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Position Paper

Clinical utility of biochemical markers in colorectal cancer: European Group on Tumour Markers (EGTM) guidelines

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Abstract

In recent years, numerous serum and cell/tissue-based markers have been described for colorectal cancer (CRC). The aim of this article was to provide guidelines for the routine clinical use of some of these markers. Lack of sensitivity and specificity preclude the use of any available serum markers such as carcinoembryonic antigen (CEA), CA 19-9, CA 242, CA 72-4, tissue polypeptide antigen (TPA) or tissue polypeptide-specific antigen (TPS) for the early detection of CRC. However, preoperative measurement of CEA is desirable as this may give independent prognostic information, help with surgical management and provide a baseline level for subsequent determinations. For patients with stage 2 (Dukes' B) and 3 (Dukes' C) disease who may be candidates for liver resection, CEA levels should be measured every 2-3 months for at least 3 years after diagnosis. For monitoring treatment of advanced disease, CEA should also be tested every 2-3 months. Insufficient evidence is presently available to recommend the routine use of other serum markers for monitoring purposes. Similarly, the new cell and tissue-based markers (e.g, *ras*, *P53*) cannot yet be recommended for routine clinical use.

Keywords: Colorectal cancer, CEA; Guidelines; Tumour markers; EGTM

1. Introduction

Biochemical markers for colorectal cancer (CRC) are potentially useful in screening for early disease, aiding diagnosis, determining prognosis, predicting likely response to specific therapies, surveillance of patients undergoing curative resection and monitoring the treatment of advanced disease. Although multiple markers have been described for CRC, confusion remains about how best to use these factors.

The European Group on Tumour Markers (EGTM) is an *ad hoc* group of scientists and physicians from universities, hospitals and the diagnostics industry with an interest in tumour markers [1]. The group was founded in 1997, one of its aims being the development of guidelines for the use of tumour markers. Adherence to these guidelines is of course voluntary since the ultimate decision regarding marker use must be made by the treating clinician. It is also important to note that the present guidelines are intended for routine clinical use and do not necessarily apply to clinical trials, which may have a different remit.

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In 1999, the Gastrointestinal Focus Group of the EGTM published a review on the use of markers in colorectal, stomach, oesophageal, pancreatic and liver cancers [2]. The aim of this paper was to present guidelines for the clinical use of markers in CRC, focusing primarily on carcinoembryonic antigen (CEA), the most widely used marker for this malignancy.

2. CEA

2.1. Structure and function

CEA is a high molecular weight glycoprotein belonging to the immunoglobulin superfamily of molecules (for review, see Refs. 3, 4). The molecule consists of a series of Ig-like domains including a 108 amino acid variable or v region at the amino terminus, and 3 pairs of constant-2 (C-2)-like domains, each containing 178 amino acids. The carboxy-terminal contains a hydrophobic region that is modified to provide a glycosyl phosphatidylinositol link to the cell membrane.

CEA has been postulated to play a role in a number of biological processes including cell adhesion, immunity and apoptosis (for review, see Refs. 3, 4). Because of its involvement in both homophilic and heterophilic adhesion, CEA has been implicated in cancer metastasis [3]. Evidence for this is derived from experiments showing that injection of CEA into mice enhanced experimental metastasis. Other work has shown enhanced metastatic potential following the transfection of colorectal cancer cells with cDNA for CEA [3].

2.2. CEA as a marker for colorectal cancer

2.2.1. Screening

CRC is the second most common malignancy of both males and females in developed countries [5]. Because of its high prevalence, its long asymptomatic phase and the presence of premalignant lesions, CRC meets many of the criteria for screening. The most widely used screening procedures for this malignancy are faecal occult blood (FOB) testing, flexible sigmoidoscopy and colonoscopy [6]. A number of randomised controlled trials have now provided convincing evidence that screening with either FOB or sigmoidoscopy can reduce mortality from CRC (for review, see Ref. 7). However, both these approaches are limited by poor rates of compliance and are less sensitive than colonoscopy [7].

