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Position Paper

Tumour markers in colorectal cancer: European Group on Tumour Markers (EGTM) guidelines for clinical use

M.J. Duffy[a,b,*], A. van Dalen[c], C. Haglund[d], L. Hansson[e], E. Holinski-Feder[f], R. Klapdor[g], R. Lamerz[h], P. Peltomaki[i], C. Sturgeon[j], O. Topolcan[k]

[a]Department of Pathology and Laboratory Medicine, Nuclear Medicine Laboratory, St Vincent's University Hospital, Elm Park, Dublin 4, Ireland
[b]School of Medicine and Medical Science, Conway Institute of Biomolecular and Biomedical Research, University College, Dublin 4, Ireland
[c]Institute of Tumour Marker Oncology, Van Strijenstraat 44, 2801 TG Gouda, The Netherlands
[d]Department of Surgery, Helsinki University Central Hospital, Helsinki, Finland
[e]Department of Clinical Chemistry and Pharmacology, Akademiska Hospital, Uppsala, Sweden
[f]Department of Medical Genetics, Ludwig Maxmilians University, Munich, Germany
[g]Centre for Clinical and Experimental Tumour Diagnosis and Therapy, Hamburg, Germany
[h]Klinikum Großhadern, Med. Klinik II, Ludwig Maximilians Universität, München, Germany
[i]Department of Medical Genetics, University of Helsinki, Helsinki, Finland
[j]Department of Clinical Biochemistry, Royal Infirmary of Edinburgh, Edinburgh, UK
[k]Second Department of Internal Medicine, University Hospital, Pilsen, Czech Republic

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ABSTRACT

The aim of this article is to present updated guidelines for the use of serum, tissue and faecal markers in colorectal cancer (CRC). Lack of specificity and sensitivity preclude the use of all existing serum markers for the early detection of CRC. For patients with stage II or stage III CRC who may be candidates for either liver resection or systemic treatment should recurrence develop, CEA should be measured every 2–3 months for at least 3 years after diagnosis. Insufficient evidence exists to recommend routine use of tissue factors such as thymidylate synthase, microsatellite instability (MSI), *p*53, K-*ras* and deleted in colon cancer (DCC) for either determining prognosis or predicting response to therapy in patients with CRC. Microsatellite instability, however, may be used as a pre-screen for patients with suspected hereditary non-polyposis colorectal cancer. Faecal occult blood testing but not faecal DNA markers may be used to screen asymptomatic subjects 50 years or older for early CRC.

1. Introduction

Colorectal cancer (CRC) is the third most common cancer worldwide with an estimated 1 million new cases and a half million deaths each year.[1] It is now clear that CRC results from the cumulative effects of sequential genetic alterations in proto-oncogenes, tumour suppressor genes and DNA repair genes (for review, see Ref. [2]). In sporadic CRC, these altera-

* *Corresponding author:* Address: Department of Pathology and Laboratory Medicine, Nuclear Medicine Laboratory, St Vincent's University Hospital, Elm Park, Dublin 4, Ireland. Tel.: +353 1 2094378; fax: +353 1 2696018.
E-mail address: Michael.J.Duffy@ucd.ie (M.J. Duffy).
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Table 1 – Summary of EGTM (2003) guidelines on the use of serum markers in CRC[9]

- Lack of sensitivity and specificity precludes the use of CEA and all other existing serum markers for the early detection of CRC
- Preoperative levels of CEA provide a baseline value for subsequent serial determinations and may also provide independent prognostic information
- For patients with stages II and III disease that may be candidates for liver resection, CEA should be assayed every 2–3 months for at least 3 years after diagnosis
- For monitoring therapy in advanced CRC, CEA should be measured every 2–3 months
- Insufficient evidence exists at present to recommend routine use of other serum markers such as CA 19-9, CA 242, TPA, TPS or TIMP-1 in the management of patients with CRC

Table 2 – Summary of EGTM (2007) guidelines for the clinical use of markers in CRC together with their level of evidence (LOE) (as defined in Ref. [10])

Marker	Proposed use/uses	EGTM guideline	LOE
Serum			
CEA	Determining prognosis	May be used in combination with standard prognostic factors	III
	Surveillance following curative resection	Should be used for stages II and III patients who may be candidates for liver resection **or systemic treatment**, should recurrence develop	I[a]
	Monitoring therapy in advanced disease	Should be used, especially in patients with non-evaluable disease using standard criteria. Should be measured prior to start of treatment and at 2–3 monthly intervals during therapy[b]. Ideally, should be used in combination with radiology.	III
CA19.9	Determining prognosis	Not recommended	III
	Surveillance following curative resection	Not recommended	IV
CA 242	Determining prognosis	Not recommended	III
TIMP-1	Determining prognosis	Not recommended	III
Tissue			
TS	Determining prognosis	Not recommended	I
	Predicting response to chemotherapy	Not recommended	III
MSI	Determining prognosis	Not recommended	I
	Predicting response to chemotherapy	Not recommended	III
DCC	Determining prognosis	Not recommended	I
Ras	Determining prognosis	Not recommended	I
P53	Determining prognosis	Not recommended	I
Faecal			
FOBT	Screening for early CRC	Yes, for screening subjects 50 years or older	I
DNA-based	Screening for early CRC	Not recommended at present	III/IV[c] (for most studies)
Tests for genetic susceptibility to CRC			
APC	For identifying subjects at high risk of developing FAP	Yes, should be used	
MSI/MMRE IHC	Prescreen for HNPCC	Yes, should be used	
MLH1/MSH2/MSH6	For identifying subjects at high risk of developing HNPCC	Yes, should be used	

LOE, are not provided for the CRC genetic susceptibility tests as the grading system used[10] was devised for standard tumour markers. [TIMP-1, tissue inhibitor of metalloproteinase type 1; TS, thymidylate synthase; MSI, microsatellite instability; DCC, deleted in colon cancer; FOBT, faecal occult blood testing, FAP, familial adenomatous polyposis, MMRE, mis-match repair enzymes, IHC, immunohistochemistry and HNPCC, hereditary non-polyposis colorectal cancer].

