Serological Markers of Digestive Tract Cancers

15

Jorge L. Sepulveda

Introduction

In this chapter we will consider circulating protein markers for esophageal (EC), gastric (GC), and colorectal (CRC) cancers. Specific nucleic acids and circulating tumor cells have shown considerable potential as markers for gastrointestinal (GI) tumors and are covered in other chapters. We will focus first into the well-established, commonly available markers, namely the glycoprotein carcinoembryonic antigen (CEA) and the sialylated glycoprotein CA 19-9, and then we will review emerging markers for each GI tumor, discussing potential practical approaches for developing clinically useful applications.

In current clinical practice, serum markers are used mainly for staging and post-therapy surveillance of gastrointestinal tumors. The only recommendation for clinical use of these markers, in guidelines published by several expert group organizations, is that CEA measurements should be used for staging, therapy monitoring, and recurrence prediction in patients with colorectal cancer (see Table 15.1). No specific markers are currently recommended for routine use in gastric or esophageal cancer. Despite this paucity of widely accepted biochemical markers for GI tumors, there is a great need for reliable, sensitive, and specific markers for the following clinical applications:

1. *Screening* for early stage cancers in the general population: the main obstacle to generalized screening tests is that all of the commercially available markers and many of the markers in development tend to be negative in the great majority of early stage, localized cancers, and attain acceptable sensitivity only in advanced, widespread tumors. Additionally, specificity needs to be very high to screen a general population; otherwise, given the low prevalence of tumors in the population, most positive results will be false positive. Given their low sensibility at early stages and less than desirable specificity, none of these markers is recommended for screening in populations with low pretest probabilities of the cancer.
2. *Diagnosis* of cancer when symptoms or other signs increase the pretest probability of cancer and in patients at high risk for cancer development: given the same lack of sensitivity at early stages mentioned above, these markers should not be used to rule out tumors; however, in conjunction with other diagnostic modalities, certain positive tumor marker measurements may help to point the diagnostician in the right direction.
3. *Tumor sizing and staging*: for many of the tumor markers, there is a good correlation with tumor size, especially for those markers that are released from the tumor (in contrast with "host response" markers), and this is one reason why these markers attain higher levels in more advanced stages. However, currently, there is no generally accepted staging protocol involving GI tumor markers.
4. *Prognosis evaluation*: while there is a good correlation with survival for some of the markers, there is no widely accepted prognostic evaluation algorithm incorporating any of the GI tumor markers because of poor accuracy of the prediction or lack of sufficient data.
5. *Predict response to therapy*: as rational therapies targeting pathogenic mechanism are developed, markers will be needed to predict response to these often highly expensive treatments. An example is the measurement of her2/neu amplification for predicting response to *Herceptin* in breast cancer. The plasma protein markers currently available cannot be used effectively to predict response to therapy in GI tumors.
6. *Monitor effectiveness of therapy*: This is an accepted use of GI tumor markers such as CEA and CA19-9, as patients with elevations of these markers produced by the tumor will show a significant decrease in levels (typically greater than 50%) with effective therapy. Complete remission

J.L. Sepulveda, M.D., Ph.D. (✉)
Department of Pathology & Cell Biology,
Columbia University, New York, NY, USA
e-mail: jls2282@columbia.edu

A.R. Sepulveda and J.P. Lynch (eds.), *Molecular Pathology of Neoplastic Gastrointestinal Diseases*, Molecular Pathology Library 7,
DOI 10.1007/978-1-4614-6015-2_15,

Table 15.1 Recommendations by various expert groups for clinical use of CEA in colorectal cancer

Application	ASCO[126,161–164]	EGTM[165–167]	ESMO[168–172]	NCCN[86]	NACB[67,173]	LOE	SOR
Screening	No	No	None published	None published	No	IV/V	A
Preoperative staging, prognosis	Yes, if it could assist in staging or surgical treatment planning	Yes, in combination with standard factors	Yes	Yes, as part of a complete staging workup	Yes, combined with other factors, if it would aid planning surgical treatment	III	C
Postoperative surveillance (Stage II–III)	Yes, if a patient is candidate for surgery or systemic therapy	Yes, candidates for aggressive surgical resection or chemotherapy if recurrence is detected	Yes	Yes, candidates for aggressive surgical resection if recurrence is detected	Yes, if patient is candidate for liver resection or systemic chemotherapy	I	A
Frequency after surgery	q3 Mo×3y	q2-3 Mo×3y		q3–6 Mo×2 y→q6 Mo×3 y	q3 Mo×3y		
Monitoring advanced disease (Stage IV)	Yes	Yes, ideally in combination with radiology	Yes, if initially elevated, after 2-3Mo of therapy	Yes, for T2 or greater lesions	Yes, especially for disease that cannot be evaluated by other modalities	III	B
Frequency for advanced disease	q1–3 Mo during active treatment	q2–3 Mo during therapy	q2–3 Mo during therapy	q3 Mo×2 y→q6 Mo×3–5 y			

Modified from[67]

ASCO American Society for Clinical Oncology, *EGTM* European Group on Tumor Markers, *ESMO* European Society for Medical Oncology, *NCCN* National Comprehensive Cancer Network, *NACB* National Academy of Clinical Biochemistry. *Mo* months, *y* years, *LOE* Level of evidence: I, evidence from prospective, controlled, high-powered studies specifically designed to test the marker, or from a meta-analysis, pooled analysis or overview of level II or III studies; II, marker data obtained from a prospective therapeutic trial; III: large prospective studies; IV, small retrospective studies; V, small pilot studies., *SOR* Strength of Recommendation from the NACB: A—(A) high (further research is very unlikely to change the panel's confidence in the estimate of effect); (B) moderate (further research is likely to have an important impact on the panel's confidence in the estimate of effect and is likely to change the estimate); (C) low (further research is very likely to have an important effect on the panel's confidence in the estimate of effect and is likely to change the estimate

Table 15.2 CEACAM genes expressed in humans

Gene	Synonyms	Expression	PM
CEACAM1	Biliary glycoprotein, BGP1, CD66a	Ubiquitous, especially GI epithelia	TM
CEACAM3	CGM1, W264; W282; CD66d;	Granulocytes	TM
CEACAM4	NCA; CGM7	Granulocytes	TM
CEACAM5	CEA, CD66e, DKFZp781M2392	Epithelia	GPI
CEACAM6	NCA; CEAL; CD66c; CEACAM6	Epithelia, lung, spleen, granulocytes	GPI
CEACAM7	CGM2	Epithelia	GPI
CEACAM8	CD67; CGM6; CD66b; NCA-95	Granulocytes	GPI
CEACAM16	CEAL2	Cerebellum (mouse)	Sec
CEACAM18		Widespread	Sec
CEACAM19	CEAL1; MGC105097	Squamous epithelia (mouse)	TM
CEACAM20	UNQ9366	Intestine, thymus (mouse)	TM
CEACAM21	FLJ13540; R29124_1; MGC119874	Hypothalamus	TM

PM plasma membrane insertion mode, *TM* transmembrane, *GPI* glycophosphatidylinositol linked, *Sec* secreted

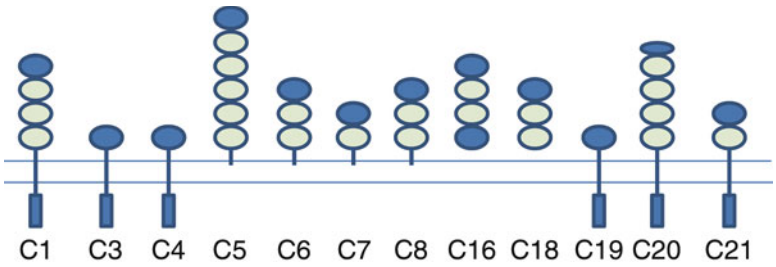

Fig. 15.1 Structure of CEACAM proteins expressed in humans. Each ellipse represents a Ig-like extracellular domain, dark IgV-like, light IgC-like (adapted from ref.240)

cannot be established by tumor marker measurements, but persistent elevation should be considered as evidence of tumor persistence, provided that alternative explanations for the marker elevation can be excluded.

7. *Screen for cancer recurrence*: This is also a widely accepted use of current GI tumor markers, as tumors that expressed the biomarkers before therapy most often re-express them upon relapse. The Working Group on Tumor Marker Criteria suggests that an increase of at least 25% or a linear increase in a marker's level in three consecutive samples is consistent with progressive disease and tumor recurrence.[1] However, due to significant false-positive and false-negative tumor marker results, complementary recurrence screening methods, including various imaging modalities, should be used.

Carcinoembryonic Antigen (CEA)

The carcinoembryonic antigen was first described by Gold and Freedman after immunizing rabbits with an extract from a colon cancer patient.[2] Since then, it has become the most widely used marker for CRC, and the only serum protein marker incorporated in current recommendations for diagnosis, treatment and follow-up of GI cancers.

Biochemistry

The carcinoembryonic antigen, also named CEACAM5, gp180 or CD66e is a member of the CEA family of genes. The CEA family is part of the immunoglobulin superfamily, and consists of the CEA subgroup and the pregnancy-specific glycoprotein (PSG) subgroup. The CEA family in humans comprises 23 genes located within a 1.2 Mb cluster on the long arm of chromosome 19, of which 18 are expressed and 11 are pseudogenes.[3,4] The CEA subgroup in humans (Table 15.2 and Fig. 15.1) comprises 6 transmembrane proteins, 4 membrane glycosylphosphatidyl inositol (GPI) -anchored glycoproteins (CEACAM5-CEACAM8), and 2 secreted proteins (CEACAM16 and 18), while the PSG group consists of 10 soluble members (PSG1–PSG10), secreted from trophoblast cells. GPI-anchoring results from a hydrophobic signal peptide coding for attachment of the phosphatidyl-glycol moiety that allows insertion of the protein into the membrane and more fluid movement to areas of interest, as demonstrated by the apical location of CEA. GPI moieties are typically linked to sugar moieties on the protein, most commonly through an alpha-6 linkage between the inositol of GPI and a glucosamine residue. The GPI tail can be cleaved with GPI phospholipase D expressed in colon cancer cells, resulting

in solubilization of CEA.[5] There is some experimental evidence that CEA released from tumor cells may have a role in metastatic spreading to the liver.[6] Interestingly, the GPI-anchored CEACAM proteins tend to be upregulated in tumors, while the transmembrane CEACAMs are typically downregulated.

The CEA protein consists of 641 aminoacid residues and 45–55% carbohydrate, resulting in heterogeneous molecules with molecular masses ranging from 150 to 300 kDa, and is encoded by mRNAs 2.6 kb transcribed from five to six exons with alternative splicing and polyadenylation sites. Meconium CEA (NCA-2) differs from colon CEA in reactivity with various monoclonal antibodies, probably reflecting different post-translational modifications.[7]

Expression and Regulation

CEA is widely expressed in fetal GI tract and other tissues, including meninges, cartilage and bone, blood vessel walls, placenta, dermis, muscle layers of the stomach and intestine and bronchioles.[8] In the adult, CEA is shutdown in most cells, although expression is maintained at low levels in some adult tissues such as colon mucosa,[9] squamous esophageal mucosa,[10] squamous uterine cervical mucosa,[11] rare thymic epithelial cells in Hassall's corpuscles,[12] tracheal, bronchial, and bronchiolar epithelium and alveolar type I pneumocytes,[13] and sweat and sebaceous glands.[14]

High-level reexpression of CEA in epithelial carcinomas provides a mechanism for selective identification and targeting of these cancer cells. This tight regulation can be reproduced with a fragment containing the CEA basic promoter (−266 to +102 bp around the transcription start site), and robust expression with preserved tissue specificity can be achieved by adding the CEA enhancer located −6.1 to −4.0 kb upstream of the start site.[15] Specific targeting of a suicide gene to xenographed colon cancer was achieved using calcium phosphate nanoparticle mediated delivery of fusion construct containing the CEA promoter and the cytosine deaminase enzyme cDNA, therefore rendering the cells more susceptible to 5-fluorocytosine.[16] The promoter apparently works even in tumors without detectable CEA.[17]

CEA expression is stimulated by transforming growth factor beta (TGFβ), possibly as a regulator of cell adhesion and differentiation during embryonic development, and its expression in embryonic development mimics that of TGFβ.[8,15] The effects of TGFβ signaling on CEA expression appear to be mediated by *Smad* transcription factors, as suggested by low CEA expression in TGFβ-unresponsive gastric cancer cells and in *Smad3* knockout mice.[18]

Functional Aspects of CEA

Adhesion

Extracellular domains are involved in homotypic and heterotypic interactions between CEACAM family members, and in general these interactions play an important role in binding targets, including adhesion to other mammalian cells and bacteria. For example, CEA cooperates with CD44 variants to bind to E- and L-selectin ligands on endothelial cells and resisthigh shear stress, which may be important for the metastatic ability of colon cancer cells.[19] Binding of bacteria to the apical surface of enterocytes expressing CEACAM molecules, followed by shedding of microvesicles or cleavage of GPI linkages provides a mechanism for regulating the amount of bacteria attached to the mucosa.[20]

Cell Differentiation

Expression of CEA (and the related CEACAM6 GPI-linked molecule) generally inhibits differentiation in a variety of cell types, including myoblasts[21] and pre-adipocytes,[22] and overexpression of CEACAM5 or CEACAM6 in colonic epithelial cells results in loss of cell polarity and tissue architecture in culture and in a nude mouse model of colonic differentiation.[23] These anti-differentiation effects appear to have been selected most likely for embryologic purposes during evolution of the ancestral transmembrane-anchored CEACAM1, which does not have these effects, to the GPI-linked CEACAM5 and CEACAM6.[22]

Immunomodulation

CEA is expressed at low levels in the apical surface of adult colonic enterocytes and goblet cells,[4,24] where it may bind bacteria, regulating bacterial colonization and promoting the immune response.[20,25] Expression at the basolateral surface is found mostly in embryonic and tumor cells and may impart an immunosuppressive function by binding CD8, in conjunction with CD1d, and activating suppressor T-cells.[26] Since tumor cells frequently have loss of polarity and express CEA in the entire cell surface,[24] CEA may play a similar immunosuppressive function in colon cancer cells. Additionally, CEA was shown to decrease killing of colon cancer cells by natural killer (NK)[27] and lymphocyte-activated killer (LAK) cells.[28] It has been speculated that immunoinhibitory CEACAM molecules appeared during mammalian evolution to play a role in fetal tolerance in species with invasive trophoblastic growth.[29]