Clearly, a serum tumour marker which has acceptable sensitivity and specificity would be more suitable for screening than either FOB testing or endoscopy.

The original study describing the measurement of CEA in serum found that the marker was elevated in almost all patients with CRC, but rarely in healthy subjects [8]. Subsequent reports, however, failed to confirm these early findings [2,4,9]. For example, using a cut-off point of 2.5 μg/l, the sensitivity of CEA for early CRC (i.e., Dukes' A and B disease) was only 30–40% (9). At this cut-off point, the specificity using healthy subjects was reported to be 87% [9]. Based on a prevalence of 1 in 1000 cases of CRC in a healthy population, a sensitivity of 40% (i.e., for Dukes' A and B disease) and a specificity of 90%, Fletcher [9] calculated that there would be 250 false-positive tests for every patient with cancer. Furthermore, 60% of the cancers would not be detected. In accordance with the conclusions of both a National Institute of Health (NIH) Consensus Conference [10] and an American Society of Clinical Oncology (ASCO) Expert Panel [11], the EGTM Panel recommends that CEA should not be used to screen for early CRC.

2.2.2. Diagnostic aid

As with screening, inadequate sensitivity severely limits the value of CEA for the diagnosis of early or low-stage CRC. In addition, as CEA can be elevated in the absence of malignancy, (e.g., in patients with benign liver disease and in subjects who smoke cigarettes [4,9]), specificity is also impaired. However, in patients with appropriate symptoms, a high serum CEA (e.g., >5 times the upper limit of normal) is highly suggestive of an adenocarcinoma [10]. In this situation, further testing is necessary to confirm the presence of malignancy and locate the disease site. Although preoperative determinations of CEA are usually of little diagnostic value, the EGTM Panel recommends that the marker should be assayed at this point in patient management, i.e., in order to establish a baseline value and for assessing prognosis (see below).

2.2.3. Prognosis

The gold standard for determining prognosis in patients with CRC is the extent of disease as defined by the Dukes', TNM or other staging systems. If a marker such as CEA is to be used for predicting outcome, it must [4]:

- Provide stronger prognostic information than that offered by existing staging systems,
- Provide information independent of the existing systems or
- Provide prognostic data within specific subgroups defined by existing criteria, e.g., in Dukes' B or node-negative patients.

Several small-scale retrospective studies have demonstrated that CRC patients with high preoperative levels of CEA have a worse prognosis than those with low levels (for reviews, see Refs. 12, 13). Furthermore, in many of these reports, the prognostic impact of CEA was found to be independent of the traditional staging

systems [12,13]. Preoperative CEA thus has the potential to supply independent prognostic data in patients with CRC.

While the traditional staging systems are highly predictive of outcome at the extremes, e.g., in Dukes' A and D disease, they are less informative for the intermediate groups, i.e., Dukes' B and C disease. Although the prognosis of patients with Dukes' C disease is variable, a NIH Consensus Statement recommended that "barring mitigating factors, all stage 3 (Dukes' C) colon cancer patients who have been resected for cure should receive adjuvant chemotherapy" [14]. For stages 2 and 3 rectal cancer patients, adjuvant therapy should be radiation as well as chemotherapy [14].

For patients with stage 2 colon cancer, the role of adjuvant chemotherapy is less clear. While 40-50% of these patients have aggressive disease and thus might benefit from adjuvant therapy, there is no consistent evidence that chemotherapy enhances outcome for all patients within this subgroup. What is required is a marker able to differentiate patients with aggressive from those with indolent disease. The former group could then receive adjuvant chemotherapy while patients effectively cured by surgery could avoid the costs and the side-effects of this treatment.

