggested (225). Taking into account that intraindividual biological variation may range up to 20% and that analytical variation for PSA assays is 5%, it has been suggested that the baseline PSA level has to change by 50% to be significant at $P < .05$ (223).

NACB Prostate Cancer Panel Recommendation 10: Post-Analytical Requirements for PSA: Intraindividual Biological Variation

The contribution of within-individual biological variation must be taken into account when interpreting clinical results [LOE, not applicable; SOR, A].

It is prudent to include with the PSA result a reminder that a single screening blood test result should not be used as the sole evidence of the presence or absence of malignant disease. The laboratory report should include the manufacturer of the PSA assay used, draw attention to any relevant clinical decision limits, and where necessary warn that the results cannot be used interchangeably with those generated by other assays unless the interchange of assay values has previously been validated (212, 220).

NACB Prostate Cancer Panel Recommendation 11: Post-Analytical Requirements for PSA – Information to Be Included on Clinical Reports

Clinical reports should include the name of the assay, relevant clinical decision limits, and a reminder that a single screening blood test result should not be used as the sole evidence of the presence or absence of malignant disease [LOE, not applicable; SOR, A].

FUTURE DEVELOPMENTS

Use of Experimental Assays to Measure Circulating Tumor Cells in Blood to Detect and Assess Progression of (Micro) Metastatic Stages of Prostate Cancer

Assays detecting circulating tumor cells (CTCs) in the peripheral blood have been developed and cleared for clinical use by the FDA to provide prognostic information in women with node-positive breast cancer (226). However, our current ability to detect and profile (micro) metastatic prostate cancer is limited. Multiple techniques have been developed and tested to isolate and characterize CTCs. Reverse transcription polymerase chain reaction (RT-PCR) assays are sensitive and highly specific when the expression of the target gene is limited to the malignant tumor cells. Flow cytometry can be used to detect and verify the identity of the cells as CTCs, but does not allow assessments of morphology and does not discriminate molecular changes at a subcellular level. Immobilization (eg, to magnetic beads) of antibodies to the epithelial cell adhesion molecule (EpCAM) allows enrichment and inspection by microscopy of circulating epithelial derived tumor cells from peripheral blood. A semi-automated system was recently developed, which uses EpCAM antibody-based immunomagnetic capture and staining methods (227). Factors predictive of detection of CTCs in prostate cancer have been reported, and for patients with metastatic prostate cancer, the detection of > 5 CTCs per 7.5 mL of blood predicts shorter progression-free survival and shorter overall survival, with CTC counts found to be more predictive of outcome than standard clinical parameters (228). For prostate cancer, preliminary analysis of the correlation of CTC counts with mRNAs for PSA or prostate specific membrane antigen and available clinical predictors (229) are encouraging but are not yet sufficiently evaluated or validated to warrant recommendations for any use in routine clinical practise.

NACB Prostate Cancer Panel Recommendation 12: Measurement of Circulating Prostate Cancer Cells in Peripheral Blood

While initial results are encouraging, these techniques are not yet sufficiently validated to warrant recommendations their application in routine clinical practice [LOE, IV; SOR, C].

KEY POINTS: TUMOR MARKERS IN PROSTATE CANCER

Measurements of serum PSA markers clearly have an important role in both diagnosis and treatment of patients with prostate cancer. Further improvement in understanding of the natural history of the disease should enable better use of these markers in the future.

Chapter **4**

Tumor Markers in Colorectal Malignancy

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BACKGROUND

Colorectal cancer (CRC) is the third most common cancer, worldwide with an estimated 1 million new cases and half a million deaths each year (230). In the USA, it is also the third most common malignant disease with an estimated 154,000 new cases diagnosed in 2007 (118). Most CRC are detected in the rectum (38%), followed by sigmoid (29%), cecum (15%), transverse colon and flexures (10%). Only approximately 5% are found in the ascending colon and 3% in the descending colon (231).

Symptoms of colon cancer may include intermittent abdominal pain, nausea, vomiting, or bleeding. A palpable mass may be found in patients with right-sided colon cancer. Rectal and rectosigmoid cancer are more likely than colonic cancer to be symptomatic prior to diagnosis as these patients frequently have rectal bleeding. It is important to point out that early colon cancers are rarely symptomatic and that the above-mentioned symptoms are non-specific.

Patient stage at initial diagnosis is the most widely used prognostic indicator for patients with CRC. Although the original Dukes' staging system has been modified several times, the extent of cancer invasion through the bowel wall and extent of regional lymph node invasion is still the mainstay of staging systems. In practice, the most widely used staging system is the TNM system of the International Union Against Cancer (UICC) (232) and the American Joint Committee on Cancer (233). In the TNM system, T refers to the local extent of the untreated primary tumor at the time of initial diagnosis; N refers to the status of the regional lymph nodes, and M refers to the presence of distant metastasis at initial presentation (234).

Although surgery is the first-line treatment for most patients with CRC, some patients with rectal cancer may receive radiation and/or chemotherapy prior to surgery. In 1990, a National Institute of Health (NIH) Consensus Conference recommended that stage III colon cancer patients should be treated with adjuvant chemotherapy (235). A subsequent pooled analysis of patients with stage III CRC confirmed that adjuvant chemotherapy increased both the probability of remaining free of tumor recurrence after 5 years and the probability of surviving for 5 years (236).

The value of adjuvant chemotherapy after resection of stage II (Dukes' B) colon cancer is however, unclear. In 2004, an American Society of Clinical Oncology (ASCO) expert panel recommended that adjuvant chemotherapy should not, in general, be given to patients with stage II colon cancer (237). However, the panel also stated that "there are populations of patients with Stage II disease that could be considered for adjuvant treatment including patients with inadequately sampled nodes, T4 lesions, perforation or poorly differentiated histology" (237).

The 1990 NIH Consensus Conference recommended combined adjuvant chemotherapy and high-dose external-beam radiotherapy for patients with stage II or III rectal cancer (235). Although radiation therapy does not appear to affect overall survival, it decreases local recurrence, which is a cause of considerable morbidity in patients with rectal cancer.

Despite potentially curative surgery, 40% to 50% of patients with CRC develop recurrent or metastatic disease (238). In an attempt to detect these relapses when they are resectable, most patients with either stage II or stage III disease currently undergo follow-up or surveillance. Surveillance strategies may include one or more of the following: clinical examination, radiology (eg, chest X-ray, ultrasound, computed tomography [CT], and magnetic resonance imaging), endoscopy, clinical chemistry testing, and the use of tumor markers.

CRC was one of the first cancers in which a tumor marker (ie, carcinoembryonic antigen [CEA]) was used to aid management. The aim of this Chapter is to present NACB guidelines on the use of CEA, as well as other markers, in the detection and treatment of patients with CRC. In doing so, we also summarize the guidelines from other expert panels on the use of tumor markers in CRC.

In order to prepare these guidelines, the literature relevant to the use of tumor markers in CRC 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 COLORECTAL CANCER

Table 9 lists the most widely investigated tumor markers for colorectal cancer. Also listed is the phase of development of each marker and the LOE for its clinical use.

Table 9. Currently Available Markers for Colorectal Cancer

Cancer Marker	Proposed Use/Uses	Phase of Development	LOE*	Reference
Blood-Based Markers				
CEA	Determining prognosis	Preoperative levels may provide prognostic information but this is rarely used for clinical purposes	III	(239-241)
	Surveillance following curative resection	In clinical use, usually in combination with radiology and clinical history	I	(251-255)
	Monitoring therapy in advanced disease	In clinical use, usually in combination with radiology and clinical history	III	(239-241)
CA19.9	Determining prognosis	Undergoing evaluation	III	(264-269)
	Surveillance following curative resection and monitoring therapy in advanced disease	Undergoing evaluation	IV	(262, 263)
CA 242	Determining prognosis	Undergoing evaluation	III	(270, 271)
TIMP-1	Determining prognosis/Screening high risk populations	Undergoing evaluation	III	(274, 275)
Tissue-Based Markers				
TS	Determining prognosis	Undergoing evaluation, a meta-analysis suggested that high levels of TS predicted poor outcome (279). Assay not standardized	I	(276-279, 564)
	Predicting response to chemotherapy (5-FU) in advanced disease	Undergoing evaluation. High levels may predict lack of response to 5-FU in advanced disease. Some studies suggest that TS should be determined on metastatic site to be treated	III	(276-280, 564)
MSI	Determining prognosis	Undergoing evaluation, a pooled analysis showed that MSI-tumors were associated with a 15% better prognosis compared with MS-stable tumors (285). Overall, data conflicting	I	(282-284, 565)
	Predicting response to chemotherapy	Results conflicting, undergoing further evaluation	III	(284, 285, 565, 566)
DCC/18q phenotype	Determining prognosis	Undergoing evaluation, prognostic value validated in a meta-analysis. Assay not standardized.	I	(286-288)
uPA/PAI-1	Determining prognosis	Undergoing evaluation	III	(289-291)
Ras	Determining prognosis	A pooled analysis showed that a mutant ras gene was weakly prognostic in Dukes' C but not in Dukes' B disease. Unlikely to be used for clinical purposes	I	(292)
	Predicting benefit from therapy	May be of value in predicting benefit from the anti-EGFR antibodies, cetuximab and panitumumab	III	(294-297)
P53	Determining prognosis	A meta-analysis showed that abnormal p53 was weakly associated with poor outcome. Unlikely to be used for clinical purposes	I	(293)

Fecal Markers				
FOBT	Screening asymptomatic populations	Shown in randomized trials that screening with FOBT reduced mortality from CRC. Used for ad hoc CRC screening. Feasibility screening trials underway in a number of countries. Lacks sensitivity for early CRC and advanced adenomas and gives rise to many false-positive results	I	(300, 302-306)
DNA Panels	Screening asymptomatic populations	A large study on asymptomatic subjects showed that a DNA panel was more sensitive than FOBT for detecting both advanced adenomas and invasive CRC (79)	III/IV for most panels. I for a specific panel 28(317)	(313-317)
Genetic Markers				
APC	For identifying subjects at high risk of developing FAP	In clinical use in specialised centers	Expert opinion	(322, 323, 326, 567, 568)
MSI	Pre-screen for HNPCC	In clinical use in specialised centers	III	(322, 323, 567-569)
MLH1/MSH2/ MSH6/PMS2	For identifying subjects at high risk of developing HNPCC	In clinical use in specialised centers	III/IV	(322, 323, 326, 567-569)

Abbreviations: TIMP-1, tissue inhibitor of metalloproteinase type 1; TS, thymidylate synthase: uPA, urokinase plasminogen activator; MSI, microsatellite instability: uPA, urokinase plasminogen activator; PAI, plasminogen activator inhibitor 1; 5-FU, 5-fluorouracil; DCC, deleted in colon cancer; FOBT, fecal occult blood testing; FAP, familial adenomatous polyposis; HNPCC, hereditary non-polyposis colorectal cancer and CRC, colorectal cancer.

*LOE, (120): level 1, evidence from a single, high-powered, prospective, controlled study that is specifically designed to test the marker, or evidence from a meta-analysis, 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 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. [LOE are not included for genetic tests.]

TUMOR MARKERS IN CRC: NACB RECOMMENDATIONS

Table 10 presents a summary of recommendations from representative guidelines published on the use of tumor markers in colorectal cancer. This Table also summarizes the NACB guidelines for the use of markers in this malignancy. Below, we present a more detailed discussion of the most widely investigated markers listed in Table 10.

CEA

CEA in screening. Lack of sensitivity and specificity when combined with the low prevalence of CRC in asymptomatic populations preclude the use of CEA in screening for CRC (239-241). In agreement with ASCO (242-244) and EGTM recommendations (245, 246), the NACB panel states that CEA cannot be used in screening healthy subjects for early CRC.

NACB Colorectal Cancer Panel Recommendation 1: Serum CEA in Screening Healthy Subjects

CEA cannot be used in screening of healthy subjects for early CRC [LOE, IV/V; SOR, A].

CEA in determining prognosis. As mentioned earlier, disease stage at initial diagnosis is universally used to determine prognosis in patients with CRC. Several studies, however, have demonstrated that preoperative concentrations of CEA can also provide prognostic information, which in some situations was found to be independent of stage (239-241, 247). This has been confirmed by two systematic reviews (248, 249). The NACB panel therefore states that preoperative concentrations of CEA might be used in combination with other factors in planning surgical treatment. Preoperative CEA concentrations, however, should not be used at present to select patients for adjuvant therapy. These guidelines are broadly in agreement with those previously published by ASCO and EGTM (242, 244-246).

It is of interest that a College of American Pathologists (CAP) expert panel recently ranked preoperative serum CEA together with TNM stage, regional lymph node metastasis, blood or lymphatic vessel invasion, and residual tumor after surgery with curative intent as a category I prognostic marker for CRC (250). According to the CAP panel, category I prognostic factors are those "definitely proven to be of prognostic importance based on evidence from multiple statistically robust published trials and generally used in patient management".

NACB Colorectal Cancer Panel Recommendation 2: Serum CEA in Prognosis and Prediction

Preoperative CEA concentrations of CEA might be used in combination with other factors in planning surgical treatment. Patients with elevated concentrations of CEA (eg, $>$ 5 μg/L) should be evaluated for the presence of distant metastases [LOE, III; SOR, C]. Preoperative CEA concentrations should not be used at present to select patients for adjuvant chemotherapy [LOE, III; SOR, C].

CEA in post-operative surveillance. The main aims of surveillance after curative resection for CRC are to provide reassurance, address possible complications due to therapy, and identify resectable recurrences or metastases. Six separate meta-analyses have compared outcome in patients with intensive follow-up versus those with minimal or no follow-up (251-256). All studies concluded that the use of an intensive follow-up regime resulted in a modest but statistically significant improved outcome when compared with regimes with minimal follow-up. In one of these meta-analyses, it was shown that only the studies including CEA demonstrated a significant impact on survival (254).