Metastasis and Tumor Survival

Injection of nude mice with CEA enhances growth of colon CA tumors.[30,31] Expression of the human chromosomal region containing CEA and CEACAM6 in transgenic mice induced enlarged colons with severe hyperplasia, dysplasia, and serrated adenomas.[32] Overexpression of CEA and

CEACAM6 in colonic cells lines disrupted their ability to form glandular structures and increased their tumorigenicity in nude mice.[23] Interestingly, in contrast with the GPI-anchored CEA and CEACAM6 molecules, CEACAM1 appears to have antitumor[33] and pro-apoptotic[34] effects and shows decreased expression in about a quarter of human colon cancers.[35]

In addition to the above-mentioned role of CEACAM molecules in adhesion and immune modulation, mechanisms of enhanced tumorigenesis potentially include:

1. Induction of interleukin-10 and resulting inhibition of the up-regulation of the inducible nitrogen oxide synthase (iNOS) in Kupfer cells.[36] Up-regulation of iNOS results in ischemic injury to the circulating tumor cells as they enter the liver microvasculature and CEA may prevent this effect.
2. Interaction of CEA with Kupfer cells also leads to release of cytokines such as IL-1β, IL-6, and TNFα, which increase expression of adhesion molecules (primarily ICAM-1) by sinusoidal endothelial cells, resulting in increased attachment of tumor cells.[37,38]
3. Inhibition of colon CA anoikis, a form of apoptosis induced by cellular detachment from the extracellular matrix, by binding to and blocking the pro-apoptotic effect of TRAIL-R2 (DR5) receptor.[39] The importance of this mechanism is highlighted by the failure of a CEA construct lacking the TRAIL-R2 binding domain to enhance experimental liver metastasis of colon cancer cells.
4. CEA and CEACAM6 modulate clustering of integrin alpha-5/beta-1 resulting in increased binding to fibronectin, enhanced cellular adhesion to the extracellular matrix with a fibronectin "cocoon" around the cells, and resistance to anoikis. Interaction of CEA with the integrins initiated signal transduction through integrin linked kinase, protein kinase B (PKB/Akt), and the mitogen-activated protein kinase cascade and appears to lead to inactivation of the intrinsic apoptosis pathway.[40-42]
5. Colon cancer apoptosis under different conditions (confluence, treatment with 5-fluorouracil, UV light or IFNγ, and in vivo) was significantly increased by selective inactivation of CEA expression with a ribozyme.[43,44]

CEA as Target

Imaging

Given its association with CRC, in particular more advanced and metastatic tumors, it makes sense to use CEA as targeting marker for localizing tumors by imaging. For example, positron emission tomography (PET) and single-photon emission computed tomography (SPECT) with pre-targeted anti-CEA antibodies identified human colon tumors in mice lungs, while ^{18}F-fludeoxyglucose labeling failed.[45] Pre-targeting with antibody, followed by addition of the radioisotope improves imaging in humans.[46] Imaging with fluorescent-labeled antibodies against CEA was used to visualize CEA-expressing xenographed tumors in mice[47] and may soon be used to help surgeons distinguish residual tumor tissue during colon cancer resections. CEA-Scan (a ^{99m}Tc-labeled anti-CEA $F_{ab'}$ fragment) has been approved by the United States Food and Drug Administration (FDA) for cancer imaging and should facilitate further studies of CEA as an imaging tumor marker.[48]

Circulating Tumor Cell Capture

Beads coupled to anti-CEA antibodies can be used to capture circulating tumor cells (CTC) expressing CEA, allowing quantification of CTC levels as well as subsequent molecular analysis on the purified cells.[49,50] Currently, most capture methods, including the FDA-approved CellSearch® test from Veridex, LLC (Raritan, NJ), use pan-epithelial-specific markers, such as cytokeratins and Er-B4, but CEA and other tumor markers have the potential to increase the specificity of the assay.

CEA as Target for Therapy

Given its role in tumor progression and survival, it is encouraging that ribozyme mediated inactivation of endogenous CEA expression in HT29 human CRC cells was followed by apoptosis and inhibition of metastatic growth in nude mice.[43,44] In addition to its specific inhibition to counter tumor promoting activities, CEA has been used as a homing target for more aggressive, nonspecific experimental therapies. Promising results have been seen with CEA-targeted radiation therapy in mice[51,52] and in humans,[53] immunotherapy with CEA-DNA vaccines,[54] CEA-stimulated dendritic cells[55] or T-cells,[56,57] and gene therapy using viral vectors expressing CEA binding domains.[58] However, these therapies need to carefully modulate the balance between anti-cancer effectiveness and toxicity due to CEA expression in normal tissues.[59]

Further refinements of ribozyme technology allow a combination of both approaches by simultaneous inactivation of CEA and expression by trans-splicing of the "suicidal gene" thymidine kinase, thereby conferring increased susceptibility of CEA-expressing cells to gancyclovir treatment.[60] Another ingenious approach uses an anti-CEA single chain antibody fused to cytosine deaminase to target colon cancer cells for 5-fluorocytosine therapy.[61] An important consideration for the use of CEA as target for therapy is that CEA expression in tumors does not necessarily correlate with CEA serum levels,[62] and therefore serum levels should not be used to select patients for CEA-directed therapy.

Assays

CEA was first detected in serum with a radioimmunoassay in 1969, which demonstrated levels above 20 ng/mL in 15 of 15 recurrent or metastatic colon CA patients, levels above the 2.5 ng/mL detection limit in 19 of 20 with preoperative or residual cancer, and undetectable levels in patients with no residual colon cancer, non-GI cancers, non-cancer GI diseases, and normal subjects including pregnant women.[63] Although these results were overly optimistic compared to subsequent studies, they launched the foundation for the use of CEA to detect cancer colon recurrence.

Most commonly used current CEA assays are performed on high throughput, automated, random-access analyzers using electrochemiluminescence detection, which have replaced manual, labor-intensive, and expensive radioimmunoassays. In general, a capture antibody is immobilized in a solid phase, such as magnetic beads, and CEA is purified from the sample after binding to the capture antibody and washing of unbound materials. Detection uses another anti-CEA antibody, which is coupled to an enzyme such as alkaline phosphatase. For chemiluminescence detection, a substrate such as Lumi Phos 530 is added and upon reaction with the enzyme generates light that can be measured with high sensitivity and low background by luminometers. Alternatively, the detection antibody may be coupled to a chemiluminescent chemical such as an acridinium ester. Other antibody-coupled enzymes may generate a colored product that can be measured with a spectrophotometer, but these are subject to higher background and more interference and are not commonly used in routine assays for clinical purposes. More experimental detection methods are pushing further the limits of detection of CEA. For example, a microchip assay using beads coated with a CEA capture antibody and a second anti-CEA antibody coupled to gold beads and thermal lens microscopy for detection achieved analytical sensitivities several times lower than conventional enzyme-linked immunoassays.[64] It is important to note that CEA is a complex molecule, with multiple glycosylation sites and alternative epitopes, and results from one method cannot be directly compared to another method, especially if different antibodies and calibrator materials are used.

Typically, the capture and/or the detection antibodies are mouse monoclonal antibodies, therefore, these assays may be subject to interference by human anti-mouse antibodies (HAMA). Excess turbidity in the sample, such as highly lipemic serum, can also interfere with assays using colorimetric or chemiluminescent detection. Another source of potential confounding results is the hook effect, which results from excessive amounts of antigen interfering with the formation of detectable antigen–antibody complexes and therefore resulting in falsely low measurements. In all these cases, dilution of the sample often removes some or all of the interfering substances. If the CEA levels after adjustment for the dilution factor are higher than the undiluted levels, an interference is possibly present. Other strategies involve removal of interfering immunoglobulins, e.g., with blocking reagents, polyethylene glycol precipitation, or anti-immunoglobulin columns.

Use of CEA in Colon Cancer

The major clinical use of CEA measurements is as an adjunct to assessing and monitoring the extent of colon cancer disease. While initially hoped to be tumor specific, it soon became evident that individuals with several non-neoplastic conditions, including chronic smokers, had elevations in CEA levels. Therefore, this marker has limited utility for general screening, but as a quantitative test, it has been shown to correlate with the extent of colon cancer growth, with higher levels being seen in more advanced cancers with worse prognosis. The serum levels of CEA depend on the amount synthesized by the tumor, the number of CEA-expressing cells in the tumor and their degree of differentiation, CEA release from tumors by secretion, GPI-cleavage, and cell death, the vascularization of the tumor, amount of necrosis, catabolism of CEA by the liver, and renal elimination. As a general rule, benign conditions express lower level of CEA (typically < 10 ng/mL) and tend to remain stable over time, whereas CEA levels often increase with tumor progression. Recommendations for the use of CEA and other tumor markers in clinical care of patients with colon cancer are summarized in Fig. 15.2.

Screening

In a review of colon cancer markers, Hundt et al[65] summarized performance characteristics of CEA from 19 studies published before July 2006. Overall sensitivity varied from 43 to 69% but was highly dependent on Dukes stage, ranging from 8 to 52% for Dukes A, 22 to 59% for Dukes B, 38 to 72% for Dukes C, and 69 to 96% for Dukes D. CEA was more often than not below cutoff in non-metastatic colon cancers (stages A–C). Specificity was dependent on the CEA analytical cutoff and on the selected population of controls, and ranged from 55 to 100%. Lower specificity was observed with cutoffs below 4 ng/ml and in benign GI disorders. In general, the *use of CEA for screening and detection of early colon cancer in healthy individuals is not recommended because of poor sensitivity and optimal specificity.*[66,67] Even though it is not recommended for screening healthy, asymptomatic individuals, a study in Singapore found that up to 7.4% of asymptomatic patients whose only indication for endoscopy was an elevated CEA had a malignancy, including colon, stomach, lung and ovarian cancer.[68] Once an elevated CEA is found, it is probably best to investigate the patient for possible malignancy.

- **CEA cannot be used in screening of healthy subjects for early CRC [LOE, IV/V; SOR, A]**
- **Preoperative CEA concentrations might be used in combination with other factors in planning surgical treatment.**
- **Patients with increased concentrations of CEA (e.g., >5 μg/L) should be evaluated for the presence of distant metastases [LOE, III; SOR, C].**
- **Preoperative CEA concentrations should not be used at present to select patients for adjuvant chemotherapy [LOE, III; SOR, C].**
- **CEA should be measured every 3 months in patients with stage II or III CRC for at least 3 years after diagnosis if the patient is a candidate for surgery or systemic therapy of metastatic disease [LOE, I; SOR, A].**
- **In patients with advanced CRC undergoing systemic therapy, regular CEA determinations should be carried out.**
- **A confirmed CEA increase (e.g., >30%) suggests progressive disease provided the possibility of false-positive elevations can be excluded [LOE, III; SOR, B].**
- **Routine measurement of CA19.9, CA 242, or TIMP-1 is not recommended [LOE, III/IV; SOR, B/C].**

Fig. 15.2 National Academy of Clinical Biochemistry Laboratory Medicine Practice Guidelines for use of tumor markers in colorectal cancer (created from data in [67]). LOE and SOE as in Table 15.1

Planning Therapy, Staging, and Evaluating Prognosis

CEA can be used conjunctly with other diagnostic modalities to evaluate extension of disease and plan surgical treatment. However, CEA should not be used to select patients for adjuvant therapy.[67] While there is no formal prognostic evaluation algorithm incorporating CEA, the College of American Pathologists[69] and The American Joint Committee on Cancer[70] have determined that CEA is a category I prognostic factor for CRC and should be incorporated in staging protocols, together with tumor TNM stage and residual tumor following surgery. Category I includes "factors definitively proven to be of prognostic import based on evidence from multiple statistically robust published trials and generally used in patient management".[69] For example, pre-op CEA>5 ng/ml was significantly associated with worst prognosis in colon cancer (5-year disease free survival 71% vs 82%), particularly in stage II tumors,[71] and CEA may be useful to select patients with stage II tumors that may be benefited from adjuvant chemotherapy.[72] *All patients with CEA elevations* (e.g., *>5 ng/mL) should be evaluated for the presence of distant metastases*.

Postoperative Surveillance

A major role of CEA measurement is in the evaluation of the CRC patient postsurgery, with the goals of monitoring effectiveness of therapy, providing reassurance to patients and health providers, and detecting recurrence or metastases. After curative resection, the overall 5-year recurrence rate of CRC is about 40%, including 12% local recurrence, nearly 20% liver metastasis, 8% pulmonary metastasis, and 30% other recurrences.[73] After curative surgical resection of CRC, CEA levels tend to revert to normal, while persistently elevated CEA is associated with residual tumor. Quantitative meta-analysis of 20 studies examining the performance of CEA in 4,285 patients following resection of colon CA arrived at an overall sensitivity of 64% for detection of cancer recurrence with 90% specificity. Using weighted

Table 15.3 Diagnostic accuracy of CEA for detection of recurrences of CRC following curative surgery, based on data from[73]

Outcome	Sensitivity	Specificity	PPV	Number of Patients
Local recurrence	60	86	2.8	1,305
Liver metastasis	73	91	8.3	1,293
Lung metastasis	56	83	3.4	525
Other recurrence	70	73	2.6	380

PPV Positive predictive value. Numbers are in percentages

meta-regression, the optimal cutoff to achieve optimal diagnostic yield was 2.2 mg/dL, corresponding to derived specificity and sensitivity of 84%, still insufficient for use in isolation.[74] Another meta-analysis determined the diagnostic accuracy of CEA for detection of local recurrence and liver, pulmonary, and other recurrences[73] (Table 15.3). It is important to note that while the specificities range from 73 to 91%, the positive predictive values are very low, around 3% for detection of local recurrences and 8% for detection of liver metastasis (Table 15.3).

Other meta-analysis studies have also concluded that intensive follow-up including CEA testing resulted in a small but statistically significant improvement in survival. In two of these studies, intensive surveillance was associated with improved outcome only if CEA was included.[75,76] In general, early surgical intervention in patients with recurrent disease detected by increases in CEA levels may improve survival in up to 35% of patients.[77] A nomogram incorporating patient age, tumor location, preoperative CEA, T stage, numbers of positive and negative lymph nodes, lymphovascular invasion, perineural invasion, and use of postoperative chemotherapy was developed to help predict post-operative cancer recurrence and showed a concordance index of 0.77, better than the categorical staging system of the American Joint Committee on Cancer.[78] Another nomogram incorporating CEA was developed to predict survival after hepatic resection for metastatic colon CA.[79] Note that there is no correlation of serum CEA with tumor CEA or histopathologic features, even though high serum CEA predicts worse outcome,[80] and therefore both CEA and morphological findings should be used for outcome prediction.