Although mostly retrospective in design and heterogeneous with respect to numbers of patients studied and the length of follow-up, the majority of published reports conclude that CEA measurements provide prognostic information in Dukes' B or equivalent stage patients (Table 1) [4]. Importantly, 2 prospective studies have confirmed a prognostic value for CEA in this subgroup of patients [15,16]. In one of these involving over 500 node-negative patients, CEA provided prognostic data that was independent of age, site of tumour, lymphatic vessel invasion, tumour size and tumour grade [16]. Serum CEA levels may thus identify a subgroup of node-negative or Dukes' B colon cancer patients that have a poor prognosis and therefore define a subset of patients that could benefit from adjuvant chemotherapy. It is important to state that there are no studies at present showing a benefit from adjuvant chemotherapy where patients have been selected for treatment based on a high CEA level. The EGTM Panel therefore recommends that patients entering prospective randomised trials aimed at evaluating adjuvant chemotherapy for Dukes' B colon cancer patients should be selected or stratified according to their preoperative CEA level.

Recently, an American Joint Committee on Cancer (AJCC) Consensus Conference suggested that CEA should be added to the TNM staging system for colorectal cancer [17]. The CEA level should be designated as follows: CX, CEA cannot be assessed; CO, CEA not elevated (<5 μg/l) or CEA1, CEA elevated (>5 μg/l). It should be pointed out that these suggestions were for the purpose of discussion only and are not yet formal proposals [17].

A College of American Pathologists Expert Groups has ranked preoperative serum CEA level as a category I prognostic marker for colorectal cancer [18]. Category I factors include those "definitely proven to be of prognostic import based on evidence from multiple statistically robust published trials and generally used in patient management". In addition, included in the Category I group were the local extent of tumour assessed pathologically (i.e., TNM staging), regional lymph node metastasis, blood or lymphatic vessel invasion and residual tumour following surgery with curative intent [18].

Since preoperative CEA levels may provide independent prognostic information and may also help with surgical management, the EGTM Panel recommends its measurement. The serum CEA level at this stage of patient management may also provide a baseline level that may be of value in the interpretation of subsequent serial results. CEA alone, however, should not be used to determine which patients should receive adjuvant chemotherapy.

Table 1
Studies evaluating preoperative serum carcinoembryonic antigen (CEA) as a prognostic marker in low risk (Dukes' B or node-negative) colorectal cancer patients

Authors	No of patients	*P* value	Type of study
Wanebo and colleagues	50	<0.02	R
Blake and colleagues	30	<0.001	R
Moertel and colleagues[a]	162	NS	R
Chu and colleagues	126	0.03	R
Carpelan-Holmstrom and colleagues	100	NS	R
Harrison and colleagues[b]	572	0.001	P
Carriquiry and colleagues	57	0.03	P

NS, non significant: R, retrospective study: P, prospective study.

[a] Analysis confined to patients with Dukes' B2 disease only, i.e., tumours invading into or through the serosa or perirectal fat. In this study, only multivariate analysis was used.

[b] Prognostic value confirmed using both univariate and multivariate analyses. Reproduced from Clinical Chemistry with permission (Ref. 4).

Postoperative CEA levels also provide prognostic information in patients with CRC. An elevated preoperative concentration should return to normal within approximately 6 weeks following complete surgical resection of a CRC [19]. Failure to do so suggests residual or metastatic disease, especially if other factors likely to give rise to an elevated value (e.g., benign liver disease) can be excluded.

2.2.4. Surveillance

Approximately 50% of patients who undergo surgical resection aimed at cure, later develop recurrent or metastatic disease [20]. Most of these relapses occur within the first 2-3 years of diagnosis and are usually confined to the liver, lungs or locoregional areas. Since recurrent/metastatic disease is invariably fatal, considerable research has focused on its identification at an early and thus potentially treatable stage. Consequently, many of these patients undergo a postoperative surveillance programme which frequently includes regular monitoring with CEA.

Serial monitoring with CEA has been shown to detect recurrent/metastatic disease with a sensitivity of approximately of 80%, specificity of approximately 70% and provides an average lead-time of 5 months (for review, see Refs. 4, 9). Furthermore, CEA was the most frequent indicator of recurrence in asymptomatic patients [21,22] and was more cost-effective than radiology for the detection of potentially curable recurrent disease [23]. As regards sites of recurrence/metastasis, CEA was most sensitive (almost 100%) for the detection of liver metastasis [22,24]. On the other hand, CEA was less reliable for diagnosing locoregional recurrences, the sensitivity being only approximately 60% [24].

