



WJG 20th Anniversary Special Issues (5): Colorectal cancer

Colorectal cancer: From prevention to personalized medicine

Gemma Binefa, Francisco Rodríguez-Moranta, Àlex Teule, Manuel Medina-Hayas

Gemma Binefa, Cancer Prevention and Control Program, Catalan Institute of Oncology, IDIBELL, CIBERESP, Hospitalet de Llobregat, 08908 Barcelona, Spain

Gemma Binefa, Department of Clinical Sciences, University of Barcelona, Hospitalet de Llobregat, 08908 Barcelona, Spain

Francisco Rodríguez-Moranta, Endoscopy Unit, University Hospital of Bellvitge, IDIBELL, CIBERESP, Hospitalet de Llobregat, 08908 Barcelona, Spain

Àlex Teule, Genetic Counseling Unit, Catalan Institute of Oncology, Hospitalet de Llobregat, 08908 Barcelona, Spain

Manuel Medina-Hayas, Department of Morphological Sciences, Universitat Autònoma de Barcelona, Bellaterra, 08193 Barcelona, Spain

Author contributions: All of the authors contributed in each phase of the paper (design, acquisition and interpretation of data); all authors revised the draft of the manuscript and approved the final version.

Supported by Partially funded by the Carlos III Health Institute No. PI11/01593

Correspondence to: Gemma Binefa, MD, MPH, Cancer Prevention and Control Program, Catalan Institute of Oncology, IDIBELL, CIBERESP, Hospitalet de Llobregat, Av. Gran Via 199-203, 08908 Barcelona, Spain. gbinefa@iconcologia.net

Telephone: +34-93-2607351 Fax: +34-93-2607956

Received: September 28, 2013 Revised: January 16, 2014

Accepted: March 6, 2014

Published online: June 14, 2014

Abstract

Colorectal cancer (CRC) is a very heterogeneous disease that is caused by the interaction of genetic and environmental factors. CRC develops through a gradual accumulation of genetic and epigenetic changes, leading to the transformation of normal colonic mucosa into invasive cancer. CRC is one of the most prevalent and incident cancers worldwide, as well as one of the most deadly. Approximately 1235108 people are diagnosed annually with CRC, and 609051 die from CRC annually. The World Health Organization estimates an increase of 77% in the number of newly diagnosed cases of CRC

and an increase of 80% in deaths from CRC by 2030. The incidence of CRC can benefit from different strategies depending on its stage: health promotion through health education campaigns (when the disease is not yet present), the implementation of screening programs (for detection of the disease in its early stages), and the development of nearly personalized treatments according to both patient characteristics (age, sex) and the cancer itself (gene expression). Although there are different strategies for screening and although the number of such strategies is increasing due to the potential of emerging technologies in molecular marker application, not all strategies meet the criteria required for screening tests in population programs; the three most accepted tests are the fecal occult blood test (FOBT), colonoscopy and sigmoidoscopy. FOBT is the most used method for CRC screening worldwide and is also the primary choice in most population-based screening programs in Europe. Due to its non-invasive nature and low cost, it is one of the most accepted techniques by population. CRC is a very heterogeneous disease, and with a few exceptions (*APC*, *p53*, *KRAS*), most of the genes involved in CRC are observed in a small percentage of cases. The design of genetic and epigenetic marker panels that are able to provide maximum coverage in the diagnosis of colorectal neoplasia seems a reasonable strategy. In recent years, the use of DNA, RNA and protein markers in different biological samples has been explored as strategies for CRC diagnosis. Although there is not yet sufficient evidence to recommend the analysis of biomarkers such as DNA, RNA or proteins in the blood or stool, it is likely that given the quick progression of technology tools in molecular biology, increasingly sensitive and less expensive, these tools will gradually be employed in clinical practice and will likely be developed in mass.

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Key words: Colorectal cancer; Prevention; Mass screen-

ing; Biological markers; Drug therapy

Core tip: Although there are different strategies for screening, the number of which is increasing due to the potential of emerging technologies in molecular marker application, not all strategies meet the criteria required for screening tests in population programs; the three most accepted tests are fecal occult blood test, colonoscopy and sigmoidoscopy.

Binefa G, Rodríguez-Moranta F, Teule À, Medina-Hayas M. Colorectal cancer: From prevention to personalized medicine. *World J Gastroenterol* 2014; 20(22): 6786-6808 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v20/i22/6786.htm> DOI: <http://dx.doi.org/10.3748/wjg.v20.i22.6786>

COLORECTAL CANCER PATHOGENESIS

Colorectal cancer (CRC) is a very heterogeneous disease that is caused by the interaction of genetic and environmental factors and can be classified based on the importance of each of these factors. The majority of CRCs are sporadic (70%-80%), with age being the most important risk factor. Only a small proportion of cases are due to inherited forms, either familial adenomatous polyposis (less than 1%), non-polyposis hereditary CRC or Lynch syndrome (2%-5%) or *MYH*-gene associated polyposis (< 1%)^[1]. An additional 20%-25% of cases are estimated to have an associated hereditary component, which has not yet been well established and is known as familial CRC^[2].

CRC develops through a gradual accumulation of genetic and epigenetic changes, leading to the transformation of normal colonic mucosa into invasive cancer. Most CRC develops from adenomas (adenoma-carcinoma sequence), and the neoplastic transformation time is considered approximately 10-15 years, which represents the available time to detect and remove these adenomas before their progression to invasive carcinoma.

Three main routes of CRC carcinogenesis are currently considered. The first known was proposed by Fearon *et al.*^[3] and is called the suppressor pathway or pathway of chromosomal instability. This route involves the accumulation of mutations that leads to oncogene activation (*KRAS*) and suppressor gene inactivation (*DCC*, *APC*, *SMAD4*, *TP53*). The accumulation of these molecular alterations, regardless of the order in which they are acquired, is responsible for neoplastic transformation^[4]. A second mechanism involves the accumulation of errors during DNA replication due to the presence of mutations in genes responsible for its repair (*MSH2*, *MLH1*, *MSH6*, *PMS2*, *MLH3*, *MSH3*, *PMS1* and *Exo1*)^[5]. These errors accumulate predominantly in repetitive DNA fragments (microsatellites) scattered throughout the genome, resulting in mutations in various target genes. This mutator pathway or microsatellite instability is involved in Lynch syndrome and in 15%-20% of sporadic CRCs^[4]. The mutator pathway tumors occur more frequently

in older women and locations proximal to the splenic angle^[6]. These tumors are histologically characterized by an increased lymphocyte infiltration (Crohn-like) and as being mucinous and poorly differentiated tumors^[7].

Finally, a third route of carcinogenesis has been recently identified in the field of epigenetics: aberrant hypermethylation, a mechanism to silence gene function. Dinucleotide methylation in the promoter region of many genes has been referred to as the CpG island methylator phenotype (CIMP). The CIMP is responsible for 15%-20% of sporadic CRC. A tumor is considered to be CIMP-positive if it exhibits methylation of at least 3 of the following markers: *CACNA1G*, *IGF2*, *NEUROG1*, *RUNX3* and *SOCS1*^[8]. Methylating pathway tumors occur more frequently in women and elderly people, are preferably located in the right colon and do not benefit from treatment with 5-fluorouracil (5-FU). These tumors are histologically poorly differentiated tumors with mucinous differentiation or signet rings, exhibit microsatellite instability and are *BRAF* mutation carriers. The precursor lesions of these CIMP tumors are sessile serrated adenomas^[9]. A better understanding of carcinogenesis pathways has allowed the development of diagnostic and prognostic markers as well as the investigation of new therapeutic targets and predictors of response to cancer treatments.

