## PREFACE

The document appearing below includes guidelines for the use of Tumor Markers originally developed by a committee of the NACB and publicly presented and discussed in 1998 at a meeting in Rye Brook, N.Y. Due to internal Academy issues, this document never completed the Academy's normal review and consensus process. Eventually, however, the Academy effort was merged with a separate set of guidelines developed by the European Group on Tumor Markers (EGTM) and published as Chapter 5, "Practice Guidelines and Recommendations for Use of Tumor Markers in the Clinic," (M Fleisher, AM Dnistrian, CM Sturgeon, R Lamerz, and JL Wittliff, authors) in a book, "Tumor Markers: Physiology, Pathobiology, Technology, and Clinical Applications" (EP Diamandis, HA Fritsche, H Lilja, DW Chan, MK Schwartz, editors), published in 2002 by AACC Press. Subsequently, the Academy obtained permission from AACC Press, the editors of the book, and the authors of the chapter to reprint the chapter in the format of previous NACB LMPG monographs. That reprinted document follows this page.

The Academy does not consider these published guidelines to be official "approved" NACB guidelines. For that reason, the Academy has initiated a project to develop new guidelines for the use of Tumor Markers with the first public presentation to be in 2005 as an EduTrak session at the joint AACC/IFCC meeting in Orlando, Florida. The NACB committee developing this LMPG is chaired by Eleftherios Diamandis, MD, PhD, FACB. Contact information for Dr. Diamandis and the members of his committee can be accessed by **clicking here**. To support the 2005 Tumor Marker LMPG project, however, the Academy has made these original guidelines available to the public through this link to encourage wider participation and interest by the worldwide scientific community and to promote thoughtful comment from scientists to members of the committee. When the first draft guidelines are developed by the committee, they will replace the older document published here.

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# The National Academy of Clinical Biochemistry

*Presents*

LABORATORY MEDICINE PRACTICE GUIDELINES

# PRACTICE GUIDELINES AND RECOMMENDATIONS FOR USE OF TUMOR MARKERS IN THE CLINIC

# PRACTICE GUIDELINES AND RECOMMENDATIONS FOR USE OF TUMOR MARKERS IN THE CLINIC

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An approved Laboratory Medicine Practice Guideline of the National Academy of Clinical Biochemistry (NACB). This document was first published as Chapter 5 in Tumor Markers: Physiology, Pathobiology, Technology, and Clinical Applications, edited by EP Diamandis, HA Fritsche, H Lilja, DW Chan, and MK Schwartz, AACC Press, Washington, DC, 2002, 542 pages. It is reproduced here by the NACB with the permission of the authors of the chapter, the editors of the book, and the AACC Press.



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# FOREWORD

In May 1998 at Rye Brook, NY, the National Academy of Clinical Biochemistry sponsored a consensus presentation of proposed Laboratory Medicine Practice Guidelines (LMPG) entitled "Guidelines for the Analytical Performance and Clinical Utility of Tumor Markers." This meeting was held in conjunction with the annual meeting of the Clinical Ligand Assay Society, many of whose members share an interest in tumor markers. This joint forum thus provided an opportunity for greater review and discussion. Under the leadership of Martin Fleisher, Ph.D., F.A.C.B., and James L. Wittliff, Ph.D., F.A.C.B., an expert panel of clinical scientists and physicians (whose names are contained within this monograph) developed, reviewed, and refined these proposed guidelines to develop a consensus document. The LMPG guidelines that were developed were then merged with other guidelines developed by the European Group on Tumor Markers (EGTM) and were published in 2002 as a book chapter with the following citation:

Fleisher M, Dnistrian AM, Sturgeon CM, Lamerz R, and Wittliff JL, Practice Guidelines and Recommendations for Use of Tumor Markers in the Clinic, Chapter 5 in Tumor Markers: Physiology, Pathobiology, Technology, and Clinical Applications, edited by EP Diamandis, HA Fritsche, H Lilja, DW Chan, and MK Schwartz, AACC Press, Washington, DC, 2002, 542 pages.

With the permissions of AACC Press, the European Group on Tumor Markers, the authors of the chapter, and the editors of the book, the NACB is pleased to be able to publish this reprint of Chapter 5 containing the Laboratory Medicine Practice Guidelines developed by the Academy from that important 1998 conference. The NACB is also pleased to announce that a committee has been appointed under the leadership of Dr. Eleftherios Diamandis to update and expand these LMPG guidelines, with public presentation and discussion anticipated to be in conjunction with the joint meeting of the AACC and IFCC in 2005. Comments or suggested updates to these guidelines are invited and may be sent to either Dr. Fleisher (fleishem@mskcc.org) or Dr. Diamandis (ediamandis@mtsinai.on.ca).

Charles D. Hawker, Ph.D., F.A.C.B. President
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Laboratory Medicine Practice Guidelines

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# PRACTICE GUIDELINES AND RECOMMENDATIONS FOR USE OF TUMOR MARKERS IN THE CLINIC

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Rolf Lamerz, and James L. Wittliff

The information represented in this chapter is based on recommendations formulated by the National Academy of Clinical Biochemistry (NACB) in the United States and by the European Group on Tumor Markers (EGTM). The U.S. recommendations were prepared at a National Academy of Clinical Biochemistry, NACB Laboratory Medicine Practice Guidelines (LMPG) Conference, and the European Recommendations EGTM Guidelines were developed independently by a group of scientists and clinicians meeting regularly in Europe.

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# INTRODUCTION

The definition of a tumor marker has evolved considerably over the past two decades, principally as a result of advances in technology and redefinition by federal regulatory agencies. In general, a tumor marker is a naturally occurring molecule that is measured in serum, plasma, or other body fluids or in tissue extracts or paraffin-embedded tissues to identify the presence of cancer, to assess patient prognosis, or to monitor a patient's response to therapy with the overall goal of improving the clinical management of the patient. Tumor markers are found inside cells, both in the cytoplasm and nuclei, and they are associated with cell surface membranes; they circulate in blood.

Since most tumor markers are measured by immunochemical techniques, there are numerous pre- and postanalytical concerns regarding the type, handling, and storage of clinical specimens. In addition, in the United States, the Food and Drug Administration (FDA) has reclassified these molecules under the 1982 medical devices ruling (Classification Regulation 21, CFR Part 866, Subpart G) as "tumor-associated antigen immunological systems." Although numerous tumor markers have been introduced throughout the past two decades, only a few have received FDA approval, and the specific clinical applications have been limited as a matter of policy and clinical documentation. As a result, many other important clinical applications of tumor markers may be precluded in the United States. It is nevertheless the joint obligation of the clinician and the laboratory medicine specialist to thoroughly evaluate the reagents, to recommend reference intervals, and to establish clinical utility of the analyte in patient screening, diagnosis, therapeutic monitoring, or prognostic evaluation.

The use of diagnostic tests in the clinical setting is highly regulated by federal agencies, but tumor markers have been particularly identified for special consideration. Agencies that control the use of tumor markers in the United States include the FDA as mandated by the Medical Devices Amendment of 1976 and by the Clinical Laboratory Improvement Act (CLIA) of 1988 regulating clinical laboratory improvement; the Centers for Medicare and Medicaid Services (CMS) through management of Medicare reimbursement; the Joint Commission on Accreditation of Healthcare Organizations (JCAHO) via hospital accreditation; state and local regulations, which may be more stringent than those of CLIA; and insurance companies that carefully review new tests and their reimbursements. Complementing these efforts are those of professional societies and consumer groups concerned with the ethics of test performance and clinical application.

This chapter reviews consensus recommendations made by two such groups, the National Academy of Clinical Biochemistry (NACB) in the United States and the European Group on Tumor Markers (EGTM) in Europe.

## National Academy of Clinical Biochemistry (NACB) Working Group

In May 1998, the National Academy of Clinical Biochemistry (NACB) sponsored a consensus conference to develop guidelines for the analytical performance and clinical utility of tumor markers (1). The guidelines, referred to as Laboratory Medicine Practice Guidelines (LMPG), presented recommendations on the analytical and clinical utility of tumor markers. These recommendations were reviewed by an expert panel comprised of clinical scientists and physicians who refined the recommendations and prepared a consensus document on the clinical use of tumor markers. The recommendations focused on pre- and postanalytical concerns, the use of reference intervals, and the manner in which tumor markers should be used clinically with specific attention to screening, diagnosis, monitoring, or prognosis.

## European Group on Tumor Markers (EGTM)

In the mid-1980s, radioimmunoassays for new tumor markers including CA125, CA15-3, and CA19-9 became available. Under the initiative of Professor Rainer Klapdor, discussion of the clinical application of these tests was encouraged by bringing together German scientists and physicians from universities, hospitals, and the diagnostics industry in the annual Hamburg Symposia. These meetings soon became more international and a working group was subsequently established to consider the quality control and standardization of tumor markers. In 1993, this group published a consensus statement on the criteria for use of tumor markers with respect to clinical relevance, analytical methods, and manufacturing requirements (2). The group was more formally constituted as the European Group on Tumor Markers (EGTM) in 1997, with its constitution and bylaws accepted in 1999. Seven focus groups were formed to consider different types of cancer, and the initial conclusions of these groups were published in 1999.

# OVERVIEW

The approach of both the NACB and EGTM groups has been similar, each group considering appropriate use of tumor markers for specific types of cancer. The EGTM also considered broad quality requirements relevant to all tumor markers. In this chapter we summarize the conclusions of both groups, highlighting differences in outlook where these are signicant. In this context it is important to note that guidelines should evolve as knowledge increases: they should be reviewed and updated regularly.

# QUALITY REQUIREMENTS AND CONTROL

Once the decision to request any tumor marker measurement has been made, the quality of the result eventually reported will reflect events during the preanalytical, analytical, and postanalytical phases of analysis. Aspects of particular practical importance to those either providing or using a tumor marker laboratory service were considered by the EGTM (3) and are reviewed briefly here.

## Preanalytical Requirements

Relevant preanalytical requirements identified by the EGTM were published in another chapter of the book in which this monograph appeared as Chapter 5.* It is important to note that reporting of erroneous tumor marker results (especially when they are incorrectly high) is more likely to cause undue alarm and distress to the patient than is the case for many other laboratory tests. This places a major responsibility on the laboratory to ensure correct results, as well as to provide clinicians with readily available information relevant to specific tests; e.g., avoiding measuring prostate-specific antigen (PSA) in a patient with prostatitis, or CA125 during menstruation.

## Analytical Requirements

Satisfactory measurement of any analyte requires that the correct and appropriate specimen be analyzed by a method meeting defined quality requirements for both Internal Quality Control (IQC) and External Quality Assessment (EQA) (4). IQC and EQA issues including assay reproducibility, acceptance criteria, matrix effects, dynamic range, interferences, stability, target values, and interpretive exercises are addressed in Table 1.

While automated immunoassays generally achieve very good intra- and inter-assay variability (e.g., <5% and <10% respectively), this might not be possible with manually performed immunoassays. In keeping with recent international proposals (6), criteria for desirable precision performance can alternatively be based on biological variation.*

The laboratory should also be aware of analytical pitfalls specific to the given analyte. These are discussed in greater detail elsewhere,* but three of the most important potential causes of erroneous tumor marker results are also summarized in Table 2.

## Postanalytical and Reporting Requirements

Advances in automation have markedly increased the analytic reliability of the most frequently used tumor markers, allowing more time and attention to be focused on how to achieve most effective clinical use of these important tests. This requires attention to events in the postanalytical phase, as outlined in the recommendations of the EGTM (Tables 3 and 4).

Interpretive exercises and surveys undertaken through EQA schemes can have an important role in auditing the advisory service that laboratories provide for their clinical users. Participants are asked to provide clinical interpretation of results obtained for routine EQA specimens, and questions relating to other laboratory services may also be included; e.g., how results are reported to clinicians (10). Surveys carried out through the UK National External Quality Assessment Schemes have identified a need for improved consensus about the appropriate timing of tumor marker measurements, what constitutes a clinically significant change in tumor marker level, and reference ranges (11,12). Such surveys can enhance the educational value of EQA by facilitating between-laboratory comparison of practice and encouraging improved consensus. Perhaps this can also contribute to the much wider area of clinical audit.

* Sturgeon, CM Limitations of Assay Techniques for Tumor Markers, pp 65-81, Chapter 6 in Tumor Markers: Physiology, Pathobiology, Technology and Clinical Applications, edited by EP Diamandis, HA Fritsche, H Lilja, DW Chan, and MK Schwartz, AACC Press, Washington, DC, 2002, 542 pages

**Table 1: Analytical Considerations of Particular Relevance to the Quality Control of Tumor Marker Measurements**

| | |
|---|---|
| **Requirements of Internal Quality Control (IQC)** | |
| Assessment of reproducibility | Demonstration of intra-assay variability <5% (desirable); inter-assay variability <10% (desirable). (Manual and/or research assays may be less precise.) |
| Established criteria for assay acceptance | Selection of appropriate criteria for acceptability of IQC, preferably based on logical criteria (5). |
| Specimens closely resembling authentic patient sera | In general it is inadvisable to rely exclusively on QC materials supplied with the kit, and an authentic serum matrix control from an independent source should be included. |
| IQC specimens of concentrations appropriate to the clinical application | Negative and low positive controls should be included for all tumor markers, but there is also a need to cover the broader concentration range, and to assess accuracy of dilution steps required for high concentration specimens. |
| Assessment of assay interferences | Occasionally checking for interferences (e.g., from heterophilic and other antibodies, clotting agents in blood clotting tubes) is desirable. |
| **Requirements of External Quality Assessment (EQA)** | |
| EQA specimens of appropriate analyte concentration | Concentrations covering the working range of assays are adequate, although occasional inclusion of higher concentrations to check behavior on dilution is desirable. Issue of normal analyte-free serum to check baseline security important for some analytes (e.g., AFP, hCG). |
| Assessment of assay "stability" | Assessment of the "stability" of results within a laboratory can readily be accomplished by issuing repeat specimens of the same pool and comparing results over time (e.g., 6–12 months). |
| Demonstrating accuracy and stability of target values | These are usually consensus means, as reference methods are not available for these analytes. The validity of the consensus means should be demonstrated by assessing their<br>1. Stability, by repeat distribution of the same pool.<br>2. Accuracy, by recovery experiments undertaken by supplementing pools with known amounts of the relevant International Standard (IS). [Such validation is possible for AFP, hCG, CEA, and PSA, for which there are currently accepted IS (BS 72/225, IS 75/537, IRP 63/601, and IS 96/670 respectively). The lack of IS for other tumor markers is currently being addressed.] |
| Interpretive exercises | These provide a valuable means of comparing practice (e.g., reference intervals, cumulative reporting of results) in different laboratories. |

Table from the EGTM Consensus Recommendations (3). Used with permission.