a LOE 1 here relates to the role of CEA as part of an intensive follow-up regime in improving patient outcome compared to a minimal follow-up strategy.

b Following the initiating of therapy in advanced CRC, CEA may exhibit transient increases.

c A specific DNA panel has been evaluated in a large population-based study.[32] Text in bold is an update of the 2003 EGTM guidelines[9] on the use of CEA in postoperative surveillance.

tions are acquired, and are likely to be caused by exogenous and endogenous carcinogens. In contrast, in cancer syndromes such as familial adenomatous polyposis (FAP) and hereditary non-polyposis CRC (HNPCC), critical genetic alterations that predispose to malignancy are inherited.[3] For example in FAP, a germline mutation in the APC gene which occurs in every cell predisposes to adenomatous polyps, while in HNPCC, mutations in DNA repair genes result in a more rapid accumulation of genetic alterations which increases the risk of polyp formation.

In recent years a multiplicity of markers have been proposed for CRC (for review, see Refs. [4–8]). These markers can be measured in serum, tissue or stools. In 2003, the European Group on Tumour Markers (EGTM) published guidelines on the use of tumour markers in CRC[9] (see Table 1 for a summary). These guidelines focussed almost exclusively on serum markers, especially CEA. The aims of this article are to present guidelines on tissue and faecal markers as well as to update the previous EGTM guidelines on serum markers. We also summarise existing published guidelines on genetic testing for inherited susceptibility to CRC. A summary of the updated EGTM guidelines together with the level of evidence[10] for their clinical application is outlined in Table 2.

These guidelines should be particularly helpful to surgeons, physicians and nurses involved in the management of patients with CRC and to laboratory personnel undertaking measurement of tumour markers. Their adoption is of course voluntary and the ultimate decision regarding use of any marker should be made by the treating clinician, i.e. the guidelines are intended to aid rather than replace clinical judgement.

2. Serum markers

2.1. CEA in postoperative surveillance

Although the oldest, CEA remains the most widely used serum marker in patients with CRC. EGTM guidelines for the use of CEA in CRC were previously published in 2003 and are summarised in Table 1. The main use of CEA in CRC is in surveillance following curative resection for primary cancer. Five independent meta-analyses have compared outcome in patients undergoing intensive follow-up versus minimal or no follow-up.[11–15] The first 2 of these 2 studies[11,12] included both non-randomised and randomised trials, whereas the other 3[13–15] included only randomised trials. Although the frequency and modalities of screening used in the individual studies varied, all five meta-analyses concluded that use of an intensive follow-up regime resulted in a modest but statistically significant improvement in outcome compared with a minimal follow-up strategy.

The Cochrane Review[15] concluded that there was an overall survival benefit at 5 years of follow-up for patients undergoing more intensive surveillance (OR = 0.67, 95% confidence interval, 0.53–0.84). The absolute numbers of recurrences however were similar in the 2 groups. No data were presented on quality of life, harm or cost-effectiveness of the intensive follow-up.

Two of these meta-analyses investigated the specific contribution of CEA to the improved outcome. In the first of these, Bruinvels et al.[11] concluded that intensive follow-up was associated with an improved outcome, but only if regular CEA determinations were carried out. Similarly, Figueredo et al.[14] found that trials using serial CEA measurements had a significant impact on survival, whereas those not using CEA failed to impact on outcome. It should be stated that compared to other follow-up tests for CRC patients (e.g. radiology and endoscopy), measurement of CEA is relatively inexpensive and causes minimal inconvenience for patients.

2.2. Early detection of metastatic disease in candidates for liver resection

One of the main aims of intensive follow-up is the early detection of resectable recurrences and metastases, especially liver metastasis. Twenty five to 50% of patients undergoing resection for primary CRC develop liver metastasis within 5 years of diagnosis.[15] Hepatic resection offers the only potential curative therapy for these patients. Curative resection, however, is possible in less than 25% of patients with metastasis confined to liver. Nevertheless, the 5-year survival for these patients is approximately 30% with about two-third of these being disease-free.[16] This 5-year survival rate is in striking contrast to historical cases of unresected patients in which the median survival was 6–9 months with few surviving for 5 years.[17]

Because of the relative success of surgery for treating CRC metastases to liver, previous guidelines recommended that for stages II and III CRC patients who are suitable candidates for liver resection, CEA should be determined every 2–3 months for at least the first 3 years after diagnosis.[9,18]

2.3. Early detection of metastatic disease in candidates for chemotherapy

While surgical resection offers the best prospect of long-term survival in patients with liver metastases from CRC, major progress is being made in the treatment of metastatic CRC with chemotherapy. Until relatively recently, the main form of systemic therapy for advanced CRC was 5-fluorouracil (5-FU) and leucovorin. This treatment reduced tumour size by at least 50% in about 20% of patients and prolonged median survival from about 6 to 11 months.[19] The addition of either irinotecan or oxaliplatin to 5-FU and leucovorin increased median survival to about 15 months.[20] Median survival increased to about 20 months if all three cytotoxic agents were used at some point in treatment or if combined cytotoxic and antibody (e.g. bevacizumab or cetuximab) therapy was administered.[20] Thus, patients with advanced CRC treated with systemic therapy may now survive approximately twice as long as they did 10 years ago.[20]

As a result of these developments with systemic therapy, the EGTM Panel has updated its guidelines on the use of CEA in postoperative surveillance. The new guidelines state that for stages II and III CRC patients, CEA should be measured every 2–3 months for at least 3 years, not only for patients who are suitable candidates for liver resection, but also for patients who are candidates for receiving systemic therapy. This inclusion of systemic therapy is in line with the recently updated guidelines from the American Society of Clinical Oncology.[21] The updated ASCO guidelines also

state that an elevated CEA, if confirmed following retesting, requires further evaluation for the presence of metastasis, but does not justify initiation of adjuvant therapy or systemic treatment for presumed metastatic disease. The EGTM Panel supports this statement. Because of the low risk of recurrence in patients with stage 1 CRC, regular surveillance following surgery may not be necessary.

