# National Academy of Clinical Biochemistry Guidelines for the Use of Tumor Markers in Colorectal Cancer

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**Abbreviations:** CRC, colorectal cancer; FOBT, fecal-occult-blood testing; TIMP-1, tissue inhibitor of metalloproteinases type 1; CEA, carcinoembryonic antigen; HNPCC, hereditary non-polyposis colon cancer; APC, adenomatous polyposis coli; EGTM, European Group on Tumor Markers; ASCO, American Society of Clinical Oncology; NCCN, National Cancer Center Network.

**INTRODUCTION**

Colorectal cancer (CRC) is the third most common cancer, worldwide with an estimated one million new cases and half a million deaths each year (1). In the USA, it is also the third most common malignant disease with an estimated 145,290 new cases diagnosed in 2005 (2). Most CRC are detected in the rectum (38%), followed by sigmoid (29%), cecum (15%), transverse colon and flexures (10%). Only approximately 5% are found in the ascending colon and 3% in the descending colon (3).

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

Patient stage at initial diagnosis is the most widely used prognostic indicator for patients with CRC. Although the original Dukes' staging system has been modified several times, the extent of cancer invasion through the bowel wall and extent of regional lymph node invasion is still the mainstay of staging systems. In practice, the most widely used staging system is the TNM system of the International Union Against Cancer (UICC) (4) and the American Joint Committee on Cancer (5). In the TNM system, "T" refers to the local extent of the untreated primary tumor at the time of initial diagnosis. The designation "N" refers to the status of the regional lymph nodes and "M" refers to the presence of distant metastasis at initial presentation (6).

Although surgery is the first-line treatment for most patients with CRC, some patients with rectal cancer may receive radiation and/or chemotherapy prior to surgery. In 1990, a National Institute of Health (NIH) Consensus Conference recommended that Stage III colon cancer patients should be treated with adjuvant chemotherapy (7). A subsequent pooled analysis of patients with Stage III CRC confirmed that adjuvant chemotherapy increased both the probability of remaining free of tumor recurrence after 5 years and the probability of surviving for 5-years (8).

The value of adjuvant chemotherapy following resection of Stage II (Dukes' B) colon cancer is however, unclear. In 2004, an American Society of Clinical Oncology (ASCO) Expert Panel recommended that adjuvant chemotherapy should not, in general, be given to patients with Stage II colon cancer (9). However, the Panel also stated that "there are populations of patients with Stage II disease that could be considered for adjuvant treatment including patients with inadequately sampled nodes, T4 lesions, perforation or poorly differentiated histology" (9).

The 1990 NIH Consensus Conference recommended combined adjuvant chemotherapy and high dose external-beam radiotherapy for patients with Stage II or III rectal cancer (7).

Although radiation therapy does not appear to affect overall survival, it decreases local recurrence which is a cause of considerable morbidity in patients with rectal cancer.

Despite potentially curative surgery, 40-50% of patients with CRC develop recurrent or metastatic disease (10). In an attempt to detect these relapses at a resectable stage, most patients with either Stage II or Stage III disease currently undergo follow-up or surveillance. Surveillance strategies may include one or more of the following, clinical examination, radiology (e.g. chest X-ray, ultrasound, computed tomography (CT), and magnetic resonance imaging), endoscopy, clinical chemistry testing and the use of tumor markers.

CRC was one of the first cancers in which a tumor marker, i.e., carcinoembryonic antigen (CEA), was used to aid management. The aim of this article is to present NACB guidelines on the use of CEA as well as other markers in the detection and management of patients with CRC. In doing so, we also summarize the guidelines form other Expert Panels on the use of tumor markers in CRC.

## CURRENTLY AVAILABLE MARKERS FOR COLORECTAL CANCER

Table 1 lists the most widely investigated tumor markers for colorectal cancer. Also listed is the phase of development of each marker and the level of evidence (LOE) for its clinical use. The levels of evidence grading system used is based on that described by Hayes et al (11).

## TUMOR MARKERS IN COLORECTAL CANCER: NACB RECOMMENDATIONS

Table 2 presents a summary of recommendations from representative guidelines published on the use of tumor markers in colorectal cancer. This table also summarises the National Academy of Clinical Biochemistry (NACB) guidelines for the use of markers in this malignancy. Below, we present a more detailed discussion of the most widely investigated markers listed in Table 2.

## CARCINOEMBRYONIC ANTIGEN (CEA)

### CEA in screening

Lack of sensitivity and specificity when combined with the low prevalence of CRC in asymptomatic populations preclude the use of CEA in screening for CRC (12-14). In agreement with ASCO (15,16) and European Group on Tumor Markers (EGTM) recommendations (17,18), the NACB Panel states that CEA cannot be used in screening healthy subjects for early CRC.