An unequivocal answer as to whether monitoring with CEA enhances patient outcome requires a level 1 evidence (LOE1) study [25], i.e., either a large randomised prospective trial comparing follow-up with and without CEA monitoring or a meta-analysis of small-scale prospective or retrospective trials addressing the same question. Although data from a high-powered randomised trial are unavailable, a number of meta-analyses have been performed. In one of these, Rosen and colleagues [26] analysed the literature published between 1972 and 1996 comparing outcome in patients with intensive follow-up versus those with no follow-up. Intensive follow-up included physical examination and CEA determination at least 3 times per year for the first 2 years after examination. The control group had no follow-up with physicians responding only when symptoms developed. In total, 2 randomised and 3 comparative cohort studies, yielding 2005 patients, met the above criteria. Meta-analysis showed that the intensively followed-up patients had a significantly better survival rate than the control group.

In a further meta-analysis, Bruinvels and colleagues [27] combined the data from 7 published non-randomised studies comprising 3283 patients. In this study, patients who underwent intensive follow-up had a 9% better 5-year survival rate than those with minimal or no follow-up, only when the intensively followed-up cohort had CEA testing.

In a third meta-analysis, Renehan and colleagues [28] identified 5 published randomised controlled trials ($n=1342$) which compared outcome following intensive versus control follow-up. Intensive follow-up was found to be associated with a significant reduction in all cause mortality (combined risk ratio, 0.81, $P=0.007$). The reduced mortality was most pronounced in the 4 trials that used computed tomography (CT) and frequent assay of CEA. Furthermore, intensive follow-up was associated with significant earlier detection of all recurrences and an increased detection rate for isolated local recurrences.

These meta-analyses when taken together provide strong evidence that intensive follow-up that includes the use of CEA monitoring enhances outcome in patients with CRC. The question therefore arises as to whether all patients with CRC should undergo regular testing with CEA. As the recurrence rate for patients with Dukes' A disease is low (approximately 5–10%), the EGTM Panel suggests that monitoring with CEA in this subgroup may not be justified. For patients with both Dukes' B and C disease that may be candidates for liver resection should recurrences develop, follow-up with regular CEA determinations for at least 3 years is recommended. The main aim of CEA testing in these patients is the early detection of operable liver metastases.

Of those patients with CRC who undergo potentially curative resection for their primary cancer, but later recur, approximately 80% develop liver metastases [29]. Hepatic resection for isolated liver metastases from CRC achieves 5-year survival in 25–50% of cases and is currently the only potentially curative therapy for metastatic CRC [29]. As serum levels of CEA are elevated in almost all patients with liver metastases from CRC [22,24], monitoring with this marker should lead to the earlier detection of metastasis. We would like to point out that an ASCO Panel also recommended regular testing with CEA for the early detection of liver metastases from CRC [11].

Two major questions arise when using CEA in the surveillance of patients with CRC. These are: what is the optimum frequency of testing and what increase in CEA values constitutes a clinically significant change? Little or no work has been carried out to address these questions. As a compromise between patient convenience, costs and efficiency of disease detection, the EGTM Panel like the ASCO Panel (11) suggests that CEA testing be carried out every 2-3 months for at least

3 years after the initial diagnosis. After 3 years, testing could be carried out less frequently, e.g., every 6 months. No evidence exists, however, to support this frequency of testing.

There is no universally accepted definition of what constitutes a clinically significant rise in a tumour marker level. Generally, however, increases of 25–30% are thought to be significant. We therefore define a clinically significant increase in CEA as an increase in concentration of at least 30% over the previous value. This increase must be confirmed by a second sample taken within 1 month. If this latter sample is similarly elevated, the patient should undergo further investigations to detect or exclude malignancy. This definition of a clinically significant increase in CEA levels should not be regarded as exclusive. For example, small percentage increases (15–20%) maintained over at least 3 successive determinations could also prompt further investigations.