2.4. *Other serum markers in CRC*

EGTM guidelines on the use of other serum markers in CRC are as previously published.[9]

3. Stool-based markers

3.1. *Faecal occult blood testing and screening for CRC*

Faecal occult blood testing (FOBT) is the most widely used screening modality for CRC.[22] Two main types of FOBT exist, i.e. the guaiac test which is based on the peroxidase-like activity (i.e. pseudoperoxidase) of haem in haemoglobin and the immunochemical test which detects the globin moiety in haemoglobin. Of these 2, the guaiac test has been the more widely evaluated. Four randomised trials have shown that screening with the guaiac-based FOBT reduced both the incidence and mortality of CRC (for review, see Ref. [22]). Meta-analysis of these trials concluded that FOBT reduced CRC incidence by approximately 20% and CRC mortality by about 16%.[22]

There is now a consensus amongst Expert Panels[23–26] that all average-risk subjects, age 50 years or older, should be offered screening for CRC and adenomatous polyps. The EGTM Panel supports these recommendations. As well as FOBT, potential screening options for CRC include flexible sigmoidoscopy, colonoscopy and double contrast barium enema.[23–26] As the most effective screening modality remains to be established, the method chosen is likely to depend on risk of CRC, local availability and personal preference. According to the American Gastroenterology Association, individuals should be offered options for screening as well as information about the advantages and disadvantages of each approach.[26] Some of the advantages and limitation of FOBT in screening for CRC are listed in Table 3.

Table 3 – Some advantages and disadvantages of FOBT in screening for CRC

Advantages
• Potentially examines the entire colorectal tract
• Non-invasive
• Requires no patient preparation (unlike endoscopic investigations)
• Simple and affordable
• Can be carried out in privacy of home
• Most extensively validated screening test for CRC
Disadvantages
• Low sensitivity for both adenomas (≈10%) and CRCs (40–85%)
• Low specificity for both CRCs and adenomas (90–98%)
• Ingestion of certain foods (red meats, fruits and vegetables) and medicines (non-steroidal anti-inflammatory drugs) can yield false-positive results with the guaiac-based assay[a]
• Multiple stool samples are necessary
• Must be performed annually to increase chances of detecting intermittent bleeding

a These limitations do not apply to the immunochemical FOBT.

3.2. *Faecal DNA-based tests and screening for CRC*

Faecal DNA tests detect mutant or abnormal DNA shed from neoplastic colorectal lesions and excreted in the stool. Since no single gene has been identified that is altered in all CRCs, a panel of DNA markers is usually employed. The most frequently measured markers in stool include mutant *K-ras*, mutant *APC*, mutant *p*53, *BAT-26* (long adenine tract 26) and long DNA (Table 4).[27–30]

Following a systematic review of the literature, Haug and Brenner[31] concluded that DNA marker panels detected CRC with a specificity of 95% or greater. However, sensitivity varied from 60% to 90%. In order to directly compare the use of a specific DNA panel with use of FOBT for detecting CRC, Imperiale et al.[32] carried out a large population-based study. Overall, 5486 subjects were enrolled with 4404 completing the study. Of the 31 invasive cancers found, the DNA panel detected 16, whereas FOBT detected only 4 ($p = 0.003$). Of the 71 invasive cancers and adenomas diagnosed with high grade dysplasia, the DNA panel detected 29 while FOBT detected only 10 ($p < 0.001$). In subjects with negative findings on colonoscopy, the DNA panel had a specificity of 94.4% with FOBT giving a specificity of 95.2%. It is clear from this study that although neither techniques detected the majority of neoplastic lesions, the DNA panel displayed a higher sensitivity than FOBT without reduced specificity.[32]

Table 4 – Stools-based DNA markers under investigation for screening for CRC

Marker	Frequency of alteration in CRC	Comment
K-*ras*	40–60%	May be present in non-neoplastic hyperproliferating cells such as pancreatic hyperplasia and colonic aberrant crypt foci, little use for proximal lesions
APC	~70%	Involved in the initiation of CRC, thus may be useful in detecting early lesions
*p*53	40–60%	Involved in late stages of CRC formation, rarely found in adenomas
L-DNA	?	Thought to reflect decreased apoptosis occurring in CRC
BAT 26	?	Widely used as a MSI marker. MSI is present in >90% of carcinomas and >80% of adenomas in HNPCC and in about 15% of sporadic cancers and 5% of sporadic adenomas

Data summarised from Refs. [27–31]. [HNPCC, hereditary polyposis non-colorectal cancer; MSI, microsatellite instability].

Table 5 – Advantages and disadvantages of DNA markers in screening for CRC

Advantages
• No restriction in diet or medication
• More accurate than FOBT
• May detect cancers of stomach and pancreas as well as CRC and
• DNA is released continuously rather than intermittently via bleeding, thus obviating the need for multiple samples
Disadvantages
• Requires large volume of faecal sample
• Expensive, may not be cost-effective
• Laborious
• Some genes, e.g. *K-ras*, may be mutated in normal-appearing colon, pancreatic hyperplasia and crypt foci cells and
• No evidence that screening with DNA markers reduces mortality from CRC at this stage (although this is likely)

Despite the better performance of the DNA panel as compared with FOBT, its overall relatively low sensitivity in asymptomatic subjects, coupled with relatively high cost and assay complexity, makes it unlikely that molecular markers will replace the FOBT as widely used screening tests for CRC in the near future. However, as certain DNA panels are superior to FOBT in screening for CRC, it would be expected that their use would decrease mortality from CRC.