EPIDEMIOLOGY

CRC is one of the most prevalent and incident cancers worldwide, along with lung and breast cancers, and is one of the most deadly. Approximately 1235108 people are diagnosed annually with CRC, and approximately 609051 die from CRC annually^[10].

CRC is more frequent and causes more deaths in men than in women worldwide, except in the Caribbean. CRC is the third most common cancer in men (663000 cases/year) and the second most common cancer in women, after breast cancer, with 571000 cases a year.

Approximately 60% of CRC cases are diagnosed in developed countries, and after Japan, Europe represents one of the regions with the highest rates both in incidence and mortality.

Japan is one of the countries with the highest incidence rate, especially in men (41.7 cases per 100000); despite this fact, CRC mortality rates are below those of Europe^[10]. This low mortality rate is due, in part, to the effect of the screening program implemented since 1992, one of the first in the world, along with Italy and Israel^[11].

In Europe, CRC is the third most common cancer and is one of the leading causes of cancer death. An estimated 432414 new cases and 212219 deaths occur each year due to CRC, which represents an age-standardized rate of 29.6 and 13.3 per 100000, respectively^[12].

Although historically the incidence and mortality rates in the US have remained above those in Europe, this relationship has recently changed. According to the latest GLOBOCAN data^[10], the standardized incidence rate

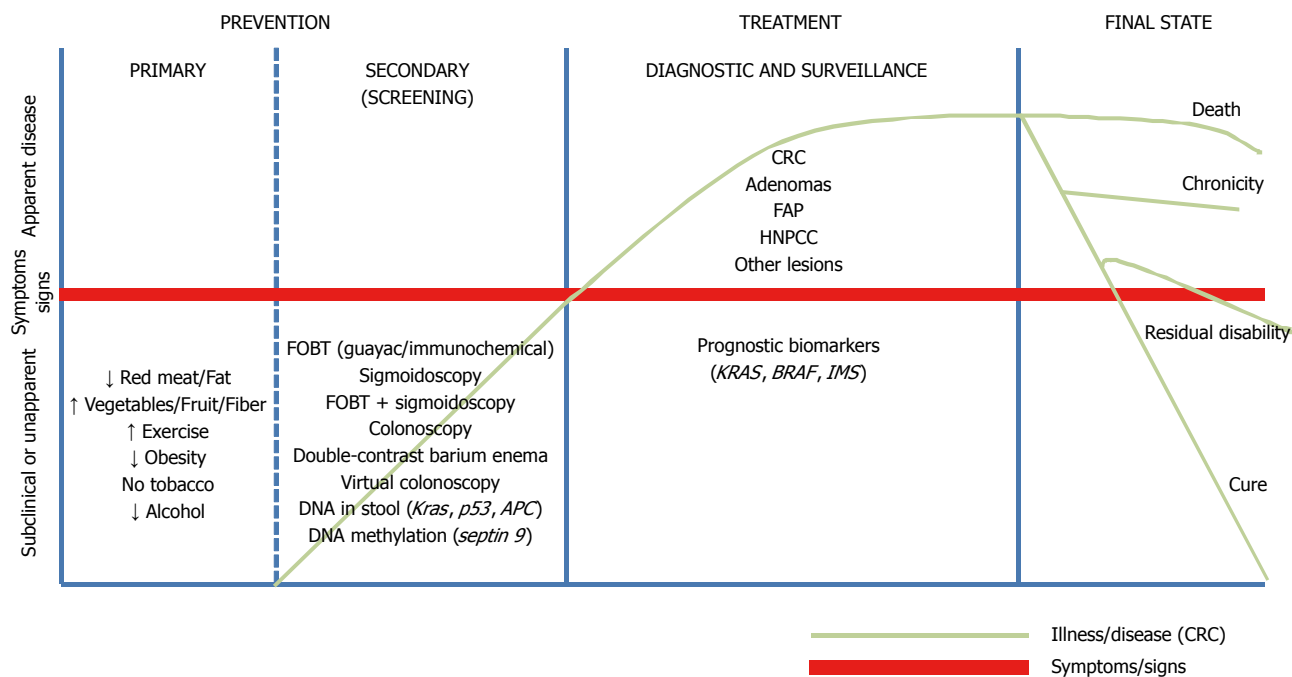


Figure 1 Colorectal cancer: From prevention to treatment. The figure shows the different alternatives to prevent and treat colorectal cancer (CRC). When the disease is not present, the best alternative is to have a healthy lifestyle (primary prevention). To detect CRC in its early stages without symptoms, screening programs are the paramount option (secondary prevention); and finally, when symptoms appear, the treatment to be considered will depend to the existence of prognostic biomarkers and the personal or family history. All these factors will be decisive in the evolution of the disease. FOBT: Fecal occult blood test; FAP: Familial adenomatous polyposis; HNPCC: Hereditary nonpolyposis colorectal cancer.

by age in the US stands at 29.2 cases per 100000, with a mortality rate of 8.8. It has been estimated that Europe is undergoing a minimum annual increase of 0.5% in CRC incidence.

The European countries with the highest incidence rates of CRC in men are Slovakia, Hungary and Czech Republic, all with results greater than 50 cases per 100000. In women, the highest rates (> 30 cases per 100000) are observed in Norway, Denmark and the Netherlands^[12,13].

Within Europe, Spain is positioned slightly above the European average in terms of incidence rate (30.4 cases per 100000)^[12,14], although the mortality rate is average (13.3 per 100000). CRC is the most common tumor in Spain when considering both sexes together and is the second leading cause of cancer death in both men and women. Estimations for next year predict a pattern similar to the present one, with increased mortality in men and a stabilization in women^[15]. There is a marked geographic variation in CRC rates, with Catalonia presenting the highest incidence of this tumor, with an adjusted rate above the European average in men^[16].

Based on current incidence and mortality rates as well as on projected demographic changes in the world population for the coming decades, the World Health Organization (WHO) estimates an increase of 77% in the number of newly diagnosed CRC cases and an increase of 80% in deaths from CRC by 2030^[13,17]. Most of the additional incidence and mortality would occur in the world's less developed regions. This estimation could be higher if developing countries continue with an increasingly Westernized lifestyle^[18].

Since the 1990s, there has been an improvement in CRC relative survival at 5 years in both sexes that can be explained by an early diagnosis at initial stages, a breakthrough in the treatment of stage II and III disease and also a decrease in postoperative mortality. Different theories have been proposed to explain the difference in the CRC mortality rate between men and women, one of which is that the use of hormone replacement treatment in women may be a protective factor^[19]. Other factors that could explain the different patterns of mortality are an increased access to health care and the adoption of healthier lifestyles by women.

Current data confirm the need to urgently incorporate measures to improve the situation, taking into account both the accelerated aging process and estimates of an increase in incidence rates. As shown in Figure 1, CRC can benefit from different strategies depending on its stage: health promotion through health education campaigns (when the disease is not yet present), the implementation of screening programs (for the detection of the disease in its early stages), and the development of nearly personalized treatments according to both patient characteristics (age, sex) and the cancer itself (gene expression).

PRIMARY PREVENTION (RISK FACTORS)

As previously mentioned, over 70% of CRC cases are sporadic and thus related to lifestyle. Despite being one of the most common cancers, CRC is also one that could benefit most from prevention through primary and secondary prevention strategies, and it is estimated that be-

tween 66% and 75% of CRC cases could be avoided with a healthy lifestyle^[20].

The known risk factors for CRC are as follows: a diet low in fruit and vegetables, excessive intake of red meat and saturated fat, alcohol intake, a sedentary lifestyle, tobacco and being overweight^[21]. However, the main risk factor is age. From 50 onwards, CRC is much more frequent, and the incidence increases exponentially with age.