2.2.5. Monitoring chemotherapy in patients with advanced disease

The main aims of chemotherapy in patients with advanced CRC are to prolong survival, control symptoms and improve quality of life [30]. Recently, the Colorectal Cancer Collaborative Group carried out a systematic review and meta-analysis of all published controlled trials comparing palliative chemotherapy with support care, in patients with advanced CRC [30]. In total, 13 randomised trials involving 1365 patients were included in the study. Analysis of the data showed a 35% reduction in the risk of death and a median survival of 11.7 months in treated patients compared with a median survival of 8 months in those patients who received the best supportive care [30]. These findings are likely to lead to the increased use of chemotherapy in patients with advanced CRC.

A number of reports have described the use of CEA in monitoring the treatment of patients with advanced CRC (for review, see Refs. 4, 9, 11). Again, most of these were retrospective, non-randomised and contained small numbers of patients. These studies suggested: (a) that patients with a decrease in CEA levels while receiving chemotherapy generally had a better outcome than those patients whose CEA levels failed to decrease and (b) for almost all patients, increases in CEA levels were associated with disease progression.

Although no study has shown that monitoring patients with advanced CRC improves survival, reduces costs or increases quality of life, serial testing may lead to the earlier detection of progressive disease. This information should allow the cessation of ineffective therapy which should result in reduced costs and a better quality of life.

For monitoring the treatment of patients with advanced CRC, the EGTM Panel recommends serial CEA measurements every 2 to 3 months while on therapy. It should be noted that certain treatments, e.g., 5-fluorouracil and levamisole can cause transient elevations in CEA levels in the absence of disease progression [31].

The ASCO Expert Panel has also recommended that CEA should be used to follow the treatment of metastatic CRC. These guidelines stated that "if no other simple test is available to indicate a response, CEA should be measured at the start of treatment for metastatic disease and every 2–3 months during active treatment" [11]. Two values above the baseline were regarded as adequate to document progressive disease even in the absence of corroborating evidence [11].

Assay of CEA is also recommended by our Panel for the monitoring of patients undergoing surgical resection of liver metastases from colorectal cancer. By measuring CEA levels both pre and postoperatively, the success of surgery in removing the metastasis can be assessed. Furthermore, both pre and postoperative levels of CEA in this situation may provide prognostic information [32]. Finally, CEA concentrations may be of use in stratifying patients for additional therapy after the removal of liver metastases [33].

3. Other serum markers

Although the oldest, CEA is still the best available serum marker for CRC. One of its major limitations in monitoring, however, is that 20–30% of patients with CRC fail to produce elevated serum levels, despite the presence of advanced disease. For the follow-up of these patients, other markers are therefore necessary. Markers that are of potential value for CRC patients not expressing high levels of CEA include CA 19-9, CA 242, CA 72-4, CA 50, TPA (tissue polypeptide antigen), TPS (tissue polypeptide-specific antigen) and tissue inhibitor of metalloproteinase 1 (TIMP-1). Some of these markers are briefly discussed below.

3.1. CA 19-9

After CEA, CA 19-9 is the most widely investigated gastrointestinal tumour marker. The CA 19-9 assay detects a mucin containing the sialated Lewis—a pentasacharide epitope, fucopentaose II (for review, see Ref. 34). Although the best available marker for pancreatic adenocarcinoma, CA 19-9 is less sensitive than CEA for the detection of CRC [34].

Despite being of little value in the early diagnosis of CRC [31], elevated preoperative levels of CA 19-9 have been found to correlate with adverse patient outcome [35,36,37,38]. In the largest study ($n = 495$ patients), the prognostic impact of CA 19-9 was found to be independent of both Dukes' stage and CEA concentration

[38]. Furthermore, CA 19-9 was a stronger prognostic factor than CEA and predicted outcome in the Dukes' B/C subgroup. In a separate study using 62 patients with Dukes' B/C disease treated with adjuvant chemotherapy, elevated preoperative CA 19-9 levels were also associated with a poor prognosis [39]. Although these results are preliminary, this is one of the first studies to investigate the predictive value of a serum marker in patients with CRC receiving adjuvant chemotherapy.