Some advantages and disadvantages of faecal DNA markers in screening for CRC are summarised in Table 5. Although promising, the EGTM Panel does not recommend the use of DNA markers in general population screening for CRC, but recommends further research in this area. This should focus on increasing the sensitivity of the assays, simplifying and automating the tests and making them available at reduced costs.

4. Tissue-based markers

While serum markers are primarily used for postoperative surveillance and stools-based markers are most likely to be used for screening, tissue-based markers have been investigated for potential prognostic and predictive value. The potential prognostic and predictive value of the most widely studied tissue markers in CRC is discussed below.

4.1. *Thymidylate synthase*

Thymidylate synthase (TS) is the rate limiting enzyme involved in the conversion of deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP).[33] This reaction provides the only *de novo* source of thymidylate which is essential for DNA synthesis. TS has been widely investigated in CRC as both a prognostic and a therapy predictive marker.

The rationale for using TS as a therapy predictive marker in CRC is that it acts as the key target for several cytotoxic agents used to treat this disease such as the fluoropyrimidines, 5-fluorouracil (5-FU) and 5-fluorodeoxyuridine and the antifolate agent, tomudex.[33] Although widely used to treat CRC, only about 20% of patients with advanced disease respond to 5-FU.[34] Clearly, it would be desirable to have a predictive marker in order to select the minority of patients likely to benefit while sparing those unlikely to benefit from the side effects and costs of chemotherapy.

Preclinical studies show a correlation between high TS expression and resistance to 5-FU.[35] Consistent with these findings are multiple retrospective clinical trials suggesting that high TS levels in CRC tissue are associated with either relative resistance to 5-FU or poor outcome following treatment with 5-FU.[36–38] Most of these studies, however, contained relatively small numbers of patients and thus were underpowered to establish a possible significant relationship. Furthermore, a variety of different assays were used to determine TS levels.

In order to establish a more precise relationship between TS levels and patient outcome, Popat et al.[38] performed a systematic review of the literature and meta-analysis. For patients with advanced CRC, 13 studies containing a total of 887 patients were identified. Of these, 12 were regarded as suitable for pooling of data. All of the patients in these trials were treated with TS inhibitors. Following a pooled-analysis, the overall hazard ratio (HR) associated with high levels of TS for overall survival was 1.74 (95% CI, 1.34–2.26). The impact of TS levels on outcome, however, depended on whether the TS assay was performed on the primary cancer or on a metastatic lesion. For example, if TS expression was determined on the metastatic lesion, the HR was 2.39 (95% CI, 1.43–4.01). On the other hand, if TS was measured on the primary cancer, the HR was only 1.33 (95% CI, 1.07–1.61). It thus appears that for predicting outcome in patients with advanced colorectal cancer treated with TS inhibitors, TS levels must be determined on the relevant metastatic lesion.

For patients with early CRC, 7 studies with a total of 2610 patients were identified.[38] The pooled HRs for overall survival and progression-free survival were 1.35 (95% CI, 1.07–1.8) and 1.24 (95% CI, 0.98–1.56), respectively. In the three eligible studies in which patients were treated with surgery only, the pooled HRs were 1.92 (95% CI, 1.12–3.32) and 1.9 (95% CI, 1.35–2.67), respectively. On the other hand, in the three studies in which patients were treated with both surgery and adjuvant 5-FU, the pooled HR for overall survival and progression-free survival were 0.93 and 1.0, respectively. These results, however, should be interpreted cautiously due to the small number of published studies.[38]

Although the above findings appear to be consistent with the hypothesis that high TS expression correlates with poor outcome in patients with CRC, there was significant evidence of heterogeneity between the contributing studies. Additionally, there was evidence of publication bias in the studies focussing on patients with advanced disease. A further problem with TS is the lack of a standardised assay for its measurement. At present therefore, assay of TS cannot be recommended for routinely determining prognosis or predicting response to therapy in patients with CRC.

4.2. *Microsatellite instability*

Microsatellites (MS) are stretches of DNA in which short sequences (usually 1–5 nucleotides long) are repeated.[39] The most common MS in human DNA is a dinucleotide repeat of cytosine and adenine (CA_n) that occurs at tens of thousands of locations in the human germline. MS instability (MSI) oc-

curs when a germline MS allele gains or loses a repeat unit. This gain or loss of MS alleles results from the absence of a functional mismatch repair (MMR) enzyme, i.e. an enzyme that repairs errors occurring during DNA replication.

Testing for MSI has a number of potential applications in CRC, including

- use as a surrogate marker for hereditary non-polyposis colorectal cancer (HNPCC) [see later discussion on genetic testing],
- determining prognosis in patients with sporadic CRC and
- predicting response to adjuvant chemotherapy in patients with sporadic CRC.

Popat et al.[40] carried out a systematic review and pooled analysis of published studies relating MSI to prognosis in patients with CRC. Of the 43 studies identified, 32 were found to be eligible for analysis, giving a total of 7642 patients including 1277 with MSI. Following a pooled analysis, patients with MSI had a 15% better outcome compared to those without MSI. The benefit of MSI was seen in patients with both locally advanced (stages II and III disease) and advanced CRC (stage IV). The reason for the association between MSI and favourable prognosis may be related to a protective role provided by functionally active lymphocytes which infiltrate MSI-positive CRCs.[41]

Although available data suggest that testing for MSI may differentiate between indolent and aggressive CRCs, Popat et al.[40] caution that prior to introduction of routine MSI testing as an aid to determine prognosis, validation in a prospective trial is essential. The EGTM Panel supports this, and does not currently recommend routine MSI testing for determining prognosis in patients with CRC.