Primary prevention is the best strategy to avoid CRC, but health promotion programs, aimed at changing dietary and hygiene habits, have long-term results and should therefore be complemented by other more immediate impact strategies such as secondary prevention.

SECONDARY PREVENTION OR SCREENING

Among the different CRC prevention options, secondary prevention is considered one of the most appropriate, as it can detect cancer precursor lesions (reducing incidence and mortality) and/or early-stage disease, when treatment is more effective (also reducing mortality).

Due to its high morbidity and mortality, its well-known natural history, the diagnostic methods to detect the disease in early stages or as even precursor lesions, and treatments that can improve survival if implemented in early stages, CRC meets the main requirements established by the WHO to be screened^[22].

It is well known that the main CRC prognostic factor is the stage at diagnosis. CRC survival at 5 years is between 50% and 60%^[23] and is higher in the initial stages (75%-90%) than in advanced stages (< 15%)^[24]. Many CRCs are detected from the presence of signs or symptoms, which typically appear in advanced phases; in these cases, a quick diagnosis does not ensure a better prognosis, as the presentation of any symptoms may indicate the presence of advanced CRC. Thus, early detection and implementation, ideally, of CRC population-screening programs is of paramount importance. However, not everyone is likely to benefit from the programs; target population are men and women over 50 years (as this is the age from which developing CRC is more common) who are asymptomatic and lack a personal or family history of CRC, the so called average risk population. There are rapid diagnostic circuits for cases with symptoms or a high suspicion diagnosis and genetic counseling units or high risk clinics for cases with a family history.

In 2000, the Advisory Committee on Cancer Prevention recommended to European Union member states the use of CRC screening in the asymptomatic population from 50 years of age^[25].

Although there are different strategies for screening, the number of which is increasing due to the potential of emerging technologies in molecular marker application, not all strategies meet the criteria required for screening tests in population programs: (1) acceptable sensitivity and specificity; (2) a demonstrated reduction in CRC mortality; (3) acceptable by the target population; and (4)

low cost. In this sense, the three most accepted tests are the fecal occult blood test (FOBT), colonoscopy and sigmoidoscopy.

European population screening programs typically use the biennial FOBT as a screening test and colonoscopy as a confirmatory exploration (in positive FOBT cases)^[26]. In contrast, in Japan, the interval is an annual FOBT. In the US, recommendations are either of one annual FOBT, sigmoidoscopy, double-contrast barium enema (DCBE) or CT colonography every 5 years or colonoscopy every 10 years. Among the various implemented programs, there are other differences in addition to the screening intervals, such as the target population to which they are addressed or the type of test used^[27,28] (Table 1).

However, there are many other techniques that, although far from being incorporated into screening programs, are extremely useful for the early diagnosis of CRC and for determining the course of treatment.

All tests or techniques (screening or not) can be divided into two groups as detailed below: (1) explorations to visualize the large intestine; and (2) analyses of biological samples.

EXPLORATIONS TO VISUALIZE THE LARGE INTESTINE

Colonoscopy

Colonoscopy is considered the gold standard of excellence for the diagnosis of colorectal pathologies.

Although its use is clearly associated with a reduction in CRC-related deaths, there are no results from randomized controlled clinical trials demonstrating its effectiveness. However, other studies (prospective observational, case-control) have reported a significant reduction in CRC mortality and incidence, reaching, in some cases, up to 65% and 67%, respectively^[29,30], some of them highlighting the differences according to the lesion location, being more favorable for the left colon^[31,32].

Two major multicenter randomized clinical trials are currently being conducted in Europe to assess colonoscopy effectiveness. The NordICC study is being conducted in the Nordic countries, the Netherlands and Poland and compares colonoscopy (22000 people between 55 and 64 years old are invited to undergo once-only colonoscopy) with no screening (44000 people of the same age)^[33]. The COLONPREV is a Spanish study in which over 8 regions collaborate and in which the performance of a once-only colonoscopy (group of 26703 subjects from 50 to 69 years old) is compared with a biennial immunological fecal occult blood test (FIT) (26599 subjects)^[34]. The final results of both studies are expected in 2026 and 2021, respectively.

However, colonoscopy has some disadvantages. Because it is an invasive test, the procedure is not exempt of complications, with perforation and post-polypectomy bleeding being the most serious. According to the European Guidelines for quality assurance in CRC screening

Table 1 Characteristics of some colorectal cancer screening programs worldwide

Country	Test	Periodicity	Target population (age)	Year ²
Germany ¹	FOBT	Annual	50-54	1971
	FOBT or CS	Biennial/Every 10 yr	≥ 55	
Italy	FOBT	Biennial	50-69/74	1982
	FS	Once-only	58-60	
Israel	FOBT	Annual	50-74	early 1990s
Japan	FOBT	Annual	≥ 40	1992
United States ¹	FOBT	Annual	50-75	1994
	FS/FOBT	Every 5/Every 3 yr		
	CS	Every 10 yr		
Taiwan	FOBT	Biennial	50-69	1995
Spain	FOBT	Biennial	50-69	2000
Poland ¹	CS	Periodic	50-66	2000
Czech Republic ¹	FOBT	Annual	50-54	2000
	FOBT/CS	Biennial/Every 10 yr	≥ 55	
France	FOBT	Biennial	50-74	2002
Finland	FOBT	Biennial	60-69	2004
South Korea	FOBT	Annual	≥ 50	2004
Latvia ¹	FOBT	Annual	≥ 50	2005
Australia	FOBT	Biennial	55-74	2002
England	FOBT	Biennial	50-74	2006
The Netherlands	FOBT	Biennial	60-69	2006
Canada				2007
Ontario	FOBT	Biennial	≥ 50	
Manitoba	FOBT	Biennial	50-74	
Croatia	FOBT	Biennial	50-74	2007
Scotland	FOBT	Biennial	50-74	2007
Sweden	FOBT	Biennial	60-69	2008

¹Most countries have population-based screening programs (national or regional) except Czech Republic, Germany, Latvia, Poland and United States (which all have opportunistic screening). ²It refers to the year which some kind of screening began (pilot, population-based or opportunistic). FOBT: Fecal occult blood test; FS: Flexible Sigmoidoscopy; CS: Colonoscopy.

and diagnosis^[28], major complications occur in 3‰ to 16‰ of examinations, depending on whether the colonoscopy is chosen as a screening test or as a confirmatory test after a positive FOBT.

Furthermore, a lesion miss rate ranging between 6% and 12% for large polyps and 5% for cancers has been described^[35-38]. Thus, it is important to ensure good colonic cleansing and to use experienced endoscopists with an extensive history of examinations performed over his or her career and a minimum of annual procedures^[28,39,40].

Other limitations of the use of colonoscopy as a screening test are its cost and its lower acceptance by the population (because of the requirement of a specific diet and the intake of a bowel cleansing preparation, the fear of anesthesia and the exploration itself or shame)^[41-44]. In the COLONPREV study previously mentioned^[34], the participation rate was higher in the FIT group than in the colonoscopy group (34.2% *vs* 24.6%). In an Italian trial in 2007, the results were very similar, with an attendance rate of 32.3% for FIT and 26.5% for colonoscopy^[44]. These data demonstrate the clear preference of the population.

As the gold standard test for the detection of colorectal pathology, colonoscopy exhibits the best results, exceeding 98% sensitivity and 99% specificity for lesions > 6 mm^[45-47].

Assuming an exploration under perfect conditions (excellent or good colonic cleansing that allows a view of

> 90% of the mucosa and cecal intubation) and taking into account the natural history of CRC, the different guidelines currently available recommend a colonoscopy every 10 years for an average-risk population starting at an age of 50 years^[28,48,49]. The cases with family or personal history with a high risk of developing CRC must follow different controls to the average-risk population, usually by reducing the interval between surveillance colonoscopies.