**CEA in determining prognosis**

As mentioned above, patient stage at initial diagnosis is universally used to determine prognosis in patients with CRC. Several studies, however, have demonstrated that preoperative concentrations of CEA can also provide prognostic information which in some situations was found to be independent of stage (12-14,19). Indeed, in some studies CEA was found to be prognostic in patients with Stage II disease (12-14). Preoperative concentrations of CEA might thus be combined with other factors in order to identify those Stage II colonic cancer patients who are candidates for adjuvant chemotherapy. There is however, no evidence at present for a beneficial effect of adjuvant chemotherapy in either Stage II patients, as a whole, or in those with Stage II disease and high preoperative serum CEA concentrations.

In 2000, a College of American Pathologists Expert ranked preoperative serum CEA together with TNM stage, regional lymph node metastasis, blood or lymphatic vessel invasion and residual tumor following surgery with curative intent as a category I prognostic marker for colorectal cancer (20). According to this group, category I prognostic factors are those “definitely proven to be of prognostic importance based on evidence from multiple statistically robust published trials and generally used in patient management”.

The 1996 and 2000 American Society of Clinical Oncology guidelines recommended that preoperative CEA concentrations should be measured “if it could assist in staging and surgical treatment planning” (15,16). More recently, the EGTM guidelines stated that “preoperative measurement of CEA is desirable as it may give independent prognostic information, help with surgical management and provide a baseline level for subsequent determinations” (17,18). The EGTM also stated that patients entering prospective randomized trials aimed at evaluating adjuvant chemotherapy for Stage II colon cancer patients should be selected or stratified according to their preoperative CEA level (18).

In agreement with other Expert Panels (15-18), the NACB Panel states that preoperative CEA levels should be measured in newly diagnosed CRC patients. CEA levels may be combined with histopathological parameters in order to determine which patients with Stage II colon cancer should receive adjuvant chemotherapy. However, as mentioned above, there is currently no evidence that Stage II colon cancer patients with elevated concentrations benefit from adjuvant chemotherapy. A further reason for measuring preoperative CEA is that it provides a baseline concentration for subsequent patient management (18) (see below).

**CEA in post-operative surveillance**

The main aims of surveillance following curative resection of CRC are to provide reassurance, address possible complications due to therapy and identify resectable recurrences or metastases. Five separate meta-analyses have compared outcome in patients with intensive

follow-up versus those with minimal or no follow-up (21-25). All concluded that the use of an intensive follow-up regime resulted in a modest but statistically significant better outcome than those with minimal follow-up. In one of these meta-analyses, it was shown that only the studies using CEA demonstrated a significant impact on survival (24).

The updated (i.e., 2005) ASCO guidelines stated that CEA should be measured every 3 months in patients with Stage II or III CRC for at least 3 years after diagnosis, if the patient is a candidate for surgery or systemic therapy (26). The NACB Panel therefore supports this recommendation.

Although serial measurements of CEA are widely used in surveillance, no agreement exists as to the magnitude of concentration change that constitutes a clinically significant increase in CEA during serial monitoring. According to the EGTM Panel, a significant increase in CEA occurs if the elevation is at least 30% over that of the previous value. This increase, however, must be confirmed by a second sample taken within one month. If this latter sample is also elevated, the patient should undergo further investigations (18). This definition however, has not been clinically validated. Furthermore, it should not be regarded as exclusive. For example, small increases, e.g. of 15-20%, maintained over at least three successive assays, may also prompt intervention (18). It should also be remembered that low concentrations of CEA concentrations do not necessarily exclude progression, and in patients with clinical symptoms of disease recurrence, additional tests such as CT-scan, x-rays, and colonoscopy are required, irrespective of the CEA concentration (18).

**CEA in monitoring therapy in advanced disease**

The prognosis for patients with advanced colorectal cancer has greatly improved in recent years due to the introduction of new cytotoxic agents such as irinotecan and oxaliplatin and monoclonal antibodies such as bevacuzimab (Avastin$^{R}$) and cetuximab (Erlotinib$^{R}$) (for review, see refs. 27,28). Indeed, the median survival for patients with metastatic colorectal cancer has almost doubled in the past 10 years as a result of these new treatments (27,28). As these treatments are potentially toxic as well as expensive, it is important to establish as quickly as possible that they are effective in halting tumor progression.