The most recent ASCO guidelines state that CEA should be measured every 3 months in patients with stage II or III CRC for at least 3 years after diagnosis, if the patient is a candidate for surgery or systemic therapy of metastatic disease (244, 257). The NACB panel supports this recommendation.

Although serial measurements of CEA are widely used in surveillance, no agreement exists as to the magnitude of concentration change that constitutes a clinically significant increase in CEA during serial monitoring. According to the EGTM panel, a significant increase in CEA occurs if the elevation is at least 30% over that of the previous value. However, this increase must be confirmed by a second sample taken within 1 month. If this latter sample is also elevated, the patient should undergo further examinations (246). However, this 30% increase has not been clinically validated. Furthermore, it should not be regarded as exclusive. For example, small increases in CEA (eg, 15% to 20%, maintained over at least three successive assays) may also prompt intervention (246). It should also be remembered that low concentrations of CEA concentrations do not necessarily exclude progression, and in patients with clinical symptoms of disease recurrence, additional tests such as CT scan, X-rays, and colonoscopy are required, irrespective of the CEA concentration (246).

NACB Colorectal Cancer Panel Recommendation 3: Serum CEA in Postoperative Surveillance

CEA should be measured every 3 months in patients with stage II or III CRC for at least 3 years after diagnosis, if the patient is a candidate for surgery or systemic therapy of metastatic disease [LOE, I; SOR, A].

CEA in monitoring therapy in advanced disease. The prognosis for patients with advanced CRC has greatly improved in recent years due to the introduction of new cytotoxic agents such as irinotecan and oxaliplatin and monoclonal antibodies, such as bevacuzimab (Avastin; Genentech, South San Francisco, CA) and cetuximab (erlotinib), which have recently been reviewed

Table 10. Recommendations for Use of Markers in Colorectal Cancer by Different Expert Groups

Marker	Application	ASCO (242, 244, 257, 324, 325)*	EGTM (245, 246, 570)	NACB 2002 (15)	ESMO (571-574)	NCCN (575)	ACS (311)	USPSTF (310)	NACB 2008	Strength of recommendation**
CEA	Screening	**No** (257)	**No**	**No**	None published	None published	None published	None published	**No**	**A**
	Determining prognosis	**Yes**, if it could assist in staging or surgical treatment planning (257)	**Yes**	None published	**Yes**	**Yes**, as part of a complete staging work-up	None published	None published	May be combined with other prognostic factors, if this would aid in the planning of surgical treatment	**C**
	Post-operative surveillance	**Yes**, if patient is a candidate for surgery or systemic therapy (257)	**Yes**, for the early detection of liver metastasis	**Yes**, if resection of liver metastasis would be clinically indicated	**Yes**	**Yes**, if the patient is a candidate for aggressive surgical resection, should recurrence be detected	None published	None published	**Yes**, if patients is a suitable candidate for undergoing liver resection or receiving systemic chemotherapy	**A**
	Monitoring advanced disease	**Yes** (257)	**Yes**	**Yes**, especially in metastasis difficult to measure by other means	NR	NR	None published	None published	**Yes**, especially for disease that cannot be evaluated by other modalities	**B**
APC gene	Screening for FAP	See ASCO general guidelines for genetic testing for cancer susceptibility (324, 325)	None published	None published	**Yes**	**Yes**	None published	None published	**Yes**	**B**
MSI	Initial screening test for HNPCC	None published	None published	None published	None published	**Yes**	None published	None published	**Yes**	**B**

(*Continued*)

Table 10. Recommendations for Use of Markers in Colorectal Cancer by Different Expert Groups *(Cont'd)*

Marker	Application	ASCO (242, 244, 257, 324, 325)*	EGTM (245, 246, 570)	NACB 2002 (15)	ESMO (571-574)	NCCN (575)	ACS (311)	USPSTF (310)	NACB 2008	Strength of recommendation**
MMR genes, e.g. MLH1, MSH2, MSH6, PMS2	Screening for HNPCC	See general guidelines for genetic testing for cancer susceptibility (324, 325)	None published	None published	**Yes**	**Yes**	None published	None published	**Yes**	**B**
FOBT	Screening asymptomatic subjects	None published	**Yes**, for subjects ≥ 50 years old	None published	None published	None published	**Yes**, for subjects ≥ 50 years old	**Yes**, for subjects ≥ 50 years old	**Yes**, for subjects ≥ 50 years old	**A**

Abbreviations: ASCO, American Society of Clinical Oncology; EGTM, European Group on Tumor Markers; NACB, National Academy of Clinical Biochemistry; ESMO, European Society of Medical Oncology; AGA, American Gastroenterology Society, ACS, American Cancer Society; NCCN, National Comprehensive Network; USPSTF, US Preventive Services Task Force and NR, no recommendation published; FOBT, fecal occult blood testing and MMR, mis-match repair.

*Ref (325) was a joint study published by ASCO and the Society of Surgical Oncology.

**Strength of Recommendation: A=High [Further research is very unlikely to change our confidence in the estimate of effect]; B=Moderate [Further research is likely to have an important impact on our 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 our confidence in the estimate of effect and is likely to change the estimate; D=Very low [Any estimate of effect is very uncertain].

(258, 259). Indeed, the median survival for patients with metastatic CRC has almost doubled in the past 10 years as a result of these new treatments (258-260). However, because these treatments are potentially toxic as well as expensive, it is important to establish as quickly as possible that they are effective in halting tumor progression.

According to the 2006 ASCO guidelines, CEA is the marker of choice for monitoring metastatic CRC during systemic therapy (244). CEA should be measured at the start of treatment for metastatic disease and every 1 to 3 months during active treatment. Persistently increasing concentrations suggest progressive disease even in the absence of corroborating radiographs (242, 243). In 2003, the EGTM panel recommended that serial CEA concentrations should be measured every 2 to 3 months while patients are receiving systemic therapy (246). Both the ASCO and EGTM guidelines stated that caution should be used when interpreting increasing CEA concentrations during the early phase of systemic treatment (16, 18). This is because certain treatments (eg, 5-fluorouracil and levamisole; oxaliplatin) can cause transient elevations in CEA levels in the absence of disease progression (246).

For monitoring patients with advanced CRC undergoing systemic therapy, the NACB panel recommends that regular CEA determinations should be carried out. In agreement with the ASCO panel (242, 243), a confirmed CEA increase (eg, $>$ 30%) may be regarded as evidence of progressive disease. Of course, it should be established that the increases are not false-positive elevations due to either chemotherapy-mediated release of marker or the development of a benign disease that produces CEA.

NACB Colorectal Cancer Panel Recommendation 4: Serum CEA in Monitoring Patients With Advanced Disease

In patients with advanced CRC undergoing systemic therapy, regular CEA determinations should be carried out. A confirmed CEA increase (eg, $>$ 30%) suggests progressive disease provided the possibility of false-positive elevations can be excluded [LOE, III; SOR, B].

OTHER SERUM MARKERS

CA 19-9

The CA 19-9 assay detects a mucin containing the sialated Lewis-a pentasacharide epitope, fucopentaose II (for review, see (261). CA 19-9 is a less sensitive marker than CEA for CRC (262, 263). Preliminary findings suggest that like CEA, preoperative concentrations of CA 19-9 are also prognostic in patients with CRC (264-268). Based on available data, routine measurement of CA 19-9 cannot be recommended for patients with CRC.

CA 242

The CA 242 assay also detects a mucin-like molecule. Although less sensitive than CEA for CRC, assay of CA 242 may complement CEA in the surveillance of patients with CRC (263, 269). Furthermore, a number of preliminary reports suggest that preoperative concentrations of CA 242 are prognostic in CRC (270, 271). Routine determinations of CA 242 should not be used at present in patients with CRC.

Tissue Inhibitor of Metalloproteinases Type 1

Tissue inhibitor of metalloproteinases type 1 (TIMP-1) is a 25 kDa glycoprotein with multiple activities including inhibition of matrix metalloproteinases, promotion of cell proliferation, and inhibition of apoptosis. Using a research enzyme-linked immunosorbent assay (ELISA), which detects total TIMP-1 (ie, the non-complex form as well as TIMP-1 complexed to matrix metalloproteinases), plasma concentrations of the inhibitor were found to be significantly higher in patients with CRC than in healthy controls, subjects with inflammatory bowel diseases, subjects with adenomas or patients with breast cancer (272, 273). For patients with Dukes' A and B colon cancers, TIMP-1 appeared to be more sensitive than CEA for the detection of cancer (ie, 58% vs 40% at 95% specificity and 56% vs 30% at 98% specificity). For patients with early rectal cancer, TIMP-1 and CEA had similar sensitivity (272). Other studies have shown that preoperative plasma TIMP-1 concentration is an independent prognostic factor in patients with CRC (ie, independent of Dukes' stage and tumor location (274, 275)). Of particular note was the finding that stage II patients with low plasma TIMP-1 concentrations (dichotomized at the 70% percentile) exhibited a survival pattern similar to an age and sex-matched background population.

Although these preliminary findings with TIMP-1 are promising, the marker cannot be recommended at present either for detecting early CRC or for evaluating prognosis in patients with this malignancy.

NACB Colorectal Cancer Panel Recommendation 5: CA19.9, CA 242, and TIMP-1 in CRC

Routine measurement of CA19.9, CA 242, or TIMP-1 is not recommended [LOE, III/IV; SOR, B/C].

TISSUE MARKERS

Several tumor tissue markers have been evaluated for potential prognostic and predictive value in patients with CRC. These include thymidylate synthase (TS) (276-280), microsatellite instability (MSI) (281-285), deleted in colon cancer (DCC) (286-288), urokinase plasminogen activator (uPA)/**plasminogen activator inhibitor 1 (**PAI-1) (289-291), mutant ras (292), and mutant/overexpression of *p53* (293). Based on available evidence, none of these markers can at present be recommended for routinely determining prognosis or for therapy prediction. However, emerging evidence suggests that the presence of wild type k-ras is associated with benefit from the anti-epidermal growth factor receptor (EGFR) antibodies, cetuximab, and panitumumab (294-297).

NACB Colorectal Cancer Panel Recommendation 6: Tissue Markers in CRC

The use of TS, MSI, DCC, uPA, PAI-1, or p53 for determining prognosis or predicting response to therapy is not recommended [LOE, III; SOR, B]. Determination of the mutation status of k-ras may in the future be used for predicting benefit from specific anti-EGFR antibodies.

FECAL MARKERS

The most widely used fecal marker involves testing for occult blood (ie, the fecal occult blood test [FOBT]). Two of the most widely described FOBTs are the guaiac test and the fecal immunochemical test (FIT) (298-301). The guaiac test measures the pseudoperoxidase activity of heme in hemoglobin while the immunochemical test detects human globin. As peroxidase activity is also present in certain fruits and vegetables, intake of these foods may give rise to false-positive results in the guaiac test. Certain medicines such as non-steroidal anti-inflammatory drugs can also interfere with this test. Despite these limitations, a number of large randomized trials have shown that screening with the guaiac test reduced mortality from CRC (302-306).

The efficacy of the FIT in reducing either the incidence or mortality form CRC has not yet been investigated in large population-based studies. However, based on available evidence, it should be at least as accurate if not more accurate than guaiac-based tests, in screening for CRC (298, 301, 307). The advantages of the immunochemical test over the guaiac tests include the following (for review, see (298, 299, 307).

FITs have better sensitivity and specificity; FITs are not affected by diet or medications; some FITs can be automated; evidence suggests that the use of FITs increases patient participation in screening for CRC; FITs can be quantitated, enabling adjustment of sensitivity, specificity, and positivity rates; as digested blood from the upper gastrointestinal tract is not usually detected by FITs, the latter are better for detecting bleeding from the lower gastrointestinal tract.

In agreement with other expert panels (308-310), the NACB recommends that all subjects 50 years or older should undergo screening for CRC. Multiple screening procedures for CRC exist however (306-308), and to date no one procedure has been shown to be significantly superior to the others. The option chosen may therefore depend on availability, personal preference, and risk of developing CRC (311).

According to the NCCN, FOBT should be performed on three successive stools specimens that are obtained while the patient adheres to a prescribed diet (308). This organization specifically recommends the Haemoccult SENSA (Beckman Coulter GmbH, Krefeld, Germany) as the testing method. Both the NCCN and the American Cancer Society recommend against use of FOBT of a specimen obtained at digital rectal examinations (308, 311).

Although screening has been shown to result in reduced mortality from CRC (302-305, 312), it may be associated with certain harmful effects. These include the psychosocial consequence of false-positive results, potential complications of colonoscopy, a false-negative result, or the possibility of overdiagnosis (312). Overdiagnosis could give rise to unnecessary investigations or treatment.

Because of the lack of sensitivity and specificity of FOBT for adenomas and early CRC, a considerable amount of research in recent years has focused on other fecal markers, especially on the genes that undergo mutation during CRC carcinogenesis. Amongst the most widely investigated DNA markers are mutant ras, mutant p53, mutant APC, specific methylated genes, MSI, and long DNA (231, 313-316). Almost all of the studies published to date on fecal DNA markers contained small numbers of patients. After an overview of the literature, Allison and Lawson (298) found that the sensitivities of the different DNA panels for invasive CRC varied from 52% to 98% (mean, 64%) while the specificity varied from 93% to 97% (mean, 95%).