While the period between CEA elevation and detection of recurrence (lead time) may reflect the speed of tumor growth, there was no statistically significant correlation between lead time and re-resection rate or survival in a study of 4,841 resected colon cancer patients, probably because the average CEA lead time (about 5 months) is too short to have a significant impact.[81] Markers that can detect recurrence with improved lead time relative to CEA, allowing more timely intervention before relapsing tumor or metastases become extensive, may offer better opportunity for improvements in outcomes.

These studies lead to the recommendation that CEA be measured every 2–3 months in stage II-III patients for at least 3 years, if the patients are candidates for surgery or systemic treatment in the event of cancer recurrence or metastatic disease (Table 15.1). Monitoring after 5 years is not advised, as over 80% of the recurrences occur during the first 3 years, and therefore the positive predictive value of CEA elevations will considerably decrease.[82] Monitoring of early stage CRC with CEA or imaging does not appear cost effective, as less than 1% of the patients would benefit from such approach.[73,83] In stage II, CEA levels >5 ng/mL together with stage T4 and lymphovascular or perineural invasion identified patients with lower 5-year survival that should potentially be considered for adjuvant chemotherapy.[84] Stage IV (Dukes D) patients are also poor candidates for follow-up, as surgery is rarely effective.[73]

Monitoring should include at a minimum imaging and CEA measurements for optimal sensitivity in detecting CRC recurrences. In one study, the combination of CEA and CT imaging detected over 90% of the asymptomatic recurrences post-resection of CRC.[85] If CEA is used alone, a lower cutoff (e.g., 2.2 ng/mL or a 30% increase) should be used for increased sensitivity, followed by imaging and endoscopy for confirmation. The NCCN Colon Cancer Panel recommends that *patients with confirmed elevations of CEA post-surgical resection,* e.g., *a 30% increase confirmed a month later, should undergo physical examination, colonoscopy, and chest, abdominal, and pelvic CT scans.*[86] PET-CT should be considered but it is extremely rare to detect CRC recurrence if CT scans are negative. Imaging studies, if initially negative, should be repeated every 3 months until recurrence is identified or CEA stabilizes or decreases. With negative imaging studies, "blind" laparotomy or laparoscopy is not indicated solely based on the CEA elevation, as most elevations will be false positives.[73]

Similarly, patients that fail to decrease CEA within 3–4 weeks postsurgery may be at high risk for early recurrence and should undergo aggressive follow-up. A study of 600 patients with CRC and elevated preoperative CEA levels found that persistently elevated postoperative CEA levels were a strong predictor of recurrence, particularly as liver metastases.[87] In another study of CRC patients with preoperative CEA levels >5 ng/mL showed that a drop ≥60% in CEA levels was associated with better 5-year survival rates.[88]

Compliance with these recommendations appears poor. For example, a recent study of nearly 10,000 patients with curative resection for colon cancer found that only 17% had appropriate follow-up at the recommended frequency, while 60% were tested less frequently and 23% above guideline recommendations.[89] Under testing was more frequent in older individuals and minorities.

Monitoring Response to Chemotherapy

In patients with advanced or metastatic disease, CEA levels are often elevated, and a sustained decrease in CEA levels correlates with good response to treatment. For example, a CEA doubling time of >13.8 days had a sensitivity of 86% with 85% specificity to detect disease progression, while patients that responded to therapy had CEA half-life of <3.4 days.[90] The doubling time or half-life is calculated from an exponential-regressive curve connecting a minimum of three consecutive, semilogarithmic-transformed CEA values, which can be implemented in widely available worksheet programs.

CEA is the marker of choice for monitoring the response of metastatic disease to chemotherapy and should be used in conjunction with imaging and other clinical assessment modalities. Testing should be done for the duration of chemotherapy with a testing interval of 2–3 months. An increase of at least 30%, when confirmed by a repeat measurement within 1 month, is considered evidence of cancer recurrence or metastatic disease, after ruling out early effects of chemotherapy or another cause for CEA elevation.

Caution: certain treatments (such as 5-FU, levamisole, irinotecan, and oxaliplatin) can cause temporary elevations of CEA, probably as a consequence of release by dying tumor cells, which may be associated with better outcomes.[91-93] For example, a CEA flare, defined as a ≥15% increase of ≥4 μg/L followed by a decrease of ≥15%, had an objective response rate of 73%, compared to 11% for patients with CEA increasing ≥15% in two consecutive measurements.[94] Other studies have supported these findings.[91,95] *Early CEA elevations should not be interpreted as failure to respond, and changes in therapy should not be based on CEA alone in the first 4–6 months of chemotherapy*.

Monitoring Colon Cancer with Negative CEA

CEA is elevated only in about 50% of patients with colon cancer, ranging from 0 to 15% in stage A to 65 to 80% in stage D. Therefore, other markers are necessary to monitor patients with CEA levels below cutoff. In addition to the use of CA19-9, other promising markers are discussed below.

Use of CEA in Gastric Cancer

CEA is actually expressed in the vast majority of gastric cancers (over 90%), particularly of the intestinal type, but expression in the tumor tissue by immunohistochemistry has little correlation with serum levels.[96] The National Academy of Clinical Biochemistry states that "although carcinoembryonic antigen and CA 19-9 have been proposed for use in gastric cancer,… none of these markers can currently be recommended for routine clinical use".[97]

Screening

CEA is insensitive for early detection of GC, with positivity rates below 20%. In advanced GC, the positivity rates are higher but usually below 50%. Highly elevated CEA in non-metastatic GC appears to be associated with signet ring morphology and poorly differentiated tumors with massive local infiltration.[98] Another study showed good correlation between CEA elevation and serosal invasion.[99]

Monitoring

A study of 258 patients post-gastrectomy for GC showed low sensitivity and specificity of both CEA and CA19-9 for prediction of recurrence of gastric cancer.[100] These authors observed a false-positive rate of 15% in GC patients post-gastrectomy, especially in patients with conditions that tend to elevate CEA, such as smoking, and liver, renal, or pulmonary diseases. Positive predictive values were particularly low after gastrectomy for early stage GC, with recurrence rates below 3%, while the sensitivity for recurrence even in advanced stage GC was not very high (37% in GC, compared to 80% for colon cancer). A different study confirmed a low positivity rate of CEA for GC (around 20%) but showed higher sensitivity (79%) for detection of recurrences in CEA-positive tumors with a specificity of 94% with the same cut-off of >5 ng/ml.[101] As in CRC, about 20% of patients undergoing chemotherapy for GC demonstrate early surges in CEA levels, occurring in the first few months of treatment, which should not be interpreted as indicators of progressive disease.[102]

Prognostication

The value of CEA levels in predicting gastric cancer prognosis is a topic of controversy. An older multicentric study of 2,768 GC patients showed that pre-gastrectomy CEA levels strongly correlated with stage, lymph node metastases, and histopathology and had independent prognostic value.[103] A study of 810 patients in Korea[96] showed significantly worse prognosis in 9% of patients with serum CEA >7 ng/mL (5-year survival of 80.7% vs 48%). In a study of 549 Japanese patients with GC undergoing gastrectomy, CEA levels above 5 ng/mL were found in 19.5% of the patients, and the levels significantly correlated with depth of invasion, hepatic metastases, and rates of curative resection.[104] In multivariate analysis, CEA>10 ng/mL, nodal involvement, and depth of invasion were significant predictors of prognosis.[104] In another study, CEA>10 ng/mL correlated with worse survival, lymph node metastases, and depth of tumor invasion, although the difference between CEA-positive and CEA-negative tumors was not large enough to be useful as a single prognostic factor in an individual patient.[105] In a separate study, patients with ascites fluid CEA (aCEA) levels >5 ng/ml had an average survival of 2.3 months compared to 7.4 months with aCEA below the cutoff; no such correlation was

found with serum CEA in these patients.[106] Peritoneal lavage CEA >0.4 ng/mL also correlated with worse survival in a study of 229 Japanese patients.[107] CEA and CA19-9 levels were of no independent prognostic value in predicting survival of GC patients,[108] although combination with pro-inflammatory proteins IL6 and CRP increased their predictive value.[109] Combination of CEA with CA19-9 and CA125 showed increased sensitivity and specificity for predicting worse prognosis.[99] A comparison of CEA, CA 19-9, CA 72-4, and AFP showed correlation of individual markers with metastasis locations, but only CA72-4 showed specific independent prognostic value, with a 3.8-fold higher risk of death.[110] *In summary, while more research is needed to determine the appropriate combination of markers for GC prognostic evaluation and none of the markers are currently recommended for follow-up of GC, it seems reasonable to provide more intensive follow-up for GC patients determined to have elevated levels of CEA, CA19-9, CA72-4, or CA125.*

CEA in Esophageal Cancer

CEA is expressed in about 60% of squamous cell tumors and most adenocarcinomas of the esophagus, and although elevations in the serum are found in only a minority of those patients, it can be used as a marker to monitor effectiveness of therapy.[111] For example, while CEA >5 ng/mL was present in only 19% of the patients with EC before resection, elevations above the threshold occurred in 55% of the patients with recurring disease, with 90% specificity.[112] Squamous cell carcinomas (ESCC) are better followed with squamous cell markers, such as squamous cell cancer antigen (SCC), but CEA elevations are seen in a few cases and may be useful to monitor response to therapy. For example, while only 4.2% of the patients with ESCC and CEA elevations above 3.3 ng/ml responded to chemotherapy, the complete response rate for those patients with low CEA was 48% in one study.[113] Interestingly, measurement of CEA mRNA in the serum of patients with ESCC had higher sensitivity and specificity than serum CEA or SCC protein levels for detection of postoperative recurrences.[114]

CEA in Non-GI Tumors

CEA is nonspecific for the gastrointestinal tract, and many other tumors can result in increased levels of CEA in the serum:

- Non-small cell Lung carcinomas (65%)
- Small cell Lung carcinomas (30%)
- Pancreatic carcinomas (25–55%)
- Biliary carcinomas (50%)
- Breast carcinomas (40%)
- Squamous uterine cervical carcinomas (40%)
- Ovarian tumors (25%)
- Thymomas[12]
- Medullary thyroid carcinomas[115,116]
- Salivary gland tumors[117]

False-Positive CEA Results

In a study in a central laboratory in Sweden, the overall incidence of false-positive and false-negative CEA results in the general population was 4%. However, in patients with GI tumors, elevations in CEA unrelated to tumor progression are more frequent. For example, CEA (and CA19-9) remained elevated in 14% of 151 patients that had curative gastrectomy.[118] Some of the patients had benign conditions associated with elevated CEA (see below), while in other patients the levels of CEA and CA19-9 spontaneously decreased 1–2 months after the operation. These false-positive elevations are less common with curative CRC resections, enhancing their value for monitoring of recurrence in these tumors, compared to GC.[100]

It is important to distinguish biological false positives, which represent true elevations of CEA not resulting from a neoplastic condition, from analytical false-positive results, which are caused by instrument malfunction or interferences with the assay and were discussed above. CEA elevations in benign diseases rarely exceed 10 ng/mL. In the following list of CEA biologic false-positives, numbers indicate approximate frequency of CEA elevations:

- Benign GI diseases
  - Rectal polyps (5%)
  - Inflammatory bowel diseases (15–90% depending on activity)
  - Diverticulitis (20%)
  - Gastric ulcer (15%)
  - Atrophic gastritis (25%)
  - Pancreatitis (20–50%)
- Various renal and hepatic diseases may affect CEA levels, as these organs are involved in its metabolism and elimination
  - Acute hepatitis (50–85%)
  - Chronic Hepatitis (20–30%)
  - Cirrhosis (15–80%)
  - Alcoholic liver disease (50–90%)
  - Biliary obstruction (50%)
  - Chronic renal failure (40%)
- Benign lung diseases
  - Pulmonary emphysema (15–30%)
  - Chronic bronchitis (15–70% depending on activity)
  - Cystic fibrosis (50%)
  - Pneumonia (45%)
  - Tuberculosis (35%)
  - Sarcoidosis[119]
  - Eosinophilic bronchiolitis[120]

- Benign fibrocystic breast disease (15%)
- Hypothyroidism following chemoradiation therapy involving the thyroid—CEA decreased after thyroxine supplementation[121]
- Circulating immune complexes with CEA can falsely elevate the CEA levels because of the reduction in clearance
- Anecdotally, high levels of CEA can persist post-curative resection of colon cancer for several years without any evidence of tumor development or any of the causes above[122]

Importantly, mild elevations of CEA can be seen in healthy individuals, correlating with smoking and advanced age. In a study of 276 healthy volunteers, smokers had higher mean levels of CEA (2.7 vs 1.9 ng/mL) and nearly 5% of the smokers had levels >5 ng/mL, although the CEA levels declined to nonsmoker range within 3 months of smoking cessation.[123] In both groups, CEA levels increased with age.

False-Negative CEA Results

About 50% of the patients with CRC have normal levels of CEA, particularly patients with localized disease and poorly differentiated tumors. Among patients with truly elevated CEA, there are some conditions that may result in falsely decreased CEA levels, including hemodilution, such as parenteral nutrition and blood transfusion, and the presence of CEA containing immune complexes in the plasma.[124] Analytic interferences by anti-mouse antibodies can cause either false increases or decreases in CEA levels.

Other Glycoprotein Markers

The CA series of antigens are carbohydrate moieties of glycoproteins with complex patterns of glycosylation, recognized by specific antibodies (Table 15.4). The most commonly used marker for monitoring of GI tumors is CA19-9, although CA 72-4 has shown some promising characteristics. In general, sensitivities above 50% were observed only for advanced and metastatic GI cancers.

