Tumor markers in colorectal cancer

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Abstract

Introduction: In 2008, colorectal cancer (CRC) represented the third most commonly diagnosed tumor in Spain and the second tumor with more deaths. Despite the new potential biomarkers in CRC, there are many challenges that need to be overcome. Standardization of its determinations is necessary.

Discussion: The continuous advance in tumor disease knowledge makes this review a summary of the current accepted, recommended and studied tumor markers for the diagnosis and monitoring of CRC such as fecal markers, tissue markers and serological markers, and various prognostic markers for which there are different lines of treatment in CRC.

Conclusions: Oncological guidelines recommend only a minority of tumor markers for routine use, such as the study of fecal occult blood, carcinoembryonic antigen determination in the postoperative follow-up, microsatellite instability to identify persons susceptible to hereditary nonpolyposis CRC, and mutation of APC in the diagnosis of familial adenomatous polyposis.

Key words: tumoral markers, colorectal cancer.

Introduction

The tumor marker can be defined as an “identifiable” component that is present or that is secreted by the tumor cell.1 The presence of a tumor marker in supraphysiological amounts indicates the presence of neoplastic disease, being potentially useful in screening for early diagnosis (diagnostic markers); determination of prognosis (prognostic markers); prediction of the effectiveness of treatment (follow-up markers); primary clinical control after tumor surgery; and monitoring of treatment in advanced disease.2,3 Their objective shall be, at all times, obtaining favorable clinical results that enable increased disease-free period, increase survival and improve quality of life.4

However, both the lack of sensitivity and specificity in the early stages of neoplastic disease strictly limits the systematic use of most of the markers in the screening of asymptomatic patients. Disease staging at the time of diagnosis, determined by tumor size, degree of cytologic differentiation and lymph node involvement constitutes the most widely used prognostic indicator in patients with colorectal cancer (CRC). However, it also is used complementary to the valuation of certain prognostic markers, quantified and determined both in tumor tissue as in peripheral blood. The prognostic value of these tumor markers will lie in the relationship established with the tumor size or with the presence of distant metastases.2,5

According to Duffy in 1981, the National Health Information Center (NHIC) established that carcinoembryonic antigen (CEA) monitoring was the best available noninvasive technique for the determination of recurrence in patients with diagnosis of prior CRC.4 Locker et al., in 1996, for the American Society of Clinical Oncology (ASCO), published clinical guidelines based on evidence for the use of tumor markers in CRC, having carried out two reviews in 2000 and 2006.6 Duffy et al. in 2007, in the European Group of Tumor Markers (EGTM), updated the guidelines for the use of serum, tissue and fecal markers in CRC.7

Despite multiple tumor markers having been studied, only a few are routinely recommended. Summarizing the markers established by the different oncology guidelines, an occult blood study of the stool is proposed for early di-
agnosis in persons >50 years of age, determination of CEA in the postoperative period, follow-up of patients amenable to systemic chemotherapy or surgical resections, instability of microsatellites for identification of those persons who should have genetic study carried out for MLH1, MSH2, MSH6, PMS2 for identification of nonpolyposis hereditary CRC; and APC mutation in the diagnosis of the familial adenomatous polyposis (FAP).

This study is intended to be a review of the medical literature (PubMed) of the principal tumor markers recommended by the principal oncology guidelines (in stool, tissue and serological). A search was undertaken with the following terms: tumor markers or tumor biomarkers and colorectal cancer or colon cancer.

**Tumor Markers in Colorectal Cancer**

**Fecal Markers**

In CRC, study of tumor markers in stools has an eminently diagnostic function. The most widely used determination is the fecal occult blood or the guaiac test or by immunological tests. According to the National Comprehensive Cancer Network (NCCN), quantification of fecal occult blood should be performed in three successive samples obtained during the execution of a pre-established diet.

Given that the sensitivity and specificity of fecal occult blood are 40-80% and 70%, respectively, various investigations have focused on the identification of certain fecal mutated genes in the early stages of neoplastic disease. These genes, such as K-ras, TP53, APC, L-DNA, BAT-26, specific methylated genes and microsatellite instability achieved a specificity of 95% and a sensitivity of 60-90%, although the latter is lower in asymptomatic patients.