Although most of the studies that evaluated DNA markers for the detection of CRC included only small numbers of patients, a specific panel was recently investigated as a screening test for CRC in a large asymptomatic population (317). Of the 31 invasive CRCs detected, the DNA panel diagnosed 16, whereas FOBT detected only four (51.6% vs 12.9%, $P = .003$). Of the 71 invasive cancers and adenomas with high-grade dysplasia, the DNA panel diagnosed 29, while FOBT detected only 10 ($P < .001$). Although the DNA panel displayed a higher sensitivity than FOBT, clearly neither test detected the majority of advanced adenomas or carcinomas (317). However, as the DNA-based test was superior to FOBT, it might be expected to be at least as good as the latter in reducing mortality from CRC. However, it should be pointed out that compared to FOBTs, measurement of fecal DNA markers is more expensive and technically demanding. Furthermore, it is not clear which combination of DNA markers provides the optimum balance of sensitivity and specificity (231).

One of the main arguments against the use of a DNA panel at present, especially when applied to large populations, is the relative cost vis-à-vis FOBT (318, 319). In 2004, Song et al (318), using a modelling approach, compared the cost-effectiveness of fecal DNA to that of standard CRC screening methods. The main conclusions were as follows:compared with no screening, all screening strategies increased life expectancy at what was regarded as reasonable cost; compared with no screening, the use of fecal DNA testing gained 4,560 life-years per 100,000 persons at an incremental cost of $47,700/life-year gained; the use of colonoscopy and FOBT/flexible sigmoidoscopy were more effective strategies, gaining an incremental 6,190 and 6,270 life-years per 100,000 persons compared to no screening, at incremental costs per life-year gained of $17,010 and $17,000; andall of the conventional approaches gained more life-years at lower cost than fecal DNA testing.

Despite their relatively high costs, the technically demanding nature of the assays, and the fact that these tests have not been validated in a prospective randomized trial, recent joint guidelines from the American Cancer Society, the U.S.

Multi-Society Task Force, and the American College of Radiology state that there is now sufficient data to include fecal DNA "as an acceptable option for CRC screening" (320, 321).

NACB Colorectal Cancer Panel Recommendation 7: Use of Fecal Markers in Screening for CRC

The NACB recommends that all subjects 50 years or older should undergo screening for CRC. As the most effective screening test is unknown, the method chosen is likely to depend on risk of CRC, local availability, and personal preference. Although FOBT is the best-validated stool-based method for screening for CRC [LOE, I; SOR, A], fecal DNA testing may also be an option. Potential harmful consequences of screening include complications due to colonoscopy and treatment, the possibility of over-diagnosis leading to unnecessary examinations, and false-negative and false-positive results.

GENETIC TESTS

For genetic testing for CRC susceptibility (ie, familial adenomatous polyposis coli and hereditary non-polyposis colorectal cancer), the NACB panel supports previously published guidelines (308, 322-326).

NACB Colorectal Cancer Panel Recommendation 8: Genetic Testing for CRC

Screening for genetic susceptibility to CRC should commence with a detailed family history. Prior to undergoing testing, subjects should receive genetic counselling. For subjects with suspected familial adenomatous polyposis, genetic testing can be used both to confirm diagnosis in a suspected proband and to assess risk in pre-symptomatic family members. Provided the mutation responsible for familial adenomatous polyposis within a family is known, testing for *APC* mutations can be considered for at-risk family members. [LOE, expert opinion; SOR, B].

MSI testing and/or IHC for specific mismatch repair enzymes can be used as a prescreen for hereditary non-polyposis CRC. If an individual is found to possess high MSI, genetic testing for mutations in *MLH1*, *MSH2*, *MSH6*, or *PMS2* genes should be carried out [LOE, III/IV; SOR, B].

KEY POINTS: TUMOR MARKERS IN COLORECTAL CANCER

Although many different markers have been evaluated for CRC, only a small number can be recommended for clinical use. These include CEA in the postoperative surveillance of patients that may be suitable candidate for either surgical resection or systemic chemotherapy, FOBT in screening for early CRC in subjects 50 years or older, MSI as a surrogate marker for identifying subjects who should undergo genetic testing for *MLH1/MSH2/MSH6/PMS2* to identify hereditary nonpolyposis colorectal cancer (HNPCC) and adenomatous polyposis coli gene (*APC*) to identify familial adenomatous polyposis. One of the most promising new plasma markers is TIMP-1. As mentioned above, preliminary findings suggest that this marker may be more sensitive than CEA in detecting early CRC as well as being an independent prognostic factor for CRC. These findings now need to be confirmed in large prospective studies. One of the most promising fecal CRC screening tests is a fecal DNA panel (317). This test should be simplified, made available at reduced costs, and subjected to further investigations.

Chapter **5**

Tumor Markers in Breast Cancer

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BACKGROUND

Breast cancer is by far the most common cancers affecting women worldwide with approximately 1 million new cases diagnosed each year (327). In 2007, an estimated 180,000 women were diagnosed with breast cancer in the United States and approximately 41,000 died from the disease (118). Currently, there are more than 2 million women in the United States who are living with a history of breast cancer (328). While the worldwide incidence of the disease appears to be increasing, mortality rates are now declining in a number of Western countries including the United States and the United Kingdom (329).

The main presenting features in women with symptomatic breast cancer include a lump in the breast, nipple change or discharge, and skin contour changes. Definitive diagnosis requires biopsy and histopathology. Currently available blood-based biomarkers are of no value in the early diagnosis of breast cancer.

The primary treatment for localised breast cancer is either breast-conserving surgery and radiation or mastectomy. After primary treatment, most women with invasive breast cancer receive systemic adjuvant therapy such as chemotherapy, hormone therapy, or a combination of chemotherapy and hormone therapy. Both adjuvant chemotherapy and hormone therapy have been shown to reduce systemic recurrence and mortality from breast cancer (330). For example, a meta-analysis of approximately 145,000 women participating in 194 randomized trials of adjuvant systemic therapy concluded that anthracycline-based polychemotherapy reduced the annual breast cancer death rate by about 38% for women younger than 50 years of age when diagnosed and by about 20% for those age 50 to 69 years when diagnosed (330). For estrogen receptor (ER)-positive patients, 5 years of adjuvant tamoxifen reduced annual breast cancer death rates by 31% (330). Patients with ER-negative tumors however, did not benefit from adjuvant tamoxifen (331).

Because not all patients with breast cancer may need adjuvant treatment [eg, approximately, 70% of lymph node-negative patients are cured of their disease by surgery and radiotherapy (332)] and not all patients benefit from this treatment, rational management requires the availability of reliable prognostic and predictive markers. Recommendations regarding the use of currently available prognostic and predictive markers for breast cancer are discussed below.

Subsequent to primary therapy, patients with a diagnosis of breast cancer usually receive follow-up at regular intervals. Historically, surveillance has included clinical history, physical examination, mammography, chest X-ray, biochemical testing, and the use of tumor markers. This practice is based on the assumption that the early detection of recurrent disease leads to a better outcome. However, at present, the clinical benefit of close surveillance is unclear (333).

Although adjuvant therapy improves patient outcome, 25% to 30% of women with lymph node-negative and at least 50% to 60% of those with node-positive disease develop recurrent or metastatic disease (334). Therapy options for metastatic breast cancer include chemotherapy (eg, anthracycline or taxane based), hormone therapy, or targeted therapies, such as trastuzumab (Herceptin; Genentech, South San Francisco, CA), lapatinib, or bevacizumab, alone or combined with chemotherapy (334, 335). Currently, metastatic breast cancer is regarded as incurable and thus the goal of treatment is generally palliative. In this context, the use of serial levels of serum tumor markers is potentially useful in deciding whether to persist in using a particular type of therapy, terminate its use, or switch to an alternative therapy.

Based on the above, it is clear that optimal treatment of patients with breast cancer requires the use of a number of tumor markers. The aim of this Chapter is to present new NACB guidelines on the use of both tissue- and serum-based tumor markers in breast cancer. A summary of guidelines published by other expert panels on this topic is also provided.

In order to prepare these guidelines, the literature relevant to the use of tumor markers in breast 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 BREAST CANCER

Table 11 lists the mostly widely investigated tissue-based and serum-based tumor markers for breast cancer. Also listed is the phase of development of each marker as well as the LOE for its clinical use.

Table 11. Useful and Potentially Useful Markers for Breast Cancer

Cancer Marker	Proposed Use/Uses	Phase of Development	LOE*	Ref
Tissue-Based Markers				
Estrogen receptor (ER)	For predicting response to hormone therapy in both early and advanced breast cancer	In clinical use	I	(330, 331, 576)
	In combination with other factors for assessing prognosis in breast cancer. ER alone is a relatively weak prognostic factor	In clinical use	III	(576, 577)
Progesterone receptors (PR)	Usually combined with ER for predicting response to hormone therapy	In clinical use	I/II	(578, 579)
HER-2	Determining prognosis, most useful in node-positive patients. Conflicting data in node-negative patients	In clinical use in some centers	II-III	(580)
	For selecting patients with either early or metastatic breast cancer for treatment with Trastuzumab (Herceptin)	In clinical use	I	(581-583)
	For predicting resistance to tamoxifen therapy in breast cancer, may be predictive of relative resistance to tamoxifen in patients with early breast cancer	Results conflicting, undergoing further evaluation	III	(348, 349)
	For predicting resistance to CMF in early breast cancer, may be predictive of relative resistance to CMF in patients with early breast cancer	Results conflicting, undergoing further evaluation	III	(348, 349)
	For selecting response to anthracycline-based therapy in early breast cancer, HER-2 may be associated with an enhanced response to anthracycline-based therapy**	Undergoing further evaluation	II/III	(348, 349, 351, 352)
Urokinase plasminogen activator (uPA)	For determining prognosis in breast, cancer, including the subgroup with axillary node-negative disease	Prognostic value validated in both a prospective randomised trial and a pooled-analysis. In clinical use in parts of Europe, e.g. Germany.	I	(361-363)
	For predicting resistance to hormone therapy in advanced breast cancer	Undergoing evaluation	III-IV	(584, 585)
	For predicting enhanced response to chemotherapy in early breast cancer	Undergoing evaluation	III	(364, 365, 586)

PAI-1	Usually assayed in combination with uPA, i.e. for determining prognosis in breast cancer including the subgroup with node-negative disease. Provides prognostic information additional to that of uPA	Prognostic value validated in both a prospective randomised trial and a pooled-analysis. In clinical use in parts of Europe, e.g. Germany.	I	(361-363)
	In combination with uPA may be of value for predicting enhanced response to adjuvant chemotherapy and resistance to hormone therapy in advanced disease	Undergoing further evaluation	III	(364, 365, 584-586)
Cathepsin D	For determining prognosis in breast cancer	Results conflicting. However, using a specific ELISA, most reports show a prognostic value. Prognostic value in node-negative breast cancer validated by meta-analysis. Not in clinical use	I (Only in node-negative disease)	(587-589)
p53	For evaluating prognosis in breast cancer	Results conflicting when p53 protein is determined by IHC. Specific mutations in the p53 gene however, correlate with adverse outcome. Undergoing further evaluation	III (with IHC), I (with mutation testing)	(590, 591)
	For predicting response to chemotherapy or hormone therapy in breast cancer	Results conflicting. Undergoing further evaluation	III	(591, 592)
DNA ploidy/S-phase	For assessing prognosis in breast cancer	Results conflicting. Undergoing further evaluation	III	(593, 594)
Gene expression microarray	For assessing prognosis	Undergoing evaluation. For one of these profiles (62,63), a prospective multicenter validation study is planned	III	(385-389)
Oncotype DX™ (A multiplex RT-PCR assay)	For predicting recurrence in lymph node-negative, ER-positive patients receiving adjuvant tamoxifen. May also predict benefit from adjuvant chemotherapy in node-negative, ER-positive patients	Validated in prospectively designed studies, assay can be carried out on paraffin-embedded tissue. In clinical use. A prospective multicenter validation of the chemopredictive utility is underway	II (for patients receiving adjuvant tamoxifen)	(391-395)

(*Continued*)

Table 11. Useful and Potentially Useful Markers for Breast Cancer (*Cont'd*)

Cancer Marker	Proposed Use/Uses	Phase of Development	LOE*	Ref
Serum-Based Markers				
CA 15-3	Post-operative surveillance in patients with no evidence of disease	In clinical use, but value of changing therapy for patients with rising levels not validated in a high-level evidence study	III	(381, 595)
	Monitoring therapy in advanced disease	In clinical use, but value not validated in a high-level evidence study	III	(381, 595)
	Assessing prognosis. High preoperative levels (e.g. > 30 U/L) predict adverse outcome	Not in clinical use	III	(596-599)
BR 27.29	Provides similar information to CA 15-3 but not as widely investigated as CA 15-3	In clinical use, but value not validated in a high-level evidence study	III	(600, 601)
CEA	Post-operative surveillance in patients with no evidence of disease. Overall, appears to be less sensitive than CA 15-3/BR 27.29	In clinical use, but value not validated in a high-level evidence study	III	(377, 602-604)
	Monitoring therapy in advanced disease, especially if CA 15-3/BR 27.29 is not elevated	In clinical use, but value not validated in a high-level evidence study	III	(377, 602-604)
	Assessing prognosis. High preoperative levels predict adverse outcome	Not in clinical use	III	(596, 598, 604)
TPA	Post-operative surveillance in patients with no evidence of disease	In clinical use in some countries, but value not validated in a high-level evidence study	III	(377, 603)
	Monitoring therapy in advanced disease. May be useful if CA 15-3, BR 27. 29 or CEA are not elevated	In clinical uses in certain countries, but value not validated by a high-level evidence study.		(595, 603)
TPS	As for TPA	As for TPA	III	(605, 606)
HER-2 (shed form)	Determining prognosis; predicting response to hormone therapy, chemotherapy and Trastuzumab; post-operative surveillance and monitoring therapy in advanced disease. Less sensitive than either CA 15-3 or CEA but may be useful in monitoring CA 15-3, BR 27.29 or CEA are not elevated. if Preliminary results suggest that serum HER-2 may be of value in monitoring Trastuzumab therapy in patients with advanced breast cancer	Undergoing evaluation	III-IV	(353, 607)

Proteomics	Detecting early disease and monitoring	Undergoing evaluation, results to date conflicting	IV/V	(608, 609)
Tumor Cells (detected by other than haematoxylin and eosin staining)				
Tumor cells in bone marrow	For assessing prognosis	Prognostic value validated in a pooled analysis. Not in widespread clinical use. Not clear if of value in otherwise favourable prognostic patients	I	(610-612)
Tumor cells in axillary nodes	For assessing prognosis	Most studies conclude that the detection of tumor cells in axillary nodes predicts adverse prognosis but prognostic impact appears relatively weak. Undergoing further evaluation	II-III	(613, 614)
Tumor cells in sentinel lymph nodes	For assessing prognosis	Undergoing evaluation. Two prospective trials are currently in progress	IV/V	(615, 616)
Tumor cells in circulation	For assessing prognosis and monitoring therapy in advanced disease	Undergoing evaluation. Available but not widely used in clinical practice. Prospective randomised trial underway	III	(226, 617, 618)
Genetic Markers				
BRCA1	For identifying individuals who are at high risk of developing breast or ovarian cancer in high risk families	In clinical use in specialised centers	Expert opinion	(324, 347, 382-384)
BRCA2	As for BRCA1	In clinical use in specialised centers	Expert opinion	(324, 347, 382-384)

Abbreviations: TPA, tissue polypeptide antigen; TPS, tissue polypeptide specific-antigen; CMF, cyclophosphamide, methotrexate, 5-fluorouracil; IHC, immunohistochemistry. RT-PCR, reverse transcriptase polymerase reaction.