CA 19-9

This antigen is related to the Lewis^{a} red blood cell antigen, structurally a sialylated Lewis^{a} lacto-fucopenteose II ganglioside, and was discovered in 1981 by Koprowski et al., in patients with gastric, colon, and pancreatic cancer.[125] Since it requires the Lewis gene product, 1,4-fucosyl-transferase, it is absent in Le^{a-b-} individuals, which comprise approximately 5% of the general population, and therefore cannot be used as a tumor marker in this population. The main application of CA19-9 measurement is for detection of recurrences of pancreatic cancer, since it is elevated in about 80% of patients with pancreatic adenocarcinoma. Patients with locally advanced or metastatic pancreatic cancer receiving active therapy should be monitored every 1–3 months as recommended by ASCO.[126] CA19-9 is also elevated in a variety of other tumors, including hepatobiliary, gastric, colorectal, breast, endometrial, and salivary carcinomas, and in a variety of benign conditions that include lung, renal, and liver disease, and up to 20% of patients with pancreatitis. In one report, persistent elevation of CA19-9 ranging from 112 to 1,338 IU/ml was observed in patients followed for up to 7 years without biliary or pancreatic tumors but with pulmonary fibrosis, diabetes, non-ulcer dyspepsia, obesity, acute diarrhea, colon diverticula, or gastric ulcer.[127]

Colorectal Cancer

While only present in a minority of patients with resectable CRC (around 20%), CA19-9 elevation is an independent predictor of adverse prognosis in CRC and may complement CEA for that purpose.[128,129] A study in Japan concluded that computed tomography (CT), CA19-9, and CEA were the first abnormal test in 73, 25, and 22% of recurrences of resected CRC, respectively.[130] While imaging was superior to the serum markers, CA19-9 was able to detect recurrence earlier than CT in 27% of the patients. In patients with liver metastases of CRC, elevated CA19-9 (but not CEA) is a good predictor of extrahepatic metastases.[131]

Gastric Cancer

Similarly to CRC, the positivity rate for CA19-9 is low in GC (around 18%), but the sensitivity for detection of recurrences in CA19-9-positive tumors is 60%, with a specificity of 93% at a cutoff of 100 U/ml.[101] A multicenter, prospective study in Japan of 321 patients with resected GC showed that the combination of CEA and CA19-9 had a sensitivity of 85% for detection of tumor recurrence, compared to 66% for CEA alone.[132] Even in patients with preoperative-elevated CEA and/or CA19-9 (45%), the levels increased again at recurrence. CA19-9 correlated well with lymph node metastasis, clinical stage, vascular invasion, and tumor size but not with survival in a study of 75 resectable, non-metastatic GC patients, suggesting that recurrence after surgical removal of non-metastatic GC is not predictable from preoperative CA19-9 levels.[108] Another study in 166 patients showed that preoperative CA19-9 correlated with clinical stage and was an independent prognostic factor in resected GC.[99] CA19-9 has moderate sensitivity (38%) to detect peritoneal metastasis of GC,[133] and appears more sensitive than CEA for that purpose, while CEA is more sensitive to detect liver metastasis.[134] In another study, elevated CA19-9 had an odds ratio of 4.4–4.5 to predict liver and lymph node metastasis in GC.[135]

Table 15.4 Glycoprotein tumor markers

	Chemical structure	Breast	Ovary	Lung	Prostate	Hepato-biliary	Pancreas	Colon	Stomach	Esophagus	Other cancers
CA 15–3	MUC-1 (Epsialin)	6–83[a]	64	71		28	80	63			
CA 27.29	MUC-1 (Epsialin)	6–86[a]									
CA 549	MUC-1 (Epsialin)	11–83[a]	50	33	40						
CA 125	Mucin MUC16	30[a]	80–90	5–25			50–60	35	40[a]		Endometrial/ cervix
MCA (B12)	Mucin-like	25–90	Few		Few						Endometrial/ cervix
DU-PAN-2	Positive in Le^{a-b-}		Few	Few		44	48–72		Few		
CA 195	Sialylated $Le^a + Le^a$		Few				76–82	71	Few		
CA 19-9	Sialylated Le^{xa}	15				30–67	70–100	18–65	13–47	30	Endometrial
CA 242	Sialylated Le^a-like			30–65			57–82	33–85	44		
CA 50	Sialylated Le^a + afucosyl sialylated Le^a	Few	Few	Few	Few	14–78/58–70	80–97	24–67	41–78	41–71	Renal, bladder
CA 72-4	Sialylated Tn	Few	24	36			70	25–43	40–84	50	

Numbers indicate percentage of tumors with elevation in the specific marker. Adapted from[174]
[a]in metastatic disease

As with CEA, early surges in the levels of CA19-9 in GC patients treated with chemotherapy should not be interpreted as treatment failures.[102]

CA 72-4

The CA72-4 glycoprotein is a mucin-like molecule with a molecular mass of over 1,000 kDa carrying the sialylated Tn blood group antigen.[136] It appears more specific and less sensitive than CEA for GI malignancies, with sensitivities of 56%, 32%, and 18% for CRC, GC, and EC, respectively, in one study[137] and specificities of more than 95%.[138] Addition of CA72-4 to CEA significantly increased the detection of CRC[139] but not of CRC recurrences.[140] A recent study using a time-resolved immunofluorometric assay showed a sensitivity of 84% with a specificity of 99% of CA72-4 for newly diagnosed GC.[141] Another study showed sensitivity of 48% for GC, which increased to 61% when combined with CEA and CA19-9.[142] While preoperative CA72-4 was elevated in only 20% of GC, post-gastrectomy recurrences showed elevations in 51% of the cases with a specificity of 97%.[143] CA72-4 is elevated in about 1–7% of benign GI conditions. While the low sensitivity of CA72-4 precludes its use as the sole marker for detection of GI cancers, its high specificity allows its addition in combination with other markers and may provide an useful target for molecular imaging and directed chemotherapy.[144]

CA 125

The CA125 antigen is present in mucin 16 (MUC16), a cell-surface associated single-pass type I membrane protein that can be cleaved and secreted into the extracellular space following phosphorylation of its intracellular domain. The main use of CA125 is to monitor epithelial ovarian cancer, but there are a few studies showing limited utility in GI cancers. For example, elevations of CA125 have a sensitivity of 39% and specificity of 98% to detect peritoneal metastasis of GC.[133] In another study, all GC patients with CA125 >35 U/ml had peritoneal metastasis compared to only 23% of patients with CA125 <35 U/ml.[145] The main problem with this assay is its lack of specificity, as up to 64% of patients with liver cirrhosis, and 20–40% of patients with other GI and liver diseases have elevated CA125.

CA 242

This antigen is similar to CA19-9 but consists of a different sialylated Le^a epitope. Following radical gastrectomy for GC, CA242 may be more sensitive to detect lung metastases, while CA19-9 is a better predictor of peritoneal metastasis, and CEA appears more sensitive for liver metastases.[134] It has a slightly better AUC than CA19-9 as an adverse prognosis factor in CRC, with a 5 year recurrence rate of 77% for CA 242-positive cases vs. 44% for CA 242-negative.[129] Use of CA 242 in combination with CEA increased the overall sensitivity for metastases, e.g., from 84% with CEA alone to 88% with the combination of CEA and CA 242.[146] CA 242 is elevated in 5–33% of benign GI conditions.

CA50

This antigen is also recognized by the CA19-9 antibody and is composed of sialylated Le^a and the afucosyl form of sialylated Le^a. It can be elevated in a variety of tumors, including pancreas, CRC and GC, but it is also elevated in 12–46% of benign diseases involving pancreas, liver, or biliary tract, limiting its usefulness as a tumor marker.

Promising Markers for Colon Cancer

A meta-analysis of CRC biomarkers published in 2007 reported 52 serum protein markers with overall sensitivity ranging from 18% to 65%,[65] many listed in Table 15.5 together with other potential biomarkers for which sensitivity and specificity data were available. No single marker is clearly superior for detection of CRC, and further study of new markers and possible marker combinations are necessary to achieve sensitive biochemical detection of CRC. For example, combining CEA measurement with detection of six autoantibodies achieved 92% sensitivity and 96% specificity for CRC detection.[147]

As an illustration of the slow progress in CRC biomarker development, the DR-70® (FDP) test (AMDL Diagnostics, Tustin, CA) is the first assay cleared by the FDA for monitoring CRC since the approval of CEA in 1982. Sensitivity and specificity are comparable to CEA, but this can be a useful assay in patients with CRC and low levels of CEA.[148]

Promising Markers in Gastric and Esophageal Cancer

Gastric and esophageal carcinomas have also been the subject of several studies examining potential biomarkers, a few examples of which are listed in Table 15.6. The standard biomarker for monitoring esophageal squamous cell carcinomas is the squamous cell antigen (SCA), which is elevated in a variety of squamous cell carcinomas, including those affecting the esophageal mucosa. It can be elevated in about 40–50% of the patients with squamous cell carcinoma of the

Table 15.5 Promising biomarkers associated with colorectal cancer

Marker	Sensitivity	Specificity	Pubmed 2009–2011	Reference
CA 19-9	18–65	80–100	68	65a
VEGF	36–91	61–100	66	65a 175
Antitumor antigen panel	61	90	23	158,176
TIMP-1	55	95	10	177,178
u-PA	76	80–96	8	179
sCD26	80–100	72–90	7	180,181
α-Defensins 1-3	69	100	7	182,183
M2-PK	56–85	76–90	6	184,185
OPN	30–65	56–85	5	186
CA 72-4	25–43	95–98	4	65a
CA 242	33–55	89–96	4	65a
TPA-M	70	96	4	187
SLEX	25	96	3	188
TATI	74	34	3	189
Laminin	89	88	3	190
Nicotinamide *N*-Methyltransferase	51	95	3	191
Anti-CEA	79	90	3	192
GM-CSF	80	70	3	193
Fibrin degradation DR-7	65–80	67–93	3	148,194
CCSA-2	78	97	3	195
sP-selectin	21	94–99	2	196
Prolactin	77	98	2	197
α-Defensin 6 (DEFA6)	69	83	2	198
Cystatin SN	28	95	2	199
Migration inhibitory factor	47	91	2	200
SIMA	36	90–95	1	201
SIMA-I	27	89	1	202
SIMA-II	19	89	1	202
Anti-p53	15–28	100	1	65a
IL-3	55	80	1	203
Progesterone	57–64	37–40	1	204
Dermokine βγ	29–36	92	1	205
Seprase	42	95	1	151
Desmin	55	80	1	156
Anti-DDX-48	10	100	0	206
Anti-Fas	33	100	0	207
Anti-NCC-ST 439	27	94	0	208
BSP	88–96	100	0	186
CA 195	71	71–100	0	209
CA 50	24–67	51–99	0	65a
CA M26	22	99	0	210
CA M29	12	99	0	210
CA M43	42–74	92–99	0	211,212
Cancer Procoagulant	86	82	0	213
CO 29.11	41	95–97	0	214
Free PSA (women)	35	93	0	215
GST enzymes	89	77–85	0	216
NCA50-90	35	95	0	217
PA 8-15	45	87–95	0	218
SCF	89	17	0	203
Tenascin	25	95	0	219

(continued)

Table 15.5 (continued)

Marker	Sensitivity	Specificity	Pubmed 2009–2011	Reference
Villin	51	87–97	0	[220]
α-L-fucosidase	69	85	0	[221]

"Pubmed" refers to the number of articles published between 2009 and 2011 referring the specific marker
[a]Data from Hundt et al.[65]

Table 15.6 Examples of promising biomarkers for detection of gastric (GC) and esophageal (EC) carcinomas

Marker	Outcome	Sensitivity	Specificity	References
CA72-4 + M2-PK	EC Detection	74	95	[222]
MMP-9	EC Detection	70	60	[223]
sVEGF-C	EC Detection	60	78	[224]
anti-CDC25B	EC Detection	57	91	[225]
MMP-9 (serum)	GC Detection	83	66	[226]
TIMP-1	GC Detection	17–89	97	[227,228]
MG7	GC Detection	84	87	[229]
MIF	GC Detection	84	92	[230]
M2-pyruvate kinase	GC Detection	62	89	[231]
IL18	GC Detection	52	83	[232]
MUC1/5 AC alternative glycosylation	GC Detection	25–42	90	[233]
IPO-38	GC Detection	57	90	[234]
Pepsinogen I/II + hsCRP	GC Detection — Early	74	70	[235]
ITIH3	GC Detection — Early	96	66	[236]
IL6	GC Detection — Advanced	82	67	[237]
IL6	GC Lymph node metastasis	87	58	[237]
Reg4 + Olfactomedin 4	GC Detection (Stage 1–4)	52–88	95	[238]
Soluble E-cadherin	GC Detection -Recurrence	47–59	75–81	[239]

esophagus and is associated with worse prognosis.[149,150] It is also elevated in a variety of benign diseases of the skin (such as psoriasis, pemphigus, and eczema), lungs (tuberculosis, sarcoidosis, and pleural effusions), and other tissues with squamous epithelia, limiting its use for diagnostic purposes.

Proteomic Approaches to GI Tumor Markers

It is evident that single markers are of insufficient diagnostic accuracy to screen for GI tumors, especially at early stages. Combinatorial approaches using several protein markers, which can be labeled as low-multiplex proteomics, have been shown to improve sensitivity and specificity for tumor detection.[151] For example, a protein chip using 12 markers (CEA, alpha-fetoprotein, CA 19-9, CA 242, CA 15-3, CA 125, prostate specific antigen, free-PSA, neuron-specific enolase, human chorionic gonadotropin-beta, human growth hormone, and ferritin) detected GC with sensitivities varying from 37% in stage I to 50% in stage IV tumors.[152] More comprehensive, unbiased proteomic approaches aim at identifying additional biomarkers differentially expressed by tumors. However, the approach using comprehensive proteomics has been somewhat disappointing. Most of the studies with serum proteomics identified peptides derived from secondary alterations in abundant serum proteins induced by tumor-associated proteases. These approaches are unlikely to result in useful markers because of the lack of specificity. For example, surface-enhanced laser desorption/ionization (SELDI) based proteomics identified peptides derived from complement C3a des-Arg, alpha1-antitrypsin, and transferrin, all nonspecific to colon cancer, as having diagnostic potential.[153] Another SELDI study in gastric cancer identified five peaks that predicted survival with 84% sensitivity and 85% specificity, but the nature of the peptides was not further specified.[154] A separate study using four unidentified peaks revealed a sensitivity of 93% and specificity of 90% for detection of GC.[155] Despite these promising results, good reproducibility of these findings has not yet been achieved.

In contrast to serum proteomics, differential proteomic analysis of tumor vs. non-tumor tissue samples can reveal tumor-associated proteins of potential diagnostic use. An example is the identification of desmin and ZF protein 829 by 2D gel comparison of normal tissue and CRC.[156]

Unfortunately, the delivery of tumor proteins to the plasma is affected by many factors, including tumor vascularization, degree of inflammation, necrosis, and fibrotic response. The study of tumor-associated membrane-expressed proteins may obviate some of these limitations and identify biomarkers more likely to be released in circulation. For example, differential labeling of membranes from CRC versus normal mucosa, using the iTraq procedure, identified CEA, CEACAM6, claudin-1, HLA class I histocompatibility antigen A-1, tapasin, and mitochondrial solute carrier family 25A4 as differentially expressed in CRC.[157]

An alternative approach to identify diagnostically useful markers is the detection of autoantibodies against tumor-enriched/modified proteins. For example, Liu et al. used enzyme-linked immunosorbent assay (ELISA) to identify antibodies against Imp1, p62, Koc, p53, and c-myc full-length recombinant proteins in CRC, achieving a combined sensitivity of 61% and a specificity of 90%.[158] Adding CEA to the panel increased the sensitivity to 83%. A proteomic approach using 2D-gel electrophoresis followed by immunoblotting with sera from GC patients resulted in the identification of GRP78 as a target for autoantibodies in 28% of GC patients versus 0/20 controls.[159] A recent study used a high density protein array containing 37,830 clones expressing recombinant proteins to identify patterns of autoantibodies that distinguished symptomatic from asymptomatic CRC patients.[160]

Conclusions

Serum protein biomarkers offer the potential to diagnose and monitor GI tumors with simple, quantitative, easily automated, and inexpensive assays. Unfortunately, the diagnostic accuracy of current and most prospective markers is insufficient to recommend their use in isolation for tumor detection, especially in the general population. In contrast, the role of serum protein markers in monitoring the response to treatment is well accepted, particularly for CEA and CRC. The list of newer, potential markers is large and likely to expand at an increasing rate, especially in consequence of large-scale "omic" approaches. While comprehensive proteomic approaches are unlikely to be used in the near future, combinatorial panels of selected markers offering increased sensitivity and specificity are expected to replace single biomarkers in the evaluation of patients with GI tumors.