Sigmoidoscopy

With respect to colonoscopy, sigmoidoscopy has the advantage of requiring less preparation and is typically performed without sedation. However, sigmoidoscopy has the great disadvantage of only detecting distal colon neoplasms.

The decision to perform a colonoscopy if a neoplasia is detected with sigmoidoscopy is controversial and must be individualized. Factors associated with an increased risk of proximal neoplasia include age > 65 years, villous histology in the distal lesion, distal adenoma ≥ 1 cm or multiple adenomas in the distal colon, and family history of CRC^[50,51]. Several studies demonstrate that the prevalence of advanced adenomas in the proximal colon in patients without distal lesions is only 2%-5%. Moreover, evidence also suggests that the risk of proximal advanced neoplasia in individuals with only hyperplastic polyps in the distal colon is comparable to the risk of people with-

out distal polyps^[52].

Unlike colonoscopy, there are results from randomized clinical trials assessing sigmoidoscopy. The most favorable results are those of a US study^[53], in which a 33% reduction in CRC incidence and a 43% reduction in mortality were obtained. United Kingdom^[54] and Italy (SCORE)^[55] trials also achieved satisfactory results (decreased incidence and mortality of 21% and 26%). In contrast, in the Norwegian study, there was no benefit after 7 years of follow-up^[56].

The sensitivity for detecting advanced neoplasia is lower than that of colonoscopy. Results between 78% and 83% have been reported^[50-52].

A sigmoidoscopy is recommended every five years in an average-risk population. Sigmoidoscopy can be performed alone or combined with an FOBT annually or biennially^[28,48,49].

DCBE

There is little evidence regarding DCBE, and the existing few results do not belong to randomized trials. In a case-control study, a reduction in CRC mortality of 33% was detected^[57]. In a substudy of the popular National Polyp Study, DCBE detected 53% of polyps between 6 and 10 mm, 48% of polyps larger than 10 mm and 32% of those under 6 mm^[58]. In a non-randomized study conducted in general clinical practice, the sensitivity for detecting adenomas > 7 mm and CRC with DCBE was 73% and 85%, respectively^[59].

DCBE is not a very frequently used first choice test for screening due to its cost and its low acceptance by the population. DCBE is often used as a complementary test to endoscopic techniques.

CT colonography

Although CT colonography exhibits superior patient acceptability than colonoscopy in symptomatic patients^[60], this method is very recent and remains little explored for screening. No sedation is required, but as in conventional colonoscopy, preparation is needed, and in the case of finding lesions, an additional exploration has to be performed to resect or biopsy the lesions.

Two meta-analyses^[61,62] have reached the same conclusion regarding the use of CT colonography: its sensitivity and specificity are high for the identification of polyps > 10 mm (82%) but not for smaller polyps (56% for polyps < 5 mm and 63% for lesions between 6 and 10 mm).

One of the disadvantages described is the high percentage of extracolonic lesions detected (to 66%), nearly a third of them requiring further testing and monitoring, generating an unexpected additional cost^[63,64].

Although its use as a first choice for CRC screening is unclear, the American Association of Gastroenterologists recommends CT colonography every five years^[48].

Other exploratory techniques

There are some new endoscopic techniques (wide angle colonoscopy, endoscopy capsule, narrow band imaging,

autofluorescence imaging system, *etc.*), still under development but very promising for CRC screening, with the goal of increasing population acceptance as well as the detection rate of neoplasia, especially in the proximal colon. It will be necessary to wait a few years to understand the results of the different ongoing studies^[65,66].

BIOLOGICAL SAMPLE ANALYSIS

Feces

Fecal hemoglobin: Most cases of CRCs develop in advanced adenomatous polyps (AA) (greater than 10 mm in diameter, with high-grade dysplasia or with more than 20% villous component). These preneoplastic lesions and CRC are characterized by presenting intermittently inappreciable blood loss in stool, which can be detected by FOBT before becoming clinically visible.

FOBT is the most used method for CRC screening worldwide and it is also the choice in most population-based screening programs in Europe. Due to its non-invasive nature and low cost, FOBT is one of the most accepted techniques by the population.

There are two basic types of FOBT: those based on guaiac resin (mostly biochemical qualitative) and those that are immunologically based (qualitative or quantitative).

The guaiac test (gFOBT) is the most studied by randomized clinical trials, demonstrating its effectiveness in reducing both CRC incidence and mortality^[67-71]. These studies demonstrate a reduction in mortality between 15% and 33%, primarily due to the higher proportion of early-stage diagnoses; a decreased incidence is also confirmed thanks to preneoplastic lesion (adenomas) detection and their removal to avoid CRC progression. The largest reduction is observed when offering the test annually (33% after 13-years follow-up). A biennial test obtained a reduction between 15%-21% after 8-years follow-up and between 18%-21% after 10 years^[67-71].

In gFOBT, peroxidase activity in the hemoglobin heme subunit is detected. These tests are not specific to human hemoglobin and can react with blood in the diet (*e.g.*, from red meat) and with the presence of peroxidase in some vegetables. Therefore, and to avoid false positives, dietary restriction few days before the test is recommended^[72]. In addition, gFOBT are not specific for lower gastrointestinal bleeding and may give false positives secondary to a bleeding pathology in the upper digestive tract. The test is performed by collecting two small stool samples from three separate bowel movements.

The two main types of gFOBT are the standard and the sensitive methods, differing by their ability to detect peroxidase activity (being better for the sensitive test).

The tests are qualitative tests, whose results are obtained using a reagent that changes the color of the stool sample; therefore, the result is exposed to a subjective assessment by the lab technician.

gFOBT sensitivity for neoplasia is very wide, from 6.2% to 83.3%, depending on the test used. Specificity is more constant, exceeding 80% in all cases and reaching

Table 2 Main colorectal cancer screening tools

Test	Sensitivity	Specificity	Considerations
Colonoscopy		90%	It requires a bowel cleansing preparation and sedation.
Adenoma ≤ 5 mm	70%-79%		Risk of severe complications.
Adenoma 6-9 mm	80%-92%		Not well accepted by population.
Adenoma ≥ 10 mm	92%-99%		
CRC	92%-99%		
Sigmoidoscopy ¹		92%	It requires less preparation.
Adenoma ≤ 5 mm	70%-79%		Sedation it is not necessary.
Adenoma 6-9 mm	80%-92%		Proximal lesions are not detected
Adenoma ≥ 10 mm	92%-99%		
CRC	90%-92%		
gFOBT standard		95%-99%	Dietary and pharmacological interactions.
Adenoma ≤ 5 mm	1%-5%		3 samples
Adenoma 6-9 mm	5%-13.7%		
Adenoma ≥ 10 mm	8.9%-27.5%		
CRC	25%-50%		
gFOBT sensitive		90%-95%	Dietary and pharmacological interactions.
Adenoma ≤ 5 mm	5%-10%		3 samples
Adenoma 6-9 mm	10%-26.2%		
Adenoma ≥ 10 mm	17.7%-49.4%		
CRC	50%-87%		
FIT		92.5%-98%	The sample needs to be refrigerated.
Adenoma ≤ 5 mm	2%-7.5%		
Adenoma 6-9 mm	7.5%-24.0%		
Adenoma ≥ 10 mm	16%-48%		
CRC	50%-87%		

¹Sensitivity and specificity only for distal lesions. Data based on the report "Evaluating test strategies for colorectal cancer screening: a decision analysis for the US Preventive Services Task Force". Available from: URL: <http://www.uspreventiveservicestaskforce.org/uspstf08/colocancer/cartzaubtab2.htm>. DCBE: Double-contrast barium enema; gFOBT: Guayac fecal occult blood test; FIT: Immunochemical fecal occult blood test; CRC: Colorectal cancer.