In vitro studies show that colorectal cancer cells displaying MSI are less responsive than MS-stable (MS-S) cells to 5-fluorouracil (5-FU).[42,43] Consistent with these findings, Ribic et al.[44] reported that adjuvant 5-FU plus levamisole was of no significant benefit in stages II and III patients with MSI-positive tumours (defined as instability at 30% or more of loci screened). In contrast, chemotherapy improved overall survival in patients lacking MSI (hazard ratio, 0.72, $p = 0.04$). Carethers et al.[45] also found that the benefit of 5-FU was different in patients with and without MSI. Thus, patients with non-MSI tumours who received this drug had better survival than those not treated. Conversely, patients with MSI-positive tumours who were treated with 5-FU had a similar survival pattern to those without treatment. In contrast to these findings, other investigators found that the presence of MSI was a significant predictor of a survival benefit from adjuvant 5-FU-based chemotherapy.[46,47] Because of these conflicting findings, MSI testing cannot be recommended at present for predicting response to 5-FU in the adjuvant treatment of CRC.

4.3. *p53*

The *p*53 tumour suppressor gene encodes a transcription factor that regulates the expression of genes involved in apoptosis, angiogenesis, cell cycle and genome maintenance.[48,49] Mutations in the *p*53 gene are found in approximately 50% of CRCs, with most mutations occurring in exons 5–8. These mutations appear to be formed relatively late in the genesis of CRC, i.e. during the conversion of dysplastic adenomas to invasive carcinomas.

*p*53 has been widely investigated both as a prognostic and as a therapy predictive marker in CRC. As with the tissue markers discussed above, most of these studies are retrospective and insufficiently powered to establish a meaningful relationship with patient outcome. Furthermore, a multiplicity of methods was used to determine p53 abnormalities. These methods have generally employed either immunohistochemistry to detect p53 protein or DNA sequence analysis to detect gene mutations.

Munro et al.[50] carried out a systematic review of published studies that investigated the relationship between *p*53 abnormality and outcome in patients with CRC. In total, 168 eligible studies comprising survival data on 18,766 patients were identified. The key findings were as follows:

- Patients with abnormal *p*53 whether detected by immunohistochemistry (IHC) or DNA sequence analysis had an increased risk of death. The relative risk (RR) with IHC was 1.32 (95% CI, 1.23–1.42) and with sequence analysis was 1.31 (95% CI, 1.19–1.90).
- The adverse impact of *p*53 abnormality was stronger in patients likely to have a good outcome compared to those likely to have poor outcome.
- *p*53 had no impact on outcome in patients treated with chemotherapy.
- Abnormal *p*53 correlated with failure of response to radiotherapy in patients with rectal cancer (RR, 1.49, 95% CI, 1.25–1.77).

In a similar study, Russo et al.[51] pooled data from 25 different groups ($n = 3583$) invited to participate in a multicenter study aimed at evaluating the potential prognostic and predictive value of *p*53 mutations in CRC. Unlike the analysis of Munro et al.[50] mentioned above, only gene mutations were investigated in this study. The main findings were

- in patients with proximal tumours, mutations in exon 5 of *p*53 were associated with adverse outcome,
- in patients with distal colon tumours, gene deletions resulting in loss of amino acids were associated with poor survival,
- patients with wild-type *p*53 gene displayed a better outcome when treated with chemotherapy compared to those treated with surgery alone, and
- patients with rectal tumours containing wild-type *p*53 derived a significant survival benefit from 5-fluorouracil (5-FU), irrespective of whether or not radiotherapy was administered.

Although the above studies suggest that altered *p*53 may have an impact on outcome in patients with CRC, this relationship is at best only modest. Consequently, at present, routine determination of *p*53 status cannot be recommended for either assessing prognosis or predicting response to therapy in CRC. Furthermore, as with other tissue-based markers in CRC, standardised assays are not available for determining *p*53 abnormalities.

4.4. *K-ras*

K-*ras* is one of the most frequently mutated c-oncogenes in human cancer. It functions as a guanine nucleotide binding protein involved in signal transduction.[52] This signalling may result in increased cell proliferation, enhanced cell survival or induction of apoptosis.

Mutant K-*ras* is present in approximately 50% of CRCs and appears to play a role in the relatively early stages of CRC carcinogenesis.[53] Conflicting data exist on the relationship between the presence of mutant K-*ras* and prognosis in patients with CRC.[53] Based on an early meta-analysis of published data, Andreyev et al.[54] concluded that K-ras mutations correlated with poor outcome in patients with CRC. A more recent meta-analysis involving 4268 patients from 42 different institutions however, concluded that only a specific type of K-*ras* mutation predicted poor outcome, i.e. only patients with a G–T transversion at codon 12 had an adverse outcome.[55] This specific mutation was detected in less than 10% of tumours and was prognostic in Dukes' C but not in Dukes' B patients.

Based on the available data, K-*ras* cannot be recommended for determining prognosis or for predicting response to chemotherapy in patients with CRC.

5. Tests for susceptibility to CRC

Approximately 15% of CRC are thought to be due to an inherited or familial predisposition.[3] The most common hereditary conditions giving rise to an increased risk of CRC are hereditary non-polyposis colorectal cancer (HNPCC) and familial adenomatous polyposis (FAP).

5.1. *Familial adenomatous polyposis*

FAP is an autosomal dominant condition characterised by hundreds to thousands of adenomas in the colon and rectum. It has an incidence of approximately 1 per 8000 to 1 per 14,000 of the population and accounts for about 0.5% of all CRCs.[56] CRC is an almost inevitable consequence of classical FAP, if untreated, with an average of onset of about 39 years of age. FAP may be associated with extra-colonic manifestations such as congenital hypertrophy of retinal pigment epithelium (CHRPE), desmoid tumours, osteomas, teeth abnormalities, thyroid cancer and hepatoblastoma.