**Table 2: Potential Causes of Erroneous Tumor Marker Results**

| | |
|---|---|
| High-dose hook effect | Tumor marker concentrations routinely encountered range over several orders of magnitude. Protocols permitting identification of high-dose "hooking" are essential to avoid reporting misleadingly low results, particularly in patients for whom markers are being measured for the first time. [Hook effects can be minimized by using solid-phase antibodies of higher binding capacity, by assaying specimens at two dilutions, or by using sequential assays that include a wash step.] |
| Specimen carry-over | Potentially a problem whenever very high concentration specimens assayed, so should occasionally be checked. |
| Interference from heterophilic or human anti-mouse antibodies (HAMA) | Falsely high or low results may be obtained for patient specimens containing anti-immunoglobulin G (IgG) antibodies capable of reacting with antibodies used in the assay. Presence of HAMA, frequently induced in cancer patients who have undergone treatment with mouse monoclonal antibodies for imaging or therapeutic purposes, may also give erroneous results. [Such interference can be detected by re-assaying the specimen after treatment with a blocking agent (commercially available immobilized on tubes), by adding further non-immune mouse serum to the reaction mixture, or by re-assaying the specimen by a different method.] |

Table from the EGTM Consensus Recommendations (3). Used with permission.

**Table 3: Postanalytical Requirements of Particular Importance to Provision of a Comprehensive Tumor Marker Service**

| Factual requirements | |
|---|---|
| Clinical information from the requesting doctor | Encouraging clinicians to provide very brief clinical information (eg "postoperative", "post-chemotherapy number 5") is essential if any interpretation is to be provided, and may help to identify occasional laboratory errors (e.g., mis-sampling on an analyzer). |
| Availability of appropriate reference ranges | Usually derived from an appropriately matched healthy population, reference ranges for tumor markers are usually most relevant for cancer patients before the initial treatment. Subsequently, the patient's own "baseline" provides the most important reference point for interpretation of marker results. If this is well established, increases even within the reference range may be clinically significant. |
| Knowledge of what constitutes a significant or clinically relevant change | Should include contributions of both biological variation and analytical variation. A confirmed increase or decrease of ± 25% is frequently considered to be of clinical significance, but further work is required in this area (7,8). |
| Defined protocol when changing methods | It may be helpful to the laboratory to indicate changes of method on tumor marker reports, but it is more helpful if the laboratory highlights whether any change is likely to have affected interpretation of the trend in marker result. [This may necessitate analyzing the previous specimen by the new method, for example, or requesting a further specimen to re-establish the baseline and/or confirm the trend in marker level.] |
| Knowledge of tumor marker half-lives | Defined as the time to 50% reduction of circulating tumor marker concentrations following complete removal of tumor tissue (i.e., distinct from definition as used in other settings). Of most relevance to interpretation of serum concentrations of certain tumor markers, e.g., AFP and hCG. |
| Objective comparison of tumor marker utility | The need to obtain objective clinical information about tumor marker utility remains a priority, and is being considered by a number of professional organizations (9). |

Table from the EGTM Consensus Recommendations (3). Used with permission.

**Table 4: Reporting Requirements for Provision of a Comprehensive Tumor Marker Service**

| |
|---|
| Provision of fully cumulated results, since it is always the trend in marker concentration that is most informative. Minimal clinical details should appear on the report, to facilitate interpretation. Graphical representations may also be very helpful. |
| Recommendations as to the appropriate frequency of marker measurements. |
| Recommendations as to when confirmatory specimens should be requested. An apparent rise in marker concentration should always be confirmed by repeat measurement. |
| Good communication between laboratory and clinical staff, which facilitate appropriate use of these (and other) tests. |

Table from the EGTM Consensus Recommendations (3). Used with permission.

# BREAST CANCER

In 2002 it is expected that more than 203,500 new cases of breast cancer will be diagnosed in the United States and 39,600 women will die of the disease (13). The average American woman has an approximately 11% chance of developing breast cancer during her lifetime. Currently, more than one million women in the United States are living with Stage II, III, or IV breast cancer. Although medical science has not established a means of preventing breast cancer, advances in diagnosis and therapy can arrest the disease in many patients. Furthermore, a significant number of deaths due to breast cancer can be avoided by early detection; i.e., screening by breast self-examination, mammography, and/or clinical examination. Although tumor markers have not proven useful as screening tests, they provide clinically useful information for the management of these patients, primarily as predictive indicators for selection of certain therapies for primary breast cancer and as markers to monitor the clinical course of the disease.

The following have been used or suggested as markers for breast cancer:

- Estrogen and progestin receptors
- CA15-3
- BR27.29 (CA27.29)
- CEA
- HER-2/neu (c-erb B2) oncoprotein

## Estrogen and Progestin Receptors

The main tumor markers used in breast cancer tissue are steroid receptors, p53, c-erb B2 (HER-2/neu), S phase, and ploidy. Steroid receptors are at present the only tissue markers accepted in standard practice (14–17). Although too recent to be considered by either the NACB or EGTM when originally drafting their current guidelines, assay of HER-2/neu is now mandatory in deciding which patients with metastatic breast cancer should receive treatment with the monoclonal antibody, Herceptin® (trastuzumab) (15).

**Clinical utility of estrogen and progestin receptors.**

In addition to a number of characteristics of breast cancer, such as size, pathologic category, and axillary lymph node status, certain protein biomarkers are useful in assessing differences in growth rates and invasive and metastatic potential (18,19). Estrogen and progestin receptors have been used as predictive indicators of response to endocrine therapies such as tamoxifen, toremifene, and droloxifene (“anti-estrogens”) (20,21), and to medroxyprogesterone acetate and megestrol acetate (progestin mimics) (22). As predictive factors, receptor levels have been used to assess potential for endocrine therapy response in both the adjuvant setting and for metastatic disease. In addition, sex hormone receptors are used to assess the likely clinical course of breast cancer patients; i.e., they are prognostic factors (19,22).

In general, patients with estrogen receptor-positive tumors have a better prognosis, at least in the short term, than those with estrogen receptor-negative malignancies (23). Approximately 30% of node-negative and up to 75% of node-positive breast cancer patients will relapse with metastatic disease and die within 10 years. Estrogen and progestin receptors have been employed as prognostic tests with other factors to distinguish breast cancer patients in both nodal classes at high risk for recurrence (poor prognosis) from those at low risk (good prognosis) (19,22). Levels of estrogen and progestin receptors should only be quantified in primary and metastatic breast cancers of both pre- and postmenopausal patients if the results would influence treatment decisions, in agreement with American Society of Clinical Oncology (ASCO) clinical practice guidelines (15). Immunohistochemical determinations should only be performed if the amount of the tissue biopsy precludes receptor quantification or if fresh tissue is unavailable.

**Reference intervals.**

Ethical considerations mean that estrogen and progestin receptors are rarely determined in normal breast tissue from women without breast carcinoma, so well-documented reference ranges are not available. However, a number of bio

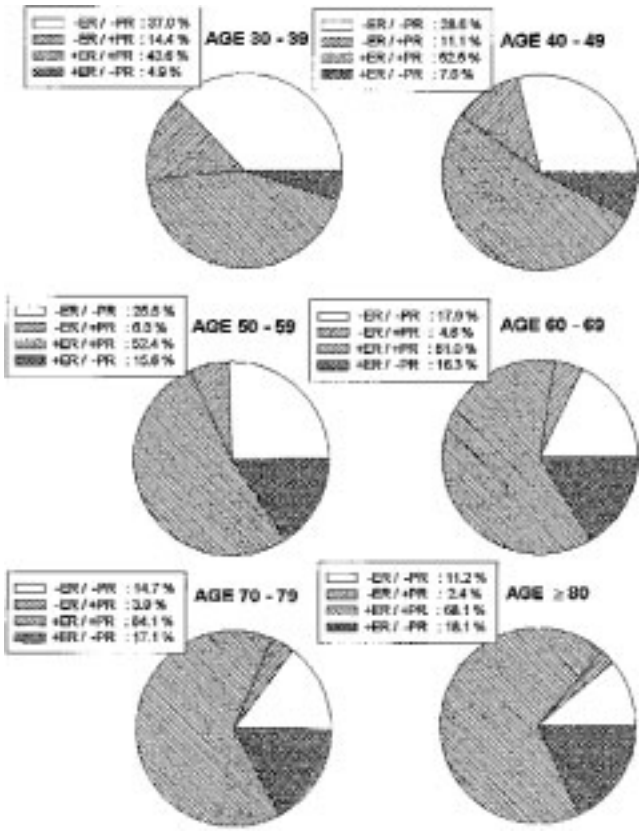


**Figure 1** Distribution of sex-hormone receptor status in breast carcinoma biopsies as a function of potential for endocrine responsiveness. The four combinations of estrogen and progestin receptor status of the biopsies are given as the percentage of the total patient population examined for each age group. Reprinted with permission from Wittliff JL, Pasic R, Bland KI. Steroid and peptide hormone receptors: methods, quality control and clinical use. In: Bland KI, Copeland III EM, eds. The breast: comprehensive management of benign and malignant diseases. Philadelphia, PA: WB Saunders Co, 1998; 458–498.

chemical studies using ligand-binding techniques or enzyme immunoassays have indicated that non-cancerous breast tissue adjacent to a carcinoma contains very low (i.e., <15 fmol/mg extract protein) or undetectable levels of the sex-hormone receptors (24). This has been confirmed in many studies in which estrogen and progestin receptors have been determined immunohistochemically using well-characterized antibodies. Values of 10 fmol/mg extract protein (by ligand binding) or 15 fmol/mg protein (by enzyme immunoassay) are generally used as cut-off levels (20,21,25). The presence of estrogen and progestin receptors in a metastasis of unknown origin at preliminary pathologic review often indicates the presence of a hormone-responsive primary breast carcinoma or other sex hormone-responsive primary tumor. Levels and distribution of estrogen and progestin receptors in biopsies of breast carcinomas are influenced by the age and menopausal status of the patient (Figure 1) (26). In general, biopsies from premenopausal patients with breast cancer contain lower levels of these receptors than do those from postmenopausal women with breast cancer. Since the response of breast cancer patients given administrative hormone therapy is related to estrogen and progestin receptor content in their tumor biopsies (19,20,22), quantification of receptor levels should be performed.

**Estrogen receptor-beta and other sex-hormone receptor variants.**

Investigations have indicated that these regulatory proteins exhibit polymorphism (27). For example a new estrogen receptor gene, estrogen receptor-beta (ER-β), has been discovered, which shows considerable sequence homology with the widely studied estrogen receptor-alpha (ER-α) in both the DNA and ligand-binding domains (96% and 58% homology, respectively) (28,29). In addition the ligand-binding properties and biological activities of these two isoforms appear to be different. The co-expression of ER-β with ER-α and progesterone receptor (PR), as well as its association with the other indicators of low biological aggressiveness of breast cancer, suggest the ER-β-positive tumors are likely to respond to anti-estrogen therapy (30). The independent predictive value of ER-β remains to be established. Furthermore, almost 20 sex hormone-receptor variants discovered in the last decade show defects due to truncations, exon deletions, and point mutations (24). In order to investigate the clinical significance of these naturally occurring isoforms and variant receptors, new reagents and reference specimens must be developed.

**Preanalytical concerns and specimen storage.**

Regardless of the chosen type of assay technique, proper selection and handling of breast tissue biopsies are essential for reproducible results. First, the tissue must be frozen at a temperature of –20 °C or less shortly after surgical excision and transported frozen to the laboratory. Optimum cutting temperature (OCT) compound used in the preparation of frozen sections must be completely removed prior to the tissue extraction process (31). If dyes such as those of the Davidson Dye Marking Kit are present, involved tissue should be removed. Presence of large amounts of normal and hemorrhagic tissue should also be avoided.

Sex hormone receptors are unstable in tissue extracts at room temperature and lose activity at 4 °C within 2–4 hours (19). Extracts should be frozen at –70 °C immediately after assay. The stability of receptor content in these frozen extracts varies depending upon the type of tumor, involvement of hemorrhagic and normal tissue, and other factors. A fresh extract of frozen residual tissue should be prepared if the original extract is not re-analyzed within 48 hours.

**Analytical concerns.**

The analytical precision of the currently available radioligand binding assay and enzyme immunoassay methods (which use standardized reagents and stable reference specimens) is considerably better [coefficients of variation (CVs) 4–15%] than that required for clinical decision-making (22,25). The total imprecision required to satisfy medical decision-making is 10–20% at estrogen and progestin levels of 30–50 fmol/mg extract protein (20,32). The total imprecision required at estrogen and progestin receptor levels of >100 fmol/mg protein is 30–40%.

**Reporting concerns.**

Data on assay precision from cooperative clinical trials suggest that estrogen and progestin receptor values of less than 10–15 fmol/mg extract protein represent a clinically insignificant quantity of these analytes in human breast cancer biopsies (20–22,32). $K_d$ values of 1 to $9 \times 10^{-10}$ M to 1 to $9 \times 10^{-11}$ M are indicative of high-affinity estrogen receptor-α, and $K_d$ values of 1 to $9 \times 10^{-9}$ M to 1 to $9 \times 10^{-10}$ M are indicative of the presence of high-affinity progestin receptors (19). The ligand-binding characteristics of estrogen receptor-β and other variants of both sex-hormone receptors are being established. The type of assay, which determines the cut-off value, should be reported. Certain groups recommend inclusion of a receptor status distribution by decades of age since the receptor level expressed is age-related (Figure 1).

## CA15-3

The CA15-3 antigen is a high-molecular-weight mucin glycoprotein termed MUC-1 (33). It has been characterized with monoclonal antibodies developed against purified extracts of a membrane fraction isolated from a human breast cancer metastasis to liver (clone DF3) (34) and against human milk fat-globule (clone 115D8). The mucin-type markers for breast cancer include BR27.29, CA-549, MCA, CA-M26, and CA-M29 (for review, see reference 35). The sensitivity and specificity of these mucins are similar and use of more than one mucin marker does not provide additional information (36,37). Only CA15-3 and BR27.29 have been approved by the FDA for following the clinical course of breast cancer patients with advanced metastatic disease.

**Clinical utility.**

Although carcinoembryonic antigen (CEA) has been used for the detection of metastatic breast cancer (38–40), numerous recent studies have established the comparative superiority of CA15-3 (35,36). Briefly, levels of CA15-3 are elevated in 60–80% of patients with metastatic breast cancer and these levels correlate with changes in clinical status and tumor therapy response (40–45). CA15-3 also appears effective for evaluating the clinical course of patients being treated for metastatic disease. A number of studies have demonstrated that the tumor marker is useful in monitoring disease progression and regression following treatment for breast cancer (35,46,47).

The EGTM guidelines (48) suggest that by using both CA15.3 and CEA it is possible to increase the sensitivity obtained as well as the clinical utility (49,50). Some groups report that simultaneous use of both CA15-3 and CEA

allows the detection of early recurrence in more patients than the use of CA15-3 alone (49,50) but there is some conflicting evidence in the literature (51). Serial determinations of markers are particularly sensitive for early detection of bone and liver metastases (49,50). Despite the ability of markers to detect recurrent disease preclinically, the long-term benefit of early detection on therapy response and patient survival has yet to be demonstrated.

It is now clear that the main use of CA15-3 is as a monitor of breast cancer activity. An objective for future work should be to provide consensus for guidelines to interpret CA15-3 sequential measurements during therapy and follow-up. There is evidence that CA15-3 monitoring of breast cancer may be more complicated than hitherto recognized (52–54). Computer simulation suggests that criteria (which must be sufficiently robust to false-positive signals) can be developed to allow rational assessment of possible recurrence and progression (55,56). However, it is clear that such criteria remain to be clinically validated.