98.4% in some cases^[73-75].

FITs are based on the use of monoclonal or polyclonal antibodies specific for human hemoglobin. The tests detect the presence of globin through immunochemical reactions^[76]. The most commonly used methods are latex agglutination (turbidimetry), enzyme immunoassay (ELISA) and immunochromatography.

FITs do not require dietary restriction, as they are specific for human hemoglobin. The tests are also specific for lower gastrointestinal bleeding. The tests can be qualitative and semi-quantitative, being able to set the cutoff point at convenience. These features make it increasingly often the chosen test in screening programs across Europe (such as Italy, France, Holland, Spain, Slovenia), Japan, New Zealand and Australia. The United Kingdom, a faithful user of gFOBT, is considering changing to FIT shortly.

FIT is better accepted by the population because it does not require a special diet, is easier to perform and requires fewer samples than gFOBT. However, it has the disadvantage that samples should be stored in a refrigerator, as high temperatures can alter the outcome, increasing the number of false negatives^[77].

Results from the literature regarding FIT sensitivity are highly variable, including tests with very little sensitivity (5.4%) and those that reach nearly 98%. Specificity ranges from 77% to 99%^[73,78]. Significant differences were reported according to the lesion location, with higher sensitivity for distal colon neoplasms^[79].

Several studies have demonstrated the superiority of

FIT to gFOBT in AA and CRC detection rates as well as a higher participation rate^[80-83].

Regardless of the FOBT used (gFOBT, FIT), a colonoscopy should be performed in all patients with a positive FOBT.

One of the main disadvantages of FOBT is its false positive and negative results. False positives lead to unnecessary explorations (with their associated complications), and false negatives give a false tranquility^[84-86]. Currently, a test can be chosen from a vast variety in the market (over 70 tests)^[87].

Thus far, we have seen CRC early detection techniques most frequently used in the context of population programs. Table 2 summarizes their main characteristics.

Fecal DNA and RNA: The spectacular improvement in the knowledge of the genome^[88-90], methylome^[91], transcriptome^[92,93] and proteome^[94] has led to the exploration of new methods for CRC diagnosis.

CRC is a very heterogeneous disease, and with a few exceptions (*APC*, *p53*, *KRAS*) most genes involved are observed in a small percentage of cases^[89,90]. Furthermore, hypermethylation of suppressor gene promoters is an early event in carcinogenesis^[95] detectable in the majority of CRCs, although this phenomenon is not universal^[96]. Therefore, the design of genetic and epigenetic marker panels able to give maximum coverage in the diagnosis of colorectal neoplasia seems a reasonable strategy. In recent years, the use of DNA, RNA and

Table 3 Fecal DNA markers for advanced adenoma and colorectal cancer *n* (%)

Marker	Study	Sensitivity		Specificity
		CRC	Adenoma > 1 cm	
Meth <i>vimentin</i>	Chen <i>et al</i> ^[166]	43 (46)	-	178 (90)
	Itzkowitz <i>et al</i> ^[167]	29 (73)	-	106 (89)
	Li <i>et al</i> ^[168]	9 (41)	9 (45)	63 (95)
Meth <i>SFRP2</i>	Huang <i>et al</i> ^[169]	49 (94)	11 (53)	23 (96)
	Wang <i>et al</i> ^[170]	60 (87)	21 (62)	28 (93)
Meth <i>TFPI2</i>	Glöckner <i>et al</i> ^[171]	36 (76)	4 (21)	-
Meth <i>ITGH4</i>	Ausch <i>et al</i> ^[172]	-	9 (69)	22 (79)
Meth <i>Spastic paraplegia-20</i>	Zhang <i>et al</i> ^[173]	77 (80)	-	30 (100)
Meth <i>PHACTR3</i>	Bosch <i>et al</i> ^[174]	(50-60)	(17-29)	(92-98)
Meth <i>TFPI2</i> , long DNA	Zhang <i>et al</i> ^[175]	52 (87)	4 (44)	25 (83)
<i>APC</i> , <i>KRAS</i> , <i>p53</i> , long DNA	Imperiale <i>et al</i> ^[102]	16 (52)	84 (12)	1344 (94)
<i>APC</i> , <i>KRAS</i> , <i>p53</i> , long DNA	Alhquist <i>et al</i> ^[103]	3 (25)	47 (8)	2246 (96)
<i>APC</i> , <i>KRAS</i> , Meth <i>vimentin</i>	Alhquist <i>et al</i> ^[103]	11 (58)	55 (45)	63 (84)
Meth <i>SFRP2</i> , <i>HPPI</i> , <i>MGMT</i>	Huang <i>et al</i> ^[169]	50 (96)	15 (71)	23 (96)
Meth <i>APC</i> , <i>ATM</i> , <i>hMLH1</i> , <i>sFRP2</i> , <i>HLTF</i> , <i>MGMT</i> , and <i>GSTP1</i>	Leung <i>et al</i> ^[176]	15 (75)	17 (68)	27 (90)
Meth <i>vimentin</i> , long DNA	Itzkowitz <i>et al</i> ^[167]	68 (83)	6 (86)	298 (82)
Meth <i>RASSF2</i> or <i>SFRP2</i>	Nagasaka <i>et al</i> ^[177]	63 (75)	25 (44)	101 (89)
Meth <i>BMP3</i> , <i>hDNA</i> , <i>KRAS</i> , <i>APC</i>	Zou <i>et al</i> ^[100]	67 (91)	21 (78)	85 (85)
Meth <i>vimentin</i> , <i>MLH1</i> , <i>MGMT</i>	Baek <i>et al</i> ^[178]	45 (75)	31 (60)	32 (87)
Meth <i>RARB2</i> , <i>p16INK4a</i> , <i>MGMT</i> , <i>APC</i>	Azuara <i>et al</i> ^[179]	16 (62)	8 (40)	20 (100)
<i>KRAS</i> , a actina Meth <i>NDRG4</i> , <i>BMP3</i> , <i>vimentin</i> , <i>TFPI2</i>	Ahlquist <i>et al</i> ^[107]	214 (85)	72 (54)	264 (90)
β -actin, <i>KRAS</i> , meth <i>BMP3</i> and <i>NDRG4</i> , fecal hemoglobin	Lidgard <i>et al</i> ^[105]	91 (98)	48 (57)	139 (90)

Meth: Methylation; CRC: Colorectal cancer; APC: Adenomatous polyposis coli gene.

protein markers in different biological samples has been explored^[92,94] as strategies for CRC diagnosis.

These tests have been the non-invasive molecular tests most extensively evaluated. CRC cells exhibit a high mitotic index and a low adhesion to the basal membrane, which facilitates their continuous exfoliation into the intestinal lumen^[97], unlike intermittent blood loss detected by FOBT. This constant exfoliation may be used for molecular analysis.

Only 0.01% of total fecal DNA tests (sDNA) is human; the rest comes from diet and bacterial flora^[98]. Furthermore, the tumor sDNA is a small percentage of all human sDNA^[99,100] and is even smaller in the case of AA. This implies that marker detection techniques must exhibit high sensitivity (Table 3).