An attenuated form of the syndrome (AFAP) is characterised by fewer adenomas (<100). Subjects with this attenuated form of FAP also have a high risk of developing CRC, i.e. approximately 80% by the age of 70 years. For patients with this form of APC, CRC is diagnosed approximately 12 years later than in classical FAP.[56]

A further variant of the FAP syndrome results from biallelic inherited mutations in the *BER* (base excision repair) gene, known as *MutYH* or *MYH* (for review, see Ref. [57]). This syndrome, which is now referred to as MAP or MYH-associated polyposis, is often indistinguishable in its clinical manifestation from classic or attenuated forms of FAP. In one study, biallelic mutations in the MYH gene accounted for approximately 30% of families with multiple adenomas (15–100) who failed to exhibit an autosomal pattern of inheritance or a germline mutation in the *APC* gene.[58]

Approximately 70–80% of patients with classical APC harbour germline mutations in the *APC* gene. Although at least 800 different mutations in the APC gene have been found to cause FAP, the vast majority of these mutations are nonsense or frameshift that gives rise to a truncated protein. Approximately one third of the so far identified FAP-causing mutations occur at codons 1061 and 1309.[59] Other mutations are spread between codons 200 and 1600. The attenuated form of FAP usually results from mutations at the extreme 5′ or 3′ ends of the *APC* gene or from an alternatively spliced region of exon 9.[60]

Screening for FAP should commence with a detailed family history. For individuals with suspected FAP, genetic testing can be used both to confirm diagnosis in a suspected proband and to assess risk in pre-symptomatic family members. Provided the mutation responsible for FAP within a family is known, testing for *APC* mutations can be considered for at-risk family members.[23] Most expert panels recommend that for families with classic FAP, *APC* gene testing should be considered at 10–12 years of age.[23,61–63]

While it is generally recommended that *APC* testing should start at 10–12 years of age, some authors suggest that screening for hepatoblastoma should be carried out in children of FAP patients.[64–67] Hepatoblastoma is a rare malignant embryonal tumour of the liver that occurs in children usually between 6 months and 3 years of age. The risk of hepatoblastoma is considerably increased in children of patients with FAP.[65,68] The best available screening tests for hepatoblastoma are serum AFP levels and liver ultrasound.[65,68] Interpretation of AFP levels in newborn infants, however, may be difficult as serum levels are increased in the first few months of life. These high levels quickly decline with adult concentrations being reached after about 10 months.

It is important to state that genetic testing for cancer susceptibility syndromes such as FAP should only be performed following appropriate counselling.[69] The counselling should involve discussion of possible risks and benefits of the early detection of cancer as well as prevention modalities.[69] Individuals found not to carry the specific APC gene mutation responsible for FAP in that family can be spared intensive surveillance but should still undergo similar screening to that of an average risk individual.[23,61,62] On the other hand, if an *APC* gene mutation is identified, prophylactic surgery should be performed once adenomas are found. The main surgical options include colectomy and ileorectal anastomosis, proctocolectomy with ileostomy and proctocolectomy with ileal pouch-anal anastomosis[23,61,62] The EGTM Panel supports these recommendations for the detection and management of patients with FAP.

Approximately 20–30% of families with classic FAP are *APC* mutation-negative using existing testing methods. The NCCN guidelines state that if an APC mutation consistent with recessive inheritance cannot be found in a family, testing for MYH should be performed.[23] As heterozygote MAP carriers appear to have an increased risk of developing CRC, regular surveillance should be carried out on these subjects.

For patients with the attenuated form of APC, treatment depends on patient age and number of adenomas. According to the NCCN[23], for subjects 21 years or younger with a low number of adenomas, colonoscopy and polypectomy should be performed every 1–2 years. On the other hand, for individuals >40 years of age and for those with large numbers of polyps that cannot be easily managed by polypectomy, a colectomy and ileorectal anastomosis should be performed.

It should be noted that a potential benefit from genetic testing in FAP is presumed and is not based on evidence from high-level studies such as prospective randomised trials.

5.2. *Hereditary non-polyposis colorectal cancer*

HNPCC is clinically defined by the fulfillment of the Amsterdam Criteria (Table 6). HNPCC includes affected families with disease causing mutations in DNA mis-match repair (MMR) genes displaying an MSI-H phenotype in their corresponding tumours (a subgroup also called Lynch syndrome) and families with MSS tumours and no mutations in DNA MMR genes. The genetic pathogenesis of the latter group is currently unclear.

Lynch syndrome is an autosomal dominant disorder characterised by the early onset of CRC in the setting of relatively few polyps. The syndrome has an incidence of approximately 1:1000 in the general population and accounts for 1–5% of CRCs.[70,71] It is characterised by a 70–80% lifetime risk of developing CRC and, for women, a 40–60% lifetime risk of developing endometrial cancer. Carriers also have an increased risk of developing ovarian, upper gastrointestinal, urological and central nervous system cancers but this is usually less than 15%.[70–72]

The genes primarily responsible for Lynch syndrome are involved in MMR and include *MSH2*, *MLH1* and *MSH6*.[39,70,71] Mutations in *MSH2* and *MLH1* are thought to be responsible for at least 80% of the reported mutations in Lynch syndrome that involve defective mismatch repair.[70,71] Over 90% of patients with germline mismatch repair mutations exhibit MSI.[70,71] MSI is thus regarded as a hallmark of Lynch syndrome and consequently is widely used in selecting individuals for genetic testing.

Various criteria have been published in order to help identify HNPCC families. In 1991, the International Collaborative Group on HNPCC proposed the Amsterdam I criteria in an attempt to standardise the approach for selecting cases for research purposes[73] (Table 6). These criteria were later revised in order to take into consideration the presence of extra-colonic tumours[74] (Table 6). The revised criteria became known as the Amsterdam II criteria. The Amsterdam criteria were intended primarily for research purposes rather than for clinical use.