Different studies have undertaken the determination of fecal DNA to quantify the values of different microRNAs in feces such as overexpression of miR-21 and miR-106a in colorectal neoplastic lesions and hypermethylation of the promoter miR-34b/c in the stool, present in up to 75% of patients with CRC in correlation with tumor stage. Similarly, in the stool, overexpression of miR-20a, miR-21, miR-92, miR-96, miR-106a, miR-203 and miR-326, and low levels of miR-16, miR-125b, miR-126, miR-143, miR-144, miR-145, miR-320 and miR-484-5p have been evidenced.

In the study by Koga et al., for miR-17-92 and miR-135 the sensitivity values of 70% and 46%, with a specificity of 81% and 95%, respectively, were determined.

The National Academy of Clinical Biochemistry (NACB) recommends that all individuals aged ≥50 years undergo procedures for early diagnosis of CRC. Even though the occult blood determination in the stool is a more valid method, DNA determination of the stool could be another option regarding risk, local availability and personal preferences.

**Tissue Markers**

Tissue markers are mainly used for establishing a prognosis of CRC among which are included: thymidylate synthase enzyme participating in DNA synthesis, which is quantified to determine the prognosis and to predict therapeutic response by being a target of different cytotoxins such as overexpression of thymidylate synthase, which leads to greater resistance to treatment with 5-fluorouracil. Dihydropyrimidine dehydrogenase, an enzyme involved in the metabolism of 5-fluorouracil, and thymidine phosphorylase have been studied in determining the therapeutic response in CRC.

Determination of microsatellite instability in the study of activity of the repair system of the defects of the DNA have been used to establish the benefit of adjuvant chemotherapy to surgery in initial stages of CRC because of its relationship with the therapeutic response and association with a better prognosis. It has been determined that tumor lesions with high microsatellite instability have a better prognosis than tumor lesions with low microsatellite instability, correlating K-ras and of TP53 mutations. The TP53 mutation is correlated with the degree of tumor differentiation, with the risk of developing metastasis and with poor response to radiation therapy in rectal neoplasms.

The loss of heterozygosity of the long arm of chromosome 18 has shown to be an important step in the development of many cases of CRC, being related with a poor prognosis. Among the genes localized in this level are SMAD-4, SMAD-2 and DCC that codify a receptor involved in apoptosis, with cellular adhesion and tumor suppression. It has been shown that stage II patients associated with 18q(-) have a prognosis similar to patients with stage III disease, whereas patients in stage II and 18q(+) behave in a manner similar to patients in stage I. It has also been proven that the loss of DCC expression is associated with liver metastasis: patients with stage II disease that express DCC have a 5-year survival of 94.3%, whereas loss of DCC implies a survival rate of only 61.6%. Similarly, it has been resorted to ploidy valuation to determine the prognosis of the neoplastic disease. The presence of aneuploidy and high DNA index has been related with a greater recurrence of the tumor and lower survival rate in stage III CRC. Another parameter of CRC survival is the analysis of proliferation, expressed by the percentage of phase S cells of the cellular cycle.

It is known that tissue inhibitors of the metalloproteinases type I (TIMP-I), whose function lies in promoting cel-
cular proliferation and inhibition of protein metabolism and apoptosis, demonstrate elevated concentrations in CRC, in intestinal inflammatory disease, in adenomas and in breast neoplasms. The consideration that they are associated with a poor prognosis has led to propose its clinical use—jointly—with the CEA for identification of patients at risk for recurrence.5,7,14

Since Michael et al.15 demonstrated the decrease in the expression of miR-143 and of miR-145 in CRC, the medical bibliography has gathered different microRNAs over-expressed in colorectal neoplasms: miR-15b, miR-17-5p, miR-19a, miR-20, miR-21, miR-29a, miR-31, miR-92, miR-96, miR-135b, miR-148a, miR-181b, miR-182, miR-183, miR-191, miR-200b, miR-200c and miR-212. In contrast, in values lower than those that exist in normal conditions are miR-1, miR-9-1, miR-30a-3p, miR-30a-5p, miR-30c, miR-34a-c, miR-126, miR-129, miR-133a, miR-133b, miR-137, miR-139, miR-143, miR-145, miR-195, miR-342, miR-422a, miR-422b and let-7a-1.16,17

Nevertheless, the NACB does not recommend the determination of tissue markers to establish the prognosis or to predict the therapeutic response, asserting that only exceptionally it could proceed to the determination of the K-ras mutation to establish those patients who could benefit from anti-EGFR (endothelial growth factor receptor) therapy. The analysis of patients with established diagnosis of CRC has determined that ~60% have non-mutated K-ras (wild type), with a great probability of benefitting from anti-EGFR therapies.3,5,18 K-ras mutations are characterized for being simple mutations that affect the amino acids that intervene in intracellular transduction.