The first sDNA tests investigated mutations in *KRAS*^[101] in CRC patients. Imperiale *et al*^[102] assessed a population-based cohort comparing a gFOBT with an sDNA panel that analyzed 21 mutations. The sensitivity for CRC was 13% and 53%, respectively ($P = 0.003$). First-generation sDNA tests did not incorporate stabilizing buffers, and so studies using stool samples sent by mail^[102,103] exhibited a low sensitivity but one far superior to gFOBT. Stabilizing buffers were subsequently incorporated^[100,104], avoiding marker degradation during transport and storage, as well as new, more sensitive detection techniques such as the digital melt curve method^[100] and beads, emulsion, amplification, and magnetics (BEAMing)^[99], enabling the detection of < 0.1% of mutated copies (the first generation detection threshold was > 1%). A technique called allele-specific quantitative real-time target and signal amplification (QuARTS) detected less frequent mutations significantly improving sensitivity for AA compared with

previous sDNA techniques^[100]. A trial comparing sDNA testing with gFOBT^[102] obtained a sensitivity for AA of 46% and 16%, respectively.

Although *APC* and *p53* are mutated in the majority of CRCs, mutations are distributed through hundreds of positions, making mutational analysis non-viable for trials in clinical practice. Abnormal methylation of specific suppressor gene promoters is an early event in CRC carcinogenesis^[95], thus making it an attractive target for the identification of biomarkers. The methylation status of various genes has exhibited remarkable diagnostic accuracy for CRC and AA, which has been improved by analyzing multimarker panels (Table 3). Sensitivity for adenoma detection increases with the size of the lesion^[104]. Serrated lesions may also be detected by these sDNA marker panels^[105].

One limitation of FOBT is its lower sensitivity for proximal lesions^[78]. In addition, colonoscopy has been recently questioned in the detection of proximal lesions^[106]. sDNA tests may not be affected by the neoplasia location^[106]. Ahlquist *et al*^[107] evaluated a panel of markers that identified 85% of patients with CRC and 54% with AA, without sensitivity differences depending on the location.

A recent technique is fluorescent long DNA (FL-DNA), which allows the identification of tumor DNA fragments > 150-200 base pairs. Neoplastic cells are characterized by not undergoing apoptosis, unlike normal cells that typically initiate cleavage and degradation of DNA, producing small identifiable fragments. FL-DNA has exhibited a sensitivity of 80% in the detection of CRC^[108].

The major disadvantage of sDNA tests is their cost. Song *et al*^[109] concluded, from a Markov model, that

Table 4 Fecal RNA markers for colorectal adenoma and colorectal cancer *n* (%)

Marker	Sample	Study	Sensitivity		Specificity
			CRC	Adenoma	
CDA, MGC20553, BANK1, BCNP1, MS4A1	Blood	Han <i>et al</i> ^[138]	30 (88)	-	27 (64)
ANXA3, CLEC4D, LMNB1, PRRG4, TNFAIP6, VNN1, IL2RB	Plasma	Marshall <i>et al</i> ^[180]	145 (72)	-	146 (70)
ANXA3, CLEC4D, TNFAIP6, LMNB1, PRRG4, VNN1, IL2RB	Plasma	Yip <i>et al</i> ^[181]	60 (61)	-	85 (77)
MicroRNA (miRNA-21, miRNA-106a)	Fecal	Link <i>et al</i> ^[113]	(74)	-	(79)
MicroRNA-L6	Plasma	Schiedeck <i>et al</i> ^[182]	145 (79)	-	45 (100)
MicroRNA (miRNA-92)	Plasma	Ng <i>et al</i> ^[142]	80 (89)	-	35 (70)
MicroRNA (miRNA-92a, miRNA-21)	Fecal	Wu <i>et al</i> ^[183]	63 (72)	32 (56)	74 (73)
ANXA3, CLEC4D, LMNB1, PRRG4, TNFAIP6, VNN1, IL2RB	Plasma	Chao <i>et al</i> ^[184]	215 (78)	-	215 (66)
COX2, matrix metalloproteinase 7	Fecal	Takai <i>et al</i> ^[111]	56 (90)	-	29 (100)

FOBT and colonoscopy were more cost-effective than fecal sDNA analysis. In the future, the use of new generation sDNA platforms with fewer markers could reduce costs and improve their viability in clinical practice^[110].

RNA expression levels in stool can also be quantified to identify CRC patients^[111]. An mRNA analysis of COX-2 in feces yielded a sensitivity of 87% for CRC with a specificity of 100%. mRNA of *matrix-7 metalloproteinase* detected 65% of CRC cases. The combination of these two markers reached a sensitivity of 90%. However, mRNA levels in stool exhibited a very low sensitivity for AA (4%)^[111] (Table 4).

MicroRNAs (miRNAs or miR) are small non-coding RNA molecules of 18-22 nucleotides that regulate gene expression at a posttranscriptional level. CRC exhibits a unique and identifiable pattern of miRNA expression^[112] that can be detected in feces. A pilot study assessed an miRNA expression profile, detecting overexpression of miR21 and miR106 with a sensitivity and specificity of 74.1% and 79.0%, respectively^[113]. Koga *et al*^[114] observed clusters miR17-92 and miR-135 overexpressed but could not confirm miR21 overexpression. Kanaoka *et al*^[115] evaluated *prostaglandin-synthase 2* in a small group of patients and reported a sensitivity between 50% to 90% and a specificity of 93% in the CRC diagnosis.

Protein in feces: Some plasma protein markers (albumin, lactoferrin, calprotectin, haptoglobin)^[116] are detectable in feces, although with a very low sensitivity for AA. However, detection of proteins derived from CRC and AA could have a greater discriminant value. A pilot study employing magnetic resonance spectroscopy used feces from patients with CRC exhibiting a sensitivity and specificity of 92%^[117].

Pyruvate kinase isoenzyme type M2 (M2-PK) is the dimeric form of pyruvate kinase isoenzyme glycolysis. M2-PK is overexpressed in all proliferating cells^[118,119] and is measurable in feces. A meta-analysis evaluated the concentration of M2-PK in stool samples from 704 patients with CRC and 11412 healthy controls from 17 independent studies. The sensitivity for CRC and adenomas > 1 cm was 80% and 44%, respectively, with a specificity of 95%.

Other mutated proteins detected in feces include S100

calcium binding protein A12 and *metallopeptidase inhibitor 1*. The latter exhibits a sensitivity for CRC of 85% and a specificity of 95% compared with controls^[120].

Blood and plasma

Unlike stool, all of the blood/plasma DNA proceeds from the host, but the number of altered DNA copies in early CRC or preneoplastic lesions may be absent or at levels below the detection limit^[121]. Some plasma enzymes could affect the marker stability^[122]. Therefore, a processing optimization of the sample to remove PCR inhibitors will be required to improve its sensitivity^[121].

Tumor cells can get into the blood through blood vessel invasion^[123,124], where they then circulate in the blood and release detectable markers in plasma samples or circulating phagocytes. However, such vessel invasion occurs more frequently in advanced stages of the disease and is absent in AA^[125-127]; thus, the detection of circulating tumor cells could be of prognostic value but would not be useful in the context of CRC screening. Moreover, the presence of free nucleic acids of tumor origin in plasma has been documented, and although these nucleic acids exhibit a small representation in plasma^[128,129], their use as biomarkers could be possible^[130]. However, certain tumor markers could be phagocytosed by inflammatory cells, allowing their detection in blood at any disease stage.

DNA in plasma: A variety of DNA markers have been assessed in plasma (Table 5). With respect to the detection of mutations, by BEAMing assay (Beads, Emulsification, Amplification, and Magnetics), *APC* mutations in tumor tissue and plasma samples were detected with a sensitivity of 73%, but the sensitivity was only 9% in the case of AA^[121].