*LOE (120): level 1, evidence from a single, high-powered, prospective, controlled study that is specifically designed to test the marker, or evidence from a meta-analysis, 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 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.

**This effect may be due to amplification of the topoisomerase IIa gene (619, 620).

Table 12. Recommendations for Use of Markers in Breast Cancer by Different Expert Groups

Marker(s)	Application	ASCO (242, 243, 375)	EGTM (371)	Joint EGTM/ NACB (15)	ESMO (372, 373)	St Gallen Conference (350, 374)	NCCN (621)	NACB 2008	Strength of recommendation*
ER + PR	For predicting response to hormone therapy	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**A** (for ER) **B** (for PR)
	For prognosis	**Should not be used alone in determining prognosis**	**Yes**, in combination with other factors	None published	None published	**Yes**	**Yes**	**Yes**, in combination within existing factors	**B**
HER-2	For predicting response to trastuzumab in early and advanced disease	**Yes**	**Yes**	None published	**Yes**	**Yes**	**Yes**	**Yes**	**A**
	For prognosis	**No**	**Yes**, in combination with other factors	None published	None published	**Yes**	**Yes**	**Yes**, in combination with other factors	**B**
	For predicting response to hormone therapy	**No**	**No**	None published	None published	None published	None published	**No**	**C**
	For predicting response to adjuvant CMF	**No**	**No**	None published	None published	None published	None published	**No**	**C**
	For predicting response to adjuvant anthracycline-based therapy	**Yes**	**Yes**	None published	None published	None published	**Yes**, may be used for predicting superiority of anthracycline-based over non-anthracycline-based adjuvant therapy	**Yes**, as per NCCN	**B**
uPA/PAI-1	For determining prognosis.	**Yes,** may be of value for determining prognosis in newly diagnosed node-negative patients	**Yes**	None published	None published	None published	None published	**Yes**	**A** (if ELISA used for assay)

Oncotype DX test	For determining prognosis	**Yes,** for predicting risk of recurrence in patients treated with adjuvant tamoxifen	**No**	None published	None published	**No**	May be an option in specific subgroups for estimating probability of recurrence and benefit from chemotherapy (622).	**Yes,** for patients treated with adjuvant tamoxifen.	**A**
CA 15-3/ BR27-29	Surveillance following surgery	**No**	**Yes**	**Yes**	**No**	****No**	**No**	May provide lead-time for early detection of metastasis but clinical value of lead-time unclear	**C**
CA 15-3/ BR27.29	Monitoring therapy in advanced disease	**Yes**, in selected cases, e.g., in absence of measurable disease	**Yes**	**Yes**	**Yes**, in non easily measurable disease	None published	None published	**Yes**, especially in patients with non-evaluable disease	**C**
CEA	Surveillance following surgery	**No**	**Yes**	None published	**No**	****No**	None published	**No**	**C**
	Monitoring therapy in advanced disease	**Yes**, in selected cases, e.g., in absence of measurable disease	**Yes**	None published	**No**	None published	None published	**Yes**, as per ASCO and EUSOMA	**C**
BRCA1 BRCA2	For identifying women at high risk of developing breast cancer	See ref. (324) for general guidelines on genetic testing for cancer susceptibility	None published	None published	None published	**Yes**	**Yes**	NACB supports documents of CGSC, ASCO, USPSTF and St Gallen Consensus Group (324, 350, 382-384)	**B**

Abbreviations: ASCO, American Society of Clinical Oncology; EGTM, European Group on Tumor Markers; NACB, National Academy of Clinical Biochemistry; ESMO, European Society of Clinical Oncology; NCCN, National Comprehensive Cancer Network; NR, no recommendation published; CGSC, Cancer Genetics Studies Consortium and US Preventive Services Task Force.

*Strength of Recommendation (520): A = High [Further research is very unlikely to change our confidence in the estimate of effect]; B = Moderate [Further research is likely to have an important impact on our 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 our confidence in the estimate of effect and is likely to change the estimate; D = Very low [Any estimate of effect is very uncertain].

**Recommendations state tumor markers without referring to specific markers.

TUMOR MARKERS IN BREAST CANCER: NACB RECOMMENDATIONS

Table 12 presents a summary of recommendations from various expert panels on the use of tumor markers in breast cancer. This Table also summarizes the NACB guidelines for the use of markers in this malignancy. Below, we present a more detailed discussion on the most clinically useful markers listed in Table 12.

Estrogen and Progesterone Receptors

Routine assay of estrogen receptors (ER; ie, ER-α) and progesterone receptors (PR) on all newly diagnosed breast cancers has been recommended by expert panels of the ASCO, EGTM, European Society of Medical Oncology, and the St Gallen Conference Consensus Panel (Table 12). The NACB panel agrees with these recommendations. The primary purpose of determining ER and PR is to select for likely response to endocrine therapy in patients with either early or advanced breast cancer. In addition, in combination with other factors, ER and PR may also be used for prognostic purposes. However, as predictors of patient outcome, hormone receptors are relatively weak factors and are of little clinical value in lymph node-negative patients. Hormone receptors should therefore not be used alone for determining outcome in breast cancer. However, in combination with established prognostic factors, hormone receptors may be used to predict risk of recurrence. Determination of ER-beta has no clinical application at present.

Recommended Assay for ER and PR

ER (ie, ER-alpha) and PR can be measured by ligand-binding assay, ELISA or immunohistochemistry. The advantages and disadvantages of these different assays are summarized in Table 13. It is important to note that most of the clinical data relating to both ER and PR was derived from biochemical (ligand-binding and ELISA) assays. Some recent reports however, have shown that the immunohistochemical determination of ER provides clinical information at least as powerful as that obtained with the biochemical assays (336-341). Indeed, one report claimed that the use of immunohistochemistry to determine ER was superior to

Table 13. Advantages and Disadvantages of Different Assays for Hormone Receptors

Ligand-Binding Assay	ELISA	Immunohistochemistry
Advantages		
• Quantitative	• Quantitative	• Simple and relatively cheap
• Can determine functionality of receptor with respect to hormone binding	• No radioactivity required	• Can assess tissue architecture, distinguishing invasive, in situ and normal breast tissue
• Can determine K_m of receptor for ligand	• Simpler than ligand binding	• Can use small amounts of tissue including fine needle aspirates and core needle biopsies
• Should be able to detect total ER, i.e. ER-α and ER-β but does not discriminate between the two forms		• Normal breast epithelial cells in adjacent tissue provide an internal positive control, at least for ER
Disadvantages		
• Time-consuming	• Requires large amount of frozen tissue	• Semi-quantitative
• Cumbersome	• Relatively time-consuming	• Interpretation subjective
• Expensive		• Difficult to standardize
• Requires large amount of tumor tissue results		• Different antibodies can give different
• Requires frozen tissue (must be rapidly frozen in liquid nitrogen and maintained at low temperature)		
• Requires radioactivity		
• May yield false negative ER values*		

Abbreviations: ELISA, enzyme-linked immunosorbent assay; ER, estrogen receptor.

*In tumors removed from patients receiving tamoxifen, when endogenous levels of steroid ligand are high, or when insufficient breast cancer is present in the tissue mass.

that of biochemical assays, for predicting response to therapy (336). Compared to ER, fewer data are available on the clinical value of PR, as determined by immunohistochemistry (341-343). As with ER, the predictive power of PR as determined by immunohistochemistry appears to be superior to that obtained using ligand-binding assays (343).

Because of its ease of use and application to a wider range of tumors (eg, small as well as large tumors and paraffin-embedded as well as frozen tissue), the NACB panel recommends the use of IHC for the determination of both ER and PR.

The following points should be kept in mind when determining ER and PR by immunohistochemistry. Immunohistochemical assays used should have been shown to give values that correlate with biochemical assays and should be validated for both predictive and prognostic purposes. Validated antibodies include 6F11 MAb (Novocastra, Burlingame, CA, and Newcastle, UK) or antibody ID5 (Dako, Glostrup, Denmark) for ER and antibody 1A6 (Novocastra), PR88 (Biogenex, Menarini Diagnostics, Finch-Hampstead, Berkshire, UK) or monoclonal antibody 1294 (Dako, Glostrup, Denmark) for PR (336, 337, 343-345). Internal controls should be included in each examination. A tissue control with receptor-positive cancer cells and adjacent benign epithelium has been previously recommended (345). Participation in an External Quality Assessment (EQA) scheme is essential (344, 345). Scoring of stain may be based either on percentage of cells staining or on a combination of percentage of cells staining plus intensity of stain. A semi-quantitative score should be reported rather than a negative or positive value (344, 345). It is important to state that patients with low ER levels (eg, staining in 1% to 10% of the cells) have been reported to respond to endocrine therapy (336). Only nuclear staining should be evaluated The report should mention source of primary antibody as well as type of tissue used (eg, paraffin embedded or frozen) (345).

NACB Breast Cancer Panel Recommendation 1: ER and PR as Predictive and Prognostic Markers

ER and PR should be measured in all patients with breast cancer. The primary purpose of measuring these receptors is to identify patients with breast cancer that can be treated with hormone therapy [LOE, I; SOR, A].

In combination with established prognostic factors (ie, tumor stage, tumor grade, and number of lymph node metastases), ER and PR may also be used for determining short-term prognosis in patients with newly diagnosed breast cancer [LOE, III; SOR, B].

HER-2 (c-erbB-2)

In agreement with the ASCO (243), a joint ASCO/ CAP (346) and NCCN panels (347), the NACB panel also recommends determination of HER-2 on all newly diagnosed patients with invasive breast cancers (Table 12). At present, the primary purpose for determining HER-2 is to select patients who may be treated with trastuzumab in either early or advanced breast cancer. In combination with other factors, HER-2 may also be used to determine prognosis. Insufficient data are currently available to recommend HER-2 for predicting response either to adjuvant endocrine therapy or to cyclophosphamide, methotrexate, and 5-fluorouracil (CMF)-based adjuvant chemotherapy (243, 348-351). However, HER-2 may be used to predict the superiority of anthracycline-based adjuvant chemotherapy over CMF (243, 348-350, 352). Insufficient data are presently available to recommend routine use of serum HER-2 testing. Preliminary findings however, suggest that serum HER-2 may be of value in monitoring patients with advanced breast cancer undergoing treatment with trastuzumab (353).

Recommended Assays for HER-2

Two main types of assay are used to detect HER-2 in breast tumors (ie, IHC and FISH (354-360)). The advantages and disadvantages of these methods are summarized in Table 14 (354-360).

After a systematic review of the literature, a joint ASCO/ CAP panel recently published comprehensive guidelines for HER-2 testing in patients with invasive breast cancer (346). Some of the key conclusions are as follows. As presently performed, approximately 20% of HER-2 testing may be inaccurate. When properly validated assays are used, existing data does not clearly show a superiority for either IHC or FISH for predicting response to trastuzumab. HER-2 should be measured on the invasive component of the breast cancer. Laboratories performing HER-2 assays should show at least 95% concordance with another validated test. Validation of assays or modifications, the use of standard operating procedures and compliance with new testing criteria should be monitored using stringent laboratory accreditation standards, proficiency testing, and competency (346).

The ASCO/CAP panel recommended the following algorithm for defining HER-2 status: HER-2 positivity was defined as IHC staining of 3+ (uniform and intense membrane staining of $>$ 30% of invasive cancer cells), a FISH value $>$ 6 HER-2 gene copies per nucleus, or a FISH ratio (HER-2/CEP 17) of $>$ 2.2 (CEP, centromeric probe for chromosome 17); HER-2 negativity was defined as an IHC score of 0 or 1+, a FISH value of $<$ 4 HER-2 gene copies per nucleus, or a FISH ratio of $<$ 1.8; HER-2 IHC was regarded to be equivocal with a score of 2+ (ie, complete membrane staining that is either non-uniform or weak in intensity but with clear circumferential distribution in at least 10% of cells). The equivocal range for FISH was a HER-2/CEP 17 ratio from 1.8 to 2.2 or an average gene copy number from 4.0 to 6.0 for those assays without an internal probe. For samples with equivocal IHC scores, FISH should be performed. For samples with equivocal FISH results, the test should be either repeated or additional cells counted. The NACB panel supports these recommendations.