According to the 1996 and 2000 ASCO guidelines, CEA should be measured at the start of treatment for metastatic disease and every 2 to 3 months during active treatment, if no other simple test is available to indicate a response (15,16). Two values above the baseline were regarded as adequate to document progressive disease even in the absence of corroborating evidence (15,16). In 2003, the EGTM Panel recommend that serial CEA concentrations should be measured every 2 to 3 months while patient are receiving systemic therapy (18). It was however

pointed out that certain treatments (5-fluorouracil and levamisole) could cause transient elevations in CEA levels in the absence of disease progression (18).

For monitoring patients with advanced colorectal cancer undergoing systemic therapy, the NACB Panel recommends that regular CEA determinations should be carried out. In agreement with the ASCO Panel (15,16), we state that a confirmed CEA increase (eg., > 30%) should be regarded as evidence of progressive disease. Of course, it should be established that the increases are not false-positive elevations due to either chemotherapy-mediated release of marker or the development of a benign disease that produces CEA.

## OTHER SERUM MARKERS

### CA 19-9

The CA 19-9 assay detects a mucin containing the sialated Lewis-a pentasacharide epitope, fucopentaose II (for review, see ref. 29). CA 19-9 is a less sensitive marker than CEA for CRC (30,31). Preliminary findings suggest that like CEA, preoperative concentrations of CA 19-9 are also prognostic in patients with CRC (32-37). Based on available data, routine measurement of CA 19-9 cannot be recommended for patients with CRC.

### CA 242

The CA 242 assay also detects a mucin-like molecule. Although less sensitive than CEA for CRC, assay of CA 242 may complement CEA in the surveillance of patients with CRC (31,38). Furthermore, a number of preliminary reports suggest that preoperative concentrations of CA 242 are prognostic in CRC (39,40). Routine determinations of CA 242 should not be used, at present, in patients with CRC.

### Tissue inhibitor of metalloproteinases type 1 (TIMP-1)

Tissue inhibitor of metalloproteinases type 1, also known as TIMP-1, is a 25 kDa glycoprotein with multiple activities such as inhibition of matrix metalloproteinases, promotion of cell proliferation and inhibition of apoptosis. Using a research ELISA which detects total TIMP-1 (i.e., the non-complex form as well as TIMP-1 complexed to matrix metalloproteinases), plasma concentrations of the inhibitor were found to be significantly higher in patients with CRC than in healthy controls, subjects with inflammatory bowel diseases, subjects with adenomas or patients with breast cancer (41,42). For patients with Dukes' A and B colon cancers, TIMP-1 appeared to be more sensitive than CEA, i.e., 58% vs 40% at 95% specificity and 56% vs 30% at 98% specificity in the detection of the cancer. For patients with early rectal cancer, TIMP-1 and CEA had similar sensitivity (41). Other studies have shown that preoperative plasma TIMP-1 concentration is an independent prognostic factor in patients with CRC, i.e., independent of Dukes' stage and tumor location (43,44). Of particular note was the finding that Stage II patients with low plasma TIMP-1

concentrations (dichotomized at the 70% percentile) exhibited a survival pattern similar to an age and gender-matched background population.

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

**TISSUE MARKERS**

Several tumor tissue markers have been evaluated for potential prognostic and predictive value in patients with CRC. These include thymidylate synthase (TS) (45-50), microsatellite stability (MS) (51-55), deleted in colon cancer (DCC) (56,58), urokinase plasminogen activator/PAI-1 (59-61), mutant ras (62), mutant/overexpression of p53 (63). Based on available evidence, none of these markers can presently be recommended for routine clinical use.

**FECAL MARKERS**

The most widely used fecal marker involves testing for occult blood, i.e., the fecal occult blood test (FOBT). FOBTs can be of 2 main types, i.e., the guaiac test and the immunochemical test. The guaiac test measures the presence of haem in haemoglobin while the immunochemical test detects human globin. As haem is also present in certain fruits and vegetables, intake of these foods may give rise to false-positive results in the guaiac test. Certain medicines such as non-steroidal anti-inflammatory drugs can also interfere with the test. In contrast, the immunochemical test is not affected by these factors. As well as being subjected to less interference, the immunochemical test exhibits superior sensitivity and specificity compared to the guaiac test (64). Despite these limitations, a number of large randomized trials have shown that screening with the guaiac test reduced mortality from CRC (65-69).