Filella and colleagues [40] compared CEA and CA 19-9 in the follow-up of 370 patients with diagnosed colorectal cancer. While CEA was abnormal in 84% of patients with recurrence and provided a lead-time in 75%, CA 19-9 levels were elevated in only 48% and gave a lead-time in only 25%. Only one patient with a normal CEA value had a high CA 19-9 level. Similarly, in monitoring the treatment of metastatic CRC, CA 19-9 results yielded no additional information to that provided by CEA [41].

Based on published data, we conclude that CA 19-9 cannot be recommended for the early diagnosis of CRC. Furthermore, serial determinations of the marker appear to provide little extra information to that of CEA in monitoring patients with diagnosed CRC. Preoperative levels of CA 19-9 may, however, provide independent prognostic information. These preliminary findings must now be confirmed in a large prospective trial.

3.2. CA 242

As with CEA and CA 19-9, CA 242 cannot be used for the detection of early stage CRC. For determining prognosis, preliminary findings suggest that CA 242 may be more potent than CEA. For example, Carpelan-Holmstrom and colleagues [42,43] have shown using multivariate analysis, that high preoperative levels of CA 242 were a significant predictor of outcome when CEA was included in the analysis, but CEA only became an independent factor if CA 242 was excluded.

Hall and colleagues [44] compared CEA and CA 242 in the surveillance of 149 patients who had undergone apparent curative resection for CRC. For the detection of recurrent disease, CEA alone had a sensitivity of 76% and a specificity of 86%. The corresponding sensitivity and specificity for CA 242 were 60% and 87%, respectively. Combination of the 2 markers increased the sensitivity to 88%, but reduced specificity to 78%. The authors concluded that although CA 242 alone is inferior to CEA, it may complement CEA in the follow-up after curative resection for CRC. Other investigators have also reported that CA 242 can complement CEA in the surveillance of patients with diagnosed CRC [45,46,47]. A recent report found that CA 242 was superior to CEA in detecting lung metastases, but that CEA was more sensitive than CA 242 in diagnosing liver metastases [48]. These preliminary findings suggest that CA 242 may complement CEA in the surveillance of patients with diagnosed CRC.

3.3. TPA and TPS

TPA which measures fragments of cytokeratin 8, 18 and 19 and TPS which detects fragments of cytokeratin 18, have also only been subjected to limited evaluation in CRC. Due to a lack of sensitivity and specificity, neither TPA nor TPS can be recommended for the detection of early stage CRC. In a study of 202 newly diagnosed patients, Lindmark and colleagues [35] found that high levels of CEA, CA 19-9, CA 242, TPA and TPS were all associated with aggressive disease. If the analysis was restricted to the patients with potentially cured disease, only TPA provided prognostic information after adjustment for Dukes' stage. A further study has suggested that TPA may complement CEA in the detection of CRC [49].

3.4. TIMP-1

TIMP-1 is a multifunctional glycoprotein that inhibits metalloproteinase activity, stimulates cell growth and inhibits apoptosis [50]. Using a research enzyme-linked immunosorbent assay (ELISA), Holten-Andersen and colleagues [51] reported that total levels of TIMP-1 were significantly higher in patients with both colonic ($n=338$) and rectal cancer ($n=108$) than in either healthy subjects ($n=108$) or patients with inflammatory bowel disease ($n=50$). At 95% specificity, TIMP-1 detected colonic cancer with a sensitivity of 65% and rectal cancer with a sensitivity of 42%. For patients with Dukes' A and B disease, sensitivity was 58% and 35%, for colonic and rectal cancer, respectively (at the 95% specificity). Combining CEA with TIMP-1 increased sensitivity for colonic cancer from 65% to 75% and rectal cancer from 42% to 54% (at 95% specificity). Other studies have shown that high preoperative plasma levels of TIMP-1 independently predicted an adverse outcome in patients with colorectal cancer [52].