Currently, the FDA has approved a number of assays for detecting HER-2 in breast cancer. Two of these assays are based on IHC (Dako Corporation, Carpinteria, CA, and Ventana Medical Systems, Inc, Tucson, AZ) and two on FISH (Ventana Medical Systems, Inc, and Vysis Inc, Downers Grove, IL). Both IHC assays were originally approved for identifying

Table 14. Advantages and Disadvantages of Different Assays for HER-2 Immunohistochemistry

Immunohistochemistry	FISH
Advantages	
• Low cost	• Relatively more objective scoring system and easier to standardize
• Simple	• Provides a more robust signal than immunohistochemistry
• Widely available	
Disadvantages	
• Evaluation is subjective and thus difficult to standardize	• Relatively expensive
• Loss of sensitivity due to antigenic alteration due to fixation	• Less widely available than immunohistochemistry (requires fluorescent microscope)
• Wide variability in sensitivity of different antibodies and different results from the same antibody, depending on staining procedure	• May sometimes be difficult to identify carcinoma in tissues with ductal carcinoma in situ
• Borderline values (eg, 2+) require additional testing	• Requires longer time for scoring than immunohistochemistry • Unable to preserve slide for storage and review • Cut-off to establish critical level of amplification and clinical outcome uncertain

Abbreviation: FISH, fluorescence in situ hybridization.

NOTE. Data summarised from references (354-360).

women with advanced breast cancer for therapy with trastuzumab. The FISH-based tests were originally cleared for the selection of women with node-negative disease at high risk for progression and for response to doxorubicin-based therapy. More recently, these tests have also been approved for selecting women with metastatic breast cancer for treatment with trastuzumab. In 2008, the FDA gave pre-market approval for a new chromogenic in situ hybridization assay (Invitrogen Corporation, Carlsbad, CA) for identifying patients eligible for trastuzumab. A serum based-HER-2 test has been cleared by the FDA for follow-up and monitoring patients with advanced breast cancer (Siemens Healthcare Diagnostics, Deerfield, IL).

NACB Breast Cancer Panel Recommendation 2: HER-2 as a Predictive and Prognostic Marker

HER-2 should be measured all patients with invasive breast cancer. The primary purpose of measuring HER-2 is to select patients with breast cancer that may be treated with trastuzumab [LOE, I; SOR, A].

HER-2 may also identify patients that preferentially benefit from anthracycline-based adjuvant chemotherapy [LOE, II/III; SOR, B].

uPA and PAI-1

Results from a pooled analysis comprising more than 8,000 patients have shown that both uPA and PAI-1 are strong (relative risk $>$ 2) and independent (ie, independent of nodal metastases, tumor size, and hormone receptor status) prognostic factors in breast cancer (361). For axillary node-negative patients, the prognostic impact of these two proteins has been validated using both a randomized prospective trial (Chemo N_0 study) and a pooled analysis of small-scale retrospective and prospective studies (361, 362). uPA and PAI-1 are thus the first biological factors in breast cancer to have their prognostic value validated using level 1 evidence studies (363).

The NACB panel therefore states that testing for uPA and PAI-1 may be carried out to identify lymph node–negative patients that do not need or are unlikely to benefit from adjuvant chemotherapy. Measurement of both proteins should be performed as the information provided by the combination is superior to that from either alone (361, 364). Lymph node–negative patients with low levels of both uPA and PAI-1 have a low risk of disease relapse and thus may be spared from the toxic adverse effects and costs of adjuvant chemotherapy. Lymph node-negative women with high levels of either uPA or PAI-1 should be treated with adjuvant chemotherapy. Indeed, results from the Chemo N_0 trial (362) as well as data from recent large retrospective studies (364, 365) suggest that patients with high levels of uPA/PAI-1 derive an enhanced benefit from adjuvant chemotherapy.

Recommended Assays for uPA and PAI-1

Measurement of both uPA and PAI-1 should be carried out using a validated ELISA. A number of ELISAs have undergone technical validation (366) while some have also been evaluated in an EQA scheme (367). For determining prognosis in breast cancer, the NACB panel recommends use of an ELISA that has been both technically and clinically validated (eg, from American Diagnostic Inc, Stamford, CT). Extraction of tumor tissue with Triton X-100 (Sigma Aldrich, St. Louis, MO) is recommended

(368). It is important to note that in order to perform an ELISA for uPA or PAI-1, a representative piece of fresh (ie, not fixed in formalin) breast tumor (> 200 to 300 mg) must be stored in liquid nitrogen immediately after histological diagnosis.

Recently, a microassay using as little as 100 mg of tumor tissue was described for the measurement of uPA and PAI-1 (369, 370). This assay can also use material from two or three core biopsies or five to 10 90-μm thick cryosections. Although not yet clinically validated, preliminary data showed that uPA and PAI-1 levels in core biopsies correlated well with corresponding levels in surgically removed tissue. As immunohistochemical determination of uPA/PAI-1 has not yet been clinically validated, this methodology cannot be recommended, at present, for the routine determination of these proteins in breast cancer.

NACB Breast Cancer Panel Recommendation 3: uPA and PAI-1 for Determining Prognosis

uPA and PAI-1 may be used to identify lymph node–negative breast cancer patients that do not need or are unlikely to benefit from adjuvant chemotherapy. uPA and PAI-1 should be measured by a validated ELISA using extracts of fresh or freshly frozen tumor [LOE, I; SOR, A].

CA 15-3/BR 27.29

The CA 15-3 and BR 27.29 (also known as CA 27.29) serum assays detect the same antigen (ie, MUC1 protein) and provides similar clinical information. CA 15-3 has however, been more widely investigated than BR 27.29. There are conflicting views about the value of CA 15-3 and BR 27.29 in the postoperative surveillance of asymptomatic patients who have undergone curative surgery for breast cancer (15, 242, 243, 371-375). Although increasing CA 15-3 or BR 27.29 levels can pre-clinically detect distant metastatic disease in approximately 70% of asymptomatic patients, there is no high level evidence study showing that the early diagnosis of progressive disease followed by initiation of therapy positively impacts on either patient survival or quality of life. Furthermore, there is no universally accepted or clinically validated definition of a clinically significant tumor marker increase. A confirmed increase of at least 25% however, is widely interpreted to signify a clinically significant increase.

Based on current evidence, the NACB panel recommends against routine CA 15-3 (or BR 27.29) testing in asymptomatic patients after diagnosis of operable breast cancer. The panel, however, would like to note that there are a number of small studies suggesting that the early initiation of therapy based on increasing serum markers levels can lead to an enhanced outcome (376-378). Although these studies do not provide high-level evidence that early treatment based on rising tumor marker levels positively impacts on patient outcome, some doctors as well as some patients may wish to have serial levels of CA 15-3 (or BR 27.29) determined following primary surgery. The ultimate decision about whether or not to use CA 15-3 (BR 27.29) in this situation must be taken by the doctor in consultation with the patient.

According to both ASCO and NCCN, CA 15-3 (or BR 27.29) should not be used alone for monitoring therapy in advanced disease (242, 243, 347, 375). The EGTM panel recommends that for patients with metastatic disease markers should be determined prior to each course of chemotherapy and at least every 3 months for patients receiving hormone therapy (371).

The NACB panel states that CA 15-3 or BR 27.29 in combination with imaging and clinical examination may be used to monitor chemotherapy in patients with advanced breast cancer. These markers may be particularly helpful in patients with non-assessable disease. In such patients, two successive increases (eg, each > 30%) are likely to indicate progressive disease and may result in cessation of therapy, change in therapy, or entry of patient into clinical trials evaluating new anti-cancer treatments. However, as with markers during postoperative surveillance, there is no universally accepted or clinically validated definition of a clinically significant increase in marker concentration during therapy of advanced disease.

It is important to keep in mind that after the initiation of chemotherapy, a transient increase in serum marker levels may occur (379, 380). Such transient increases or spikes usually subside within 6 to 12 weeks after starting chemotherapy. Increases in markers levels unrelated to tumor progression might also occur as a result of certain benign diseases (381). These increases may be transient or progressive depending on whether the benign disease is short lived or continues to deteriorate.

Recommended Assays for CA 15-3/BR 27.29

The FDA has approved a number of commercially available CA 15-3 and BR 27.29 assays.

NACB Breast Cancer Panel Recommendation 4: CA 15-3 and BR 27.29 in Postoperative Surveillance and Monitoring Therapy in Advanced Disease

CA 15-3 and BR 27.29 should not be routinely used for the early detection of recurrences/metastases in asymptomatic patients with diagnosed breast cancer. However, as some patients, as well as some doctors, may wish to have these measurements, the ultimate decision on whether or not to use CA 15-3 or BR 27.29 must be taken by the doctor in consultation with the patient [LOE, III; SOR, B].

In combination with radiology and clinical examination, CA 15-3 or BR 27.29 may be used to monitor chemotherapy in patients with advanced breast cancer. For patients with non-assessable disease, sustained increases in marker concentrations suggest progressive disease [LOE, III; SOR, B].

CEA

As for CA 15-3 and BR 27.29, the NACB panel does not recommend routine use of CEA in the surveillance of patients with diagnosed breast cancer. For monitoring patients with

advanced disease, CEA should not be used alone. For monitoring patients with non-assessable disease, CEA may occasionally be informative when CA 15-3/BR 27.29 is not. As a marker for breast cancer, CEA is generally less sensitive than CA 15-3/BR 27.29 but on occasion, it can be informative when levels of MUC-1-related markers remain below the cutoff point.

Recommended Assay for CEA

The FDA has approved a number of commercially available CEA assays.

NACB Breast Cancer Panel Recommendation 5: CEA in Postoperative Surveillance and Monitoring of Therapy in Advanced Disease

CEA should not be routinely used for the early detection of recurrences/metastases in patients with diagnosed breast cancer. However, as some patients as well as some doctors may wish to have these measurements, the ultimate decision on whether to use CEA must be taken by the doctor in consultation with the patient [LOE, III; SOR, B].

In conjunction with radiology and clinical examination, CEA may be used to monitor chemotherapy in patients with advanced breast cancer. In patients with non-assessable disease, sustained increases in CEA concentrations suggest progressive disease [LOE, III; SOR, B].

BRCA1 and *BRCA2*

According to the Task Force of the Cancer Genetics Studies Consortium (CGSC), "early breast and ovarian cancer screening are recommended for individuals with BRCA1 mutations and early breast cancer screening for those with BRCA2 mutations" (382). However, No recommendation was made for or against prophylactic surgery (eg, mastectomy or oophorectomy). The guidelines further stated that "these surgeries are an option for mutation carriers, but evidence of benefit is lacking, and case reports have documented the occurrence of cancer following prophylactic surgery. It is recommended that individuals considering genetic testing be counselled regarding the unknown efficacy of measures to reduce risk and that care for individuals with cancer-predisposing mutations be provided whenever possible within the context of research protocols designed to evaluate clinical outcome" (382). It is important to point out that these guidelines were based on expert opinion only.

In 2003, an ASCO panel published a detailed policy statement regarding genetic testing for cancer susceptibility (324). This statement included recommendations in the following areas: indications for genetic testing, regulation of testing, insurance reimbursement, protection from discrimination, confidentiality issues associated with genetic testing, continuing educational challenges, and special research issues surrounding genetic testing of human tissues.

According to the 2005 consensus panel of the 8th St Gallen Conference, treatment decisions for women with mutations in *BRCA1* or *BRCA2* genes "need to include consideration of bilateral mastectomy with plastic surgical reconstruction, prophylactic oophorectomy, chemoprevention and intensified surveillance" (350).

The NACB panel supports the statements published by CGSC, ASCO, US Preventive Services Task Force, and the St Gallen Consensus Panel (324, 350, 382-384).

NACB Breast Cancer Panel Recommendation 6: BRCA1 and BRCA2 Mutation Testing for Identifying Women at High Risk of Developing Breast Cancer

BRCA 1 and *BRCA2* mutation testing may be used for identifying women who are at high risk of developing breast or ovarian cancer in high-risk families. For those with such mutations, screening should begin at 25 to 30 years of age. However, insufficient data exists to recommend a specific surveillance/screening strategy for young women with high risk. Appropriate counseling should be given to any individual considering *BRCA1/2* testing [LOE, expert opinion; SOR, B].

MULTIGENE GENE SIGNATURES

Gene Expression Profiling

Gene expression profiling uses microarray technology to measure the simultaneous expression of thousands of genes. At least eight gene signatures have been described for predicting outcome in patients with breast cancer [for review, see (385)]. Although these signatures contain few genes that overlap, most give similar prognostic information (386).

In one of the first clinical microarray studies, van't Veer et al (387) described a 70-gene signature that correctly predicted the later appearance of distant metastasis in 65 of 78 patients with newly diagnosed lymph node–negative breast patients younger than 55 years who had not received systemic treatment. Application of this signature to an independent set of 19 breast cancers resulted in only two incorrect classifications. This 70-gene signature was subsequently both internally (388) and externally validated (389). In both the internal and external validations studies, the prognostic impact of the gene signature was independent of the conventional prognostic factors for breast cancer.

Currently this 70-gene signature is undergoing prospective validation as part of the Microarray for Node-Negative Disease Avoids Chemotherapy trial (390). The primary objective aim of this trial is to establish if lymph node-negative breast cancer patients with low risk of recurrence based on the above gene signature but at high risk of recurrence based on clinicopathological factors, can be spared adjuvant chemotherapy safely without affecting distant metastasis-free survival.