**Preanalytical concerns and specimen storage.**

CA15-3 measurements should be performed on specimens after prompt separation of serum from the clot. CA15-3 is stable in serum stored in plain tubes or those containing thixotropic gel when stored at 4 °C for 24 hours (35,36). Storage of serum frozen at –20 °C (short-term) and at –70 °C (long-term) is recommended to ensure stability of the analyte if the need for re-analysis occurs. For long-term storage, tubes containing thixotropic gel should not be used because of apparent instability of CA15-3.

**Analytical concerns.**

The type, analytical precision, and reference interval of the CA15-3 assay selected should be validated within each institution to include the medical decision limits (41).

**Postanalytical and reporting concerns.**

Additional studies should be performed to establish the influence of gender, race, age, and menopausal status on the expression of CA15-3 in normal subjects and in patients with breast carcinoma. The implications of these for quoted reference intervals should be documented.

## BR27.29 (CA27.29)

BR27.29 is a newly developed member of the mucin-type breast cancer markers, which include CA15-3 (57,58). The assay was developed with antibodies against the same mucin antigen (MUC-1) using a different antibody termed B27.29. The reactive sequence of the B27.29 monoclonal antibody was shown to overlap with that recognized by the DF3 antibody used in the CA15-3 assay, using epitope mapping (34,35,57).

**Clinical utility.**

Numerous investigations have explored the use of BR27.29 levels in the detection and diagnosis of breast carcinoma (35,58). As was concluded from CA15-3 evaluations, BR27.29 also lacks the specificity and sensitivity required for routine detection of breast cancer. Several reports suggest that BR27.29 is a more sensitive but less specific marker than CA15-3.

Collectively, many institutions have used elevations in the levels of BR27.29 to detect relapse in breast cancer patients at high risk for recurrence and to monitor disease progression in patients with advanced disease (47). These studies provide clinical evidence of the utility of BR27.29 levels in the detection of late-stage metastatic disease and suggest that elevations in the levels of this tumor marker reflect disease activity. Several studies have reported the use of BR27.29 as an aid to predict recurrent breast cancer in patients with Stage II or Stage III disease (58). Recent studies also suggest that elevations in BR27.29 levels may predict the probability of bone metastases in breast cancer patients with bone disease (40,57).

**Preanalytical concerns and specimen storage.**

Since BR27.29 is a member of the MUC-1 family of antigens containing carbohydrate, the handling of serum or plasma samples is similar to that recommended for other tumor-associated mucin antigens. The main concern appears to be enzymatic cleavage of sequences recognized by the antibodies incorporated in the assay.

BR27.29 analysis should be performed on specimens after prompt separation of serum from the clot. Samples may be stored at 4 °C for up to 24 hours. Storage of serum frozen at –20 °C (short-term) and at –70 °C (long-term) is recommended to ensure stability of the analyte if the need for re-analysis occurs. Further research is required on the influence of freezing and thawing on the stability of BR27.29 in serum.

**Analytical concerns.**

Each laboratory analyzing BR27.29 should ensure that the sensitivity and specificity of assays meet clinical recommendations. The type, analytical precision, and reference interval of the BR27.29 assay selected should be validated within each institution to include the medical decision limits (41).

There have been no reported interferences from common matrix effects, but further study would be desirable. A need for significant quality control is evident from studies of analytical sensitivity (35,36,41). It is strongly recommended that reference specimens of BR27.29 be developed and that proficiency surveys be implemented with the aim of improving inter-laboratory agreement of results for BR27.29.

**Postanalytical and reporting concerns.**

It is recommended that a statement on the result report regarding the clinical utility of BR27.29 be restricted to the follow-up of breast cancer patients with advanced disease. Several institutions contributing to the NACB guidelines recommend that the laboratory report should state that the clinical utility of BR27.29 is restricted to the follow-up of breast cancer patients with advanced disease.

## NACB AND EGTM RECOMMENDATIONS FOR USE OF TUMOR MARKERS IN BREAST CANCER

1. Estrogen and progestin receptor status should be used to identify those breast cancer patients most likely to respond to hormone therapy (e.g., tamoxifen) given in the adjuvant setting. Levels of these receptors should be quantified in every primary breast cancer of both pre- and postmenopausal patients at the time of diagnosis, in agreement with ASCO Clinical Practice Guidelines. Immunohisto-chemical determinations should only be performed if the amount of the tissue biopsy precludes receptor quantification. Immunochemistry performed on paraffin-embedded tissue should also be the method of choice when no fresh tissue is available.
2. The total imprecision required to satisfy medical decision-making is 10–20% at estrogen and progestin receptor levels of 30–50 fmol/mg of extract protein. The total imprecision required at estrogen and progestin receptor levels of >100 fmol/mg of protein is 30–40%.
3. Levels of estrogen and progestin receptors should be quantified only in those metastatic breast cancers of both pre- and postmenopausal patients if the results would aid in treatment decisions, in agreement with ASCO Clinical Practice Guidelines. Immunohistochemical determinations should be performed only if the amount of the tissue biopsy precludes receptor quantification.
4. CA15-3 or BR 27.29 determinations are useful for the early detection of breast cancer recurrences in patients previously treated for stage II and stage III carcinomas who are clinically free of disease. High CA15-3 levels in a patient with breast cancer almost certainly indicate the presence of metastatic disease.
5. Decreasing concentrations of circulating CA15-3 are indicative of successful therapeutic response, and persistent or increasing CA15-3 levels are associated with disease progression and poor response to therapy. It is therefore recommended that CA15-3 be used with caution as an aid in following the clinical course of breast cancer patients. In addition, measurement of CEA is recommended by the EGTM for the early diagnosis of distant metastases.

6. Since elevated levels of CA15-3 are observed in a number of other malignant and nonmalignant diseases, its use is precluded in screening, diagnosis, or staging of breast cancer, in agreement with the ASCO clinical practice guidelines.
7. The use of BR27.29 in the clinical setting is restricted to the follow-up of breast cancer patients with advanced disease.
8. CA15-3 should be assayed after prompt separation of serum from the clot. Samples may be stored at 4 °C for up to 24 hours. Storage of serum at –20 °C (short-term) or –70 °C (long-term) is recommended to ensure stability of the analyte if the need for re-analysis occurs. For long-term storage, tubes should not contain thixotropic gel.

## Emerging Markers for Breast Cancer Management

**Epidermal growth factor receptors.**

Epidermal growth factor (EGF) is a single polypeptide chain of MW 6000, with a high heat stability conferred by three disulfide bridges. EGF receptor protein is a complicated molecule consisting of a large extracellular domain responsible for the association with EGF, a transmembrane portion that secures the receptor in the cytoplasmic membrane, and an internal domain that contains an ATP-binding site and exhibits tyrosine kinase activity (19).

EGF receptors are present in certain breast, endometrial, and ovarian cancers, as detected by binding studies with radiolabeled EGF (59–62). As radiolabeled EGF is expensive, most investigators prefer a ligand competition assay, which measures both specific binding capacity and EGF affinity (61–64). The overexpression (i.e., increased numbers) of EGF receptors in a breast tumor biopsy appears to correlate with a shorter disease-free interval and with a decreased overall survival (63–65). In contrast to steroid hormone receptors, high levels of EGF receptors in a breast tumor biopsy appear to indicate poor prognosis. Although the ASCO clinical practice guidelines (15) did not address the use of EGF receptors, there is growing evidence of their value as prognostic factors and as predictive tests for response to new drugs targeting EGF receptor-positive breast cancer (19,63,64).

**HER-2/neu (c-erb B2) oncoprotein.**

The *neu* oncogene, originally isolated from rat neuroblastomas, encodes a 185-kDa surface glycoprotein termed “p185 neu” and exhibits tyrosine kinase activity with a structure similar to the EGF receptor. When various molecular properties and chromosomal localization studies were conducted, it was revealed that the EGF receptor was distinct from p185 neu (19,66). The identity of the native ligand for p185 neu is the focus of research in many laboratories.

The human homologue of the *c-neu* gene, which is termed *c-erb B2*, is reportedly amplified (i.e., increased in copy number) in human breast cancer and correlates with decreased disease-free survival and lower overall survival (65,67,68). The oncogene is also called *HER-2/neu*. Furthermore, elevated levels of the HER-2/neu oncoprotein are associated with poor prognosis in breast cancer patients (69) and indicate the use of Herceptin® (trastuzumab) therapy. In 1997, the ASCO panel considered that available data were insufficient to allow recommendation of the use of *c-erb B2* (*HER-2/neu*) gene amplification or overexpression for management of breast cancer patients (15). The principal difficulties in assessing the clinical utility of this analyte are the wide variety of assays that have been used in the absence of a universally accepted reference material (61,66–68). In the ASCO 2000 update, measurements of HER-2/neu oncoprotein are recommended on every primary breast cancer either at the time of diagnosis or at the time of recurrence (15). Amplification of the *HER-2/neu* gene may also be of value (70). A recent Erratum to the ASCO 2000 update (16) contains additional recommendations relating to methods for measuring the oncoprotein and the use of results in trastuzumab therapy, and in chemotherapy selection and response. Measurements of HER-2/neu oncoprotein, either in tumor extracts or circulating extracellular domain, are not recommended for prognostic use.

**Cathepsin D.**

Cathepsin D belongs to a class of acidic lysosomal proteases, which are found in all cells (71). The digestive pattern of cathepsin D appears to be similar in breast cancer and normal breast. This interesting protease is estrogen-induced in cultured breast cancer cells, where it is secreted as a 52-kDa precursor. Pro-cathepsin D, a phosphoglycoprotein, is cleaved to mature forms of 48-kDa, 34-kDa, and 14-kDa molecules. Monoclonal antibodies have been produced

against these forms and have been assimilated into an immunoradiometric assay (IRMA) kit format (72,73). Excellent enzyme immunoassay (EIA) and enzyme-linked immunosorbent assay (ELISA) kits have also been developed for quantifying this analyte in breast cancer extracts. Evidence suggests that overexpression of cathepsin D in node-negative breast cancer correlates with shorter disease-free survival and with decreased overall survival (65,71). However, the ASCO panel concluded that there are insufficient data currently available to recommend the use of cathepsin D in the management of breast cancer patients.

**Urokinase-type plasminogen activator, its receptor, and its inhibitor.**

The serine proteases, urokinase-type plasminogen activator (uPA), and tissue-type plasminogen activator (tPA), are known to convert plasminogen to plasmin. tPA participates mainly in intravascular thrombolysis while uPA mediates pericellular proteolysis during cell migration and tissue remodeling under physiological and pathophysiological conditions (mammary gland involution, trophoblast invasion, spermatogenesis, wound healing) (for review, see reference 74).

The enzymatically inactive form, or proenzyme of uPA (pro-uPA), is secreted by the cells and is bound to its receptor (uPAR) on the cell surface. Certain proteases (e.g., plasmin, trypsin, kallikrein, cathepsin B) convert pro-uPA (411 amino acids) to an enzymatically active, high-molecular weight form (HMW uPA, MW = 52,000), which is composed of two polypeptide chains linked by a disulfide bond [A chain (158 amino acids, MW = 32,000) and B chain (253 amino acids, MW = 20,000)]. The activated uPA subsequently converts plasminogen to plasmin. Plasmin is a broad-spectrum protease that catalyzes degradation of multiple proteins in the extracellular matrix, thus allowing invasion and metastasis. Plasminogen activator inhibitors (PAI-1 and PAI-2), which are produced by normal and tumor cells, inhibit uPA even when it is bound to its receptor (75).

uPA appears to play a role in mediating tumor cell invasion in cancer. Elevated levels of uPA, PAI-1, and PAI-2 have been demonstrated in tissue extracts of various carcinomas, including breast (75), ovary (76), uterus (77), cervix, prostate, and colon. Expression of both uPA and PAI-1 in breast carcinomas correlates with the clinical course in numerous studies (75) (for review, see ref. 74). When both are overexpressed in the same biopsy, the risk of disease recurrence and decreased overall survival is more accurately predicted (78). However, this has not been tested in a rigorous, cooperative clinical trial in North America. Two Level 1 evidence studies carried out in Europe, i.e., a prospective randomized trial (79) and a pooled analysis (78), have recently validated the prognostic value of uPA/PAI-1 in node-negative breast cancer patients. uPA and PAI-1 are thus among the first biological prognostic factors to have their clinical value confirmed by two different Level 1 evidence studies. Advances accelerating these studies include the production of standardized ELISA kits for measuring uPA, uPAR, and PAI-1, and the availability of stable reference preparations for clinical trials.

# GYNECOLOGICAL CANCER

In the Western world, gynecological cancers represent approximately 15% of all cancers in women and are responsible for approximately 10% of all cancer deaths. In terms of frequency, endometrial cancers are the most common, followed by cancers of the ovary and the uterine cervix. However, ovarian cancer has the highest mortality rates, with more women dying of ovarian cancer than from cervical and endometrial cancer combined. Annually in the United States, there are approximately 23,300 new cases with about 13,900 deaths due to the disease (80).

## Ovarian Cancer

Over 90% of ovarian malignancies are epithelial tumors arising from the coelomic epithelium. Most of the remaining types are either germ cell or sex cord stromal cancers. The lack of early symptoms means that approximately 70% of patients with ovarian cancer present with advanced disease. While the overall five-year relative survival rate is on the order of 30%, the survival rate for Stage III and IV disease combined is only 10%. In contrast, a survival rate of 90% may be achieved for patients with early stage disease confined to the ovary.

At present there are no effective methods to screen for ovarian cancer in asymptomatic women. Tumor markers that would detect early ovarian cancer would be extremely useful, but available markers have not been helpful in detecting early epithelial cell cancers. The best available marker for epithelial ovarian cancer is the mucin, CA125 (81,82). Although elevated CA125 levels have been found in approximately 80% of all patients with epithelial ovarian cancer, high levels are found in only about 50% of patients with FIGO* Stage 1 disease.

**Use of CA 125 in screening.**

As a screening test, the main problems with CA125 are lack of sensitivity for early-stage disease (only about 50% of patients with Stage I have elevated levels) and lack of specificity. The EGTM guidelines state that use of CA125 cannot be recommended for general population screening to detect sporadic forms of this disease (82).
In combination with transvaginal sonography (TVS), however, CA125 may have a role in the early detection of ovarian cancer in women with a hereditary ovarian cancer syndrome. An NIH consensus statement has recommended that these women undergo at least annual rectovaginal pelvic examination and TVS, and that serum CA125 is measured (83).

**Use of CA 125 in diagnosis.**

It is possible to make some general recommendations about interpretation of CA125 results obtained in a “case-finding” setting (84,85). Doubling of CA125 levels in serum above baseline at any interval should prompt physical examination, TVS, and abdominal computerized tomography (CT) scan. Abnormalities detected by any of these indicate the need for laparoscopy or laparotomy.

In postmenopausal women, CA125 may be useful in the differential diagnosis of benign and malignant pelvic masses. Significantly elevated values of serum CA125 (>95 U/mL) in a postmenopausal woman with a pelvic mass should prompt referral to a surgeon specializing in thorough abdominal exploration, node sampling, omentectomy, and cytoreductive operations (86).