Summarising the current situation regarding the newer serum markers for CRC, the EGTM Panel concludes that:

- Insufficient data are available to recommended their use for the detection of early CRC,
- Levels of CA 19-9, CA 242, TPA and TIMP-1 at the time of initial presentation appear to provide independent prognostic information and may be stronger prognostic factors than CEA, but there is currently insufficient evidence to recommend their routine use and
- Insufficient evidence exists to recommend their use for routine monitoring patients with CRC.

4. Cell and tissue markers

4.1. Screening and early diagnosis

All the currently used markers for CRC have 2 main disadvantages, i.e., lack of sensitivity for early or premalignant disease and lack of specificity for malignancy. It is, however, now well established that multiple molecular alterations occur at the genome level, during the transition from normal mucosa to invasive carcinoma [53]. Genes undergoing mutation during this process might be expected to provide markers that are relatively specific for malignant or premalignant disease and also aid early diagnosis. These alterations occur principally in cellular oncogenes (e.g., *ras*), tumour suppressor genes (e.g., *P53* and *APC*) and microsatellite instability sequences. Cells containing these abnormal DNA sequences are shed into the lumen by exfoliation and can be detected in stools (for review, see Ref. 54).

Mutant *ras* genes have been found in approximately 50% of invasive CRCs and in approximately 60% of adenomas >1 cm in diameter [54]. In 1992, Sidransky and colleagues [55] identified a mutant *ras* gene in stools from 8/9 with a CRC expressing the abnormal gene. Overall, however, only 9/24 (38%) of randomly selected tumours possessed a mutant *ras* gene.

In an attempt to increase sensitivity, Ahlquist and colleagues [56] determined alterations in 5 different genes, i.e., K-*ras*, *APC*, *P53*, *Bat 26* (a microsatellite instability marker) and long DNA (*L-DNA*). Using stool DNA from 22 patients with CRC, 11 with adenomas $\geqslant 1$ cm and 22 apparently healthy subjects, the marker panel yielded a sensitivity of 91% for cancer, 82% for the adenomas and a specificity of 93%. Excluding K-*ras* led to no change in the sensitivity for cancer, slightly decreased sensitivity for the adenomas but increased specificity to 100%. In a similar type of study, Dong and colleagues [57] detected 36/51 (71%) patients with CRC using 3 mutant genes, i.e., *P53*, K-*ras* and *Bat26*.

It is important to point out that while these results are promising and offer for the first time molecular tests for the early diagnosis of CRC, the alterations described are unlikely to be specific for this malignancy. For example, mutations in *ras* have also been found in pancreatic adenocarcinoma and hyperplasia, aberrant crypt foci and normal-appearing colonic mucosa [58,59]. Furthermore, mutations in the *P53* gene have been found in almost all types of cancer including diverse gastrointestinal cancers [58,59].

4.2. Prognosis

Some of the genes mutated during CRC formation have also been evaluated for possible prognostic value in patients with invasive disease. A correlation between mutations in *ras* and poor prognosis has been reported by some, but not all, investigators (for review, see Ref. 60). Recently, Andreyev and colleagues [61] performed a meta-analysis of published studies relating *ras* gene mutations to patient outcome in colorectal cancer. In total, data was available on 4268 patients from 42 different centres. The study found that of 12 possible mutations on codons 12 and 13 of Kirsten *ras* gene, only one mutation, i.e., glycine to valine on codon 12, correlated with an adverse patient outcome. This mutation was found in only 8.6% of all patients and was prognostic in Dukes' C, but not in Dukes' B disease. These results suggest that measurements of *ras* mutations are unlikely to be useful for determining prognosis in patients with colorectal cancer.