References

1. Bonfrer JM. Working Group on Tumor Marker Criteria (WGTMC). *Tumour Biol*. 1990;11(5):287–288.
2. Gold P, Freedman SO. Demonstration of tumor-specific antigens in human colonic carcinomata by immunological tolerance and absorption techniques. *J Exp Med*. 1965;121:439–462.
3. Zebhauser R, Kammerer R, Eisenried A, McLellan A, Moore T, Zimmermann W. Identification of a novel group of evolutionarily conserved members within the rapidly diverging murine Cea family. *Genomics*. 2005;86(5):566–580.
4. Hammarstrom S. The carcinoembryonic antigen (CEA) family: structures, suggested functions and expression in normal and malignant tissues. *Semin Cancer Biol*. 1999;9(2):67–81.
5. Naghibalhossaini F, Ebadi P. Evidence for CEA release from human colon cancer cells by an endogenous GPI-PLD enzyme. *Cancer Lett*. 2006;234(2):158–167.
6. Pakdel A, Naghibalhossaini F, Mokarram P, Jaberipour M, Hosseini A. Regulation of carcinoembryonic antigen release from colorectal cancer cells. *Mol Biol Rep*. 2012;39:3695–3704.
7. Bjerner J, Lebedin Y, Bellanger L, et al. Protein epitopes in carcinoembryonic antigen. Report of the ISOBM TD8 workshop. *Tumour Biol*. 2002;23(4):249–262.
8. Huang JQ, Turbide C, Daniels E, Jothy S, Beauchemin N. Spatiotemporal expression of murine carcinoembryonic antigen (CEA) gene family members during mouse embryogenesis. *Development*. 1990;110(2):573–588.
9. Zimmermann W, Ortlieb B, Friedrich R, von Kleist S. Isolation and characterization of cDNA clones encoding the human carcinoembryonic antigen reveal a highly conserved repeating structure. *Proc Natl Acad Sci USA*. 1987;84(9):2960–2964.
10. Sanders DS, Wilson CA, Bryant FJ, et al. Classification and localisation of carcinoembryonic antigen (CEA) related antigen expression in normal oesophageal squamous mucosa and squamous carcinoma. *Gut*. 1994;35(8):1022–1025.
11. Sanders DS, Stocks SC, Milne DM, Milne GA, Hopwood D, Kerr MA. Membranous expression of carcinoembryonic antigen (CEA) in the normal cervical squamous mucosa. *J Pathol*. 1992;167(1):77–82.
12. Savino W, Durand D, Dardenne M. Immunohistochemical evidence for the expression of the carcinoembryonic antigen by human thymic epithelial cells in vitro and in neoplastic conditions. *Am J Pathol*. 1985;121(3):418–425.
13. Nouwen EJ, Pollet DE, Eerdekens MW, Hendrix PG, Briers TW, De Broe ME. Immunohistochemical localization of placental alkaline phosphatase, carcinoembryonic antigen, and cancer antigen 125 in normal and neoplastic human lung. *Cancer Res*. 1986;46(2):866–876.
14. Metze D, Luger TA. Ultrastructural localization of carcinoembryonic antigen (CEA) glycoproteins and epithelial membrane antigen (EMA) in normal and neoplastic sweat glands. *J Cutan Pathol*. 1996;23(6):518–529.
15. Nyati MK, Sreekumar A, Li S, et al. High and selective expression of yeast cytosine deaminase under a carcinoembryonic antigen promoter-enhancer. *Cancer Res*. 2002;62(8):2337–2342.
16. Zhang G, Liu T, Chen YH, et al. Tissue specific cytotoxicity of colon cancer cells mediated by nanoparticle-delivered suicide gene in vitro and in vivo. *Clin Cancer Res*. 2009;15(1):201–207.
17. Dabrowska A, Szary J, Kowalczuk M, Szala S, Ugorski M. CEA-negative glioblastoma and melanoma cells are sensitive to cytosine deaminase/5-fluorocytosine therapy directed by the carcinoembryonic antigen promoter. *Acta Biochim Pol*. 2004;51(3):723–732.
18. Han SU, Kwak TH, Her KH, et al. CEACAM5 and CEACAM6 are major target genes for Smad3-mediated TGF-beta signaling. *Oncogene*. 2008;27(5):675–683.
19. Thomas SN, Zhu F, Schnaar RL, Alves CS, Konstantopoulos K. Carcinoembryonic antigen and CD44 variant isoforms cooperate to mediate colon carcinoma cell adhesion to E- and L-selectin in shear flow. *J Biol Chem*. 2008;283(23):15647–15655.
20. Baranov V, Hammarstrom S. Carcinoembryonic antigen (CEA) and CEA-related cell adhesion molecule 1 (CEACAM1), apically expressed on human colonic M cells, are potential receptors for microbial adhesion. *Histochem Cell Biol*. 2004;121(2):83–89.

21. Screaton RA, Penn LZ, Stanners CP. Carcinoembryonic antigen, a human tumor marker, cooperates with Myc and Bcl-2 in cellular transformation. *J Cell Biol*. 1997;137(4):939–952.
22. Naghibalhossaini F, Yoder AD, Tobi M, Stanners CP. Evolution of a tumorigenic property conferred by glycophosphatidyl-inositol membrane anchors of carcinoembryonic antigen gene family members during the primate radiation. *Mol Biol Cell*. 2007; 18(4):1366–1374.
23. Ilantzis C, DeMarte L, Screaton RA, Stanners CP. Deregulated expression of the human tumor marker CEA and CEA family member CEACAM6 disrupts tissue architecture and blocks colonocyte differentiation. *Neoplasia*. 2002;4(2):151–163.
24. Baranov V, Yeung MM, Hammarstrom S. Expression of carcinoembryonic antigen and nonspecific cross-reacting 50-kDa antigen in human normal and cancerous colon mucosa: comparative ultrastructural study with monoclonal antibodies. *Cancer Res*. 1994;54(12):3305–3314.
25. Leusch HG, Drzeniek Z, Markos-Pusztai Z, Wagener C. Binding of Escherichia coli and Salmonella strains to members of the carcinoembryonic antigen family: differential binding inhibition by aromatic alpha-glycosides of mannose. *Infect Immun*. 1991;59(6):2051–2057.
26. Allez M, Brimnes J, Shao L, Dotan I, Nakazawa A, Mayer L. Activation of a unique population of CD8(+) T cells by intestinal epithelial cells. *Ann N Y Acad Sci*. 2004;1029:22–35.
27. Prado IB, Laudanna AA, Carneiro CR. Susceptibility of colorectal-carcinoma cells to natural-killer-mediated lysis: relationship to CEA expression and degree of differentiation. *Int J Cancer*. 1995;61(6):854–860.
28. Kammerer R, von Kleist S. CEA expression of colorectal adenocarcinomas is correlated with their resistance against LAK-cell lysis. *Int J Cancer*. 1994;57(3):341–347.
29. Kammerer R, Zimmermann W. Coevolution of activating and inhibitory receptors within mammalian carcinoembryonic antigen families. *BMC Biol*. 2010;8:12.
30. Hostetter RB, Augustus LB, Mankarious R, et al. Carcinoembryonic antigen as a selective enhancer of colorectal cancer metastasis. *J Natl Cancer Inst*. 1990;82(5):380–385.
31. Jessup JM, Thomas P. Carcinoembryonic antigen: function in metastasis by human colorectal carcinoma. *Cancer Metastasis Rev*. 1989;8(3):263–280.
32. Chan CH, Camacho-Leal P, Stanners CP. Colorectal hyperplasia and dysplasia due to human carcinoembryonic antigen (CEA) family member expression in transgenic mice. *PLoS One*. 2007;2(12):e1353.
33. Leung N, Turbide C, Olson M, Marcus V, Jothy S, Beauchemin N. Deletion of the carcinoembryonic antigen-related cell adhesion molecule 1 (Ceacam1) gene contributes to colon tumor progression in a murine model of carcinogenesis. *Oncogene*. 2006;25(40):5527–5536.
34. Leung N, Turbide C, Balachandra B, Marcus V, Beauchemin N. Intestinal tumor progression is promoted by decreased apoptosis and dysregulated Wnt signaling in Ceacam1−/− mice. *Oncogene*. 2008;27(36):4943–4953.
35. Song JH, Cao Z, Yoon JH, et al. Genetic alterations and expression pattern of CEACAM1 in colorectal adenomas and cancers. *Pathol Oncol Res*. 2011;17:67–74.
36. Jessup JM, Samara R, Battle P, Laguinge LM. Carcinoembryonic antigen promotes tumor cell survival in liver through an IL-10-dependent pathway. *Clin Exp Metastasis*. 2004;21(8):709–717.
37. Minami S, Furui J, Kanematsu T. Role of carcinoembryonic antigen in the progression of colon cancer cells that express carbohydrate antigen. *Cancer Res*. 2001;61(6):2732–2735.
38. Gangopadhyay A, Lazure DA, Thomas P. Adhesion of colorectal carcinoma cells to the endothelium is mediated by cytokines from CEA stimulated Kupffer cells. *Clin Exp Metastasis*. 1998; 16(8):703–712.
39. Samara RN, Laguinge LM, Jessup JM. Carcinoembryonic antigen inhibits Anoikis in colorectal carcinoma cells by interfering with trail-R2 (DR5) signaling. *Cancer Res*. 2007;67(10):4774–4782.
40. Camacho-Leal P, Zhai AB, Stanners CP. A co-clustering model involving alpha5beta1 integrin for the biological effects of GPI-anchored human carcinoembryonic antigen (CEA). *J Cell Physiol*. 2007;211(3):791–802.
41. Ordonez C, Zhai AB, Camacho-Leal P, Demarte L, Fan MM, Stanners CP. GPI-anchored CEA family glycoproteins CEA and CEACAM6 mediate their biological effects through enhanced integrin alpha5beta1-fibronectin interaction. *J Cell Physiol*. 2007;210(3):757–765.
42. Camacho-Leal P, Stanners CP. The human carcinoembryonic antigen (CEA) GPI anchor mediates anoikis inhibition by inactivation of the intrinsic death pathway. *Oncogene*. 2008;27(11):1545–1553.
43. Wirth T, Soeth E, Czubayko F, Juhl H. Inhibition of endogenous carcinoembryonic antigen (CEA) increases the apoptotic rate of colon cancer cells and inhibits metastatic tumor growth. *Clin Exp Metastasis*. 2002;19(2):155–160.
44. Soeth E, Wirth T, List HJ, et al. Controlled ribozyme targeting demonstrates an antiapoptotic effect of carcinoembryonic antigen in HT29 colon cancer cells. *Clin Cancer Res*. 2001;7(7):2022–2030.
45. Sharkey RM, Karacay H, Vallabhajosula S, et al. Metastatic human colonic carcinoma: molecular imaging with pretargeted SPECT and PET in a mouse model. *Radiology*. 2008;246(2):497–507.
46. Aarts F, Boerman OC, Sharkey RM, et al. Pretargeted radioimmunoscintigraphy in patients with primary colorectal cancer using a bispecific anticarcinoembryonic antigen CEA X anti-di-diethylenetriaminepentaacetic acid F(ab′)2 antibody. *Cancer*. 2010;116(4 Suppl):1111–1117.
47. Kaushal S, McElroy MK, Luiken GA, et al. Fluorophore-conjugated anti-CEA antibody for the intraoperative imaging of pancreatic and colorectal cancer. *J Gastrointest Surg*. 2008; 12(11):1938–1950.
48. Hong H, Sun J, Cai W. Radionuclide-based cancer imaging targeting the carcinoembryonic antigen. *Biomark Insights*. 2008;3:435–451.
49. Park S, Lee B, Kim I, et al. Immunobead RT-PCR versus regular RT-PCR amplification of CEA mRNA in peripheral blood. *J Cancer Res Clin Oncol*. 2001;127(8):489–494.
50. Douard R, Moutereau S, Serru V, et al. Immunobead multiplex RT-PCR detection of carcinoembryonic genes expressing cells in the blood of colorectal cancer patients. *Clin Chem Lab Med*. 2005;43(2):127–132.
51. Liu Z, Jin C, Yu Z, et al. Radioimmunotherapy of human colon cancer xenografts with 131I-labeled anti-CEA monoclonal antibody. *Bioconjug Chem*. 2010;21(2):314–318.
52. Liu Z, Jin C, Yu Z, et al. Radioimmunotherapy of human colon cancer xenografts with 131I-labeled anti-CEA monoclonal antibody. *Bioconjug Chem*. 2010;21(2):314–318.
53. Buchegger F, Roth A, Allal A, et al. Radioimmunotherapy of colorectal cancer liver metastases: combination with radiotherapy. *Ann N Y Acad Sci*. 2000;910:263–269. discussion 9–70.
54. Mori F, Giannetti P, Peruzzi D, et al. A therapeutic cancer vaccine targeting carcinoembryonic antigen in intestinal carcinomas. *Hum Gene Ther*. 2009;20(2):125–136.
55. Ojima T, Iwahashi M, Nakamura M, et al. Successful cancer vaccine therapy for carcinoembryonic antigen (CEA)-expressing colon cancer using genetically modified dendritic cells that express CEA and T helper-type 1 cytokines in CEA transgenic mice. *Int J Cancer*. 2007;120(3):585–593.
56. Shibaguchi H, Luo NX, Kuroki M, et al. A fully human chimeric immune receptor for retargeting T-cells to CEA-expressing tumor cells. *Anticancer Res*. 2006;26(6A):4067–4072.
57. Hou Y, Kavanagh B, Fong L. Distinct CD8+ T cell repertoires primed with agonist and native peptides derived from a tumor-associated antigen. *J Immunol*. 2008;180(3):1526–1534.