**Use of CA125 in prognosis and monitoring.**

The rate of decline of CA125 following initial cytoreductive surgery and during cytotoxic chemotherapy has been shown to be an independent prognostic factor in multiple trials and could be used to determine eligibility for subsequent additional chemotherapy (87,88). Following potentially curative surgery and cytotoxic chemotherapy, CA125 should be measured every three months. As above, an increase of CA125 above 35 U/mL, or a doubling of CA125 above baseline, should prompt further investigation and possible laparoscopy, if the patient is a candidate for salvage chemotherapy (89).

* FIGO, International Federation of Gynecology and Obstetrics.

Doubling of an elevated CA125 value during salvage chemotherapy is associated with disease progression in more than 90% of cases and indicates failure of that particular salvage chemotherapy. However, disease can progress without a concomitant increase in CA125 and tumor deposits should be measured by physical examination or imaging whenever possible. (For reviews see ref. 90 and ref. 91).

**Reference intervals.**

Some 95% of healthy adult women have a CA125 value of 35 U/mL or less. Postmenopausal women patients tend to have lower values (<20 U/mL in 99% of apparently healthy women). CA125 is not a specific marker for ovarian cancer and may be elevated in adenocarcinomas of the fallopian tube, endometrium, cervix, pancreas, colon, breast, and lung.

Serum CA125 levels may be elevated in benign gynecologic conditions including endometriosis, fibroids, adenomyosis, pelvic inflammatory disease, menstruation, and the first trimester of normal pregnancy. CA125 can also be elevated in plasma by benign ascites or by any condition that can inflame the peritoneum, pleura, or pericardium. Severe hepatic disease can also increase CA125 in the presence or absence of ascites. Reproducible effects of medications have not been observed.

**Preanalytical concerns.**

Serum tumor markers containing carbohydrate generally are reasonably stable at room temperature under routine laboratory conditions. However, rapid processing of samples to minimize degradation is prudent. CA125 should be measured after prompt separation of the serum from the clot. Storage of sera at 4 °C or frozen at –20 °C (short-term) and –70 °C (long-term) is recommended to ensure stability of the analyte if the need for re-analysis occurs.

**Analytical concerns.**

The NACB considers a CV of <15% desirable for sequential monitoring of CA125 values. At this CV, the range of 21–39 U/mL is within the 95% confidence interval for a mean value of 30 U/mL. The EGTM recommends a somewhat tighter inter-assay variability of <10% for automated tumor marker measurements (Table 1). Both biological variation and analytical imprecision should be taken into account (92).

**Postanalytical and reporting concerns.**

Since kit reagents and formats from different manufacturers may give slightly different values, the source of the assay kit as well as the range of normal values should be indicated on the report.

## NACB AND EGTM RECOMMENDATIONS FOR THE USE OF TUMOR MARKERS FOR OVARIAN CANCER

1. CA125 should not be used for screening a general asymptomatic population to detect sporadic ovarian cancer.
2. CA125 levels should be determined every six months with transvaginal sonography (TVS) performed annually as an aid in early detection of ovarian cancer in individuals with either a strong family history of breast or ovarian cancer, a demonstrated mutation in BRCA1, BRCA2, or a mismatch repair gene.
3. CA125 levels should be determined in women presenting with pelvic masses to distinguish benign from malignant lesions.
4. CA125 levels should be determined during primary therapy to predict prognosis.
5. CA125 levels may be used to document failure of salvage therapy.
6. Since loss of CA125 activity has been observed on repeated freezing and thawing, it is recommended that aliquots of sera be stored at –70 °C.

## Cervical Cancer

Worldwide, cervical malignancy ranks second to breast cancer as the main cause of cancer mortality in women. In the United States, the overall five-year survival for this malignancy is approximately 70%. However, for patients diagnosed with early stage disease, the five-year survival increases to almost 90%. For squamous cell carcinomas, squamous cell carcinoma antigen (SCCA) is the marker of choice. Other markers that have been investigated in cervical cancer include carcinoembryonic antigen (CEA), human chorionic gonadotropin (hCG) beta-core fragment (hCGβcf), and cytokeratins (TPA and CYFRA 21.1), but based on currently available evidence their use for cervical cancer cannot be recommended (82).

**Clinical utility of SCCA in cervical cancer.**

Results from SCCA determinations may be used with caution to balance prognostic variables employed to select patients in clinical trials or to identify individuals at sufficiently high risk to justify experimental adjuvant therapy. Elevated serum levels of SCCA at the time of diagnosis of stage IB or IIA cervical cancer indicate a three-fold increased risk of disease recurrence, independent of tumor diameter, grade, or the presence of lymph node metastases. Results of SCCA determinations may be used with caution to monitor disease recurrence.

Two consecutively rising SCCA values indicate progression or recurrence in 76% of cases, with false-positive elevations observed in 2.8–5%. Consequently, measurements of serum SCCA levels every three months in patients who are candidates for salvage radiotherapy or surgery may be helpful in therapy decisions, although a clinical benefit has not yet been documented.

**Reference intervals for SCCA.**

Some 99% of apparently healthy women have an SCCA value of 1.9 µg/L or less. Values in women are higher than in men. No consistent difference has been observed between smokers and nonsmokers. SCCA is not a specific serum marker for cancer of the uterine cervix, but it is elevated in a variety of squamous cell neoplasms including cancers of the skin, lung, head and neck, esophagus, bladder, penis and anus, benign skin diseases, lung diseases, and severe renal malfunction.

**Preanalytical concerns.**

Since large amounts of SCCA are often found in saliva, sweat, and respiratory secretions, exposure of blood to skin or saliva should be avoided.

**Analytical concerns.**

Mean daily variations of 24% have been observed in serum levels of SCCA. Cut-off values should therefore be individualized when monitoring disease recurrence.

**Postanalytical and reporting concerns.**

Manufacturer of kit reagents and limits of normal values should be reported. In the United States, results should be marked “for investigational use only.”

## Endometrial Cancer

No existing marker has the necessary sensitivity and specificity to screen for early endometrial cancer, and tumor markers contribute little to diagnosis. For monitoring patients with endometrial cancer, CA125 is the best available marker, with elevated levels found in approximately 60% of patients with recurrent endometrial cancer. However, as with SCCA in cervical cancer, there is no evidence that follow-up with serial CA125 levels enhances patient outcome. The role of CA125 in monitoring patients with uterine serous carcinoma is clinically of questionable value (93).

# PROSTATE CANCER

Prostate cancer is the most common male malignancy in the United States (excluding skin cancer) and the second leading cause of male death from cancer. According to the American Cancer Society, there will be 189,100 new cases in 2002 and 30,200 men are expected to die from this disease (13). Once prostate cancer reaches an advanced stage and becomes hormone-refractory, there is no effective therapy, and prevention of the disease is not yet possible. Thus, early detection and local treatment have been advocated in an effort to influence the significant morbidity and mortality associated with the disease.

**Clinical utility of prostate-specific antigen (PSA) in prostate cancer.**

Prostate-specific antigen (PSA) is the most important marker in the evaluation of prostate cancer and has significant application in detection and clinical management. The determination of prostatic acid phosphatase (PAP) does not add clinically useful information to PSA measurement, and is not recommended by the EGTM. PSA is associated almost exclusively with prostatic disease but is not specific for cancer and may be elevated in other conditions, including benign prostatic hyperplasia (BPH) and or prostatitis (94).

**PSA in detection of prostate cancer.**

This lack of specificity and the inability of PSA to determine tumor aggressiveness represent the most serious limitations of PSA in the setting of prostate cancer detection. The conventional reference range for PSA is between 0 and 4 ng/mL, but there is no absolute normal cut-off value applicable to all men or all PSA assays. Approximately 25% of men with known prostate cancer exhibit normal PSA levels, whereas as many as 50% of men with benign prostatic disease will have elevated PSA levels (95). Recent data from Swiss researchers demonstrate that a significant number of prostate cancer cases are diagnosed in men with PSA values between 1 and 3 ng/mL, and the indications are that most of these tumors may be clinically significant (96).

The positive predictive value of PSA [the number of positive tests in patients with the disease divided by the total number of positive tests (true positives + false positives)] in screening populations is disturbingly low (30%). Moreover, use of certain drugs and herbal remedies that decrease serum testosterone also decrease PSA levels, confounding interpretation of results.

**Reference intervals for PSA.**

Use of age-specific reference ranges is intended to increase sensitivity in younger men and increase specificity in older men. For younger men, using an upper reference limit of 2.5 ng/mL has been proposed to improve the early detection of organ-confined disease (97). However, this would result in an increased number of false-positive results in men without cancer. Conversely, use of higher PSA reference ranges in older men could result in the missed diagnosis of clinically significant tumors in a population that might potentially benefit from early treatment.

Although there is no consensus, some experts tend to favor using lower PSA values in younger men while using the conventional 4 ng/mL upper limit for older men. The EGTM view is that the use of age-specific reference ranges cannot be recommended yet, since only limited experience exists showing the efficacy of prostate biopsies for age-specific PSA decision points at concentrations lower than 4 ng/mL (98). In contrast, the NACB does endorse the use of age- and race-specific intervals for PSA (97,99,100). However, because of the controversy regarding the use of PSA for detecting very small tumors, the NACB does not recommend the use of a low cut-off value for PSA (2 ng/mL).

**PSA in screening for prostate cancer.**

Despite limitations, PSA is currently the best screening modality available for the detection of early stage prostate cancer, for which there is the greatest potential for successful treatment. Apart from the use of age-specific reference ranges, there have been several attempts to increase the specificity of PSA in the detection of prostate cancer. PSA density, PSA velocity, PSA doubling time, and percent-free PSA have also been considered, but with the exception of free PSA, these strategies have not been widely used in practice. Benign disease is associated with higher levels of free PSA versus com-

plexed PSA, and use of percent-free PSA may decrease the number of unnecessary biopsies in men with PSA concentrations in the diagnostic gray zone between 4 and 10 ng/mL (101).

Percent-free PSA may be particularly useful in identifying patients who actually have prostate cancer despite initial negative biopsy findings. In cases suspected of harboring malignant disease because the percent-free PSA indicates a high-risk profile, a cancer diagnosis may become evident with repeat biopsy (102). Some authors have reported that percent-free PSA determination also may have prognostic significance since relatively low levels of percent-free PSA have been associated with a more aggressive form of the disease (103). However reports from other authors contradict these results. The NACB and EGTM recommends the use of percent-free PSA as an aid in distinguishing prostate cancer from benign disease in highly selected subpopulations, e.g., when the total PSA level in serum is 4–10 ng/mL and digital rectal examination (DRE) is negative. This recommendation is tempered by the need to validate the medical decision limits for each combination of free and total PSA assays.

More recently, an assay has been developed to quantify the PSA complexed to alpha-1 antichymotrypsin (104,105). The measurement of this parameter results in slightly enhanced specificity as compared to total PSA (106), but no equivalence of the performance of complexed PSA to percent-free PSA has been proven yet.

**Preanalytical concerns.**

The NACB has issued recommendations for blood drawing and handling of specimens for PSA determinations. Since a number of factors may influence PSA concentrations, it is essential that blood be drawn prior to any manipulation of the prostate, after at least 24 hours following ejaculation if free PSA is being measured (if within 24 hours, the time of last ejaculation should be noted), and several weeks after resolution of prostatitis, prostate biopsy, or transurethral resection of the prostate.

Blood should be centrifuged and serum samples refrigerated within three hours of phlebotomy. Serum 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 temperatures of at least –20 °C (preferably at least –30 °C to avoid the eutectic point). Samples requiring long-term storage should be frozen at temperatures of at least –70 °C (107,108; see ref. 109 for review). Recognizing that recommendations about specimen collection may be difficult to implement in routine practice, the EGTM has suggested that specimens be taken under preanalytical conditions as similar as possible to those used in generating the reference values of the particular assays used (98).

**Analytical and reporting concerns.**

A statement should be appended to each report of results indicating that a single PSA measurement should not be used as a diagnostic tool for the detection and diagnosis of prostate cancer, but should be used in conjunction with a physical examination.

The report should indicate the sensitivity of the assay and the normal reference range for the test. It should include a statement saying that the results cannot be interpreted as evidence of the presence or absence of malignant disease, unless several measurements show rising PSA concentrations with a slope that cannot be explained by increases in benign prostatic tissue. The report should disclose the manufacturer of the PSA assay and state that results cannot be used interchangeably with any other method (98,110).

When monitoring patients post-treatment, a single PSA measurement should not be used as a diagnostic tool for the detection of recurrent prostate cancer. Demonstration of a rising PSA concentration is necessary.

Following radical prostatectomy, a sustained increase in PSA (based on several PSA measurements) indicates recurrence of disease. When an ultrasensitive assay is used for follow-up after radical prostatectomy (the only clinical indication for use of an ultrasensitive assay), the analytical sensitivity of the assay should be established and reported to the physician. It is essential that quality control material be available and determined at the ultrasensitive level.

Within-individual biological variation may be quite high at these low concentrations, and demonstration of a sustained increase in PSA level is therefore highly desirable. The EGTM view is that clinical treatment decisions should not be made on the basis of ultrasensitive PSA assay results.

**Guidelines for the early detection of prostate cancer.**

The American Cancer Society has issued guidelines for the early detection of prostate cancer (111). The guidelines recommend an annual screening with digital rectal examination (DRE) and serum PSA measurement beginning at the age of 50 in men at average risk with at least 10 years of life expectancy. PSA is the best biochemical test currently available for the detection of prostate cancer, but a DRE should be included whenever possible. Screening at an earlier age (45 years) would be appropriate in men at increased risk, including men of African-American descent and those with a family history. African-American men and those with one or more first-degree relatives with prostate cancer may develop prostate cancer several years earlier than other individuals and often develop a more aggressive type of cancer (112).

Individuals with a strong family history of prostate cancer involving first-degree relatives diagnosed at an early age could begin screening at 40 years of age. The recommendation for follow-up testing of these individuals would depend on the initial PSA result. Those with PSA levels less than 1 ng/mL would resume testing at 45 years of age, those with levels greater than 1 but less than 2.5 ng/mL would be tested annually, while those with levels of 2.5 ng/mL or greater would be evaluated further and considered for biopsy (113).

These guidelines are not an endorsement for mass screening for prostate cancer in men at average risk for the disease. Rather, the guidelines recommend that men be offered screening, but only after careful consideration of the benefits and limitations of prostate cancer testing. The essential difference in more recent recommendations for the detection of prostate cancer is the greater emphasis on informed decision-making by the patient. The revised guidelines emphasize that patients should be counseled concerning the risks, benefits, limitations, and potential harms associated with testing. It is important to indicate the disparity between incidence and mortality associated with prostate cancer and that many more men are diagnosed with prostate cancer than eventually die from the disease (114). It is therefore essential to put the proper emphasis on the risk and benefits associated with screening: the physician must not overestimate the possible benefits of early detection or underestimate the risks associated with early intervention. Men should also be informed that there are many uncertainties concerning treatment of early-stage disease and that the preferred treatment for clinically localized prostate cancer is not known (115).

**Merits of early detection of prostate cancer.**

There is still considerable debate regarding the merits of early detection of prostate cancer and not all physician organizations advocate routine screening. While the American Urological Association and the NACB endorse the American Cancer Society policy statement on the early detection of prostate cancer, other organizations differ about the benefit of prostate cancer screening. Arguments against screening are based on the fact that there is no conclusive evidence that early detection and treatment influence overall mortality, while the standard treatments for organ-confined prostate cancer are associated with significant and frequently irreversible side effects. Thus, 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. The overriding concern is that screening will result in over-diagnosis and over-treatment of early stage disease that may not be clinically significant (116). The view of the EGTM is that application of screening to the general population should depend on the results of prospective randomized studies showing that early detection and treatment can decrease prostate cancer mortality.