In 1997, a workshop held in Bethesda produced guidelines for the identification of subjects with HNPCC who should undergo MSI and/or genetic testing[75] (Table 7). The fulfillment of any one of the Bethesda criteria is sufficient to justify testing for MSI. More recently, a second Bethesda workshop simplified the earlier criteria and proposed the so-called revised Bethesda guidelines[76] (Table 7). Although the Bethesda criteria were less stringent than the Amsterdam criteria, as they include testing for MSI, they should be both more sensitive and more specific.[77]

The Bethesda guidelines recommended a panel of 5 MS markers for use in screening for HNPCC. These include 2 mononucleotides (*BAT 25* and *BAT 26*) and 3 dinucleotides (*D2S123, D5S346* and *D17S250*).[78] Tumours with no instability in any of these markers are considered to be MS stable (MSS). On the other hand, if one marker is mutated, the tumour is regarded to have low MSI (MSI-L) and if 2 or more markers are mutated, the tumour is regarded to have high MSI (MSI-H). For patients with MSI-L tumours, an additional panel of markers is required, e.g. MYCL and/or BAT 40.[76]

Mutation in genes encoding MMR enzymes generally results in abnormal or absent protein products. Consequently, in recent years, immunohistochemistry has been used in order to test for the presence or absence of specific MMR enzymes. Overall, MSI testing and immunohistochemistry appear to be almost equivalent strategies for identifying subjects who should be investigated for MMR germline mutations.[79–83] Different strategies for combining MSI testing and immunohistochemistry have been proposed[84–87], but the optimum sequence of testing remains to be established. Some of the advantages and disadvantages of MSI analysis versus immunohistochemistry as surrogate marker tests for HNPCC are summarised in Table 8.

Because of the difficulty and expense associated with mutation detection in MMR genes and because of the high prevalence of MSI in patients with HNPCC (i.e. >90%), MSI analysis and/or immunohistochemistry of MMR enzymes, are now one of the first steps in testing these patients.[23,61,62,87] According to the American Gastroenterology Association, genetic testing in HNPCC is indicated for affected subjects in families meeting either Amsterdam or modified Bethesda criteria and for first-degree adult relatives of those with known mutation.[61,62] MSI testing using the Bethesda markers[76,78] should be performed on tumour tissue of individuals putatively affected with HNPCC.[61,62] Individuals with MSI-H tumours should be considered for germline testing for

Table 6 – Amsterdam criteria for the clinical diagnosis of hereditary non-polyposis colorectal cancer (HNPCC), FAP, familial adenomatous polyposis

Original Amsterdam Criteria (Amsterdam I criteria)[73]

Three or more relatives with colon cancer plus all of the following:

- One affected patient should be a first degree relative of the other 2
- CRC should involve at least 2 generations
- At least one case of CRC should have been diagnosed before the age of 50 years
- FAP has been excluded.

Revised Amsterdam criteria (Amsterdam II criteria)[74]

Three or more relatives with HNPCC-associated cancer (CRC or cancer of the endometrium, small bowel, ureter or renal pelvis) plus all of the following

- One affected patient should be a first-degree relative of the other 2
- Two or more successive generations should be affected
- Cancer in one or more affected relative should be diagnosed before the age of 50 years
- FAP should be excluded in any case of CRC
- Tumours should be diagnosed by pathological examination

Table 7 – Bethesda criteria for testing CRC tumours for microsatellite instability

Bethesda guidelines (1997)[75]

Only one of these criteria needs to be met

- Individuals with cancer in families that meet the Amsterdam criteria
- Individuals with 2 HNPCC-associated cancers and metachronous CRC or associated extracolonic cancers (endometrial, ovarian, gastric, hepatobiliary, small bowel or transitional carcinoma of the renal pelvis or ureter)
- Individuals with CRC and a first-degree relative with CRC and/or HNPCC-associated extracolonic cancer and/or a colorectal adenoma diagnosed <40 years
- Individuals with CRC or endometrial cancer diagnosed before the age of 45 years
- Individuals with right-sided CRC with an undifferentiated pattern (solid/cribriform) on histology diagnosed at age <45 years
- Individuals with signet-ring cell-type CRC diagnosed at age <45 years
- Individuals with adenomas diagnosed at age <40 years

Revised Bethesda guidelines (2003)[76]

Only one of these criteria needs to be met

- CRC diagnosed at age <50 years
- Presence of synchronous or metachronous CRC or other HNPCC-associated tumours, regardless of age
- CRC with MSH-H histology diagnosed in a patient <60 years of age
- CRC diagnosed in one or more first-degree relatives with an HNPCC-related tumour, with one of the cancers diagnosed at age <50 years
- CRC diagnosed in 2 or more first- or second-degree relatives with HNPCC-related tumours, regardless of age

Hereditary non-polyposis colorectal cancer, HNPCC; FAP, familial adenomatous polyposis.

mutations in the *MSH2* and *MLH1* repair genes. These are the most frequently mutated MMR genes and are responsible for up to 90% of all genetically characterised cases.[70,71] Subjects with MSI-L or MS-S tumours are unlikely to have germline MMR mutations and further genetic testing is not necessary.[61,62] If MSI testing on tumour tissue is not possible, direct *MLH1/MSH2* genetic testing should be carried out.[76] As with APC testing, genetic testing for HNPCC should only be carried out following appropriate genetic counselling.

Table 8 – Advantages and disadvantages of MSI analysis and immunohistochemistry in testing for microsatellite phenotype

MSI testing

Advantages

- Currently regarded as the 'gold standard' for MS phenotype
- More sensitive than immunohistochemistry

Disadvantages

- More difficult and labour-intensive than immunohistochemistry
- Tumour must contain a sufficient number of malignant cells (preferably >50%)
- Approximately 10% of HNPCC patients do not exhibit MSI and about 15% of apparently sporadic CRC have MSI
- In some families with MSH-6 mutations, MSI may not be present.[70]

Immunohistochemistry

Advantages

- Relatively simple and widely available
- Can direct the search for the relevant gene to be screened
- Can be carried out on small amounts of tissue, e.g. from a needle biopsy

Disadvantages

- Difficult to standardise and interpretation is subjective
- May miss a small proportion of MSI-H tumours
- Immunohistochemistry cannot differentiate between active and inactive mismatch repair proteins

Data summarised from Refs. [79–83].