NACB Breast Cancer Panel Recommendation 7: Gene Expression Profiling, as Determined by Microarray, for Predicting Outcome

None of the microarray-based gene signatures currently available should be routinely used for predicting patient outcome [LOE, III; SOR, B].

Oncotype DX Test

Oncotype DX (Genomic Health, Redwood City, CA) is a multigene assay that quantifies the likelihood of breast cancer recurrence in women with newly diagnosed, early stage breast cancer (for review, see ref (391). Rather than using microarray technology, this test uses RT-PCR to measure the expression 21 genes (16 cancer associated and five control genes). Based on the expression of these genes, a recurrence score (RS) was calculated that predicted low, intermediate and high risk of distant metastasis for ER-positive patients, treated with adjuvant tamoxifen (392). The RS was prospectively validated in an independent population of lymph node–negative ER-positive patients treated with adjuvant tamoxifen, as part of the National Surgical Adjuvant Breast and Bowel Project trial B14 (392). In this validation study, the RS was an independent predictor of patient outcome. The independent prognostic impact of the RS was later confirmed in a population-based case-control study (393). While a low RS predicted good outcome in patients treated with adjuvant tamoxifen, a high RS was found to be associated with favourable outcome in patients treated with either neoadjuvant or adjuvant chemotherapy (394, 395). A particular advantage of this test is that it may be carried out on formal-fixed paraffin-embedded tissue.

Currently, the RS is undergoing prospective validation as part of the Trial Assigning Individualized Options for Treatment trial (396). In this trial, patients with intermediate RS are being randomly assigned to receive hormone therapy alone or hormone therapy plus chemotherapy. The aim is to establish if adjuvant chemotherapy improves survival in the group of patients with the intermediate score. Also, in this trial, patients with low RS after tamoxifen therapy will receive endocrine treatment while those with high RS will be given chemotherapy and hormone therapy.

NACB Breast Cancer Panel Recommendation 8: Oncotype DX Test for Predicting Outcome

The Oncotype DX test may be used for predicting recurrence in lymph node–negative, ER-positive patients receiving adjuvant tamoxifen. Patients predicted to have a good outcome may be able to avoid having to undergo treatment with adjuvant chemotherapy [LOE, I/II; SOR, A].

The Oncotype DX test may also be used to predict benefit from adjuvant chemotherapy (CMF or methotrexate and 5-fluorouracil) in node-negative, ER-positive patients (ie, patients with high recurrence score appear to derive greater benefit from chemotherapy than those with low scores) [LOE, III; SOR, B].

KEY POINTS: TUMOR MARKERS IN BREAST CANCER

The best-validated markers in breast cancer are all tissue based and include ER, PR, HER-2, uPA, and PAI-1. Assay of ER, PR, and HER-2 is now mandatory for all newly diagnosed breast cancer patients. The measurement of uPA and PAI-1, although technically and clinically validated (361-363, 366, 367), is not presently in widespread clinical use, mainly due to the requirement of a minimum amount of fresh or freshly frozen tissue. Assay of these proteins however, may be used to aid the selection of lymph node–negative breast cancer patients who do not need adjuvant chemotherapy. Similarly, the Oncotype DX test may be used for predicting recurrence in lymph node–negative, ER-positive patients receiving adjuvant tamoxifen. Although widely used in postoperative surveillance and monitoring therapy in advanced disease, the clinical value of CA 15-3, and other serum markers has not yet been validated by a level I evidence study.

Chapter **6**

Tumor Markers in Ovarian Cancer

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BACKGROUND

In the United States, ovarian cancer is among the top four most lethal malignant diseases in women, who have a lifetime probability of developing the disease of 1 in 59 (397). Worldwide, the incidence of ovarian cancer was estimated in as 204,499 cases per year with corresponding 124,860 deaths (398).

The overall mortality of ovarian cancer is still poor despite new chemotherapeutic agents, which have significantly improved the 5-year survival rate (118). The main reason is lack of success in diagnosing ovarian cancer at an early stage, as the great majority of patients with advanced stage of ovarian carcinoma die of the disease. In contrast, if ovarian cancer is detected early, 90% of those with well-differentiated disease confined to the ovary survive. Furthermore, biomarkers that can reliably predict clinical behavior and response to treatment are generally lacking. The search for tumor markers for the early detection and outcome prediction of ovarian carcinoma is therefore of profound importance and represents one of the critical subjects in the study of ovarian cancer.

Although ovarian cancer is often considered to be a single disease, it is composed of several related but distinct tumor categories including surface epithelial tumors, sex-cord stromal tumors, germ cell tumors (399). Within each category, there are several histological subtypes. Of these, epithelial tumors (carcinomas) are the most common and are divided, according to Federation of Gynecology and Obstetrics (FIGO) and WHO classifications, into five histologic types: serous, mucinous, endometrioid, clear cell, and transitional (400). The different types of ovarian cancers are not only histologically distinct but are characterized by different clinical behavior, tumorigenesis, and pattern of gene expression. Based on prevalence and mortality, the serous carcinoma is the most important, representing the majority of all primary ovarian carcinomas with a dismal clinical outcome (401). Therefore, unless otherwise specified, serous carcinoma is what is generally thought of as ovarian cancer.

The search for more effective biomarkers depends on a better understanding of the pathogenesis of ovarian cancer (ie, the molecular events in its development). Based on a review of recent clinicopathological and molecular studies, a model for the development of ovarian carcinomas has been proposed (402). In this model, surface epithelial tumors are divided into two broad categories designated type I and type II tumors which correspond to two main pathways of tumorigenesis. Type I tumors tend to be low-grade neoplasms that arise in a stepwise fashion from borderline tumors whereas type II tumors are high-grade neoplasms for which morphologically recognizable precursor lesions have not been identified, so-called "de novo" development. As serous tumors are the most common surface epithelial tumors, low-grade serous carcinoma is the prototypic type I tumor and high-grade serous carcinoma is the prototypic type II tumor. In addition to low-grade serous carcinomas, type I tumors are composed of mucinous carcinomas, endometrioid carcinomas, malignant Brenner tumors, and clear cell carcinomas. Type I tumors are associated with distinct molecular changes that are rarely found in type II tumors, such as *BRAF* and *KRAS* mutations for serous tumors, *KRAS* mutations for mucinous tumors, and β-catenin, *PTEN* mutations, and MSI for endometrioid tumors. Type II tumors include high-grade serous carcinoma, malignant mixed mesodermal tumors (carcinosarcoma), and undifferentiated carcinoma. There are very limited data on the molecular alterations associated with type II tumors, except frequent *p53* mutations in high-grade serous carcinomas and malignant mixed mesodermal tumors (carcinosarcomas). This model of carcinogenesis provides a molecular platform for the discovery of new ovarian cancer markers.

In order to prepare these guidelines, the literature relevant to the use of tumor markers in breast 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 OVARIAN CANCER

The most widely studied ovarian cancer body fluid- and tissue-based tumor markers are listed in Table 15, which also summarizes the phase of development of each marker and the LOE for its clinical use. The LOE grading system is based on a previous report describing the framework to evaluate clinical utility of tumor markers (120). The following discussion focused mainly on CA125, which is the only marker that has been accepted for clinical use in ovarian cancer. The NACB panel does not recommend clinical utilization of other biomarkers in diagnosis, detection, or monitoring of ovarian cancer as all other markers are either in the evaluation phase or in the research/discovery phase.

Table 15. Currently Available Serum Markers for Ovarian Cancer

Cancer marker	Proposed uses	Phase of development	LOE[1]	References
CA125[2]	Differential diagnosis of pelvic masses Monitoring treatment with chemotherapy	Accepted clinical use Accepted clinical use	III I, II	(407)(411) (407)(408)(411)(428)(623)(624)(625)(626)(627)
Her-2/neu	Tissue marker for prognosis prediction and treatment outcome	Evaluation	IV	(628)
Akt-2	Tissue marker for prognosis prediction	Research/discovery	V	(500)
Inhibin	Detection	Evaluation	IV	(506)(507)(508)
HLA-G	Differential diagnosis	Research/discovery	V	(629)
TATI	Tumor monitoring	Research/discovery	IV, V	(480)
CASA	Tumor monitoring, prognosis prediction	Research/discovery	IV	(473)(482)(483)(484)(630)
TPA	Tumor monitoring	Research/discovery	IV	(472)(473)
CEA	Tumor monitoring	Research/discovery	IV	(473)
LPA	Detection	Evaluation	IV, V	(474)(631)
PAI-1	Prognosis prediction	Research/discovery	V	(485)(486)(632)
Interleukin-6	Prognosis prediction	Research/discovery	IV	(487)(488)(489)
Kallikreins 5, 6, 7, 8, 9, 10, 11, 13, 14, 15	Differential diagnosis, tumor monitoring, prognosis prediction	Research/discovery	IV, V	(445)(446)(447)(448)(449)(450)(451)(452)(453)(454)(455)(456)(457)(458)(459)(460)(461)(462)(463)(464)(465)
hCGβcf	Prognosis prediction	Evaluation	III, IV	(491)(492)
Prostasin	Differential diagnosis	Research/Discovery	IV	(470)
Osteopontin	Tumor monitoring	Research/Discovery	III, IV	(468)(469)(633)(634)
HE4[3]	Differential diagnosis of pelvic masses, monitoring therapy	In clinical use in some centers	III, IV	(635)(636)(637)
Mitogen-activated protein kinase	Tissue marker for prognosis prediction	Research/discovery	V	(504)(505)
Insulin-like growth factor binding protein–2 (IGFBP-2)	Prognosis prediction	Research/discovery	IV	(638)
RSF-1	Prognosis prediction	Research/discovery	V	(512)(513)
NAC-1	Prognosis prediction	Research/discovery	V	(516)(518)

[1] LOE (120), level 1, evidence from a single, high-powered, prospective, controlled study that is specifically designed to test the marker, or evidence from a meta-analysis, 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 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.

[2] Refer to Table 16 for additional information.

[3] HE4 was recently cleared by the FDA as an aid for monitoring patients with ovarian cancer.

Table 16. Recommendations for Use of CA125 as a Tumor Marker in Ovarian Cancer by Different Expert Groups

Use	American College of Physicians (405)	EGTM 2005 (404)	ESMO (406)	NACB and EGTM 2002 (15)	NCCN (639)	NIH Panel (408)	NACB 2008		
							Recommendation	LOE*	SOR**
Screening – no family history or other risk factors	**No**	**No**	None published	**No**	None published	**No**	**No**	**III**	**B**
Early detection in hereditary syndromes – with trans-vaginal ultrasound (TVUS)	**No**	**Yes**	None published	**Yes**	None published	**Yes**	**Yes**	**III**	**B**
Differential diagnosis – suspicious pelvic mass	None published	**Yes** [Post-menopausal women only]	None published	**Yes** [Post-menopausal women only]	**Yes**	**Yes** [Post-menopausal women]	**Yes** [Post-menopausal women]	**III/IV**	**A**
Monitoring therapy	None published	**Yes**	**Yes**	**Yes**	**Yes**	None published	**Yes**	**I/II**	**A**
Detection of recurrence	None published	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**III**	**B**
Prognosis	None published	**No**	**Yes**	**Yes**	None published	**Yes**	**Yes**	**III**	**A/B**

Abbreviations: EGTM, European Group on Tumor Markers; ESMO, European Society for Medical Oncology; NACB, National Academy for Clinical Biochemistry; NCCN, National Comprehensive Cancer Network; NIH, National Institutes of Health. Recommendation: Yes or No or None published.

* LOE (120): level 1, evidence from a single, high-powered, prospective, controlled study that is specifically designed to test the marker, or evidence from a meta-analysis, 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 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.

**Strength of recommendation (520): 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 of 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.]

TUMOR MARKERS IN OVARIAN CANCER: NACB RECOMMENDATIONS

Several organizations including the EGTM (403, 404), The American College of Physicians (405), The European Society for Medical Oncology (406), and the NCCN (407) have developed guidelines for the use of CA125 as a tumor marker for ovarian cancer. In addition, an NIH Consensus Conference on screening, prevention, diagnosis, and treatment of ovarian cancer was held in 1994 (408). Recommendations from these groups are summarized in Table 16. The Table also includes previous recommendations from the NACB as well as current recommendations based on the information below and other established guidelines.

CA125

In 1981, Bast et al identified the CA125 antigen with the development of the OC 125 murine monoclonal antibody against cell line OVCA 433, which was derived from a patient with ovarian serous carcinoma (409). The CA125 molecule has since been cloned using a partial cDNA sequence originating from the peptide core of the molecule identified (410). This new mucin molecule has been designated CA125/MUC16 (gene MUC16) and consists of a 156-amino-acid tandem repeat region in the *N*-terminus and a possible transmembrane region and tyrosine phosphorylation site in the *C*-terminus.

The first immunoassay for CA125, commercialized in 1983, used the OC 125 antibody for both capture and detection (411, 412). A second-generation assay (CA125 II) was subsequently developed, incorporating M11 and OC 125 antibodies, which have distinct nonoverlapping epitopes. Assays for CA125 have since been adapted to automated platforms and although the majority of manufacturers quote a similar reference interval, concentrations of CA125 may vary among manufacturers due to differences in calibration, assay design, and reagent specificities. The lack of an international standard for CA125 hampers progress in improving between-method comparability and the clinical and laboratory communities should work toward producing and adopting such a standard. For the present, values from different methods are not interchangeable and patients who are serially monitored should be re-baselined If there is a change in methodology (413). Manufacturers should specify the standard preparation against which their method is calibrated and laboratories should indicate the CA125 method used on their clinical reports.