As an alternative to mutation analysis, the accuracy of detecting epigenetic changes in plasma was assessed. Specifically, hypermethylation of *Septine 9* (a gene belonging to a guanosine triphosphatase class) is associated with CRC^[131,132]. Methylation analysis of *Septin 9* in plasma detected between 58% and 96% of CRC patients and 18% of AA, with specificities of 86%-100%^[131-134]. Its low sensitivity for AA would force patients to take the test annually or biennially. A multicenter trial included 7941

Table 5 Serum DNA markers for adenoma and colorectal cancer *n* (%)

Marker	Study	Sensitivity		Specificity
		CRC	Adenoma	
APC, KRAS, p53	Wang <i>et al</i> ^[185]	36 (46)	-	50 (100)
APC mutation	Diehl <i>et al</i> ^[121]	16 (73)	1 (9)	33 (100)
APC, MLH1, HMTF	Leung <i>et al</i> ^[186]	3-28 (6-57)	-	37-41 (90-100)
Meth SEPT9	Lofton-Day <i>et al</i> ^[134]	92 (69)	-	154 (86)
	Grützmann <i>et al</i> ^[133]	73 (58)	3 (18)	165 (90)
	deVos <i>et al</i> ^[131]	62 (69)	-	132 (89)
	Tóth <i>et al</i> ^[187]	88 (96)	-	78 (85)
TMEF2, NGFR, SEPT9	Lofton-Day <i>et al</i> ^[134]	40-69 (30-52)	-	170 (95)
Meth on 10 genes	Lee <i>et al</i> ^[188]	210 (87)	48 (75)	254 (92)
Meth Vimentin	Li <i>et al</i> ^[168]	48 (59)	-	102 (93)

TMEF2: Transmembrane protein with EGF-like and follistatin-like and two follistatin-like domains 2; SEPT9: Septin 9; Meth: Methylation; CRC: Colorectal cancer; APC: Adenomatous polyposis coli gene; MLH1: MutL homolog 1; HMTF: Helicase-like transcription factor.

individuals ≥ 50 years with an average CRC risk. A sensitivity of 48% and a specificity of 91% were observed. However, the sensitivity for AA was low (11%)^[135]. There is currently an automated test available on the market (Epi proColon Early Detection Assay Kit[®])^[132]. The addition of new markers to this detection blood test could optimize its performance in the future^[136]. Nevertheless, its high cost compared with FOBT could limit its use.

RNA in plasma: The transcriptome of peripheral blood and plasma provides a source of potential diagnostic markers^[137]. Han *et al*^[138] designed a plasmatic marker panel (*BANK1*, *BCNP1*, *CDA*, *MGC20553* and *MS4A1*) as a result of mRNA leukocyte microarray expression profiling, capable of discriminating a CRC patient from a healthy individual. An independent analysis revealed a sensitivity and specificity of 88% and 64%, respectively.

miRNAs have been demonstrated to be stable plasma markers, reproducible and consistent^[139-141]. High miR92 levels have been identified in CRC patient plasma compared with healthy control samples^[142], and significantly elevated miR92a and miR29a levels were detected in AA and CRC patients compared with controls^[143].

Proteins in plasma: Carcinoembryonic antigen (CEA) is the most investigated protein but is not useful in CRC screening due to its low sensitivity (43% to 69%) in early CRC. Other antigens, such as CA 19.9, CA 50, CA 72.4, CO 29.11, have been studied without demonstrating acceptable diagnostic performance^[144]. Furthermore, the performance of some proteins analyzed as single markers has demonstrating promising results in a small group of patients, thus they must be validated. Among these proteins, the most prominent include CD26 (sensitivity 90%, specificity 90%)^[145], alpha-defensin 1 (sensitivity 69%, specificity 100%)^[146], colon cancer-specific antigen (CCSA)-3 and CCSA-4 (for CRC sensitivity 100%, specificity 96%; for AA sensitivity 78%)^[147], CCSA-2 (for CRC sensitivity 89%, specificity 84%; for AA sensitivity 20%)^[148]; and TIMP-1 (sensitivity 60%, specificity 98%)^[149] (Table 5).

The incorporation of new technologies in proteomics

allows the analysis of large-scale protein patterns, including chromatographic techniques based on mass spectrometry (MS) assays, surface-enhanced laser desorption/ionization time-of-flight (SELDI-TOF)-MS and matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) MS, that identify peptide patterns that discriminate CRC patients from healthy individuals with a sensitivity and specificity of over 90%^[150]. The application of these profiles has to be defined, as most of these studies are conducted in a small number of cases, and in some of them, the identified biomarkers are not CRC-specific^[151,152] but part of larger serum protein degradation products.

Circulating lymphocytes may contain traces of dysplastic lesions^[153]. A pilot study examined the possibility of detecting CD24 in circulating leukocytes, demonstrating the ability to detect CRC and AA^[154].

Another area of research is the identification of tumor-associated autoantibodies (epithelial cell adhesion molecule, p53, p62, CEA, HER-2/neu, Ras, topoisomerase II-alpha, histone deacetylase 3 and 5, ubiquitin L3, tyrosinase, tropomyosin, cyclin B1) as CRC markers^[155-157]. These markers are absent in healthy individuals, and because they are very stable molecules and can be detected by immunoassays, they suggest a promising avenue for research.

Babel *et al*^[158] used a microarray platform evaluating 8000 proteins. The authors identified 43 proteins capable of discriminating tumor serum from normal serum. The combined ELISA analysis of two of the markers (MAPKAPK3 and ACVR2B) in a CRC population and in a healthy population obtained a sensitivity of 83% and a specificity of 74%^[156]. The accuracy of these proteins was enhanced through the incorporation of two new proteins to the panel (MST1/STK4 and SULF1)^[159].

This research field, still in the experimental stage, is very interesting because it uses immunoassay techniques characterized by low cost, robustness and applicability.

Urine

Tumor markers can access urine through blood circulation. The markers are metabolized in small fragments and

Table 6 TNM classification of colorectal cancer

T = Primary tumor
TX = Primary tumor cannot be assessed
T0 = No evidence of primary tumor
Tis = Carcinoma in situ: intraepithelial or invasion of lamina propria
T1 = Tumor invades submucosa
T2 = Tumor invades muscularis propria
T3 = Tumor invades through the muscularis propria into subserosa or into nonperitonealized pericolic or perirectal tissues
T4a = Tumor penetrates to the surface of the visceral peritoneum
T4b = Tumor directly invades or is adherent to other organs or structures
N = Regional lymph nodes
NX = Regional lymph nodes cannot be assessed
N0 = No regional lymph node metastasis
N1a = Metastasis in one regional lymph node
N1b = Metastasis in two to three regional lymph nodes
N1c = Tumor deposit(s) in the subserosa, mesentery, or nonperitonealized pericolic or perirectal tissues without regional nodal metastasis
N2a = Metastasis in four to six regional lymph nodes
N2b = Metastasis in seven or more regional lymph nodes
M = Distant metastasis
MX = Distant metastasis cannot be assessed
M0 = No distant metastasis
M1a = Distant metastasis to one organ or site
M1b = Distant metastasis to more than one organ/site or the peritoneum
Staging
Stage I (T1-T2, N0, M0)
Stage II A (T3, N0, M0)
Stage II B (T4a, N0, M0)
Stage II C (T4b, N0, M0)
Stage III A (T1-T2, N1, M0 and T1, N2a, M0)
Stage III B (T1-T2, N2b, M0; T2-T3, N2a, M0 and T3-T4a, N1, M0)
Stage III C (T3-T4a, N2b, M0 and T4b, N1-N2, M0 and T4a, N2a, M0)
Stage IV A (any T, any N and M1a)
Stage IV B (any T, any N and M1b)

cross glomerular filtration^[160]. Urine sample collection is easy and non-invasive, and DNA isolation is easier than in the feces, due to its low content in foreign proteins. Urine contains products that have been excreted over a period of time; therefore, urine may be less vulnerable to the intermittent release of markers in the blood^[160,161].