According to an NCCN Panel, if a patient is found to have a MSI-H tumour, genetic testing for mutations in *MLH1*, *MSH2* and *MSH6* should be performed.[23] If HNPCC is then confirmed, colonoscopy is advised between the ages of 20 and 25 or 10 years younger than the youngest age at diagnosis in the family, whichever comes first.[23,87] Colonoscopy should be repeated every 1–2 years. According to Lindor et al.[87], however, colonoscopy may start at 30 years for those with MSH6 mutations.

Endometrial carcinoma is the second most common malignancy in Lynch syndrome carriers. The cumulative risk is up to 50% in female carriers with a mean age of diagnosis of about 50 years in *MLH1* and *MSH2* carriers and 55 years in *MSH6* families.[70,71] According to the NCCN Panel[23], for women carriers, an annual transvaginal ultrasound and endometrial aspirate, starting at ages 25–35 or 5–10 years earlier than the age of first diagnosis of those cancers in the family, should be considered. As regards ovarian cancer, screening with transvaginal ultrasound and CA 125 testing has been recommended.[23,88] Both these procedures, however, have limited accuracy in detecting early ovarian cancer. According to Lynch and de la Chapelle[70], screening for other extracolonic tumours, especially in families with an excess at a specific site, should also be considered.

The value of surveillance in families with HNPCC has been evaluated in a number of studies. After a follow-up of 15 years, Jarvinen et al.[89] concluded that screening every 3 years significantly reduced both the rates of CRC and mortality from CRC. In another study, the introduction of surveillance was also found to result in a significant decrease in mortality from CRC but not from endometrial cancer.[90] In a further study, prophylactic hysterectomy with bilateral salpingo-oophorectomy was shown to reduce endometrial and ovarian cancer in women with HNPCC and documented germline mutations in *MLH1*, *MSH2* and *MSH6* genes.[91]

Following a systematic review of the literature, Lindor et al.[87] recommended in addition to colonoscopy every 1–2

years, annual endometrial sampling and transvaginal ultrasound of the uterus and ovaries (ages 30–35 years), urinalysis with cytology (ages 25–35 years), history, examination, review of systems, education and genetic counselling regarding Lynch syndrome.

The EGTM Panel supports published recommendations[23,61–63,87] for the detection and follow-up of HNPCC patients.

While MSI testing is used as a surrogate marker for HNPCC, it cannot be used for definitive diagnosis. This is because approximately 10% of HNPCC tumours do not exhibit MSI and about 15% of apparently sporadic CRCs display MSI.[70,71] Lack of MLH1 promoter methylation and/or lack of somatic mutation in the *BRAF* (*V600E*) gene in HNPCC cancers can help differentiate most of these from sporadic MSH-H cancers.[92] The presence of MSI in apparently sporadic CRCs is not due to mismatch repair mutation but is mainly due to MLH1 gene silencing as a result of promoter hypermethylation.[93]

6. Future work

Guideline articles should not only contain recommendations on existing markers, but should also identify areas requiring further investigation. In the context of tumour markers in CRC, the following topics should be given priority:

- In order to develop a more accurate screening test for CRC, the existing panel of DNA markers should be expanded to enhance sensitivity. The main focus should be on structurally altered genes that are present in either adenomas with a high predisposition of progressing to invasive carcinoma or in early invasive carcinomas. Specific methylated genes such as vimentin might be considered for addition to the existing panel.[94]
- New prognostic markers are particularly important for patients with stage II colonic cancer. As it is unlikely that any single marker will be sufficiently predictive, gene expression microarrays should be used to identify new markers. A small panel of the strongest markers might then be selected and validated as was recently described for breast cancer.[95] Ideally, these new markers should be validated using a large prospective trial.
- Predictive markers are required for all the main forms of systemic therapy used in CRC. Again, gene expression microarrays should be used to identify these factors and validations via a prospective trial.
- The clinical use of existing markers should be optimised. For example, patients entering prospective randomised trials aimed at evaluating adjuvant chemotherapy for stage II colon cancer patients might either be selected or stratified according to their preoperative CEA level. The use of CEA as a surrogate marker for monitoring therapy in advanced CRC should also be further explored.

In the context of validating new markers, the US National Cancer Institute has recently formed an organisation known as the Early Detection Research Network (EDRN).[96] The aim of this group is to bring together experts from academia and industry in order to promote biomarker discovery and validation, while also helping transfer of this knowledge to clinical practice. The EDRN network has established criteria for the development and validation of new markers for the early diagnosis of cancer. The first phase involves preclinical exploration in order to identify promising leads. Next, there is a clinical assay and validation phase necessary to test the ability of the assay to detect established disease. The third phase is a retrospective and longitudinal study to assess the ability of the new marker to diagnose preclinical disease and to define a 'screen positive' rule. Following this, prospective screening is carried out to identify the extent and characteristics of disease detected by the test, as well as the false-positive rate. In the final phase, a definite trial, such as a prospective randomised study, is performed to evaluate the value of screening on the burden of disease in the general population. These steps should now be implemented to evaluate promising markers for the screening and early diagnosis of CRC.

Finally, for reporting diagnostic studies, the EGTM Panel recommends adherence to previously published guidelines on Standards for Reporting of Diagnostic Accuracy (STARD).[97] For the evaluation of new prognostic and predictive markers, we recommend use of the Reporting Recommendations for Tumour Marker Prognostic Studies (REMARK)[98] guidelines.

Conflict of interest statement

None declared.

Note: The EGTM is an ad hoc group of scientists and physicians from universities, hospitals and the diagnostic industry with an interest in tumour markers.[9] One of its main aims is to produce guidelines on the clinical use of tumour markers. All the authors listed are members of the Gastrointestinal Focus group of the EGTM, apart from CS EH-F and PP who were guest authors.

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