58. Li HJ, Everts M, Pereboeva L, et al. Adenovirus tumor targeting and hepatic untargeting by a coxsackie/adenovirus receptor ectodomain anti-carcinoembryonic antigen bispecific adapter. *Cancer Res*. 2007;67(11):5354–5361.
59. Bos R, van Duikeren S, Morreau H, et al. Balancing between antitumor efficacy and autoimmune pathology in T-cell-mediated targeting of carcinoembryonic antigen. *Cancer Res*. 2008;68(20):8446–8455.
60. Jung HS, Lee SW. Ribozyme-mediated selective killing of cancer cells expressing carcinoembryonic antigen RNA by targeted transsplicing. *Biochem Biophys Res Commun*. 2006;349(2):556–563.
61. Zamboni S, Mallano A, Flego M, et al. Genetic construction, expression, and characterization of a single chain anti-CEA antibody fused to cytosine deaminase from yeast. *Int J Oncol*. 2008;32(6):1245–1251.
62. Guadagni F, Roselli M, Cosimelli M, et al. Quantitative analysis of CEA expression in colorectal adenocarcinoma and serum: lack of correlation. *Int J Cancer*. 1997;72(6):949–954.
63. Thomson DMP, Krupey J, Freedman SO, Gold P. The radioimmunoassay of Circulating carcinoembryonic antigen of the human digestive system. *Proc Natl Acad Sci USA*. 1969;64(1):161–167.
64. Sato K, Tokeshi M, Kimura H, Kitamori T. Determination of carcinoembryonic antigen in human sera by integrated bead-bed immunoassay in a microchip for cancer diagnosis. *Anal Chem*. 2001;73(6):1213–1218.
65. Hundt S, Haug U, Brenner H. Blood markers for early detection of colorectal cancer: a systematic review. *Cancer Epidemiol Biomarkers Prev*. 2007;16(10):1935–1953.
66. Duffy MJ. Carcinoembryonic antigen as a marker for colorectal cancer: is it clinically useful? *Clin Chem*. 2001;47(4):624–630.
67. Sturgeon CM, Duffy MJ, Stenman UH, et al. National Academy of Clinical Biochemistry laboratory medicine practice guidelines for use of tumor markers in testicular, prostate, colorectal, breast, and ovarian cancers. *Clin Chem*. 2008;54(12):e11-e79.
68. Lim YK, Kam MH, Eu KW. Carcinoembryonic antigen screening: how far should we go? *Singapore Med J*. 2009;50(9):862–865.
69. Compton CC, Fielding LP, Burgart LJ, et al. Prognostic factors in colorectal cancer. College of American Pathologists Consensus Statement 1999. *Arch Pathol Lab Med*. 2000;124(7):979–994.
70. Compton C, Fenoglio-Preiser CM, Pettigrew N, Fielding LP. American Joint Committee on Cancer Prognostic Factors Consensus Conference: Colorectal Working Group. *Cancer*. 2000;88(7):1739–1757.
71. Huh JW, Oh BR, Kim HR, Kim YJ. Preoperative carcinoembryonic antigen level as an independent prognostic factor in potentially curative colon cancer. *J Surg Oncol*. 2010;101(5):396–400.
72. Ogata Y, Murakami H, Sasatomi T, et al. Elevated preoperative serum carcinoembrionic antigen level may be an effective indicator for needing adjuvant chemotherapy after potentially curative resection of stage II colon cancer. *J Surg Oncol*. 2009;99(1):65–70.
73. Kievit J. Follow-up of patients with colorectal cancer: numbers needed to test and treat. *Eur J Cancer*. 2002;38(7):986–999.
74. Tan E, Gouvas N, Nicholls RJ, Ziprin P, Xynos E, Tekkis PP. Diagnostic precision of carcinoembryonic antigen in the detection of recurrence of colorectal cancer. *Surg Oncol*. 2009;18(1):15–24.
75. Bruinvels DJ, Stiggelbout AM, Kievit J, van Houwelingen HC, Habbema JD, van de Velde CJ. Follow-up of patients with colorectal cancer. A meta-analysis. *Ann Surg*. 1994;219(2):174–182.
76. Figueredo A, Rumble RB, Maroun J, et al. Follow-up of patients with curatively resected colorectal cancer: a practice guideline. *BMC Cancer*. 2003;3:26.
77. Hida J, Yasutomi M, Shindoh K, et al. Second-look operation for recurrent colorectal cancer based on carcinoembryonic antigen and imaging techniques. *Dis Colon Rectum*. 1996;39(1):74–79.
78. Weiser MR, Landmann RG, Kattan MW, et al. Individualized prediction of colon cancer recurrence using a nomogram. *J Clin Oncol*. 2008;26(3):380–385.
79. Kattan MW, Gonen M, Jarnagin WR, et al. A nomogram for predicting disease-specific survival after hepatic resection for metastatic colorectal cancer. *Ann Surg*. 2008;247(2):282–287.
80. Li M, Li JY, Zhao AL, Gu J. Colorectal cancer or colon and rectal cancer? Clinicopathological comparison between colonic and rectal carcinomas. *Oncology*. 2007;73(1–2):52–57.
81. Chen CH, Hsieh MC, Lai CC, et al. Lead time of carcinoembryonic antigen elevation in the postoperative follow-up of colorectal cancer did not affect the survival rate after recurrence. *Int J Colorectal Dis*. 2010;25(5):567–571.
82. Sargent D, Sobrero A, Grothey A, et al. Evidence for cure by adjuvant therapy in colon cancer: observations based on individual patient data from 20,898 patients on 18 randomized trials. *J Clin Oncol*. 2009;27(6):872–877.
83. Chao M, Gibbs P. Caution is required before recommending routine carcinoembryonic antigen and imaging follow-up for patients with early-stage colon cancer. *J Clin Oncol*. 2009;27(36):e279-e280. author reply e81.
84. Quah HM, Chou JF, Gonen M, et al. Identification of patients with high-risk stage II colon cancer for adjuvant therapy. *Dis Colon Rectum*. 2008;51(5):503–507.
85. Chau I, Allen MJ, Cunningham D, et al. The value of routine serum carcino-embryonic antigen measurement and computed tomography in the surveillance of patients after adjuvant chemotherapy for colorectal cancer. *J Clin Oncol*. 2004;22(8):1420–1429.
86. NCCN Colon Cancer Panel. Colon Cancer. 2010 [updated 9/10/2010; cited 2010 9/30/2010]; Available from: http://www.nccn.org/professionals/physician_gls/PDF/colon.pdf.
87. Lin JK, Lin CC, Yang SH, et al. Early postoperative CEA level is a better prognostic indicator than is preoperative CEA level in predicting prognosis of patients with curable colorectal cancer. *Int J Colorectal Dis*. 2011;26:1135–1141.
88. Lee WS, Baek JH, Kim KK, Park YH. The prognostic significant of percentage drop in serum CEA post curative resection for colon cancer. *Surg Oncol*. 2012;21:45–51.
89. Cooper GS, Kou TD, Reynolds HL Jr. Receipt of guideline-recommended follow-up in older colorectal cancer survivors: a population-based analysis. *Cancer*. 2008;113(8):2029–2037.
90. Iwanicki-Caron I, Di Fiore F, Roque I, et al. Usefulness of the serum carcinoembryonic antigen kinetic for chemotherapy monitoring in patients with unresectable metastasis of colorectal cancer. *J Clin Oncol*. 2008;26(22):3681–3686.
91. Ailawadhi S, Sunga A, Rajput A, Yang GY, Smith J, Fakih M. Chemotherapy-induced carcinoembryonic antigen surge in patients with metastatic colorectal cancer. *Oncology*. 2006;70(1):49–53.
92. An X, Ding PR, Xiang XJ, et al. Carcinoembryonic antigen surge in metastatic colorectal cancer patients responding to irinotecan combination chemotherapy. *Biomarkers*. 2010;15(3):243–248.
93. Sorbye H, Dahl O. Carcinoembryonic antigen surge in metastatic colorectal cancer patients responding to oxaliplatin combination chemotherapy: implications for tumor marker monitoring and guidelines. *J Clin Oncol*. 2003;21(23):4466–4467.
94. Strimpakos AS, Cunningham D, Mikropoulos C, Petkar I, Barbachano Y, Chau I. The impact of carcinoembryonic antigen flare in patients with advanced colorectal cancer receiving first-line chemotherapy. *Ann Oncol*. 2010;21(5):1013–1019.
95. Wang WS, Lin JK, Lin TC, et al. Carcinoembryonic antigen in monitoring of response to systemic chemotherapy in patients with metastatic colorectal cancer. *Int J Colorectal Dis*. 2001;16(2):96–101.
96. Park SH, Ku KB, Chung HY, Yu W. Prognostic significance of serum and tissue carcinoembryonic antigen in patients with gastric adenocarcinomas. *Cancer Res Treat*. 2008;40(1):16–21.
97. Sturgeon CM, Duffy MJ, Hofmann BR, et al. National Academy of Clinical Biochemistry Laboratory Medicine Practice Guidelines for use of tumor markers in liver, bladder, cervical, and gastric cancers. *Clin Chem*. 2010;56(6):e1-e48.

98. Horie Y, Miura K, Matsui K, et al. Marked elevation of plasma carcinoembryonic antigen and stomach carcinoma. *Cancer*. 1996;77(10):1991–1997.
99. Zhang YH, Li Y, Chen C, Peng CW. Carcinoembryonic antigen level is related to tumor invasion into the serosa of the stomach: study on 166 cases and suggestion for new therapy. *Hepatogastroenterology*. 2009;56(96):1750–1754.
100. Ohtsuka T, Nakafusa Y, Sato S, Kitajima Y, Tanaka M, Miyazaki K. Different roles of tumor marker monitoring after curative resections of gastric and colorectal cancers. *Dig Dis Sci*. 2008; 53(6):1537–1543.
101. Qiu MZ, Lin JZ, Wang ZQ, et al. Cutoff value of carcinoembryonic antigen and carbohydrate antigen 19–9 elevation levels for monitoring recurrence in patients with resectable gastric adenocarcinoma. *Int J Biol Markers*. 2009;24(4):258–264.
102. Kim HJ, Lee KW, Kim YJ, et al. Chemotherapy-induced transient CEA and CA19–9 surges in patients with metastatic or recurrent gastric cancer. *Acta Oncol*. 2009;48(3):385–390.
103. Sakamoto J, Nakazato H, Teramukai S, et al. Association between preoperative plasma CEA levels and the prognosis of gastric cancer following curative resection. Tumor Marker Committee, Japanese Foundation for Multidisciplinary Treatment of Cancer, Tokyo, Japan. *Surg Oncol*. 1996;5(3):133–139.
104. Ishigami S, Natsugoe S, Hokita S, et al. Clinical importance of preoperative carcinoembryonic antigen and carbohydrate antigen 19–9 levels in gastric cancer. *J Clin Gastroenterol*. 2001;32(1):41–44.
105. Kim DY, Kim HR, Shim JH, Park CS, Kim SK, Kim YJ. Significance of serum and tissue carcinoembryonic antigen for the prognosis of gastric carcinoma patients. *J Surg Oncol*. 2000; 74(3):185–192.
106. Jung M, Jeung HC, Lee SS, et al. The clinical significance of ascitic fluid CEA in advanced gastric cancer with ascites. *J Cancer Res Clin Oncol*. 2010;136(4):517–526.
107. Yamamoto M, Baba H, Toh Y, Okamura T, Maehara Y. Peritoneal lavage CEA/CA125 is a prognostic factor for gastric cancer patients. *J Cancer Res Clin Oncol*. 2007;133(7):471–476.
108. Dilege E, Mihmanli M, Demir U, et al. Prognostic value of preoperative CEA and CA 19–9 levels in resectable gastric cancer. *Hepatogastroenterology*. 2010;57(99–100):674–677.
109. Lukaszewicz-Zajac M, Mroczko B, Gryko M, Kedra B, Szmitkowski M. Comparison between clinical significance of serum proinflammatory proteins (IL-6 and CRP) and classic tumor markers (CEA and CA 19–9) in gastric cancer. *Clin Exp Med*. 2011;11:89–96.
110. Ucar E, Semerci E, Ustun H, Yetim T, Huzmeli C, Gullu M. Prognostic value of preoperative CEA, CA 19–9, CA 72–4, and AFP levels in gastric cancer. *Adv Ther*. 2008;25(10):1075–1084.
111. Bhatnagar J, Heroman W, Murphy M, Austin GE. Immunohistochemical detection of carcinoembryonic antigen in esophageal carcinomas: a comparison with other gastrointestinal neoplasms. *Anticancer Res*. 2002;22(3):1849–1857.
112. Clark GW, Ireland AP, Hagen JA, Collard JM, Peters JH, DeMeester TR. Carcinoembryonic antigen measurements in the management of esophageal cancer: an indicator of subclinical recurrence. *Am J Surg*. 1995;170(6):597–600. discussion –1.
113. Yi Y, Li B, Sun H, et al. Predictors of sensitivity to chemoradiotherapy of esophageal squamous cell carcinoma. *Tumour Biol*. 2010;31(4):333–340.
114. Setoyama T, Natsugoe S, Okumura H, et al. Carcinoembryonic antigen messenger RNA expression in blood predicts recurrence in esophageal cancer. *Clin Cancer Res*. 2006;12(20 Pt 1): 5972–5977.
115. Cvejic D, Savin S, Golubovic S, Paunovic I, Tatic S, Havelka M. Galectin-3 and carcinoembryonic antigen expression in medullary thyroid carcinoma: possible relation to tumour progression. *Histopathology*. 2000;37(6):530–535.
116. Gill S, Cosolo W, Herbertson RA, Berlangieri SU, Scott AM. Medullary carcinoma of the thyroid in a patient with colon cancer and a rising carcinoembryonic antigen level. *Intern Med J*. 2009;39(4):264–265.
117. Angelov A, Klissarova A, Dikranian K. Radioimmunological and immunohistochemical study of carcinoembryonic antigen in pleomorphic adenoma and mucoepidermoid carcinoma of the salivary glands. *Gen Diagn Pathol*. 1996;141(3–4):229–234.
118. Ohtsuka T, Sato S, Kitajima Y, Tanaka M, Nakafusa Y, Miyazaki K. False-positive findings for tumor markers after curative gastrectomy for gastric cancer. *Dig Dis Sci*. 2008;53(1):73–79.
119. Gallagher DJ, Libby DM, Kemeny N. Elevated carcinoembryonic antigen and sarcoidosis masquerading as metastatic colon cancer. *Clin Colorectal Cancer*. 2009;8(3):172–174.
120. Yanagitani N, Shimizu Y, Kazama T, Dobashi K, Ishizuka T, Mori M. Eosinophilic bronchiolitis indicating eosinophilic airway disease with overexpression of carcinoembryonic antigen in sinus and bronchiole: case report. *J Biol Regul Homeost Agents*. 2010;24(1):99–102.
121. Kawaguchi G, Abe E, Sasamoto R, Sasai K. Elevation of serum carcinoembryonic antigen level in a patient with hypothyroidism after radiation therapy for cervical esophageal cancer. *Int J Clin Oncol*. 2010;15(1):104–108.
122. Hsu TC. Unusual elevation of CEA in a patient with history of colon cancer. *Jpn J Clin Oncol*. 2006;36(12):811–813.
123. Alexander JC, Silverman NA, Chretien PB. Effect of age and cigarette smoking on carcinoembryonic antigen levels. *JAMA*. 1976;235(18):1975–1979.
124. Fuchs C, Krapf F, Kern P, Hoferichter S, Jager W, Kalden JR. CEA-containing immune complexes in sera of patients with colorectal and breast cancer–analysis of complexed immunoglobulin classes. *Cancer Immunol Immunother*. 1988;26(2):180–184.
125. Koprowski H, Herlyn M, Steplewski Z, Sears HF. Specific antigen in serum of patients with colon carcinoma. *Science*. 1981; 212(4490):53–55.
126. Locker GY, Hamilton S, Harris J, et al. ASCO 2006 update of recommendations for the use of tumor markers in gastrointestinal cancer. *J Clin Oncol*. 2006;24(33):5313–5327.
127. Ventrucci M, Pozzato P, Cipolla A, Uomo G. Persistent elevation of serum CA 19–9 with no evidence of malignant disease. *Dig Liver Dis*. 2009;41(5):357–363.
128. Basbug M, Arikanoglu Z, Bulbuller N, et al. Prognostic value of preoperative CEA and CA 19–9 levels in patients with colorectal cancer. *Hepatogastroenterology*. 2011;58(106):400–405.
129. Yang XQ, Chen C, Peng CW, Liu SP, Li Y. Carbohydrate antigen 242 highly consists with carbohydrate antigen 19–9 in diagnosis and prognosis of colorectal cancer: study on 185 cases. *Med Oncol*. 2012;29:1030–1036.
130. Kawamura YJ, Tokumitsu A, Mizokami K, Sasaki J, Tsujinaka S, Konishi F. First alert for recurrence during follow-up after potentially curative resection for colorectal carcinoma: CA 19–9 should be included in surveillance programs. *Clin Colorectal Cancer*. 2010;9(1):48–51.
131. Sasaki A, Kawano K, Inomata M, Shibata K, Matsumoto T, Kitano S. Value of serum carbohydrate antigen 19–9 for predicting extrahepatic metastasis in patients with liver metastasis from colorectal carcinoma. *Hepatogastroenterology*. 2005;52(66): 1814–1819.
132. Takahashi Y, Takeuchi T, Sakamoto J, et al. The usefulness of CEA and/or CA19–9 in monitoring for recurrence in gastric cancer patients: a prospective clinical study. *Gastric Cancer*. 2003;6(3):142–145.
133. Hwang GI, Yoo CH, Sohn BH, et al. Predictive value of preoperative serum CEA, CA19–9 and CA125 levels for peritoneal metastasis in patients with gastric carcinoma. *Cancer Res Treat*. 2004;36(3):178–181.