The cut-off of 35 kU/L for the CA125 and CA125II assays was determined from the distribution of values in healthy individuals to include 99% of normals (414). Values tend to decline with menopause and aging (415). It has recently been reported that CA125II concentrations vary 20% to 50% by race in postmenopausal women, with concentrations in African and Asian women lower than in white women (415). Menstrual cycle variations can also be found (412). Elevated values may be found in 1% to 2% of normal healthy individuals, 5% of those with benign diseases, and 28% of those with non-gynecologic cancers (15, 411, 412).

It is recommended that analysis be performed shortly after prompt centrifugation of the specimen and separation of serum from the clot, and that specimens be stored at either 4°C (1 to 5 days) or –20°C (2 weeks to 3 months) in the short-term or –70°C in the long-term to ensure stability (15). Plasma is an acceptable specimen type for some assays, where indicated by the manufacturer. As in other immunoassays, assay interferences may be observed if heterophilic antibodies are present in the serum, particularly after therapeutic or diagnostic use of monoclonal antibodies.

NACB Ovarian Cancer Panel Recommendation 1: Handling of Specimens for CA125 Determination

Analysis should be performed shortly after prompt centrifugation of the specimen and separation of serum from the clot, and specimens stored at either 4°C (1 to 5 days) or –20°C (2 weeks to 3 months) in the short-term or –70°C in the long-term [LOE, not appliable; SOR, A].

The recommendations of the current NACB panel and other groups with respect to the potential clinical utility for CA125 are summarized in Table 16 and are described below.

Screening/Early Detection

For women with epithelial ovarian cancer, 80% have CA125 levels > 35 kU/L, with elevations of 50% to 60% in clinically detected stage I disease, 90% in stage II, and > 90% in stages III and IV (412, 416). Concentrations correlate with tumor burden and stage. Due to the lack of sensitivity and specificity for a single determination of the marker, CA125 is not recommended for use in screening asymptomatic women by the NACB panel as well as other authoritative organizations (15, 403, 405-408). An NIH Consensus Development Panel has concluded that evidence is not yet available that either CA125 or transvaginal ultrasonography effectively reduce mortality from ovarian cancer (408). However, the same panel did recommend annual CA125 determinations, in addition to pelvic and ultrasound examinations, for women with a history of hereditary ovarian cancer who have an estimated lifetime risk of 40%, as early intervention may be beneficial.

A number of approaches have been proposed to improve the specificity of CA125 for early detection as very high specificity (99.7%) is needed to achieve an acceptable positive predictive value of 10% with a prevalence of disease of 40 per 100,000 in women older than 50 years (417). Strategies have included sequential or two-stage strategies combining CA125 with ultrasound, longitudinal measurements of CA125, and measurement of CA125 in combination with other markers, such as OVX1, M-CSF, or other new biomarkers discovered using proteomic profiling approaches (411, 417-419). In order to evaluate the potential role for CA125 in screening for ovarian cancer in asymptomatic populations, two major prospective randomized trials are currently in progress in the United States (420) and the United Kingdom (421). In total 200,000

women will be randomly assigned to either screening with ultrasound, screening with CA125 plus ultrasound, or no screening. The studies are adequately powered to detect a significant improvement in survival among women screened with serial CA125 measurements and transvaginal sonography.

NACB Ovarian Cancer Panel Recommendation 2: CA125 in Screening

CA125 is not recommended for screening asymptomatic women [LOE, III; SOR, B].

CA125 is recommended, together with trans-vaginal ultrasound, for early detection of ovarian cancer in women with hereditary syndromes as early intervention may be beneficial [LOE, III; SOR B].

Discrimination of Pelvic Masses

In contrast to its use in early detection, CA125 is more widely accepted as an adjunct in distinguishing benign from malignant disease in women, particularly in post-menopausal women presenting with ovarian masses (407, 408, 422), facilitating triage for operations by optimally qualified surgeons. Benign conditions resulting in elevated CA125 levels may be a confounding factor in pre-menopausal women. In the United Kingdom, CA125 measurement is an integral part of the risk of malignancy index (RMI), which forms the basis of patient pathway guidelines for the management of pelvic masses and/or adnexal cysts (423). The RMI is calculated as a product of CA125 concentration multiplied by menopausal status (1 for pre-menopausal and 3 for post-menopausal) multiplied by ultrasound score (0, 1, or 3 depending on ultrasound features). A cut-off of 200 or 250 is frequently used, with patients with scores above this referred to specialist gyna-oncology teams. Sensitivities of 71% to 78% and specificities of 75% to 94% have been reported in other studies (414). Elevated concentrations of CA125 $>$ 95 kU/L in post-menopausal women can discriminate malignant from benign pelvic masses with a positive predictive value of 95% (411). Therefore, based on current evidence, CA125 is recommended as an adjunct in distinguishing benign from malignant pelvic masses, particularly in postmenopausal women. When there is a suspicion of germ cell tumor, particularly in women younger than 40 years or in older women where scan features suggest a germ cell tumor, AFP, and hCG are also important markers for triage, as for testicular germ cell tumors [see Staging, Risk Stratification, and Selection of Therapy section p. 6].

NACB Ovarian Cancer Panel Recommendation 3: CA125 in Discrimination of Pelvic Masses

CA125 is recommended as an adjunct in distinguishing benign from malignant suspicious pelvic masses, particularly in postmenopausal women [LOE, III/IV; SOR, A].

Monitoring Treatment

Serial measurement of CA125 may also play a role in monitoring response to chemotherapy. Declining CA125 concentrations appear to correlate with treatment response even when disease is not detectable by either palpation or imaging. In a meta-analysis, serial CA125 concentrations in 89% of 531 patients correlated with clinical outcome of disease (424-426). There is general consensus among current guidelines in recommending that CA125 be used to monitor therapeutic response but there is no consensus as to how best to define a CA125-based response (404, 427, 428). The Gynecologic Cancer Intergroup (GCIG) defines a response as a reduction of 50% or more in pre-treatment CA125 level that is maintained for at least 28 days (428-431). The pre-treatment sample must be at least twice the upper limit of the reference range, which means that patients with pre-treatment concentrations between the upper limit and twice the upper limit are non-assessable by this criterion. The first sample is recommended within 2 weeks prior to treatment with subsequent samples at 2 to 4 weeks during treatment and at intervals of 2 to 3 weeks during follow-up. The same assay method is required throughout and patients who received immunotherapy (ie, mouse antibodies) cannot be evaluated. In addition to monitoring initial chemotherapeutic regiments, CA125 measurements may be useful in monitoring salvage therapy, because a doubling of values is associated with disease progression and treatment failure in more than 90% of cases (411). However, disease progression may also occur without an increase in CA125, and therefore the presence of tumor should also be assessed by physical examination and imaging (15). Tuxen et al (427) suggested that interpretation of changes in serial CA125 levels should be based on a statistical estimation that takes account both of the analytical variation of the method used and of the normal background intraindividual biological variation of the marker (432, 433). The theoretical background for this statistical procedure has recently been reviewed in detail (434). Serial measurement of CA125 to aid in monitoring response to therapy is a second FDA-indicated use for the marker. Trials currently in progress, including the UK Medical Research Council OV05 trial, have been designed to evaluate the benefit of early chemotherapy for recurrent ovarian cancer, based on a raised CA125 level alone versus chemotherapy based on conventional clinical indicators (435). Pending results of these trials, practice is likely to vary.

NACB Ovarian Cancer Panel Recommendation 4: CA125 in Monitoring Treatment

CA125 measurements may be used to monitor response to chemotherapeutic response. The first sample should be taken within 2 weeks prior to treatment with subsequent samples at 2 to 4 weeks during treatment and at intervals of 2 to 3 weeks during follow-up. The same assay method should be used throughout and patients who received therapy with anti-CA125 antibodies cannot be evaluated [LOE, I/II; SOR, A].

CA125 Measurement Postoperatively: Second-Look Operation

Early studies on CA125 indicated that it was useful postoperatively in predicting the likelihood that tumor would be found at a second-look operation, therefore CA125 assays were initially approved by the FDA for this indication (412, 424). Elevations of CA125 higher than 35 kU/L after debulking surgery and chemotherapy indicate that residual disease is likely (> 95% accuracy) and that chemotherapy will be required (436). Second-look laparotomy is now considered to be controversial and suggested only for patients enrolled in clinical trials or in situations when surgical findings would alter clinical management. Monitoring with CA125 testing in women with elevated preoperative CA125 concentrations, along with a routine history and physical, and rectovaginal pelvic examination, has been advocated instead of surgery for asymptomatic women after primary therapy (408).

CA125 Measurement Postoperatively: Detection of Recurrence

Elevated, rising, or doubling CA125 concentrations predict relapse. However, it should be noted that postoperative CA125 levels below the cut-off concentration do not necessarily exclude disease presence.

The GCIG is an organization consisting of representatives from 13 international groups performing clinical trials in gynecologic cancer (437). The GCIG has defined criteria progression using serial CA125 measurements (431) as: CA125 concentrations ≥ twice the upper limit of normal on two occasions in patients with elevated CA125 levels pre-treatment that normalize, or patients with CA125 in the reference range or CA125 concentrations ≥ the nadir value on two occasions in patients with elevated CA125 levels pre-treatment that do not normalize. The two measurements must be at least 1 week apart (431).

Although monitoring intervals are as yet undefined, current practice suggests following patients every 2 to 4 months for 2 years and then less frequently (407). Elevations in CA125 can precede clinical or radiological evidence of recurrence with a median time of 2 to 6 months, although there is no evidence to date that initiating salvage chemotherapy prior to clinical recurrence improves survival (436). Early detection of recurrent disease, however, permits the timely evaluation of the multiple drugs available for salvage therapy. As only a fraction of patients will respond to any single drug and as reliable predictive tests are not yet available, chemotherapeutic agents are generally used individually and sequentially to identify those drugs that are active against a particular patient's cancer. Given the modest difference between time to recurrence and overall survival, early detection of recurrence provides time in which to identify effective palliative therapy. Therefore, measurement of CA125 at follow-up visits is recommended if values were initially elevated. Low preoperative concentrations do not exclude the possibility that CA125 concentrations may increase above the cut-off prior to clinical relapse and progressive increases in CA125 within the reference range may be predictive of recurrence (438).

NACB Ovarian Cancer Panel Recommendation 5: CA125 in Monitoring Patients After Therapy

Measurement of CA125 at follow-up visits is recommended if values were initially elevated. Although monitoring intervals are as yet undefined, current practice suggests following patients every 2 to 4 months for 2 years and then less frequently [LOE, III; SOR, B].

Prognosis

CA125 is recommended during primary therapy as a potential prognostic marker since CA125 concentrations, both preoperatively and postoperatively, may be of prognostic significance (439-442). After primary surgery and chemotherapy, declines in CA125 concentrations during chemotherapy have generally been observed to be independent prognostic factors, and in some studies the most important indicator. Persistent elevations indicate a poor prognosis. In patients who had a pre-operative CA125 concentration > 65 kU/L, the 5-year survival rates were significantly lower and conferred a 6.37-fold risk of death compared to patients who had values lower than 65 kU/L (412, 426). In addition to the measured level, the half-life of the CA125 marker indicates prognosis after chemotherapy. A half-life of fewer than 20 days was associated with significantly improved survival (28 months vs 19 months) as compared to greater than 20 days (411, 443). Improved survival also correlates with normalization of CA125 after three cycles of combination chemotherapy. These findings have been supported by a recent study suggesting that CA125 half-life and CA125 nadir during induction chemotherapy are independent predictors of epithelial ovarian cancer outcome (444).

NACB Ovarian Cancer Panel Recommendation 6: CA125 in Prognosis

CA125 measurement during primary therapy is recommended as CA125 concentrations, both preoperatively and postoperatively, may be of prognostic significance. Persistent elevations indicate poor prognosis [LOE, III; SOR, A/B].

Other Markers for Ovarian Cancer

Several other potential tumor-associated markers have been reported in body fluid and tissue of ovarian cancer patients. Although these experimental markers could represent promising new biomarkers for future ovarian cancer screening, diagnosis, and monitoring, it is uncertain whether they will become viable clinical tools (ie, their clinical usefulness needs to be validated by assessing their sensitivity and specificity in larger groups of patients with stage I disease).

The kallikrein family

Kallikreins are a subgroup of the serine protease enzyme family that play an important role in the progression and metastasis

of human cancers (445). Kallikreins 4, 5, 6, 7, 8, 9, 10, 11, 13, 14, and 15 in ovarian cancer have been shown to have value in detection, diagnosis, prognosis prediction, and monitoring of ovarian cancer (446-463). Kallikrein 4, for example, is expressed in the majority of serous carcinomas but rarely in normal ovarian surface epithelium (449, 450). Kallikrein 4 expression is associated with higher clinical stage and tumor grade in ovarian cancer: a univariate survival analysis revealed that patients with ovarian tumors positive for kallikrein 4 expression had an increased risk for relapse and death (450). Similarly, kallikrein 5 has been suggested to be a useful independent prognostic indicator in patients with stage I and II disease (451). Assessment of kallikrein 5 expression could help oncologists determine those who are at higher risk of relapse. Kallikrein 7 expression in ovarian cancer tissue is associated with poorer prognosis of ovarian cancer patients, especially those with lower grade disease and those who have been optimally debulked (464). In contrast, kallikrein 8 (neuropsin or ovasin) (452), kallikrein 9 (465), and kallikrein 11 (462) are favorable prognostic markers in ovarian cancer. Patients with higher kallikrein 8 expression in their tumors have lower-grade disease, lower residual tumor, longer survival, and low rate of recurrence. In a multivariate analysis, higher kallikrein 8 expression was significantly associated with longer disease-free survival. As well as their roles as tissue markers, kallikrein 6, 10, 11 can be detected in serum, and are potential serological markers of the disease (446, 448, 466). A recent comprehensive and parallel analysis of different secreted kallikreins in ovarian cancer has demonstrated that kallikreins 6, 7, 8, and 10 are the four most specific secreted kallikreins in ovarian cancer effusions (467). These kallikreins may have clinical implications in the differential diagnosis of ovarian carcinoma from benign controls and other cancer types.