Several prospective randomized studies are already in progress to evaluate the impact of prostate cancer screening on survival from prostate cancer, but it will be several years before the controversy regarding prostate cancer screening will be resolved. The NCI and the US Public Health Service are conducting long-term, multi-center prostate cancer screening trials, and other large prospective studies are in progress in Canada and Europe (117). In the absence of definitive data from prospective randomized trials, evidence for the efficacy of testing has depended largely on demonstration of some association between prostate cancer testing and reduced prostate cancer mortality (118).

At present, there is no clear-cut evidence that screening is effective in saving lives. One study of registry data from Tyrol, Austria (where prostate cancer screening has been widely accepted), indicates a significant recent decline in the expected death rate from prostate cancer (119). This is in contrast with other parts of Austria where mortality rates have declined, but to a much lesser extent. The decrease in observed mortality was associated with a shift towards a more favorable stage at diagnosis with an increase in the proportion of organ-confined disease at presentation (120,121). The implications are that early detection and the availability of effective treatment resulted in a corresponding improvement in disease-specific survival. Trend data from the National Cancer Institute's Surveillance, Epidemiology, and End Results (SEER) program also suggest a decrease in prostate cancer mortality associated with a more favorable stage at presentation. Another indication that screening may be effective comes from a study conducted in Olmsted County, Minnesota, where a decline in the rate of advanced disease was linked with a corresponding improvement in survival (122).

Even though recent data suggest that the apparent stage shift to early disease and subsequent treatment of localized prostate cancer detected with PSA has positively influenced mortality rates, there is no conclusive irrefutable evidence that early intervention alters the natural history of the disease (123). Observed benefits may be the result of lead-time bias. 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. It is unlikely that the decline in prostate cancer mortality is entirely attributable to PSA testing, and more aggressive treatment of localized prostate cancer may account for some of the change in the mortality statistics (124).

**PSA in patient management.**

Treatment decisions for early-stage disease are perplexing since there are controversies about optimal therapy. Options for patients include expectant management (watchful waiting), radical prostatectomy, or radiation therapy (external beam radiation or brachytherapy). Patients with advanced metastatic disease usually undergo some form of hormonal therapy to achieve androgen deprivation and those with hormone-refractory disease may be entered into experimental chemotherapy protocols. PSA plays a vital role in the follow-up of prostate cancer patients during different phases of management, including surveillance, determination of eligibility for treatment, estimation of prognosis, and post-therapeutic monitoring. (Free PSA has not been shown to offer clinically relevant information and should not be determined during follow-up of prostate cancer.)

Radical prostatectomy is an option only for patients with organ-confined disease, but evaluation of the extent of disease and predicting response to surgical removal of the prostate have proven difficult. Attempts have been made to use biochemical parameters in estimating the probability of organ-confined disease but PSA as a single marker is not useful in this regard (125). However, the combination of PSA, clinical stage, and Gleason score has proven informative in the prediction of pathological stage for localized prostate cancer; predictive tables incorporating these parameters have been published (126). Physicians have used these tables to estimate the probability of organ-confined disease and determine whether the patient is a candidate for radical prostatectomy.

Following successful surgery, PSA should decrease to undetectable levels, persistently elevated PSA providing evidence of residual disease. However, undetectable PSA following surgery does not necessarily mean that the disease is cured, since approximately 35 percent of these patients will exhibit a detectable PSA elevation within 10 years following surgery. A rising PSA level after radical prostatectomy is a sign of relapsing disease and may pre-date other signs of progression by years (127). Following endocrine therapy, PSA does not always reflect the behavior of the tumor. Anti-androgenic medication can lead to low PSA concentrations although prostate cancer is present.

The use and interpretation of serial PSA data for assessing outcomes and determining prognosis still present major challenges to the clinician. Not all patients with biochemical recurrence will progress to metastatic disease or symptoms of disease in their lifetimes and not all patients will need to be treated (128). Factors reported to predict the time course to the development of metastatic disease include time to biochemical recurrence, Gleason score, and PSA doubling time (129). These parameters have been incorporated into an algorithm to estimate the likelihood of patients

remaining free of overt metastatic disease. Use of this diagnostic tool allows 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. PSA levels provide essential information about the efficacy of surgery or radiation therapy, help establish the possibility of residual disease (local or distant), signal recurrent metastatic disease before it can be detected by other conventional diagnostic procedures, and provide a useful adjunct in the evaluation of therapeutic response.

Knowledge of post-treatment PSA values can enhance quality of life when they suggest absence of residual disease. Conversely, however, increasing PSA values can lead to diminished well being in otherwise asymptomatic patients who may anticipate the clinical progress of the disease by rising PSA values months or years prior to the appearance of symptoms. The possible drawbacks of PSA determinations following treatment should always be weighed against the therapeutic means that can be offered to the patient in case of rising PSA values. However the clinical status of prostate cancer patients requires constant reassessment, and PSA can play a central role throughout the disease process.

**Advances in patient management—use of nomograms incorporating PSA for treatment decisions.**

The development and validation of prognostic nomograms (computerized models) incorporating PSA, Gleason score, and clinical stage to assist in determining eligibility of patients for treatment and in making informed treatment decisions reflect the most recent advances in patient management. Prediction is central to strategy, and nomograms provide the most accurate predictions currently available for most clinical situations and outcomes (130). Rather than relying on physician experience or general risk assessments of patient populations with similar characteristics to determine the optimal approach, nomograms provide specific information with maximum predictive accuracy.

During initial prostate evaluation, nomograms help determine whether biopsy is indicated, whether treatment is necessary, and which treatment would offer the best prognosis. After definitive therapy, where there is uncertainty concerning efficacy of treatment, nomograms provide prognostic information by estimating risk of recurrence. For patients with biochemical recurrence (rising PSA), nomograms indicate whether the patient requires further therapy and may help guide treatment strategies.

Predictive outcomes provided by computer models are not perfect, but nomograms can be extremely useful for making treatment decisions. The difficulty arises in the selection of the appropriate device when several competing nomograms may be applicable. At present, predictive models are available for determining outcomes for patients considering radical prostatectomy, three-dimensional conformal radiation therapy, and brachytherapy. A predictive model for expectant management is under development to assist in clarifying the risks and benefits of treatment versus watchful waiting.

## NACB AND EGTM RECOMMENDATIONS FOR USE OF PSA IN PROSTATE CANCER

1. The NACB and the EGTM agree with the American Cancer Society recommendation that for diagnosis, PSA must not be used alone, but should be evaluated in conjunction with DRE.
2. Given the controversy regarding the use of PSA for detecting very small tumors, a low cut-off (2 ng/mL) is not recommended.
3. The use of age- and race-specific reference intervals for each PSA assay is strongly recommended by the NACB, but age-specific reference ranges are not recommended by the EGTM.
4. The use of percent-free PSA is recommended as an aid in distinguishing prostate cancer from BPH when the total PSA level in serum ranges from 4–10 ng/mL and DRE is negative. This recommendation is tempered by the need for proper validation of the medical decision limits for each combination of free and total PSA assays within each institution.
5. It is recommended that blood be drawn before any manipulation of the prostate and several weeks after resolution of prostatitis.

6. The following recommendations apply to sample handling:
   - Samples should be centrifuged and refrigerated within three hours of phlebotomy.
   - Samples may be stored at refrigerated temperatures for up to 24 hours.
   - Samples that will not be analyzed within 24 hours of collection should be stored frozen (at least at –20 °C, and preferably at –30 °C or less).
   - For long-term storage, samples should be frozen at –70 °C or less.
7. It is recommended that when an ultrasensitive assay is used for clinical purposes, the lowest reportable concentration should be determined by the laboratory and reported to physicians. Quality control at such levels should be established. The contribution of within-individual biological variation (which may be quite high at these low concentrations) should also be taken into account.
8. It is recommended that the following statements and information be appended to each report of results:
   - PSA used in conjunction with DRE.
   - The name of the assay.
   - The analytical sensitivity of the assay.
   - A valid reference range specifically generated for the assay used. (Ethnic or regional differences between reference range populations should be considered.)

# COLORECTAL CANCER

Colorectal cancer is a leading cause of cancer death in the United States (representing 11% of all cancer deaths), and is second only to lung cancer. According to the American Cancer Society, there will be 148,300 new cases in 2002 and 56,600 people are expected to die from this disease (13). The five-year survival is about 90% when colorectal cancer is diagnosed at an early stage, but most cases are detected only after the cancer has spread and cure is not possible (120). Screening is key to controlling colorectal cancer since early detection and removal of adenomas that give rise to cancer have a major impact on survival (111). Tumor markers have not been useful in screening because of lack of specificity, but are a useful adjunct in predicting recurrence and assessing efficacy of treatment (15).

## Guidelines for the Early Detection of Colorectal Cancer

The American Cancer Society has advocated screening for colorectal cancer for the past two decades and has issued guidelines for early detection of the disease (111). The updated guidelines for 2001 provide several options for screening patients with an average risk for colorectal cancer, as well as for screening high-risk groups (those at greater than twice the average risk). Since colorectal screening utilization among individuals is low, these guidelines allow for greater flexibility in achieving screening goals.

Most patients (75%) who develop colorectal cancer exhibit no specific risk factors for the disease and are considered to be at average risk (131). The first option for average-risk adults 50 years of age or over is annual fecal occult blood testing (FOBT), which has been shown to decrease the risk of death by about one-third when performed routinely (132). Other options for screening at intervals of five years include flexible sigmoidoscopy (with or without FOBT) or double contrast barium enema. Flexible sigmoidoscopy is considered a reliable screening test for cancer within reach of the sigmoidoscope (133,134); combined with FOBT, which screens for cancer anywhere in the colon, sigmoidoscopy is even more effective (135). Double contrast barium enema refers to a procedure that allows radiological examination of the entire colorectum; however, this method is rather insensitive for visualizing smaller neoplasms. A final option is colonoscopy, the most sensitive method (136,137), recommended once every 10 years for individuals at average risk for colorectal cancer. A shorter time interval may be desirable in certain cases.

Individuals at increased risk for colorectal cancer are those with inflammatory bowel disease, familial adenomatous polyposis, hereditary nonpolyposis colorectal cancer, or a family history of cancer (111,138). High-risk individuals are more likely to benefit from screening at an earlier age and in many cases require special screening approaches and more intense follow-up surveillance. A family history for colorectal cancer involving one first-degree relative with either colon cancer or an adenomatous polyp increases an individual's risk approximately two-fold; these individuals develop cancer approximately 10 years sooner than those of average risk. Colorectal screening guidelines therefore recommend that men and women with a family history be offered screening at 40 years of age.

## Merits of Early Detection of Colorectal Cancer

Most colorectal cancers arise from precursor adenomatous polyps, lesions that may take as many as 10–12 years to progress to the malignant state (138). This long interval for cancer development offers a substantial opportunity for intervention before cancer actually develops. There is now considerable evidence to indicate that screening, diagnosis, and removal of the polyp are effective in decreasing the incidence and mortality of colorectal cancer (131).

Procedures for early detection can at the same time identify and remove precancerous adenomatous polyps, thereby preventing the development of colorectal cancer (111). In addition to prevention of colon cancer by polypectomy, screening enables the detection of early-stage cancers that can be treated with significantly less morbidity. Most importantly, screening for colorectal cancer not only results in a shift to an earlier stage of disease, but in randomized controlled trials, screening is associated with a decrease in disease-specific mortality. Unfortunately, screening rates for

colorectal cancer are low, and the major challenge is to increase public awareness and implement effective colorectal cancer detection programs (131).

## Clinical Utility of Carcinoembryonic Antigen (CEA) in Colorectal Cancer

A panel of the American Society of Clinical Oncology (ASCO) has developed guidelines for the use of tumor markers in colorectal cancer (15). CEA is considered the marker of choice, but CEA has no role in detection and diagnosis. Although CEA may identify the occasional patient with colorectal cancer, the high false-positive rate is unacceptable (139). Furthermore, there are no data to indicate that screening with CEA would have an effect on survival. The ASCO panel therefore does not recommend CEA testing for colorectal cancer screening. It is generally agreed that the primary role of CEA in the management of colorectal patients is as an adjunct in determining prognosis, monitoring for recurrence, and assessing response to therapy. Similar proposals have been published by the EGTM (140).

**Pre- and immediately postoperative measurement of CEA.**

The ASCO panel recommends that CEA measurement may be requested prior to surgical intervention in patients with colorectal cancer because the test may be useful as a prognostic indicator to complement pathologic staging and surgical treatment planning (141). In a recent consensus conference, the American Joint Committee on Cancer has proposed that CEA be included in the TNM staging system for colorectal cancer (142).

The ASCO recommendation is based on the panel's conclusion that most studies indicate that abnormal preoperative CEA values are associated with a higher risk of recurrence and a worse prognosis (143). However, despite the increased risk, there is no evidence that patients benefit from adjuvant therapy solely on the basis of an abnormal preoperative CEA level. In certain studies, preoperative levels of CEA have been shown to be prognostic in patients with Dukes' B stage disease, approximately 40–50% of these patients having aggressive disease. Recent preliminary data suggest that adjuvant chemotherapy has a modest but detectable beneficial effect on the outcome of patients with Dukes' B stage disease. It has yet to be established, however, whether CEA measurement can identify the patients within this subgroup who could benefit from adjuvant chemotherapy. Consequently, the panel does not endorse the use of CEA to select patients for adjuvant therapy. An elevated postoperative CEA is also an adverse prognostic indicator (144), but this information is not clinically relevant in terms of any major outcomes. The recommendation is that the CEA test should not be used in the immediate postoperative period.

**Monitoring with CEA following surgery.**

There is a limited role for CEA as an indicator for asymptomatic recurrence. CEA elevations frequently precede clinical evidence of disease progression by several months (145–147) and may be the first sign of a potentially curable recurrence. For identifying recurrences in patients with diagnosed colorectal cancer, CEA reportedly has a sensitivity of about 80% (range 17–89%) and a specificity of approximately 70% (range 34–91%). CEA testing was found to be most sensitive for diagnosing hepatic or retroperitoneal disease and relatively insensitive for local, peritoneal, or pulmonary involvement.

Some investigators have reported that a slowly rising CEA usually indicates locoregional recurrence, while rapidly increasing levels usually suggest hepatic metastasis. The value of CEA in detecting local recurrence was recently evaluated in a single institution prospective randomized trial (148). In this study with 207 patients, an elevated CEA value was the most frequent indicator of local recurrence without symptoms. The authors concluded that for the diagnosis of local recurrence, CEA measurement was more cost-effective than other procedures, including computerized tomography (CT). It was also suggested that intensive follow-up with CEA might be more beneficial for patients with rectal cancer than for those with colon cancer, as local recurrence is more frequent for rectal cancer.