Although several guaiac tests exist, only 2 have been evaluated in large-scale screening, i.e., the Haemoccult II and the Haemoccult Sensa. The efficacy of the immunochemical test in reducing either the incidence or mortality form CRC has not yet been investigated in large population-based studies. However, based on available evidence, it should be at least as accurate if not more accurate than FOBT in screening for CRC (70)

In agreement with other Expert Panels (71-73), NACB recommends that all subjects 50 years or older should undergo screening for CRC. Multiple screening procedures for CRC exist however (69-71), and to-date no one procedure has been shown to be significantly superior to the others. The option chosen may therefore depend on availability, personal preference and risk of developing CRC (72).

According to the National Comprehensive Cancer Network (NCCN), FOBT should be performed on 3 successive stools specimens that are obtained while the patient adheres to a prescribed diet (71). This organization specifically recommends the Haemoccult SENSA as the testing method. Both the NCCN and the American Cancer Society recommend against use of FOBT of a specimen obtained at digital rectal examinations (71,72).

Because of the lack of sensitivity and specificity of FOBT for adenomas and early CRC, a considerable amount of research in recent years has focused on other fecal markers, especially on the genes that undergo mutation during CRC carcinogenesis. Amongst the most widely investigated DNA markers are mutant ras, mutant p53, mutant APC, specific methylated genes, MSI and long DNA (74-78).

A fecal DNA panel was recently investigated as a screening test for CRC in a large asymptomatic population. Of the 31 invasive CRCs detected, the DNA panel diagnosed sixteen, whereas FOBT detected only four ($p = 0.003$). Of the 71 invasive cancers and adenomas with high-grade dysplasia, the DNA panel diagnosed 29, while FOBT detected only ten ($p < 0.001$). Although the DNA panel displayed a higher sensitivity than FOBT, clearly neither test detected the majority of advanced adenomas or carcinomas (79). As there are no studies to-date showing that screening with fecal DNA markers reduces mortality from CRC, this test cannot be recommended, at present, to screen for CRC.

**GENETIC TESTS**

For genetic testing for CRC susceptibility, i.e., familial adenomatous polyposis and hereditary non-polyposis colorectal cancer, the NACB Panel supports the guidelines of the American Gastroenterology Association (80,81), and the National Cancer Center Network (71).

**CONCLUSIONS**

Although many different markers have been evaluated for CRC, only a small number can be recommended for clinical use. These include CEA in the postoperative surveillance of patients that may be suitable candidate for either surgical resection or systemic chemotherapy, FOBT in screening for early CRC, MSI as a surrogate marker for identifying subjects who should undergo genetic testing for HNPCC, MLH1/MSH2/MSH6 in testing for HNPCC and APC in testing for FAP. One of the most promising new plasma markers is TIMP-1. As mentioned above, preliminary findings suggest that this marker may be more sensitive than CEA in detecting early CRC as well as being an independent prognostic factor for CRC. These findings now need to be confirmed in large prospective studies. One of the most promising fecal CRC screening tests is the fecal DNA

panel (74-79). This test should be simplified and made available at reduced costs. Its potential to reduce mortality from CRC should be evaluated in a large prospective randomized trial.

**Table 1. Currently available markers for colorectal cancer.**

| Cancer Marker | Proposed Use/Uses | Phase of Development | LOE | Ref |
|---|---|---|---|---|
| *Blood-Based Markers* | | | | |
| CEA | Determining prognosis | Preoperative levels may provide prognostic information but this is rarely used for clinical purposes | III | 12-14 |
| | Surveillance following curative resection | In clinical use, usually in combination with radiology and clinical history | I | 21-25 |
| | Monitoring therapy in advanced disease | In clinical use, usually in combination with radiology and clinical history | III | 12-14 |
| CA19.9 | Determining prognosis | Undergoing evaluation | III | 32-38 |
| | Surveillance following curative resection and monitoring therapy in advanced disease | Undergoing evaluation | IV | 30,31 |
| CA 242 | Determining prognosis | Undergoing evaluation | III | 39,40 |
| TIMP-1 | Determining prognosis<br>Screening high risk populations | Undergoing evaluation | III<br>III | 43,44<br>41 |
| *Tissue-Based Markers* | | | | |
| TS | Determining prognosis | Undergoing evaluation, a meta-analysis suggested that high levels of TS predicted poor outcome (48). Assay not standardized | I | 45-49 |
| | Predicting response to chemotherapy (5-FU) in advanced disease | Undergoing evaluation. High levels may predict lack of response to 5-FU in advanced disease. Some studies suggest that TS should be determined on metastatic site to be treated | III | 45-50 |
| MSI | Determining prognosis | Undergoing evaluation, a pooled analysis showed that MSI-tumors were associated with a 15% better prognosis compared with MS-stable tumors (55). | I | 51-54 |
| | Predicting response to chemotherapy | Results conflicting, undergoing further evaluation | III | 55,55 |
| DCC/18q phenotype | Determining prognosis | Undergoing evaluation, prognostic value validated in a meta-analysis. Assay not standardized. | I | 56-58 |