134. Choi SR, Jang JS, Lee JH, et al. Role of serum tumor markers in monitoring for recurrence of gastric cancer following radical gastrectomy. *Dig Dis Sci*. 2006;51(11):2081–2086.
135. Nakagoe T, Sawai T, Tsuji T, et al. Predictive factors for preoperative serum levels of sialy Lewis(x), sialyl Lewis(a) and sialyl Tn antigens in gastric cancer patients. *Anticancer Res*. 2002;22(1A):451–458.
136. Sheer DG, Schlom J, Cooper HL. Purification and composition of the human tumor-associated glycoprotein (TAG-72) defined by monoclonal antibodies CC49 and B72.3. *Cancer Res*. 1988;48(23):6811–6818.
137. Lopez JB, Royan GP, Lakhwani MN, Mahadaven M, Timor J. CA 72–4 compared with CEA and CA 19–9 as a marker of some gastrointestinal malignancies. *Int J Biol Markers*. 1999;14(3):172–177.
138. Guadagni F, Roselli M, Cosimelli M, et al. CA 72–4 serum marker–a new tool in the management of carcinoma patients. *Cancer Invest*. 1995;13(2):227–238.
139. Carpelan-Holmstrom M, Louhimo J, Stenman UH, Alfthan H, Jarvinen H, Haglund C. Estimating the probability of cancer with several tumor markers in patients with colorectal disease. *Oncology*. 2004;66(4):296–302.
140. Carpelan-Holmstrom M, Louhimo J, Stenman UH, Alfthan H, Jarvinen H, Haglund C. CEA, CA 242, CA 19–9, CA 72–4 and hCGbeta in the diagnosis of recurrent colorectal cancer. *Tumour Biol*. 2004;25(5–6):228–234.
141. Sheng SL, Wang Q, Huang G. Development of time-resolved immunofluorometric assays for CA 72–4 and application in sera of patients with gastric tumors. *Clin Chim Acta*. 2007;380(1–2): 106–111.
142. Mattar R. Alves de Andrade CR, DiFavero GM, Gama-Rodrigues JJ, Laudanna AA. Preoperative serum levels of CA 72–4, CEA, CA 19–9, and alpha-fetoprotein in patients with gastric cancer. *Rev Hosp Clin Fac Med Sao Paulo*. 2002;57(3):89–92.
143. Marrelli D, Pinto E, De Stefano A, Farnetani M, Garosi L, Roviello F. Clinical utility of CEA, CA 19–9, and CA 72–4 in the follow-up of patients with resectable gastric cancer. *Am J Surg*. 2001;181(1):16–19.
144. Zou P, Povoski SP, Hall NC, et al. 124I-HuCC49deltaCH2 for TAG-72 antigen-directed positron emission tomography (PET) imaging of LS174T colon adenocarcinoma tumor implants in xenograft mice: preliminary results. *World J Surg Oncol*. 2010;8:65.
145. Fujimura T, Kinami S, Ninomiya I, et al. Diagnostic laparoscopy, serum CA125, and peritoneal metastasis in gastric cancer. *Endoscopy*. 2002;34(7):569–574.
146. Hall NR, Finan PJ, Stephenson BM, Purves DA, Cooper EH. The role of CA-242 and CEA in surveillance following curative resection for colorectal cancer. *Br J Cancer*. 1994;70(3):549–553.
147. Ran Y, Hu H, Zhou Z, et al. Profiling tumor-associated autoantibodies for the detection of colon cancer. *Clin Cancer Res*. 2008;14(9):2696–2700.
148. Small-Howard AL, Harris H. Advantages of the AMDL-ELISA DR-70 (FDP) assay over carcinoembryonic antigen (CEA) for monitoring colorectal cancer patients. *J Immunoassay Immunochem*. 2010;31(2):131–147.
149. Shimada H, Nabeya Y, Okazumi S, et al. Prediction of survival with squamous cell carcinoma antigen in patients with resectable esophageal squamous cell carcinoma. *Surgery*. 2003;133(5): 486–494.
150. Ikeda K, Terashima M, Ishida K, et al. Clinical significance of serum levels of SCC antigen in patients with esophageal squamous cell carcinoma. *Gan To Kagaku Ryoho*. 1988;15(4 Pt 1):677–682.
151. Wild N, Andres H, Rollinger W, et al. A combination of serum markers for the early detection of colorectal cancer. *Clin Cancer Res*. 2010;16(24):6111–6121.
152. Chen C, Chen LQ, Chen LD, Yang GL, Li Y. Evaluation of tumor markers biochip C12 system in the diagnosis of gastric cancer and the strategies for improvement: analysis of 100 cases. *Hepatogastroenterology*. 2008;55(84):991–997.
153. Ward DG, Suggett N, Cheng Y, et al. Identification of serum biomarkers for colon cancer by proteomic analysis. *Br J Cancer*. 2006;94(12):1898–1905.
154. Qiu FM, Yu JK, Chen YD, Jin QF, Sui MH, Huang J. Mining novel biomarkers for prognosis of gastric cancer with serum proteomics. *J Exp Clin Cancer Res*. 2009;28:126.
155. Lim JY, Cho JY, Paik YH, Chang YS, Kim HG. Diagnostic application of serum proteomic patterns in gastric cancer patients by ProteinChip surface-enhanced laser desorption/ionization time-of-flight mass spectrometry. *Int J Biol Markers*. 2007;22(4):281–286.
156. Ma Y, Peng J, Liu W, et al. Proteomics identification of desmin as a potential oncofetal diagnostic and prognostic biomarker in colorectal cancer. *Mol Cell Proteomics*. 2009;8(8):1878–1890.
157. Chen JS, Chen KT, Fan CW, et al. Comparison of membrane fraction proteomic profiles of normal and cancerous human colorectal tissues with gel-assisted digestion and iTRAQ labeling mass spectrometry. *FEBS J*. 2010;277(14):3028–3038.
158. Liu W, Wang P, Li Z, et al. Evaluation of tumour-associated antigen (TAA) miniarray in immunodiagnosis of colon cancer. *Scand J Immunol*. 2009;69(1):57–63.
159. Tsunemi S, Nakanishi T, Fujita Y, et al. Proteomics-based identification of a tumor-associated antigen and its corresponding autoantibody in gastric cancer. *Oncol Rep*. 2010;23(4):949–956.
160. Kijanka G, Hector S, Kay EW, et al. Human IgG antibody profiles differentiate between symptomatic patients with and without colorectal cancer. *Gut*. 2010;59(1):69–78.
161. American Society of Clinical Oncology. Clinical practice guidelines for the use of tumor markers in breast and colorectal cancer. Adopted on May 17, 1996 by the American Society of Clinical Oncology. *J Clin Oncol*. 1996;14(10):2843–2877.
162. Bast RC Jr, Ravdin P, Hayes DF, et al. 2000 update of recommendations for the use of tumor markers in breast and colorectal cancer: clinical practice guidelines of the American Society of Clinical Oncology. *J Clin Oncol*. 2001;19(6):1865–1878.
163. Desch CE, Benson AB 3rd, Somerfield MR, et al. Colorectal cancer surveillance: 2005 update of an American Society of Clinical Oncology practice guideline. *J Clin Oncol*. 2005;23(33): 8512–8519.
164. Guillem JG, Wood WC, Moley JF, et al. ASCO/SSO review of current role of risk-reducing surgery in common hereditary cancer syndromes. *J Clin Oncol*. 2006;24(28):4642–4660.
165. Klapdor R, Aronsson A, Duffy M, Hansson L, Khalifa R, Lamerz R. Tumour markers in gastrointestinal cancers–EGTM recommendations. European Group on Tumour Markers. *Anticancer Res*. 1999;19(4A):2811–2815.
166. Duffy MJ, van Dalen A, Haglund C, et al. Clinical utility of biochemical markers in colorectal cancer: European Group on Tumour Markers (EGTM) guidelines. *Eur J Cancer*. 2003;39(6):718–727.
167. Duffy MJ, van Dalen A, Haglund C, et al. Tumour markers in colorectal cancer: European Group on Tumour Markers (EGTM) guidelines for clinical use. *Eur J Cancer*. 2007;43(9):1348–1360.
168. Van Cutsem EJ. Advanced colorectal cancer: ESMO clinical recommendations for diagnosis, treatment and follow-up. *Ann Oncol*. 2007;18(Suppl 2):ii25-ii26.
169. Van Cutsem E, Oliveira J. Advanced colorectal cancer: ESMO clinical recommendations for diagnosis, treatment and follow-up. *Ann Oncol*. 2009;20(Suppl 4):61–63.
170. Van Cutsem E, Nordlinger B, Cervantes A. Advanced colorectal cancer: ESMO Clinical Practice Guidelines for treatment. *Ann Oncol*. 2010;21(Suppl 5):v93-v97.
171. Balmana J, Castells A, Cervantes A. Familial colorectal cancer risk: ESMO Clinical Practice Guidelines. *Ann Oncol*. 2010; 21(Suppl 5):v78-v81.