The value of resecting recurrent cancer is controversial since early detection may not necessarily lead to a better outcome and there are no data to indicate that initiating therapy solely on the basis of an elevated CEA improves survival. However, two meta-analyses support the potential value of early detection of recurrence (149,150). The first meta-

analysis, while not discriminating between the effect of follow-up intensity and CEA testing on survival, considered pooled data from various small-scale randomized and comparative cohort studies. A statistically significant difference in cumulative five-year survival was demonstrated for patients undergoing intensive follow-up, as compared to a control group not offered such follow-up. (Intensive follow-up as defined in this study included clinical history, physical examination, and CEA testing at least three times per year for at least two years.) The second meta-analysis indicated that intensive monitoring only improved five-year survival rates if CEA assays were included.

Resection of isolated hepatic metastasis, if detected early, may enhance survival and quality of life (151). The ASCO panel therefore recommends that if resection of liver metastases would be clinically indicated, CEA testing should be performed postoperatively every two to three months in patients with stage II or stage III disease for about two years after diagnosis. If an abnormal CEA is confirmed, further evaluation for metastatic disease is essential before any therapy is initiated.

**Monitoring with CEA following chemotherapy and in advanced disease.**

Although surgery remains the most effective therapy for colorectal cancer, chemotherapy (e.g., with 5-fluorouracil and levamisole) is finding increasing use, especially in patients with advanced disease. CEA may be measured to assess response to treatment and is particularly useful in assessing metastases without easily measurable disease (152). It is important to note that transient elevations of CEA levels may occur following administration of 5-fluorouracil/levamisole (153).

CEA alone should not be used to determine the type or duration of treatment but can be used in evaluating disease status and may contribute additional information to that afforded by other clinical and diagnostic criteria (150). In the setting of metastatic disease, CEA accurately reflects disease activity and allows the physician to recognize and discontinue ineffective therapy (154). Although there is no consensus regarding the value of CEA in the management of advanced colorectal cancer, the NACB, ASCO, and EGTM all recommend the use of CEA for monitoring therapeutic response.

The ASCO panel regards CEA as the marker of choice for monitoring patients with advanced colorectal cancer but also recognizes that the serum test alone may not be sufficient for monitoring response to treatment. In evaluating response, ASCO and the EGTM recommend that CEA should be measured before the initiation of therapy and at regular intervals every two to three months thereafter, at least for the first two years after initial diagnosis.

Progressive disease may be documented with two successive CEA values that are above baseline, even in the absence of other confirmatory diagnostic criteria. This is based on the view that the predictive value of CEA is high enough to preclude the need for confirmatory testing. These recommendations represent a useful approach to the management of patients with advanced disease; but the value of this strategy in terms of clinical outcome and cost effectiveness remains to be determined.

While a clear benefit for serial CEA determination has not yet been shown in prospective randomized trials, the EGTM view is that CEA may be of use in the follow-up of certain patients with diagnosed colorectal cancer, i.e., to detect asymptomatic recurrences that can be operated on with intention to cure. Furthermore, the use of CEA in the follow-up of patients may prolong the interval between radiological examinations and may even reduce the number of these examinations required, albeit without wholly replacing them. This may help reduce cost and improve patient compliance. However, the following caveats must always be kept in mind when serially monitoring patients with diagnosed colorectal cancer:

- CEA elevations usually only occur in patients with advanced disease.
- Not all patients with recurrent colorectal cancer will exhibit increased levels.
- High levels may occur in conditions unrelated to recurrence.
- Certain cytotoxic therapies may transiently increase CEA concentrations.

## NACB AND EGTM RECOMMENDATIONS FOR USE OF CEA IN COLORECTAL CANCER

1. CEA testing is not recommended for colorectal cancer screening.
2. CEA may be ordered prior to surgical intervention in patients with colorectal cancer to complement pathologic staging and treatment planning.
3. CEA should not be used in the immediate postoperative period.
4. CEA testing may be performed postoperatively if resection of liver metastases would be clinically indicated.
5. CEA may be measured during treatment to monitor response to therapy and to document progressive disease.

# NEUROENDOCRINE TUMORS (PHEOCHROMOCYTOMA, NEUROBLASTOMA, CARCINOID TUMORS)

Neuroendocrine tumors arise from neural crest cells and include pheochromocytoma, neuroblastoma, medullary carcinoma of the thyroid, islet cell carcinoma, carcinoid of the gut, and Merkel cell tumors (155,156). An important clinical feature of all neuroendocrine tumors is that they produce and secrete excessive amounts of physiologically active compounds that are normally found in blood and urine, but at lower nonpathologic concentrations. In many instances, pathology is associated with elevated levels of tumor markers, and this feature may be diagnostic of the disease. Tumor markers associated with different neuroendocrine tumors are listed in Table 5.

**Table 5: Neuroendocrine Tumors and the Tumor Markers Associated With Them**

| Disease | Laboratory tests used in diagnosis and monitoring |
|---|---|
| Pheochromocytoma | Urinary and plasma catecholamines<br>Epinephrine, norepinephrine, dopamine<br>Vanillylmandelic acid (VMA), homovanillic acid (HVA) |
| Medullary thyroid carcinoma | Calcitonin |
| Islet cell carcinoma | Insulin[a] (nonspecific) |
| Carcinoid of the gut | Urinary 5-hydroxyindoleacetic acid (5-HIAA) |

[a] Not recommended as a marker for this tumor.

In each cancer the tumor marker should be utilized for case finding to confirm the existence of disease. Consecutive normal levels of the tumor marker do not necessarily rule out the presence of disease. Laboratory tests, appropriately used, are important and useful adjuncts for the diagnosis of neuroendocrine tumors (157). As described in detail below, major concerns with all these tumor markers are the need for rigorous specimen collection and preservation, and the effects of iatrogenic interferences.

## Catecholamines

**Collection of urine for determination of catecholamines.**

A 24-hour urine specimen must be refrigerated immediately after collection. Sample preservation can be enhanced by maintaining an acid pH of 2–4 with the addition of 6N hydrochloric acid (HCl). The sample acidity should not be lower than pH 2 as assay interference may result. If HCl is added to the collection container prior to collection, 6 mL of 6N HCl will be satisfactory in maintaining pH for adult collections (based on urine output of 1– 2 L/24-hour period). Acid preservation should be adjusted for volume in pediatric specimen collection.

**Collection of plasma for determination of catecholamines.**

A heparinized, anti-coagulated blood specimen should be collected from the patient, who should be calm and/or in a supine position for 30 minutes prior to collection. The specimen should be chilled in ice water immediately after collection and the plasma separated as soon as possible (158). The specimen must be frozen immediately. Specimens are stable at –70 °C for 6–8 months, but the analytes are unstable at ambient temperature, or 4 °C.

**Iatrogenic-related problems influencing the determination of catecholamines.**

Urinary catecholamines may be extremely elevated (sometimes unpredictably) as a result of medication that directly interferes with the assay or due to physiologic interference (157,159,160). Medications such as alpha-methyldopa (Aldomet) interfere with the quantitation of dopamine. Monoamine oxidase (MAO) inhibitors also affect physiologic levels of catecholamines. Plasma catecholamines cannot be quantified in patients receiving isoproterenol, isoetharine, or labetalol.

**Reference ranges for catecholamines.**

High-performance liquid chromatography (HPLC) is the analytical method of choice for measurement of catecholamines (158,161,162). Age-related reference ranges obtained using this technique are shown in Table 6.

**Table 6: Age-Related Reference Ranges for Catecholamines (Measured by HPLC)**

| Age (years) | Norepinephrine (µg/total volume) | Epinephrine (µg/total volume) | Dopamine (µg/total volume) |
|---|---|---|---|
| 0–1 | 0–10 | 0–2.5 | 0–85 |
| 1–2 | 1–17 | 0–3.5 | 10–140 |
| 2–4 | 4–29 | 0–6.0 | 40–260 |
| 4–7 | 8–45 | 0.2–10 | 65–400 |
| 7–10 | 13–65 | 0.5–14 | 65–400 |
| 10–15 | 15–80 | 0.5–20 | 65–400 |

## Vanillylmandelic Acid (VMA) and Homovanillic Acid (HVA)

**Collection of urine for determination of VMA and HVA.**

The 24-hour collection procedure is the same as that for catecholamines. VMA and HVA can also be determined in random specimens and reported as a ratio of VMA or HVA to urinary creatinine.

**Iatrogenic-related problems influencing the determination of VMA and HVA.**

The effects of some drugs on VMA and HVA determinations may not be predictable. Medications that may interfere are shown in Table 7. A markedly elevated total catecholamine concentration accompanied by a normal VMA suggests that the patient is taking methyldopa, which is metabolized to α-methylnorepinephrine and is not consistent with the presence of a pheochromocytoma (163).

**Table 7: Medications That May Interfere With VMA and HVA Determinations**

| |
|---|
| Amphetamines and amphetamine-like compounds |
| Appetite suppressants |
| Bromocriptine |
| Buspirone |
| Caffeine |
| Carbidopa-levodopa (Sinemet) |
| Clonidine |
| Dexamethasone |
| Diuretics (in doses sufficient to deplete sodium) |
| Methyldopa (Aldomet) |
| MAO inhibitors |
| Nose drops |
| Propafenone (Rythmol) |
| Tricyclics |
| Vasodilators |

**Table 8: Age-Related Reference Ranges for Vanillylmandelic Acid (VMA) and Homovanillic Acid (HVA) (Measured by HPLC)**

| Age | VMA (µg/mg creatinine) | HMA (µg/mg creatinine) |
|---|---|---|
| 1 day | <17 | <42 |
| 3 months | <16 | <39 |
| 6 months | <15 | <37 |
| 9 months | <14 | <35 |
| 1 year | <13 | <33 |
| 1.5 years | <12 | <29 |
| 2 years | <11 | <26 |
| 3 years | <10 | <22 |
| 4 years | <9 | <19 |
| 5 years | <8 | <17 |
| 6 years | <8 | <14 |
| 7 years | <7 | <13 |
| 8 years | <7 | <12 |
| 9 years | <7 | <11 |
| 10 years | <6 | <10 |
| 11 years | <5 | <9 |
| 18 years and adult | 1.08–4.23 | 0.5–4.2 |

**Reference ranges for VMA and HVA.** HPLC is the analytical method of choice for measurement of VMA and HVA. Age-related reference ranges obtained using this technique are shown in Table 8.

## Hydroxyindoleacetic Acid (5-HIAA)

Measurement of the urinary excretion of 5-HIAA, the major metabolite of serotonin, has been the principal laboratory test for the diagnosis of serotonin overproduction in carcinoid tumors (164). Serotonin is one of many vasoactive substances contributing to the carcinoid syndrome of flushing, diarrhea, and cardiac valvular disease. The absence of 5-HIAA in patients suspected of having carcinoid tumor may be due to its intermittent secretion.

**Collection of urine for determination of 5-HIAA.**

The 24-hour collection procedure is the same as that for catecholamines.

**Iatrogenic and food-related problems influencing the determination of 5-HIAA.**

Patients should be instructed to abstain from certain foods rich in serotonin and other indoles as well as medications. Common foods and medications that should be avoided are listed in Table 9.

**Table 9: Foods and Medications That May Interfere With the Measurement of Urinary 5-HIAA**

| Decreased 5-HIAA values | Increased 5-HIAA values | |
|---|---|---|
| *Medications* | *Medications* | |
| Aspirin | Acetaminophen | Methamphetamine (Desoxyn) |
| Chlorpromazine | (Thorazine) | Acetanilid Naproxen |
| Corticotropin | Caffeine | Nicotine |
| Dihydroxyphenylactic acid | Coumarin acid | Phenacetin |
| Ethanol | Ephedrine | Phenmetrazine |
| Gentisic acid | Fluorouracil | Phenobarbital |
| | Glycerol | Phentolamine |
| Homogenistic acid | Guaiacolate (guaifenesin) | Rauwolfia |
| | Melphalan (Alkeran) | Reserpine |
| Hydrazide derivatives | Mephenesin | Robaxin |
| Imipramine (Tofranil) | | Valium (Diazepam) |
| Isocargoxazid (Marplan) | | |
| Levodopa ← Methocarbamol | | |
| Keto acids | *Foods* | |
| Monoamine oxidase (MAO) inhibitors | Avocados | |
| Methenamine | Bananas | |
| | Coffee | |
| Methyldopa (Aldoclor) | Eggplant | |
| Phenothiazine (Compazine) | Plums | |
| Perchlorperazine | Pineapple | |
| Promazine | Tomatoes | |
| Promethazine (Mepergan) | Walnuts | |

**Reference range for 5-HIAA.**

HPLC is the analytical method of choice for measurement of 5-HIAA. The reference range with this method is 3–15 mg/dL.

## Medullary Thyroid Carcinoma (MTC)

Medullary thyroid carcinoma (MTC) is a neoplasm of the calcitonin-secreting parafollicular or C-cells of the thyroid gland and represents approximately 10% of all thyroid cancers. While follicular cells of the thyroid gland metabolize iodine and produce and elaborate thyroid hormones (i.e., $T_3$ and $T_4$), the C-cells produce and secrete calcitonin (165).

**Indications for measurement of calcitonin.**

Calcitonin determinations should be performed on patients who are suspected to have MTC because elevations in calcitonin levels are highly specific for the diagnosis and follow-up of MTC. In most patients with MTC, the concentration of calcitonin is sufficiently elevated to be diagnostic for the tumor. Indeed, calcitonin may be elevated prior to any clinical evidence of a tumor. There is also a positive correlation between tumor mass and circulating calcitonin concentration. After treatment of MTC by total thyroidectomy, calcitonin measurements should be used to monitor the patient for recurrent disease (166,167).

**Specimen collection and handling for calcitonin.**

Calcitonin can be measured in serum or plasma (EDTA). Specimens should be processed immediately following collection and stored at 2–8 °C for a maximum of one day or frozen (–20 °C) for no more than 15 days. Rapid processing of specimens for calcitonin analysis is required. Specimens should be separated in a refrigerated centrifuge and serum (or plasma) should be frozen quickly in plastic tubes.

**Interpretation of provocative tests for MTC.**

Calcitonin is the only biochemical marker that can be used to detect the presence of the tumor before clinical symptoms have evolved in patients with occult C-cell carcinoma or suspected familial MTC (168). As a marker for provocative testing using pentagastrin or calcium, calcitonin levels exceeding four times the upper reference limit or that of the basal values are diagnostic for MTC. In normal individuals, pentagastrin stimulation will result in calcitonin levels less than four times the upper limit of normal, with calcitonin normalization within 30 minutes. Determination of basal calcitonin levels prior to therapy is essential for interpreting the results of provocative tests.

**Reference intervals for calcitonin.**

All U.S. laboratories are required by federal (CLIA) or state regulations to test and establish reference intervals for all assays. Whether the calcitonin assay employed is a radioimmunoassay (RIA) or a two-site immunometric assay (IMA) will influence results considerably. Immunometric methods, using monoclonal antibodies, generally give lower results than do RIA, which use polyclonal antibodies. The reference intervals for calcitonin therefore vary, reflecting differences in methodology and antibody specificity, as well as the purity of the standards employed. Generally, a basal calcitonin level useful for clinical interpretation is 5–19 pg/mL, with lower results observed for IMA.

In order to achieve the assay stability essential for diagnosis and monitoring of MTC, manufacturers should provide clinical laboratories with calcitonin reagents and standards that have minimal lot-to-lot variation. Provision to laboratories of kits with long shelf lives (e.g., by sequestering of lots) is also recommended.