As with the individual studies involving *ras*, conflicting findings also exist on the relationship between the presence of *P53* gene mutations and/or protein overexpression and patient outcome (for review, see Ref. 60). Preliminary findings suggest that expression of either the *DCC* (deleted in colon cancer) gene [62] or the presence of microsatellite instability [63] confers a favourable prognosis in CRC. Finally, high levels of specific proteases causally involved in metastasis such as urokinase plasminogen activator [64,65] and certain matrix metalloproteinases [66] have been shown to predict adverse outcome in patients with CRC.

4.3. Predicting response to therapy

While prognostic factors predict likely outcome in the absence of adjuvant therapy, predictive factors help determine the probability of response to a particular therapy. In recent years the use of both adjuvant and palliative chemotherapy has found increasing use in colon cancer. Overall, however, only approximately 20% of patients with advanced disease respond to 5-fluorouracil (5-FU) plus levamisole. Clearly, therefore, many patients are treated without any benefit. Consequently, the availability of a marker to prospectively predict response/resistance to therapy is desirable.

The most widely studied therapeutic predictive marker in CRC is thymidylate synthase (TS). TS catalyses the rate-limiting step in the conversion of uridine to thymidine for DNA synthesis and is the target of 5-FU and raltitrexed (Tomudex), 2 commonly used cytotoxic compounds used to treat CRC. Early data showed a correlation between TS protein levels and response to 5-FU in cell lines [67]. Using reverse transcriptase-polymerase chain reaction (RT-PCR) to measure mRNA for TS, Leichman and colleagues [68] found an inverse relationship between TS expression in metastatic deposits and response to 5-FU in patients with advanced CRC. In this study, the response rates for patients with low TS levels was greater than 50%, whereas it was less than 5% for the patients with high

TS expression. Other investigators have also reported that high levels of TS predict resistance to fluoropyrimidines in patients with metastatic CRC (for review, see Ref. 69). In contrast to these findings in advanced disease, high levels of TS were found to be associated with a benefit from adjuvant chemotherapy in patients with rectal cancer [70].

In a study of 460 patients with Stage II and III colon cancer patients, Watanabe and colleagues [71] found that both the retention of the 18q allele in cancers and mutations in the gene for *TGFBeta-1* predicted response to adjuvant fluorouracil-based chemotherapy. Patients with microsatellite-positive cancers have also been reported to have a higher probability of responding to fluorouracil-based adjuvant chemotherapy than those lacking this marker [63].

Other markers undergoing evaluation for therapy prediction in CRC include *P53*, topoisomerase 1, vascular endothelial growth factor (VEGF), thymidine phosphorylase and dihydropyrimidine dehydrogenase (for review, see Ref. 69).

In summary, preliminary data with the cell/tissue-based markers look promising. In contrast to the available serum markers, some of these molecular markers have the potential to aid the diagnosis of early CRC cancers. In addition, for the first time, markers to predict response to therapy may soon be available. At present, however, none of these markers should be used for routine clinical purposes. Rather, research should be directed at simplifying, standardising and automating these tests. The simplified and standardised assays should then be evaluated in prospective randomised trials.

5. Suggestions for future research

Using prospective randomised trials where possible, the following studies should be carried out:

- Establish whether serial levels of CEA enhance patient outcome, quality of life or cost of care,
- Investigate if elevated preoperative CEA levels can be used to select the aggressive Dukes' B (node-negative) patients that could benefit from adjuvant chemotherapy.
- Establish the variations in serial CEA levels that optimally define tumour progression and regression,
- Using multivariate analysis, compare the relative prognostic value of both preoperative CEA and the newer serum markers such as CA 19-9, CA 242, TIMP-1 and TPA,
- Establish whether the assay of other serum markers in addition to CEA enhances the early detection of recurrent CRC and see which bests complements CEA.
- Develop simple, cheap, automated and standardised assays for the detection of molecular markers. Evaluate these markers for screening, early diagnosis and assessment of prognosis.
- Using differential gene expression methodology, search for a possible colorectal-specific marker.

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