The mutated product of K-ras induces cellular proliferation and is found to be involved in the process of tumor invasion and of metastasis. The frequency of mutations at the K-ras level is high, between 15 and 68% as the size of the adenoma increases, whereas in CRC the K-ras mutation has been determined in up to 50-90%.19,20

**Serum Markers**

CEA was described for the first time in 1965 as an intracellular glycoprotein that was expressed in ~90% of colorectal tumors. CEA is present in the fetal colon as well as in adenocarcinomas of the colon, but not in the colon of healthy adults, which has caused that this structure be the most widely used tumor marker in colon cancer.21 CEA levels could be elevated in other pathological situations such as gastric, pulmonary, pancreatic, breast neoplasms or in medullary thyroid cancer and in noncancerous situations such as cirrhosis, ulcerative colitis, pancreatitis and in smokers.22

The ASCO clinical guidelines (The American Society of Clinical Oncology), EGTM (European Group on Tumour Markers) and NACB do not recommend the use of CEA in the screening of colorectal lesions.5-7 Currently, it is believed that the main prognostic factors related with postoperative survival are age, TNM staging, tumor grade, lymph and/or vascular involvement, preoperative CEA related with a poor prognosis with values >5 mg/mL. CEA may provide prognostic information although it should not be used in the selection of patients for adjuvant therapy.5,6,23

Percentages of recurrences in CRC are from 60-80% in the first 2 years and 90% in the first 4 years. It has been shown that there is a relationship between the postoperative elevation of the CEA and tumor recurrence with an 80% sensitivity and 70% specificity.4,5,7 Given that the postoperative values of the CEA normalize 4 to 8 weeks after surgery, failure to decrease the CEA numbers postoperatively should raise suspicion of an incomplete resection or of the existence of a micrometastasis. The presence of an abnormal postoperative CEA implies a relative risk of 3.778 in the evolution towards a recurrence.24 In the evaluation of the postoperative CEA for early diagnosis of metastatic disease, it has been established that a significant increase of CEA occurs if the elevation is at least 30% of the previous values, needing to be confirmed a month later. It is then necessary to confirm the diagnosis by means of additional tests such as computed tomography or colonoscopy. In evolutionary controls after curative treatment of the primary tumor by intensive monitoring of CEA, various meta-analyses have demonstrated superior clinical results, which are reflected in higher survival rates. The ASCO guidelines, as well as the NACB, establish that intensive follow-up implies determination of the CEA every 2 to 3 months for 3 years in patients with stage II or III CRC because of the possibility of developing liver metastasis that are susceptible to surgery and/or CT.2,5-7

In advanced disease, the ASCO has established the determination of the CEA as a marker of choice for monitoring during systemic therapy. Its value should be established at the start of treatment and each 3 months during treatment. Increase in the CEA values suggest disease progression even in the absence of radiological confirmation, with the elevation in CEA values in the initial phases of treatment needed to be cautiously interpreted. In patients with liver metastasis, evolution of the CEA and CA 19-9 levels have been correlated with the radiological response after preoperative chemotherapy with a sensitivity and specificity of 90%.2,5,6,25

The NACB does not recommend the determination of CEA in the early diagnosis of CRC as a screening method for healthy patients. With respect to the studies of CEA levels for the determination of prognosis and follow-up, the
NACB establishes that the preoperative determinations of CEA combined with other factors could serve as a basis for surgical treatment planning, but not so in the selection of patients for chemotherapy. In patients who present with an increase in the CEA concentrations, it is advised that they undergo testing for the presence of distant metastasis.\textsuperscript{5}

**Other Tumor Markers**

The carbohydrate antigen 19-9 (CA 19-9), sialylated Lewis antigen, has a lower sensitivity than CEA. It has been determined that concentrations of CA 19-9 have a prognostic value in CRC, related to the values of CEA and CA 19-9 with time passed, disease progression, reappearance and with overall survival.\textsuperscript{26} The ASCO and the NACB establish that there does not exist sufficient evidence to recommend its routine study in population screening, in early diagnosis, in staging, during follow-up or treatment monitoring. Similarly, CA 242 is not recommended on a routine basis, despite it being able to complement the CEA.\textsuperscript{5,6}