172. Van Cutsem E, Dicato M, Arber N, et al. Molecular markers and biological targeted therapies in metastatic colorectal cancer: expert opinion and recommendations derived from the 11th ESMO/ World Congress on Gastrointestinal Cancer, Barcelona, 2009. *Ann Oncol*. 2010;21(Suppl 6):vi1-vi10.
173. Fleisher M, Dnistrian A, Sturgeon C, Lamerz R, Witliff J. *Practice guidelines and recommendations for use of tumor markers in the clinic. Tumor Markers: physiology, pathobiology, technology and clinical applications*. Washington, DC: AACC Press; 2002:33–63.
174. Chan DW, Booth RA, Diamandis E. Tumor markers. In: Burtis C, Ashwood E, Bruns D, eds. *Tietz textbook of clinical chemistry and molecular diagnostics*. St. Louis, MI: Elsevier; 2006.
175. Tsai WS, Changchien CR, Yeh CY, et al. Preoperative plasma vascular endothelial growth factor but not nitrite is a useful complementary tumor marker in patients with colorectal cancer. *Dis Colon Rectum*. 2006;49(6):883–894.
176. Babel I, Barderas R, Diaz-Uriarte R, Martinez-Torrecuadrada JL, Sanchez-Carbayo M, Casal JI. Identification of tumor-associated autoantigens for the diagnosis of colorectal cancer in serum using high density protein microarrays. *Mol Cell Proteomics*. 2009;8(10):2382–2395.
177. Holten-Andersen MN, Christensen IJ, Nielsen HJ, et al. Total levels of tissue inhibitor of metalloproteinases 1 in plasma yield high diagnostic sensitivity and specificity in patients with colon cancer. *Clin Cancer Res*. 2002;8(1):156–164.
178. Nielsen HJ, Brunner N, Jorgensen LN, et al. Plasma TIMP-1 and CEA in detection of primary colorectal cancer: a prospective, population based study of 4509 high-risk individuals. *Scand J Gastroenterol*. 2011;46:60–90.
179. Huber K, Kirchheimer JC, Sedlmayer A, Bell C, Ermler D, Binder BR. Clinical value of determination of urokinase-type plasminogen activator antigen in plasma for detection of colorectal cancer: comparison with circulating tumor-associated antigens CA 19–9 and carcinoembryonic antigen. *Cancer Res*. 1993;53(8):1788–1793.
180. Cordero OJ, Ayude D, Nogueira M, Rodriguez-Berrocal FJ, de la Cadena MP. Preoperative serum CD26 levels: diagnostic efficiency and predictive value for colorectal cancer. *Br J Cancer*. 2000;83(9):1139–1146.
181. Cordero OJ, Imbernon M, Chiara LD, et al. Potential of soluble CD26 as a serum marker for colorectal cancer detection. *World J Clin Oncol*. 2011;2(6):245–261.
182. Melle C, Ernst G, Schimmel B, et al. Discovery and identification of alpha-defensins as low abundant, tumor-derived serum markers in colorectal cancer. *Gastroenterology*. 2005;129(1):66–73.
183. van den Broek I, Sparidans RW, Engwegen JY, et al. Evaluation of human neutrophil peptide-1, -2 and −3 as serum markers for colorectal cancer. *Cancer Biomark*. 2010;7(2):109–115.
184. Haug U, Rothenbacher D, Wente MN, Seiler CM, Stegmaier C, Brenner H. Tumour M2-PK as a stool marker for colorectal cancer: comparative analysis in a large sample of unselected older adults vs colorectal cancer patients. *Br J Cancer*. 2007;96(9): 1329–1334.
185. Hathurusinghe HR, Goonetilleke KS, Siriwardena AK. Current status of tumor M2 pyruvate kinase (tumor M2-PK) as a biomarker of gastrointestinal malignancy. *Ann Surg Oncol*. 2007;14(10):2714–2720.
186. Fedarko NS, Jain A, Karadag A, Van Eman MR, Fisher LW. Elevated serum bone sialoprotein and osteopontin in colon, breast, prostate, and lung cancer. *Clin Cancer Res*. 2001;7(12):4060–4066.
187. Fernandes LC, Kim SB, Matos D. Cytokeratins and carcinoembryonic antigen in diagnosis, staging and prognosis of colorectal adenocarcinoma. *World J Gastroenterol*. 2005;11(5):645–648.
188. Kawahara M, Chia D, Terasaki PI, et al. Detection of sialylated LewisX antigen in cancer sera using a sandwich radioimmunoassay. *Int J Cancer*. 1985;36(4):421–425.
189. Pasanen P, Eskelinen M, Kulju A, Penttila I, Janatuinen E, Alhava E. Tumour-associated trypsin inhibitor (TATI) in patients with colorectal cancer: a comparison with CEA, CA 50 and CA 242. *Scand J Clin Lab Invest*. 1995;55(2):119–124.
190. Saito N, Kameoka S. Serum laminin is an independent prognostic factor in colorectal cancer. *Int J Colorectal Dis*. 2005;20(3): 238–244.
191. Roessler M, Rollinger W, Palme S, et al. Identification of nicotinamide N-methyltransferase as a novel serum tumor marker for colorectal cancer. *Clin Cancer Res*. 2005;11(18):6550–6557.
192. Albanopoulos K, Armakolas A, Konstadoulakis MM, et al. Prognostic significance of circulating antibodies against carcinoembryonic antigen (anti-CEA) in patients with colon cancer. *Am J Gastroenterol*. 2000;95(4):1056–1061.
193. Mroczko B, Szmitkowski M, Okulczyk B, Piotrowski Z. Granulocyte-macrophage-colony stimulating factor in patients with colorectal cancer. *Folia Histochem Cytobiol*. 2001;39(Suppl 2):110–111.
194. Kerber A, Trojan J, Herrlinger K, Zgouras D, Caspary WF, Braden B. The new DR-70 immunoassay detects cancer of the gastrointestinal tract: a validation study. *Aliment Pharmacol Ther*. 2004;20(9):983–987.
195. Leman ES, Schoen RE, Magheli A, Sokoll LJ, Chan DW, Getzenberg RH. Evaluation of colon cancer-specific antigen 2 as a potential serum marker for colorectal cancer. *Clin Cancer Res*. 2008;14(5):1349–1354.
196. Ferroni P, Roselli M, Martini F, et al. Prognostic value of soluble P-selectin levels in colorectal cancer. *Int J Cancer*. 2004;111(3):404–408.
197. Soroush AR, Zadeh HM, Moemeni M, Shakiba B, Elmi S. Plasma prolactin in patients with colorectal cancer. *BMC Cancer*. 2004; 4:97.
198. Nam MJ, Kee MK, Kuick R, Hanash SM. Identification of defensin alpha6 as a potential biomarker in colon adenocarcinoma. *J Biol Chem*. 2005;280(9):8260–8265.
199. Yoneda K, Iida H, Endo H, et al. Identification of Cystatin SN as a novel tumor marker for colorectal cancer. *Int J Oncol*. 2009;35(1):33–40.
200. Lee H, Rhee H, Kang HJ, et al. Macrophage migration inhibitory factor may be used as an early diagnostic marker in colorectal carcinomas. *Am J Clin Pathol*. 2008;129(5):772–779.
201. Pinczower GD, Gianello RD, Williams RP, Preston BN, Preston H, Linnane AW. Monoclonal antibody 4D3 detects small intestinal mucin antigen (SIMA)–glycoprotein in the serum of patients with colorectal cancer. *Int J Cancer*. 1993;54(3): 391–396.
202. Eskelinen M, Pasanen P, Janatuinen E, Pettersson N, Linnane A, Alhava E. Small intestinal mucin antigen (SIMA); a novel tumour marker in colorectal cancer? *Anticancer Res*. 1995;15(5B): 2351–2356.
203. Mroczko B, Szmitkowski M, Wereszczynska-Siemiatkowska U, Okulczyk B. Stem cell factor (SCF) and interleukin 3 (IL-3) in the sera of patients with colorectal cancer. *Dig Dis Sci*. 2005;50(6):1019–1024.
204. Kuru B, Ozaslan C, Yalman K, Camlybel M. Serum progesterone levels in patients with gastric and colorectal cancers. *Acta Chir Belg*. 2002;102(2):122–125.
205. Tagi T, Matsui T, Kikuchi S, et al. Dermokine as a novel biomarker for early-stage colorectal cancer. *J Gastroenterol*. 2010;45:1201–1211.
206. Xia Q, Kong XT, Zhang GA, Hou XJ, Qiang H, Zhong RQ. Proteomics-based identification of DEAD-box protein 48 as a novel autoantigen, a prospective serum marker for pancreatic cancer. *Biochem Biophys Res Commun*. 2005;330(2):526–532.
207. Reipert BM, Tanneberger S, Pannetta A, et al. Increase in autoantibodies against Fas (CD95) during carcinogenesis in the human

colon: a hope for the immunoprevention of cancer? *Cancer Immunol Immunother*. 2005;54(10):1038–1042.
208. Yamaguchi A, Kurosaka Y, Ishida T, et al. Clinical significance of tumor marker NCC-ST 439 in large bowel cancers. *Dis Colon Rectum*. 1991;34(10):921–924.
209. Kornek G, Depisch D, Temsch EM, Scheithauer W. Comparative analysis of cancer-associated antigen CA-195, CA 19–9 and carcinoembryonic antigen in diagnosis, follow-up and monitoring of response to chemotherapy in patients with gastrointestinal cancer. *J Cancer Res Clin Oncol*. 1991;117(5):493–496.
210. Yedema KA, Kenemans P, Wobbes T, et al. Carcinoma-associated mucin serum markers CA M26 and CA M29: efficacy in detecting and monitoring patients with cancer of the breast, colon, ovary, endometrium and cervix. *Int J Cancer*. 1991;47(2):170–179.
211. Goodgame R, Kiefe C, Rose E, Sutton F, Brown J, Alpert E. Clinical evaluation of M43: a novel cancer-associated mucin epitope. *Cancer Res*. 1993;53(12):2803–2809.
212. van Kamp GJ, von Mensdorff-Pouilly S, Kenemans P, et al. Evaluation of colorectal cancer-associated mucin CA M43 assay in serum. *Clin Chem*. 1993;39(6):1029–1032.
213. Kozwich DL, Kramer LC, Mielicki WP, Fotopoulos SS, Gordon SG. Application of cancer procoagulant as an early detection tumor marker. *Cancer*. 1994;74(4):1367–1376.
214. Herlyn M, Blaszczyk M, Bennicelli J, et al. Selection of monoclonal antibodies detecting serodiagnostic human tumor markers. *J Immunol Methods*. 1985;80(1):107–116.
215. Duraker N, Can D, Parilti M. Measurement of serum total and free prostate-specific antigen in women with colorectal carcinoma. *Br J Cancer*. 2002;86(2):203–206.
216. Severini G. Glutathione S-transferase activity in patients with cancer of the digestive tract. *J Cancer Res Clin Oncol*. 1993;120 (1–2):112–114.
217. Kuroki M, Matsushita H, Matsumoto H, Hirose Y, Senba T, Yamamoto T. Nonspecific cross-reacting antigen-50/90 (NCA-50/90) as a new tumor marker. *Anticancer Res*. 1999;19(6C): 5599–5606.
218. Arai M, Sakamoto K, Otsuka H, Yokoyama Y, Akagi M. Detection of tumor associated antigen, PA8–15, in sera from pancreatic and gastrointestinal carcinoma patients. *Jpn J Clin Oncol*. 1990;20(2):145–153.
219. Riedl S, Bodenmuller H, Hinz U, et al. Significance of tenascin serum level as tumor marker in primary colorectal carcinoma. *Int J Cancer*. 1995;64(1):65–69.
220. Dudouet B, Jacob L, Beuzeboc P, et al. Presence of villin, a tissue-specific cytoskeletal protein, in sera of patients and an initial clinical evaluation of its value for the diagnosis and follow-up of colorectal cancers. *Cancer Res*. 1990;50(2):438–443.
221. Ayude D, Fernandez-Rodriguez J, Rodriguez-Berrocal FJ, et al. Value of the serum alpha-L-fucosidase activity in the diagnosis of colorectal cancer. *Oncology*. 2000;59(4):310–316.
222. Schneider J, Bitterlich N, Schulze G. Improved sensitivity in the diagnosis of gastro-intestinal tumors by fuzzy logic-based tumor marker profiles including the tumor M2-PK. *Anticancer Res*. 2005;25(3A):1507–1515.
223. Mroczko B, Kozlowski M, Groblewska M, et al. The diagnostic value of the measurement of matrix metalloproteinase 9 (MMP-9), squamous cell cancer antigen (SCC) and carcinoembryonic antigen (CEA) in the sera of esophageal cancer patients. *Clin Chim Acta*. 2008;389(1–2):61–66.
224. Kozlowski M, Kowalczuk O, Milewski R, Chyczewski L, Niklinski J, Laudanski J. Serum vascular endothelial growth factors C and D in patients with oesophageal cancer. *Eur J Cardiothorac Surg*. 2010;38(3):260–267.
225. Dong J, Zeng BH, Xu LH, et al. Anti-CDC25B autoantibody predicts poor prognosis in patients with advanced esophageal squamous cell carcinoma. *J Transl Med*. 2010;8:81.
226. Wu CY, Wu MS, Chiang EP, et al. Plasma matrix metalloproteinase-9 level is better than serum matrix metalloproteinase-9 level to predict gastric cancer evolution. *Clin Cancer Res*. 2007;13(7):2054–2060.
227. Mroczko B, Groblewska M, Lukaszewicz-Zajac M, Bandurski R, Kedra B, Szmitkowski M. Pre-treatment serum and plasma levels of matrix metalloproteinase 9 (MMP-9) and tissue inhibitor of matrix metalloproteinases 1 (TIMP-1) in gastric cancer patients. *Clin Chem Lab Med*. 2009;47(9):1133–1139.
228. Wang CS, Wu TL, Tsao KC, Sun CF. Serum TIMP-1 in gastric cancer patients: a potential prognostic biomarker. *Ann Clin Lab Sci*. 2006;36(1):23–30.
229. Jin B, Wang X, Jin Y, et al. Detection of serum gastric cancer-associated MG7-Ag from gastric cancer patients using a sensitive and convenient ELISA method. *Cancer Invest*. 2009;27(2): 227–233.
230. Xia HH, Yang Y, Chu KM, et al. Serum macrophage migration-inhibitory factor as a diagnostic and prognostic biomarker for gastric cancer. *Cancer*. 2009;115(23):5441–5449.
231. Kumar Y, Tapuria N, Kirmani N, Davidson BR. Tumour M2-pyruvate kinase: a gastrointestinal cancer marker. *Eur J Gastroenterol Hepatol*. 2007;19(3):265–276.
232. Thong-Ngam D, Tangkijvanich P, Lerknimitr R, Mahachai V, Theamboonlers A, Poovorawan Y. Diagnostic role of serum interleukin-18 in gastric cancer patients. *World J Gastroenterol*. 2006;12(28):4473–4477.
233. Xu Y, Zhang L, Hu G. Potential application of alternatively glycosylated serum MUC1 and MUC5AC in gastric cancer diagnosis. *Biologicals*. 2009;37(1):18–25.
234. Hao Y, Yu Y, Wang L, et al. IPO-38 is identified as a novel serum biomarker of gastric cancer based on clinical proteomics technology. *J Proteome Res*. 2008;7(9):3668–3677.
235. Chung HW, Kim JW, Lee JH, et al. Comparison of the validity of three biomarkers for gastric cancer screening: carcinoembryonic antigen, pepsinogens, and high sensitive C-reactive protein. *J Clin Gastroenterol*. 2009;43(1):19–26.
236. Chong PK, Lee H, Zhou J, et al. ITIH3 is a potential biomarker for early detection of gastric cancer. *J Proteome Res*. 2010;9(7): 3671–3679.
237. Ashizawa T, Okada R, Suzuki Y, et al. Clinical significance of interleukin-6 (IL-6) in the spread of gastric cancer: role of IL-6 as a prognostic factor. *Gastric Cancer*. 2005;8(2):124–131.
238. Oue N, Sentani K, Noguchi T, et al. Serum olfactomedin 4 (GW112, hGC-1) in combination with Reg IV is a highly sensitive biomarker for gastric cancer patients. *Int J Cancer*. 2009;125(10):2383–2392.
239. Chan AO, Chu KM, Lam SK, et al. Early prediction of tumor recurrence after curative resection of gastric carcinoma by measuring soluble E-cadherin. *Cancer*. 2005;104(4):740–746.
240. Kammerer R, Zimmermann W. Coevolution of activating and inhibitory receptors within mammalian carcinoembryonic antigen families. *BMC Biol*. 2012;8:12.