Osteopontin

Osteopontin was first identified by a cDNA microarray approach used to identify upregulated genes in ovarian cancer cells and osteopontin has been found as a potential diagnostic biomarker for ovarian cancer (468). In the original report, osteopontin expression was higher in invasive ovarian cancer than in borderline ovarian tumors, benign ovarian tumors, and normal ovarian surface epithelium (468). Plasma levels of osteopontin were significantly higher in patients with epithelial ovarian cancer when compared to healthy controls, patients with benign ovarian disease, and patients with other gynecologic cancers. In a more recent report (469), osteopontin has been shown to be less sensitive than CA125 in predicting clinical response to therapy. However, osteopontin increased earlier than CA125 in 90% of the study patients who developed recurrent disease, indicating that osteopontin may be a clinically useful adjunct to CA125 in detecting recurrent ovarian cancer.

Prostasin

Using gene expression profiling by cDNA microarrays, Mok et al have identified an overexpressed gene called prostasin that produces a secretory product (470). Prostasin was originally isolated from human seminal fluid and its highest levels are found in the prostate gland (471). Prostasin was detected more strongly in ovarian carcinoma than in normal ovarian tissue. The mean level of serum prostasin was 13.7 μg/mL in patients with ovarian cancer and 7.5 μg/mL in control subjects. In a series of patients with non-mucinous ovarian carcinoma, the combination of prostasin and CA125 had a sensitivity of 92% and a specificity of 94% for detecting ovarian cancer. Although this finding is promising, prostasin should be investigated further as a screening or tumor marker, both alone and in combination with CA125.

Tissue polypeptide antigen

Tissue polypeptide antigen (TPA) is a single chain polypeptide which may represent proteolytic fragments of the cytokeratins (472). Production of TPA may be associated with rapid cell turnover, and elevated TPA levels in serum have been reported in patients suffering from cancers and probably other diseases (473). In ovarian cancers of serous and mucinous type, TPA levels correlate with FIGO stage. Thirty-three percent to 50% of patients with stage I or II disease, and 88% to 96% of patients with stage IIIor IV disease, presented with elevated serum TPA. Serial TPA measurements correlated with the clinical course of ovarian cancer in 42% to 79% of the matched event. These findings suggest that TPA may be a potential marker for following ovarian cancer in patients.

Lysophosphatidic acid

Lysophosphatidic acid (LPA) was first identified in ascites of ovarian cancer patients and has since been demonstrated to play a biological role in ovarian cancer cell growth (474-477). In a preliminary study in a small number of patients (474), plasma LPA concentrations were elevated in 90% of patients with stage I disease and 100% of patients with advanced and recurrent disease compared to controls without apparent diseases, although 80% of women with other gynecologic cancers also had elevated levels. CA125 concentrations appeared to complement LPA levels.

Tumor-associated trypsin inhibitor

Tumor-associated trypsin inhibitor (TATI) was first identified from the urine of patients with ovarian cancer (478). The amino acid sequence and biochemical properties of TATI are identical to those of pancreatic secretory trypsin inhibitor (479). Elevated serum and urinary concentrations of TATI are frequently observed in postoperative patients, in severe inflammatory diseases, and in various types of cancer, especially gynecological and pancreatic cancer (473). Increased concentrations of TATI can be observed in ovarian cancers, especially the mucinous types. The elevated serum levels of TATI appear to correlate with higher stages of disease. In one report, the sensitivity is only 8% in patients with stage I-II and 62% of patients with stage III-IV (480). Several reports suggest that TATI is not a good marker for monitoring disease during therapy, as TATI had a lower sensitivity for residual tumor than CA125, and fewer than 50% of the matched clinical events are observed to correlate serum levels of TATI.

CEA

CEA is an oncofetal antigen (473) and elevated serum levels of CEA are frequently found in a variety of benign diseases and cancers, including ovarian carcinoma. The frequency of elevated concentration in ovarian carcinoma varies with the histological type and disease stage, generally being higher in patients with mucinous ovarian cancers and with metastatic disease. The sensitivity of CEA as a marker to detect ovarian cancer is approximately 25%, and the positive predictive value of an elevated CEA concentration is only 14% (473). Although CEA is not a marker for early diagnosis due to its low sensitivity, CEA can be useful in determining treatment response in ovarian cancer patients.

Cancer-associated serum antigen

Cancer-associated serum antigen (CASA) was initially defined by a monoclonal antibody that bound to an epitope on the polymorphic epithelial mucin (481). Elevated CASA levels in serum were found in individuals in the later stage of pregnancy, the elderly, smokers, and in patients with cancer. CASA is expressed in all histological types of ovarian cancer and appears to have a sensitivity of 46% to 73% in patients with ovarian cancer (473). Only a few studies have indicated that CASA is a potentially useful marker in monitoring ovarian cancer. Ward et al reported that inclusion of CASA in a diagnostic tumor panel might improve the detection of residual disease by increasing the sensitivity from 33% to 62% and the negative predictive value from 66% to 78% (482, 483). One study has demonstrated that CASA can detect more cases with small volume disease than CA125, and that 50% of patients with microscopic disease are detected by CASA alone (473). Another study has shown that the prognostic value of postoperative serum CASA level is superior to CA125 and other parameters including residual disease, histological type, tumor grade, and the cisplatin-based chemotherapy (484).

PAI-1 and -2

Fibrinolytic markers include PAI-1 and PAI-2, for which diagnostic and prognostic values have recently been reported in ovarian cancer (485). In this pilot study, PAI-1 appeared to be a poor prognostic factor (486), as plasma levels of PAI-1 are significantly higher in patients with ovarian cancer, and their levels correlate with the diseases at higher clinical stages. Whether PAI-1 can be used clinically for screening and/or monitoring ovarian cancer awaits further studies, including correlation with clinical treatment events and comparison with CA125. In contrast, expression of PAI-2 in tumors has been shown to be a favorable prognostic factor in ovarian cancer patients (485).

Interleukin-6

High levels of interleukin-6 (IL-6) have been detected in the serum and ascites of ovarian cancer patients (487). IL-6 correlates with tumor burden, clinical disease status, and survival time of patients with ovarian cancer, implying that this marker may be useful in diagnosis. Based on a multivariate analysis, investigators have found serum levels of IL-6 to be of prognostic value, but less sensitive than CA125 (488, 489).

hCG

hCG normally is produced by the trophoblast, and clinically has been used as a serum or urine marker for pregnancy and gestational trophoblastic disease (490). Ectopic hCG production, however, has been detected in a variety of human cancers. Recent studies have demonstrated that the immunoreactivity of total hCG in serum and urine (urinary β-core fragment, hCGβcf) provides a strong independent prognostic factor in ovarian carcinoma, and its prognostic value is similar to that of grade and stage (491, 492). When serum hCG is normal, the 5-year survival rate can be as high as 80%, but it is only 22% when hCG is elevated (491). In patients with stage III or IV and minimal residual disease, the 5-year survival is 75% if hCG is not detectable compared to 0% if hCG is elevated. Similarly, hCGβcf can be detected in urine in 84% of ovarian cancer patients (492). The incidence of positive urinary hCGβcf correlates with disease progression with elevations observed in a higher proportion of patients in advanced clinical stages. Although the availability of this marker before surgery could facilitate selection of treatment modalities, the clinical application of hCG and its free beta subunit (hCGβ) for screening and diagnosis is limited. Since several different types of tumors can produce hCG ± hCGβ and only a small proportion of ovarian tumors express these, detection of serum hCG ± hCGβ or urinary hCGβcf will not provide a specific or sensitive tool for screening or diagnosis in ovarian cancer.

Her-2/neu

The c-erbB-2 oncogene expresses a transmembrane protein, p185, with intrinsic tyrosine kinase activity, also known as Her-2/neu. Amplification of Her2/neu has been found in several human cancers, including ovarian carcinoma. In ovarian cancer, 9% to 38% of patients have elevated levels of p105, the shed extracellular domain of the HER-2/neu protein (493-495). According to one report, measurement of Her2/neu alone or in combination with CA125 is not useful for differentiating benign from malignant ovarian tumors (495). However, elevation of p105 in serum or the overexpression immunohistochemically of Her2/neu in tumors has correlated with an aggressive tumor type, advanced clinical stages, and poor clinical outcome (496). Screening for increased p105 levels might therefore make it possible to identify a subset of high-risk patients (494). Furthermore, the test could be potentially useful for detecting recurrent disease.

AKT2 gene

The *AKT2* gene is one of the human homologues of v-akt, the transduced oncogene of the AKT8 virus, which experimentally induces lymphomas in mice. *AKT2*, which codes for a serine-threonine protein kinase, is activated by growth factors and other oncogenes such as v-Ha-ras and v-src through phosphatidylinositol 3-kinase in human ovarian cancer cells (497, 498). Studies have shown that the *AKT2* gene is amplified and

overexpressed in approximately 12% to 36% of ovarian carcinomas (499-501). In contrast, *AKT2* alteration was not detected in 24 benign or borderline tumors.

Ovarian cancer patients with *AKT2* alterations appear to have a poor prognosis. Amplification of *AKT2* is more frequently found in histologically high-grade tumors or tumors at advanced stages (III or IV), suggesting that *AKT2* gene overexpression, like c-erbB-2, may be associated with tumor aggressiveness (500).

Mitogen-activated protein kinase

Activation of mitogen-activated protein kinase (MAPK) occurs in response to various growth stimulating signals and as a result of activating mutations of the upstream regulators, KRAS and BRAF, which can be found in many types of human cancer. Activation of MAPK activates downstream cellular targets (502, 503) including a variety of cellular and nuclear proteins. Two studies have reported that expression of active MAPK in ovarian cancer tissue or ascites cells correlates with better prognosis in the advanced stage ovarian cancer (504, 505).

Inhibin

Inhibin is a glycoprotein and member of the transforming growth factor beta (TGFβ) family. Inhibins A and B are heterodimers consisting of identical α subunits and either βA or βB subunits linked with disulfide bonds (506-508). Inhibin is primarily produced by the gonads and functions as a regulator of FSH secretion. Inhibin is associated with granulosa cell tumors and mucinous carcinomas as opposed to CA125, which is associated with serous, endometrioid, and undifferentiated tumors. In addition the α subunit may function as an ovarian tumor suppressor. Using a total inhibin ELISA in combination with CA125 has been shown to detect the majority of ovarian cancer types with 95% sensitivity and specificity (507).

Rsf-1

The clinical significance of Rsf-1 in ovarian cancer was first demonstrated by analyzing a new amplified chromosomal region, 11q13.5, in ovarian cancer genome using digital karyotyping. *Rsf-1* gene belongs to the SWI/SNF chromatin remodelling gene family and Rsf-1 protein partners with hSNF2h to form the chromatin remodelling complex, RSF (remodeling and spacing factor) (509). It has been shown that *Rsf-1* participates in chromatin remodeling (509) and transcriptional regulation (510, 511). Previous studies have demonstrated that *Rsf-1* amplification and overexpression are associated with the most aggressive type of ovarian cancer and patients with *Rsf-1* gene amplification in their carcinomas had a significantly shorter overall survival (512-514). Further multi-institutional studies are required to validate the clinical significance of *Rsf-1* gene amplification for future clinical practice.

NAC-1

The genes within the BTB/POZ family participate in several cellular functions including proliferation, apoptosis, transcription control, and cell morphology maintenance (515). The roles of BTB/POZ proteins in human cancer have been recently revealed as several of BTB/POZ proteins such as BCL-6 are involved in cancer development. Based on analyzing gene expression levels in all 130 deduced human *BTB/POZ* genes using the serial analysis of gene expression (SAGE) data, Nakayama et al have recently identified NAC-1 as a carcinoma-associated *BTB/POZ* gene (516). NAC-1 is a transcription repressor and is involved in self-renewal and maintaining pluripotency of embryonic stem cells (517). In ovarian carcinomas, NAC-1 is significantly overexpressed in high-grade carcinoma but not in borderline tumors or benign cystadenomas. The levels of NAC-1 expression correlate with tumor recurrence in ovarian serous carcinomas and intense NAC-1 immunoreactivity in primary ovarian tumors predicts early recurrence (516, 518). As the NAC-1 specific antibody is available to evaluate NAC-1 protein levels in archival paraffin sections, the marker alone or in combination with other biomarkers may hold promise for prognosis and prediction in ovarian carcinoma patients.

NACB Ovarian Cancer Panel Recommendation 7: Tumor Markers Other Than CA125

CA125 is the only marker that can be recommended for use in serous ovarian malignancies. New ovarian cancer markers offer promise, however, their contribution to the current standard of care is unknown and further investigations in properly designed clinical trials are needed [LOE, not applicable; SOR, B].

KEY POINTS: TUMOR MARKERS IN OVARIAN CANCER

The NACB panel recommends CA125 as the only marker for clinical use in ovarian cancer for the following indications: early detection in combination with trans-vaginal ultrasound in hereditary syndromes, differential diagnosis in suspicious pelvic mass, detection of recurrence, monitoring of therapy, and prognosis. The NACB panel does not recommend CA125 for screening of ovarian cancer. All other markers are either in the evaluation phase or in the research/discovery phase, therefore the NACB panel does not recommend these biomarkers for clinical use in ovarian cancer.

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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 "evidence base" 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 situations—all 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 (14)	
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).

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