The aberrant expression of microRNAs in blood, which have been found in different tumors among which is CRC, allows the use of circulating microRNAs as tumor markers, having been proposed as markers of early diagnosis. Plasma values of miR-17-3p and miR-92a that are found to be elevated in colorectal neoplasms decrease during the postoperative period. MicroRNAs that have elevated plasma concentration in patients with colon and rectal cancers are miR-29a, miR-95, miR-135b, miR-221, miR-222 and miR-141, the latter being related with stage IV tumors. It has been affirmed that the determination of miR-141 associated with the CEA would increase the detection of liver metastases.\textsuperscript{10,27-32}

**Prognostic Markers**

A large variety of tumor markers have been identified in CRC, with the intent of finding new markers to help increase disease survival in patients. Prognostic markers have been described that correlate both with disease-free survival (DFS) as with survival, thereby being predictive markers to establish a specific treatment response. The American Joint Committee on Cancer (AJCC) established the first tumor-node-metastasis (TNM) classification to express disease extension, provide a useful method for patient treatment, establish a prognosis and provide the possibility of staging the results.\textsuperscript{13}

The therapeutic guidelines of the National Cancer Institute and of the National Comprehensive Cancer Network (NCCN) include different drugs approved by the Food and Drug Administration (FDA) for chemotherapy treatment of CRC. At present, anti-tumor drugs used are oriented at inhibiting the DNA metabolism, block the endothelial growth factor receptor (EGFR) and interact with the vascular endothelial growth factor (VEGF).

The potential benefits of endothelial anti-growth treatments lie in the presence of a nonmutated K-ras such as previously described. The anti-growth factor treatments of the vascular endothelium have the objective of inhibition of tumor angiogenesis, a line of investigation where the angiogenic markers demonstrate their relationship with the prognosis and disease survival.\textsuperscript{35}

**Angiogenic Markers**

Angiogenesis is one of the main physiopathological processes implicated in disease prognosis. There are studies that seek the prognostic-predictive relationship between CRC and angiogenesis with coagulation, fibrinolysis and plasminogen systems.\textsuperscript{26} VEGF has demonstrated high values of specificity and effectiveness, which give it a key role in angiogenic regulation.\textsuperscript{26,34} The VEGF family is constituted by VEGF-A (called VEGF), VEGF-B, VEGF-C, VEGF-D, VEGF-E and PGF (placenta growth factor). Among the functions of the VEGF are vasodilation, vascular permeability, and stimulus of protease synthesis and synthesis of receptors implicated in invasion, proliferation, cell migration and tissue remodeling. In tumor angiogenesis the VEGF participates in the activation of the coagulation system, adhesion interactions between endothelial surface integrins and extracellular matrix, and control of extracellular proteolysis.\textsuperscript{26}

VEGF is found to be elevated in patients with CRC and its values decrease after surgery. VEGF levels have been related to disease stage, tumor progression, presence of distance metastasis, recurrence of the neoplasm, poor response to chemotherapy and radiotherapy in rectal cancer and with lower survival rate.\textsuperscript{35,38} For this reason, VEGF has been recommended as a prognostic marker in CRC but is exempt from diagnostic value.

In angiogenesis, VEGF and fibroblast growth factor (FGF) induce the expression of urokinase-type plasminogen activator (uPA), its receptor (uPA), tissue plasminogen activator (t-PA) and plasminogen activator inhibitor (PAI-1). PAI-1 levels associated with chemotherapy have been associated with disease progression and survival prognosis.\textsuperscript{26,37} We also studied the relationship between the coagulation system and CRC, correlating levels of von Willebrand factor, D-dimer, fibrinogen and platelets with overall survival of patients with CRC and are considered as prognostic factors for survival, predicting therapeutic response or disease progression, much more specific and earlier than CEA or CA 19-9.\textsuperscript{26,38}
In conclusion, despite having described the usefulness of various tumor markers, different constraints exist for implantation as routine clinical techniques. Manipulation of the samples and the procedures for determination of new tumor markers implies having available laboratories with the most recent technology and training. This carries the need for major financial involvement plus the added cost involved in repeated determinations of different products such as fecal DNA determinations or microRNAs.

Some tumor markers proposed by the Oncology Guidelines to be used routinely are as follows:

- **Study of fecal occult blood for early diagnosis**
- **Determination of CEA in monitoring postoperative patients after systemic chemotherapy or surgical resection**
- **Microsatellite instability to identify subjects susceptible to hereditary nonpolyposis CRC**
- **APC mutation in the diagnosis of FAP**

Due to the large variability in detection methods of new prognostic markers in CRC, it is necessary to standardize their determinations.

Conflict of interest: The authors declare that there are no conflicts related to this study.

**References**