**Performance criteria.**

As both circulating calcitonin and the antisera used in the reagents for assay are heterogeneous, it is not possible to specify performance criteria that are applicable to all assay procedures. Each laboratory must determine its own performance criteria and use internal reference standards to monitor assay quality.

Determination of calcitonin should be performed on specimens from a statistically significant number of normal volunteers in the age range of 5–17, 18–30, 31–50, and over 50 years, to obtain an approximation of normal values for fasting calcitonin.

This is particularly important if calcitonin is measured after stimulation of secretion by pentagastrin and calcium. Failure to do so may lead to the reporting of totally uninterpretable clinical data.

## NACB RECOMMENDATIONS FOR USE OF TUMOR MARKERS IN NEUROENDOCRINE TUMORS

1. Measurement of plasma catecholamines is useful in the diagnosis of a pheochromocytoma but extreme care must be taken in obtaining the samples for analysis. It must also be remembered that a plasma catecholamine determination represents a single point in time and does not have the advantage of the integrating effect of a timed urine collection. A urine catecholamine fractionation should be performed to assist in the diagnosis of pheochromocytoma.
2. All medications that are not absolutely required clinically should be discontinued for at least 72 hours prior to specimen collection.
3. Catecholamine fractionation should be performed. This is preferable to the use of total catecholamine levels for the diagnosis of pheochromocytoma.
4. Specimens should be stored between 2–8 °C to optimize preservation.
5. HPLC is recommended as the analytical method for catecholamine, VMA, and HVA determinations.
6. Serial determinations of 5-HIAA should be performed during an episode if practical, when the patient is symptomatic.
7. Urine specimens to be used for determination of catecholamines, VMA, HVA, and 5-HIAA should be refrigerated during the collection period. Specimens must be acidified between pH 2–4.

# GERM CELL TUMORS

Germ cell tumors, despite their rarity, constitute the most common cancer in males between the ages of 15 and 35 (169). This disease is highly curable, with long-term remissions being observed in more than 90% of patients following treatment, whether by surgery, radiation therapy, chemotherapy, or a combination of these (170,171).

Survival and prognosis in germ cell cancer are both highly dependent on TNM stage, vascular invasion, number and extension of visceral metastases (liver, bone, lung, brain), and the initial concentrations of the serum tumor markers α-fetoprotein (AFP), human chorionic gonadotropin (hCG) and/or its β-subunit (hCGβ), lactate dehydrogenase (LDH), and placental alkaline phosphatase (PLAP). Pre-treatment serum concentrations of these markers all influence the choice of therapy (172,173).

Germ cell tumors are divided into seminomas and nonseminomatous germ cell tumors (NSGCT). In contrast to seminoma, NSGCT can contain any combination of multiple cell types such as teratoma, embryonal cell carcinoma, choriocarcinoma, or endodermal sinus tumor. The rationale for this classification is that it correlates with clinical treatment: seminoma is highly sensitive to radiation therapy whereas NSGCT is highly radiation-resistant. The correct diagnosis is therefore critical for optimal treatment outcome (170).

**Tumor Markers in the Identification and Treatment of Germ Cell Tumors Diagnosis.**

The routine evaluation for patients who present with a testicular mass includes a CT scan of the abdomen, chest, and pelvis, as well as measurement of serum tumor markers including AFP, hCG, and LDH (174,175). The production of AFP is restricted to endodermal sinus tumor and embryonal carcinoma. Syncytiotrophoblastic cells produce hCG, and elevated levels of hCG can be found in patients with pure seminoma as well as patients with NSGCT histologies. Measurement of both intact hCG and hCGβ, both commonly detected by some commercial hCG tests, is essential, as some tumors may produce only hCGβ. It is important to note that not all commercially available assays for hCG recognize both these forms.

The EGTM also recommends measurement of placental alkaline phosphatase (PLAP), a heat-stable isoenzyme of alkaline phosphatase that is normally expressed by placental syncytiotrophoblasts (176,177). PLAP or PLAP-like activity occurs in normal tissue (e.g., testis, cervix, thymus, lung) as well as in malignant tissue (e.g., germ cell, ovarian, and lung tumors). Raised serum concentrations of PLAP are found in seminomas (sensitivity 51–90%) and NSGCT (sensitivity 20–36%), as well as in ovarian tumors. Measurement of serum PLAP, which has a half-life of 0.6 to 2.8 days, is advantageous as it is raised in up to 80% of testicular seminomas (Stage I and metastatic), while hCG is raised in fewer than 20%. Measurement of PLAP is not recommended in smokers, however, as serum levels are increased up to ten-fold relative to those of nonsmokers, with considerable inter-individual variation.

The serum level of LDH, and possibly its subunit LDH-1, is an independent prognostic factor in patients with advanced germ cell tumors (178). LDH has a relatively constant rate of degradation from patient to patient. Increases in the serum level are influenced primarily by tumor burden and growth rate, cell proliferation, and death. LDH is elevated in approximately 60% of NSGCT patients with advanced disease and 80% of patients with advanced seminoma. Historically, either hCG or AFP, or both, were elevated in approximately 80% of patients with advanced GCT (172). As a consequence of stage migration, hCG and AFP are each elevated in only 40% of nonseminoma patients in more recent series; hCG is also raised in 7–18% of seminomas. In contrast, an increased AFP is seen only in nonseminomatous histologies even if histopathology suggests pure seminoma.

**Prognosis.**

The International Germ Cell Cancer Collaborative Group (IGCCCG) studied the use of serum tumor markers as prognostic variables and analyzed data from GCT trials in Europe, North America, and Australia to determine independent prognostic factors for use in a single classification system (179). This study was performed in over 5000 GCT patients and confirmed that pre-treatment levels of LDH and hCG, the site of the primary tumor (mediastinal vs. testis or

retroperitoneal), and the presence of nonpulmonary visceral metastases were independent factors for survival. Due to the larger number of patients in this study, the pre-treatment level of AFP was confirmed as independently prognostic. Using tumor markers, histology, site of primary tumor, and extent of disease, patients with advanced disease were allocated to three prognostic strata (Table 10). Based on the finding that prognosis was dependent on pre-treatment serum tumor marker concentrations, GCT staging was completely revised in 1997 to include their use. Both the American Joint Committee on Cancer (AJCC) and the Union Internationale Contre le Cancer (UICC) have adopted the new system. AFP, hCG, and LDH each contribute to the (S) variable.

**Table 10: Contribution of Serum Tumor Marker Measurements to the Prognostic Classification of Metastatic Germ Cell Tumors (179)**

| | Tumor marker concentration | | |
|---|---|---|---|
| **Prognostic group**[a] | **AFP (ng/mL)** | **HCG (U/L)** | **LDH (Multiple of RR)**[b] |
| Good (S1) | <1000 | <5000 | <1.5 × (RR) |
| Intermediate (S2) | ≥1000 and ≤10,000 | ≥5000 and ≤50,000 | ≥1.5 × (RR) and 10 × (RR) |
| Poor (S3) | >10,000 | >50,000 | >10 × (RR) |

[a] S, serum tumor marker.
[b] LDH concentrations expressed as multiples of the upper limit of the reference range (RR).

**Post-treatment monitoring.**

Determination of the half-lives of AFP and hCG is recommended for monitoring treatment, with normalization of both markers (AFP half-life within 5 days; hCG half-life within 1–2 days) indicating favorable prognosis (180). Half-lives may be calculated using linear regression, which requires a minimum of three well-spaced measurements within a 10-day period post-orchiectomy. After two cycles of chemotherapy, patients with marker half-lives of more than seven days for AFP and/or more than three days for hCG have significantly lower survival rates than those with shorter marker half-lives (181). An abnormal serologic response, defined as failure to normalize, or a prolonged half-life by either marker can predict relapse months after chemotherapy. The majority of patients with slow marker decline will relapse despite early clinical improvement. This first experience with the rate of tumor marker decline during chemotherapy (181) has recently been confirmed to possess prognostic value independent of risk (stratified according to the classification system of the IGCCCG) (182).

By combining the prognostic criteria of the UK Medical Research Council (MRC) with analysis of marker half-lives, prognostic discrimination can be improved and three different risk groups identified. Patients in the “poor risk” category may then be selected for more aggressive chemotherapy (183).

**Guidance of post-chemotherapy surgery.**

Cure of patients with metastatic GCT is optimized by the use of surgery after chemotherapy in selected patients (173). The role of surgery in patients with advanced GCT after chemotherapy has evolved substantially in the era of combined modality therapy. Patients with residual masses after chemotherapy should undergo post-chemotherapy surgery to remove residual carcinoma or teratoma. It is essential that only patients whose serum tumor marker levels are normal be subjected to post-chemotherapy surgery.

The large majority of patients with a persistently elevated AFP or HCG after chemotherapy will probably have surgically unresectable disease. These patients should be referred for salvage chemotherapy.

**Management of germ cell tumors.**

Clinical research has resulted in the cure of the majority of patients presenting with GCT. A major advance in the diagnosis and treatment of GCT patients is the integration of serum tumor markers in staging and treatment. The use of serum tumor markers in GCT is unique in that:

- Serology may predominate over histology in treatment decisions; e.g., the presence of AFP in a patient with seminoma dictates that the patient is treated as an NSGCT even if the histology is seminoma.
- The extent of elevation of tumor marker levels defines the prognostic classification of metastatic germ cell tumors.
- Tumor marker normalization is required to assess response to chemotherapy.
- The potential for relapse months after chemotherapy can be predicted by the rapidity of decline in the serum AFP or hCG in the first six weeks of therapy.
- Normalization of serum tumor markers is a requirement for the integration of post-chemotherapy surgery.

## NACB AND EGTM RECOMMENDATIONS FOR USE OF TUMOR MARKERS IN GERM CELL TUMORS

1. Determine AFP, hCG, and LDH as aids in the evaluation and staging of germ cell tumors prior to orchiectomy.
2. Any patient with a seminoma and an elevated AFP should be considered to have a nonseminomatous tumor and treated accordingly.
3. These tumor markers should be determined immediately before orchiectomy and, if elevated, should then be measured serially after orchiectomy. The rate of fall should be compared with the normal rate of disappearance of AFP (half-life <7 days) and hCG (half-life <3 days). All GCT patients should be staged using the new TNMS system incorporating tumor marker results.
4. The surveillance schedule should include determinations of AFP, hCG, and LDH monthly in the first year, and alternating months during the second and third year. Clinical investigations should include physical examination, chest X-ray, and ultrasonography or CT scan of the abdomen and pelvis at 2- to 3-month intervals during the first three years after therapy.
5. Stage IIA and Stage IIB patients who do not receive chemotherapy immediately after surgery should undergo surveillance incorporating determinations of AFP, HCG, and LDH with a monthly physical exam and chest X-ray for the first year, every other month in the second year, and every third month in the third year.

# PLASMA CELL DYSCRASIA (MONOCLONAL GAMMOPATHIES)

The monoclonal gammopathies constitute a group of disorders characterized by the proliferation of a single clone of plasma cells that produce a homogeneous, monoclonal (M) protein (184). Each M-protein consists of two heavy polypeptide chains of the same class [gamma (γ) in IgG, alpha (α) in IgA, mu (μ) in IgM, delta (δ) in IgD, and epsilon (ε) in IgE] and two light polypeptide chains [kappa (κ) or lambda (λ)] of the same type.

## Laboratory Diagnosis and Monitoring

**Preanalytical concerns.**

These include preference for a fasting sample in order to avoid hyperlipemia. Samples should be covered to avoid evaporation if they are stored in the refrigerator. If cryoglobulin is suspected, the specimen should be kept at 37 °C. If bacterial growth appears, the specimen must be discarded. Hemolysis should be avoided because the presence of a haptoglobin-hemoglobin complex will produce an $\alpha_2$-β spike in the electrophoretic pattern. The serum should be frozen at –20 °C if it is not used immediately. An increase or decrease of 0.5 g/dL in the size of the M-spike indicates a significant change.

Sulfosalicylic acid or Exton's reagent is more reliable than dipstick tests for the detection of monoclonal light chains (Bence Jones proteins). An aliquot from a 24-hour urine specimen with no preservative is needed.

The amount of M-protein in the urine is a direct measure of the tumor mass in the patient, and thus is useful in following the clinical course.

**Electrophoresis.**

Electrophoresis is performed on agarose gel, with high voltage applied across the electrodes and gel. After staining with Ponceau S, the preparation is dried and each pattern is visually inspected. A scanning densitometer quantitates each component after the total protein has been determined (185).

Electrophoresis is useful as a screening test and as an aid to the diagnosis of multiple myeloma (MM) or related disorders. It is also useful in monitoring the course of a patient with MM or Waldenström's macroglobulinemia (WM) because the size of the M-protein is a direct reflection of the tumor mass of the patient. Consequently, one can determine whether the patient is responding to therapy or has progressive disease (186,187).

**Immunofixation or immunoelectrophoresis.**

Immuno-fixation (or immunoelectrophoresis) is necessary for determining the presence and type of the M-protein. It is particularly helpful for detecting a small M-protein in patients with primary systemic amyloidosis (AL) or in following a patient with solitary plasmacytoma or extramedullary plasmacytoma. (185,188). Immunofixation is very helpful in the recognition of biclonal or triclonal gammopathies.

**Quantitation of immunoglobulins (nephelometry).**

Nephelometry is an excellent test for quantitation of immunoglobulins. The degree of turbidity produced by antigen-antibody interaction is measured by nephelometry in the near ultraviolet (UV) regions. This technique accurately measures 7S and 19S IgM, monomers and polymers of IgA, or aggregates of IgG accurately (189). The levels of IgM may be 1,000 to 2,000 mg/dL greater with nephelometry than expected from the size of the M-protein in the densitometer tracing. The IgG and IgA levels may also be spuriously increased. Therefore, the clinician must use the same or both techniques for following the size of an M-protein in a patient (190).

**Serum viscosity.**

Viscosity is the property of fluid to resist flow. Signs or symptoms of hyperviscosity include oronasal bleeding, blurring or loss of vision, headache, vertigo, nystagmus, deafness, ataxia, paresthesias, diplopia, somnolence, stupor, and coma. There is segmental dilation of retinal veins and retinal hemorrhages. WM is the most common cause of hyperviscosity, but it can also be found in patients with IgA or, rarely, IgG myeloma (191). The Ostwald-100 viscometer is a satisfactory instrument for measurement of viscosity, but a Wells-Brookfield viscometer is preferred because it is more accurate, requires less serum, and can perform at different shear rates and at variable temperatures. In addition, the determinations can be made much more quickly, especially if the viscosity of the serum is high.

**Cryoglobulin determination.**

Cryoglobulins are proteins that precipitate when cooled and dissolve when heated. They may be classified as type I (monoclonal IgM, IgG, IgA, or rarely, monoclonal light chains); type II (mixed; two or more immunoglobulins of which one is monoclonal), and type III (polyclonal in which no M-protein is found). The specimen must be collected at 37 °C and then placed in a 37 °C thermos and delivered to the laboratory. The specimens are allowed to clot at 37 °C and are then centrifuged at 37 °C. The serum is placed in a refrigerator or an ice bath and read at 24 hours. If no precipitate occurs, the specimen is kept at 0 °C for seven days. The precipitate is washed, and immunoelectrophoresis is performed with monospecific antisera to determine the type of immunoglobulin in the cryoprecipitate (192).