| | | | | |
|---|---|---|---|---|
| uPA/PAI-1 | Determining prognosis | Undergoing evaluation | III | 59-61 |
| Ras | Determining prognosis | A pooled analysis showed that a mutant ras gene was weakly prognostic in Dukes' C but not in Dukes' B disease. Unlikely to be used for clinical purposes | I | 62 |
| P53 | Determining prognosis | A meta-analysis showed that abnormal p53 was weakly associated with poor outcome. Unlikely to be used for clinical purposes | I | 63 |
| | | | | |
| *Fecal Markers* | | | | |
| FOBT | Screening asymptomatic populations | Shown in randomized trials that screening with FOBT reduced mortality from CRC. Used for ad hoc CRC screening. Feasibility screening trials underway in a number of countries. Lacks sensitivity for early CRC and advanced adenomas and gives rise to many false-positive results | I | 64-68 |
| DNA Panel | Screening asymptomatic populations | A large study on asymptomatic subjects showed that a DNA panel was more sensitive than FOBT for detecting both advanced adenomas and invasive CRC. Undergoing further evaluation (77) | III | 72-77 |
| *Genetic Markers* | | | | |
| APC | For identifying subjects at high risk of developing FAP | In clinical use in specialised centers | Expert opinion | 80-82 |
| MSI | Prescreen for HNPCC | In clinical use in specialised centers | Expert opinion | 80-82 |
| MLH1/MSH2/ MSH6/PMS2 | For identifying subjects at high risk of developing HNPCC | In clinical use in specialised centers | Expert opinion | 80-82 |

LOE, level of evidence (as defined in ref. 11); TIMP-1, tissue inhibitor of metalloproteinase type 1; TS, thymidylate synthase: uPA, urokinase plasminogen activator; MSI, microsatellite instability: uPA, urokinase plasminogen activator; PAI, plasminogen activator inhibitor 1; 5-FU, 5-fluorouracil; DCC, deleted in colon cancer; FOBT, fecal occult blood testing; FAP, familial adenomatous polyposis; HNPCC, hereditary non-polyposis colorectal cancer and CRC, colorectal cancer.

**Table 2. Recommendations for use of markers in colorectal cancer by different Expert Groups.**

| Marker | Application | ASCO (15,16,26,81) | EGTM (17,18) | NACB 2002 (83) | ESMO (84,85) | NCCN (71,86) | ACS (72) | USPSTF (73) | NACB 2007 |
|---|---|---|---|---|---|---|---|---|---|
| CEA | Screening | **No** | **No** | **No** | None published | None published | None published | None published | **No** |
| | Determining prognosis | **Yes**, if it could assist in staging and surgical treatment planning (15,16) | **Yes** | None published | **Yes** | **Yes**, as part of a complete staging work-up | None published | None published | May be combined with other prognostic factors, especially in patients with Stage II disease |
| | Post-operative surveillance | **Yes**, if patient is a candidate for surgery or systemic therapy (26) | **Yes**, for the early detection of liver metastasis | **Yes**, if resection of liver metastasis would be clinically indicated | **Yes** | **Yes**, if the patient is a candidate for aggressive surgical resection, should recurrence be detected | None published | None published | **Yes**, if patients is a suitable candidate for undergoing liver resection or receiving systemic chemotherapy |
| | Monitoring advanced disease | **Yes** if no other simple test is available (15,16) | **Yes** | **Yes**, especially in metastasis difficult to measure by other means | NR | NR | None published | None published | **Yes**, especially for disease that cannot be evaluated by other modalities |
| APC gene | Screening for FAP | See ASCO general guidelines for genetic testing for cancer susceptibility (81) | None published | None published | **Yes** | **Yes** | None published | None published | **Yes** |
| MSI | Initial screening test | None published | None published | None published | None published | **Yes** | None published | None published | **Yes** |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | for HNPCC | | | | | | | | |
| MMR genes, e.g, MLH1, MSH2, MSH6, PMS2 | Screening for HNPCC | See general guidelines for genetic testing for cancer susceptibility (81) | None published | None published | **Yes** | **Yes** | None published | None published | **Yes** |
| FOBT | Screening asymptomatic subjects | None published | None published | None published | None published | None published | **Yes**, for subjects ≥ 50 yr | **Yes**, for subjects ≥ 50 yr | **Yes**, for subjects ≥ 50 yr |

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

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