**Capillary electrophoresis.**

Capillary zone electrophoresis is performed on fused silica capillary tubes with a large surface-to-volume ratio and high voltage. This produces rapid separation of the proteins. The densitometry tracings of the separation obtained with capillary and agarose gel electrophoresis produce very similar patterns. Better resolution with capillary electrophoresis allows for more accurate quantitation of the M-protein (189,193). Immunosubtraction with capillary electrophoresis for typing of the M-protein is performed by repeating electrophoresis with antisera to IgG, IgA, IgM, κ, and λ. The presence of an M-protein is detected by its removal with the appropriate heavy- and light-chain antisera. This is less labor-intensive and more economical than immunofixation. The latter is still the gold standard for the detection of a monoclonal protein. Capillary electrophoresis is not yet available for evaluation of M-proteins in the urine.

## NACB RECOMMENDATIONS FOR LABORATORY TESTING IN MULTIPLE GAMMOPATHIES (MYELOMA)

1. Electrophoretic studies should be performed whenever multiple myeloma (MM), Waldenström's macroglobulinemia (WM), or primary systemic amyloidosis (AL) is suspected.
2. It is essential to differentiate a monoclonal from a polyclonal increase in immunoglobulins. Immunofixation should be performed when a sharp peak or band is found on the agarose electrophoretogram or when MM, WM, AL, or a related disorder is suspected.
3. Quantitation of immunoglobulins by radial immunodiffusion is not recommended.
4. The specimen must be allowed to clot because fibrinogen appears as a distinct narrow band between the β and γ fractions in plasma and may be mistaken for an M-protein.
5. Analysis of urine is essential for patients with monoclonal gammopathies.
6. The serum viscosity should be performed when the IgM monoclonal protein value is >3 g/dL or the IgA or IgG protein value is >4 g/dL. It should also be performed on any patient with oronasal bleeding, blurred vision, or neurologic symptoms suggestive of a hyperviscosity syndrome.

# LUNG CANCER

The generally poor prognosis of patients with lung cancer and the lack of effective therapy for recurrent disease limit the application of tumor marker determinations, especially in follow-up care. Nevertheless, measurement of tumor markers may be helpful; guideline recommendations for their proper use in differential diagnosis and in monitoring the efficacy of therapy have been proposed by EGTM (194).

Most primary lung tumors can be classified into four major histological types—squamous cell carcinoma, adenocarcinoma, large cell carcinoma, and small-cell lung cancer (SCLC). SCLC accounts for 20–25% of the cases of bronchogenic carcinoma and differs clinically and biologically from the other three histological types, which are all generally referred to as non-small-cell lung cancer (NSCLC). It is now evident that many tumors have features of more than one histological type of cancer. Thus, both SCLC and NSCLC represent heterogeneous groups in which there is considerable overlap among the major histological types of lung cancer.

## Use of Tumor Markers in Lung Cancer

The tumor markers most frequently used or suggested as markers for lung cancer include neuron-specific enolase (NSE), carcinoembryonic antigen (CEA), squamous cell carcinoma antigen (SCCA), CYFRA 21-1, and pro-gastrin-releasing peptide (ProGRP). The properties of CEA and SCCA (195) are described elsewhere in this chapter.

**Neuron-specific enolase (NSE).**

The glycolytic enzyme enolase (2-phospho-D-glycerate hydrolase, EC 4.2.1.11) exists as several dimeric isoenzymes ($\alpha\alpha$, $\alpha\beta$, $\alpha\gamma$, $\beta\beta$, and $\gamma\gamma$). The $\alpha\gamma$- and $\gamma\gamma$-enolase isoenzymes are also known as neuron-specific enolase (NSE) as they are produced in central and peripheral neurons and malignant tumors of neuroectodermal origin (e.g., SCLC, neuroblastomas, intestinal carcinoid) (196).

**CYFRA 21-1.**

CYFRA 21-1 is a relatively new tumor marker for which the assay uses two monoclonal antibodies directed against a cytokeratin 19 fragment. Immunohisto-chemical studies demonstrate that cytokeratin 19 is abundant in carcinomas of the lung, thus CYFRA 21-1 is the most sensitive marker for NSCLC (197). Since CYFRA 21-1 represents only fragments of cytokeratin 19, the CYFRA 21-1 tumor marker test shows a higher specificity than that for tissue polypeptide antigen (TPA), which determines a mixture of cytokeratins 8, 18, and 19.

**Pro-gastrin-releasing peptide (ProGRP).**

ProGRP is a relatively stable precursor of the hormone gastrin-releasing peptide (GRP). In humans, GRP is found predominantly in the gastrointestinal and respiratory tract and also in the central nervous system. Several studies suggest that GRP is released by tumor cells of small cell lung cancer and may even stimulate the growth of SCLC cells. ProGRP has not yet been incorporated in the EGTM recommendations, but is discussed in more detail in Chapter 57.

## Clinical Utility of Tumor Markers in Lung Cancer

**Screening and diagnosis.**

Their lack of sensitivity and organ- and tumor-specificity means that none of the tumor markers described above is suitable for screening for lung cancer, either in asymptomatic persons or in patients at high risk of malignancy (e.g., smokers).

The diagnosis of lung cancer generally requires medical imaging, endoscopy, intra-operative findings, and histology. Although tumor marker measurements cannot replace histological results, they can be very helpful where it is not possible to establish a final diagnosis by biopsy (approximately 20% of cases). For example, release of NSE in tissue and in serum in patients where histological evidence is absent may support a diagnosis of SCLC (198,199). Similarly a

high serum level of SCCA provides a strong suspicion of NSCLC and of squamous cell carcinoma in particular (200). CYFRA 21-1 has the highest sensitivity for lung cancer in general (200–202).

Although serum CYFRA 21-1, TPA, NSE, and CEA all show a correlation with tumor burden, there is no consistent relationship between production of these markers and tumor stage. Generally, high tumor marker concentrations reflect advanced tumor stage and suggest a bad prognosis. Low or mildly increased marker concentrations never exclude malignancy or progression. Pre-treatment measurement of tumor markers at the time of primary diagnosis may be helpful (Table 11). Markers raised at that time are likely to be most relevant for monitoring. The rate at which marker levels decrease after surgery can give some indication of remaining tumor burden and the effectiveness of therapy (203–205).

**Table 11: Tumor Markers of use in Lung Cancer**

| Histology known | Baseline marker* |
|---|---|
| Adenocarcinoma | CYFRA 21-1 and CEA |
| Squamous cell carcinoma | CYFRA 21-1 |
| Large cell carcinoma | CYFRA 21-1 and CEA |
| Small cell carcinoma | NSE and CYFRA 21-1 |
| Unknown | CYFRA 21-1, NSE, CEA |

*Markers raised at baseline are likely to be most relevant for monitoring.

**Prognosis.**

Two comprehensive reviews on the prognostic use of serum tumor markers in NSCLC (206) and SCLC (207) have recently been published.

All parameters investigated by multivariate analysis in NSCLC and SCLC patients have yielded conflicting results in different studies. Nevertheless, the use of CYFRA 21-1 in NSCLC has been most frequently evaluated and found to be a significant prognostic factor in multivariate analysis (208).

There are several reasons for the contradictory results from different studies on prognostic value:
- The heterogeneity of the study populations (tumor stage, histology, etc.).
- The parameters studied.
- The procedure by which optimized cut-off values have been chosen.
- The use of different procedures to determine cut-off values within the same study.

It is particularly important that potential new markers are compared with existing markers where appropriate. Well-designed trials are essential to verify the prognostic significance of markers such as NSE or CYFRA 21-1. The effect on therapeutic decision-making has yet to be assessed in prospective randomized intervention trials.

**Post-treatment monitoring.**

An important indication for tumor marker determinations in lung cancer is in assessing the efficacy of therapy and post-operative follow-up care. As for tumor markers in other carcinomas, the rate of post-operative decrease of the relevant marker provides information regarding patient outcome. Decreasing levels after primary surgery are the first sign of curative resection and therefore an indication of good prognosis. Tumor marker concentrations that decrease slowly and/or do not fall to within the reference range are suggestive of residual disease or incorrect staging (e.g., due to occult metastases not identified at the time of initial staging).

The risk of recurrence in lung cancer is high (70–90%), and follow-up care with tumor markers should only be performed if treatment options are available. Where tumor markers are used for follow-up, determination of post-operative baseline levels is necessary to allow appropriate interpretation of later results, with a confirmed increase from this baseline providing the first sign of recurrent disease.

Tumor markers may also give some indication of the effectiveness of chemotherapy. For more than 10 years, it has been accepted that in patients with SCLC, serum levels of NSE reflect the response to chemotherapy (199,209,210). It should be noted that during chemotherapy, transient increases in NSE may occur 24 to 72 hours after treatment (i.e., tumor lysis syndrome) and have been interpreted as first sign of effective therapy. Where there is a good response to chemotherapy, elevated pre-treatment serum NSE levels generally decrease rapidly within a week or by the end of the first treatment cycle. In contrast, failure of therapy is associated with persistently elevated or transiently decreased levels. Increasing tumor markers generally indicate progressive disease, often weeks or months before this is evident from imaging techniques (211–213). Whether implementing treatment early (solely on the basis of an increasing tumor marker level) can prolong survival or increase the quality of life is not yet known.

## Preanalytical Concerns

Samples for lung cancer markers may be stored at 4 °C (short-term) and at –70 °C (long-term). When frozen samples are thawed for cytokeratin analysis, vigorous mixing of samples should be avoided, as cytokeratins may adhere to tube walls after extreme agitation.

Specimens for NSE determination should be separated from the clot within 60 minutes of collection to avoid leaking of NSE present in normal erythrocytes, and in plasma cells. NSE should not be determined on hemolyzed specimens.

When measuring SCCA, contamination of samples with skin or saliva must be avoided, as this may lead to erroneously high results. CYFRA 21-1- and SCCA values may be significantly influenced by renal failure, a condition in which higher results may be observed.

## EGTM RECOMMENDATIONS FOR USE OF TUMOR MARKERS IN LUNG CANCER

1. CYFRA 21-1, CEA, and NSE should not be used for screening purposes either in asymptomatic populations or in those at high risk for lung cancer (e.g., smokers).
2. Depending on histology, CYFRA 21-1, CEA, and/or NSE may be ordered in lung cancer patients prior to first therapy. Where no histology can be obtained before surgery, measurement of all three markers is necessary to identify the leading marker (usually that present in highest concentration).
3. Where inoperable lung cancer is suspected but no histology is available, raised serum NSE is suggestive of small-cell lung cancer.
4. Follow-up of asymptomatic patients after primary therapy of lung cancer is controversial. However, serial determinations of the leading marker may help assess the completeness of tumor removal and provide early indication of recurrence.
5. NSE can be measured during systemic treatment of SCLC to reflect response to therapy and to document progressive disease.
6. Careful attention to preanalytical factors is essential. Specimens for NSE determination should be separated from the clot within 60 minutes of collection, and hemolyzed samples should not be assayed. Vigorous mixing of serum samples after thawing should be avoided for cytokeratin measurements. Contamination of samples with skin or saliva must be avoided for SCCA measurements.
7. Samples may be stored at 4 °C (short-term) and at –70 °C (long-term).

# SUMMARY

The key guidelines recommended by the NACB (LMPG), ASCO, ACS, and EGTM for the use of tumor markers are summarized in Table 12. This information is clearly still in development and should be updated periodically, perhaps every two years. As the guidelines reflect the results of clinical research, they should inevitably change with advances in technology and their adoption in clinical practice.

The single most important caveat for the use of tumor markers in a clinical setting relates to the inter-laboratory variability of tumor marker measurement, which is discussed in greater detail elsewhere in this book. An assay performed using a specific antibody or a particular instrument may give results different from those obtained with a different method. This does not necessarily reflect poor individual methods or instrumentation, but rather differences in assay reagents and procedures. It is, therefore, essential that where tumor markers are used to monitor cancer patients, information about the method is included with the assay results.

## Table 12: Summary of Key Guideline Recommendations

| Cancer type | NACB | ASCO | ACS | EGTM |
|---|---|---|---|---|
| Breast | ER / PR on all cancers<br>CA15-3 / CA27.29 for monitoring advanced disease | Routine use of CA15-3 or CA 27.29 alone *not* recommended<br>Increasing CA15-3 or CA27.29 may be used to suggest treatment failure<br>Routine use of CEA not recommended<br>Estrogen and progesterone receptors to be determined for primary lesions<br>Steroid hormone receptors to be used to select patients for endocrine therapy<br>HER-2/neu (c-erb B2) over-expression or amplification may be used to select patients for Herceptin® (trastuzumab) therapy | None | Steroid receptors in tissue for predicting response to hormone therapy<br>CEA and one MUC1-gene-related protein in serum for prognosis, follow-up and monitoring of therapy<br>HER-2/neu in tissue for predicting response to Herceptin® (trastuzumab®) in patients with advanced disease |
| Ovarian | CA125 as a diagnostic aid and for monitoring therapy | None | None | CA125 as an aid in diagnosis, for monitoring treatment, and early prediction of recurrence |
| Prostate | PSA with DRE<br>%fPSA when PSA is between 4–10 ng/mL and DRE is negative | Guidelines under development for metastatic disease | PSA and DRE for screening and detection | tPSA with DRE for screening (studies), case finding, or prognosis. tPSA in follow-up and and monitoring of therapy if additional means of therapy can be offered in case of rising tPSA.<br>%fPSA for differential diagnosis when tPSA is 4–10 ng/mL and DRE is negative |
| Germ cell | AFP, hCG, and LDH for detecting and monitoring testicular tumors<br>AFP is diagnostic for NSGCT | None | None | AFP, hCG, LDH, and PLAP* for case-finding, staging, prognosis, follow-up, and monitoring of therapy<br>AFP is diagnostic for non-seminomatous germ cell tumors (NSGCT) |
| Colon | CEA for monitoring therapy | CEA for prognosis, detecting recurrence, and monitoring therapy | None | CEA for case-finding, prognosis, follow-up, and monitoring of therapy |
| Neuroendocrine | Urinary catecholamines, VMA, HVA as indicators for pheochromocytoma and neuroblastoma<br>Calcitonin for medullary thyroid carcinoma | None | None | None |
| Myeloma | Serum protein electrophoresis for M spike<br>Immunofixation electrophoresis to differentiate monoclonality | None | None | None |
| Lung | None | None | None | NSE in differential diagnosis<br>CYFRA 21-1, CEA, and/or NSE for follow-up and monitoring of therapy |

NACB, National Academy of Clinical Biochemistry; ASCO, American Society for Clinical Oncology; ACS, American Cancer Society; EGTM, European Group on Tumor Markers; tPSA, total PSA; fPSA, free PSA.

"None" indicates that the relevant group has not yet considered this cancer type.

*Placental alkaline phosphatase (PLAP), for monitoring of seminomas in nonsmokers only.

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# APPENDIX

## Acknowledgements

## Appendix A
**Contributors to NACB Guidelines**

The following individuals were recorded as participants in the public discussion of the draft NACB guidelines at the May 1998 meeting in Rye, New York. Their input and comments were greatly appreciated. We apologize if we have failed to note the names of other individuals who may have participated in this presentation and discussion.

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## Appendix B
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