Nucleosides that can accurately discriminate CRC patients from healthy controls have been identified in urine^[162]. One study evaluated mutated *KRAS* sequences in the tissue and urine of a small number of CRC patients and healthy controls, reporting an 83% concordance between the mutated DNA in tissue and the corresponding urine sample^[161]. This concordance of results between urine and tissue could be higher than that observed between plasma and tumor tissue^[163].

Hypermethylation detection of the *vimentin* gene in urine samples was significantly associated with CRC compared with controls^[164]. The use of SELDI-TOF MS and MALDI-TOF MS platforms to identify protein profiles in the urine of CRC patients have been evaluated by Ward *et al.*^[165], with a sensitivity of 78% and a specificity of 87%.

COLORECTAL CANCER TREATMENT

Colorectal locoregional non-metastatic cancer treatment

Surgery: Surgical treatment for non-metastatic rectal cancer should include total mesorectal excision (TME), which consists of the total excision of the rectum vascular pedicle along fascia anatomic planes^[189] with adequate distal and circumferential margins and inferior mesenteric lymphadenectomy^[190]. Sphincter-sparing surgery is possible in most cases of middle or lower rectal tumors as long as there is a distal margin at least of 1 cm. In very low tumors, an abdominoperineal resection may be required^[191].

Local excision by transanal endoscopic microsurgery is also an option in tumors that do not exceed the upper third of the submucosa if a complete excision with adequate margins is obtained^[192].

Likewise, it has been postulated that in colon cancer, surgery should be based on colectomy and bloc resection of lymph nodes^[193]. In most cases, a laparoscopic intervention is possible^[194]. The evaluation of a minimum of 12 lymph nodes is considered a measure of surgery quality^[195,196]. Resection must be complete to be curative, so other atypical nodes or ganglions outside the resection field should be biopsied or resected whenever possible.

Adjuvant chemoradiotherapy: The anatomic complexity of the pelvis adds complication to rectal cancer treatment. The locoregional recurrence risk is higher in rectal than in colon cancer and is associated with a worse prognosis. This risk is due to the absence of serosa in the rectum and its proximity to other pelvic organs and structures, making it difficult to obtain wide surgical margins^[197]. The treatment of choice for stage II and III rectal cancer, clinically T3, T4 or ganglionic affection (Table 6)^[198], includes preoperative chemoradiotherapy with conventional radiotherapy fractionation of 50.4 Gy in 28 fractions. Performing this treatment preoperatively reduces the incidence of local recurrence and toxicity, also allowing greater sphincter preservation, compared with its postoperative administration^[199]. In those patients not treated with preoperative chemoradiotherapy, postoperative administration is recommended, exhibiting no differences in 10-year overall survival (OS), in 10-year cumulative incidence for distant metastasis, or in 10-year progression-free survival (PFS)^[200].

The addition of concomitant chemotherapy to radiotherapy in locoregional rectal cancer treatment, both preoperatively and postoperatively, is due to its sensitizing effect in irradiated tissues, increasing the complete pathological responses percentage, along with its micrometastases potential eradication, improving disease systemic control^[201]. To date, conventional chemotherapeutic treatment concomitant with radiation includes 5-FU continuous infusion or, more recently, capecitabine (one fluorouracil oral prodrug), with both drugs being equivalent^[202]. The use of short hypofractionated radiotherapy

(25 Gy for 5 d) has also exhibited a decreased local recurrence rate and improved survival compared with surgery alone^[203,204] and is currently considered an alternative to conventional chemoradiotherapy treatment in elderly patients or with higher comorbidity.

Unlike rectal cancer, adjuvant treatment of CRC is more aimed at the prevention of distant metastases because the local recurrence rates are lower. Therefore, treatment with perioperative chemoradiotherapy is administered only in selected cases of T4 tumors with fixed structures impairment or recurrent disease^[205].

Adjuvant chemotherapy: Surgery is the main treatment for CRC cure, but in cases with ganglionic involvement (stage III), the administration of adjuvant chemotherapy is the treatment of choice for optimizing the chances of healing. The administration of 5-FU and folinic acid (Leucovorin) for 6 mo improves survival by approximately 10%-15%^[206]. The oral capecitabine monotherapy scheme has been shown to be equivalent to that of 5-FU with a lower percentage of adverse effects^[207]. Despite this, the treatment of choice in stage III after MOSAIC results^[208] is the scheme that uses oxaliplatin with 5-FU and Leucovorin (FOLFOX), adding a 7% 3-year disease-free survival (DFS) with respect to the scheme without oxaliplatin. Similarly, the scheme combining capecitabine with oxaliplatin (CapeOx) also proved to be superior to the traditional scheme of 5-FU and Leucovorin, increasing the 3-year DFS in 4.4%^[209]. Both schemes with oxaliplatin are considered of choice in stage III, reserving schemes without oxaliplatin for patients where this drug is considered contraindicated.

The use of adjuvant chemotherapy in stage II colon cancer is more controversial. There is a subgroup of patients at this stage with increased risk of recurrence [T4, inadequate nodal surgery (< 12 nodes removed), lymphovascular invasion, visceral peritoneum affection, bowel obstruction or poorly differentiated histology] in which the use of adjuvant chemotherapy with the previous schemes discussed can be considered^[210]. In these cases, it is recommended to discuss with the patient the pros and cons of using chemotherapy, as the survival benefit is unclear^[211,212]. However, there are good prognostic markers, such as high microsatellite instability (H-MSI), to be taken into account when assessing the use of adjuvant chemotherapy in stage II disease because there are studies reporting little benefit or even a negative impact of 5-FU adjuvant chemotherapy without oxaliplatin in these cases^[213]. On this basis, the generalized use of adjuvant chemotherapy in stage II tumors with H-MSI is not recommended.

With regard to rectum cancer, the use of adjuvant chemotherapy is recommended in both stages II and III disease, although its effectiveness in many cases is extrapolated from colon cancer studies. A meta-analysis published in 2012 and based on 21 randomized clinical trials in rectal cancer patients reported an increase in OS and PFS in patients receiving postoperative chemotherapy

with 5-FU-based schemes^[214].

No survival benefit has been reported for CRC adjuvant treatment using irinotecan^[215,216] or new drugs such as bevacizumab [humanized monoclonal vascular endothelial growth factor (VEGF) antibody]^[217] or cetuximab (murine monoclonal antibody directed against epidermal growth factor receptor, EGFR), regardless of tumor *KRAS* status^[218].

There is no evidence for the benefit of adjuvant chemotherapy in stage I CRC^[205].

Colorectal metastatic cancer treatment

Approximately half of the patients diagnosed with CRC eventually develop metastases, mainly those of metachronic presentation. The most common site for metastases occurrence is the liver. Approximately 20% of patients are diagnosed with synchronous liver metastases. However, resection of hepatic metastatic disease may achieve healing in a group of patients, with 5-year DFS of 20% in those patients where liver metastases resection was achieved. For this reason, it is essential to select those patients with resectable or potentially resectable disease. In addition, some patients with extrahepatic metastases may also benefit from their resection^[219]. This has made the metastatic CRC therapeutic approach more complex, with multiple treatment options that increasingly require a multidisciplinary medical team, which can combine locoregional treatment of metastases with systemic treatment to obtain disease resectability^[220].

Surgery: Good prognostic factors in liver metastases resection have been postulated, including the presence of a low number of metastases, lesions smaller than 5 cm, no major vasculature affection, absence or a low volume of extrahepatic disease, sufficient liver reserves and the presence of negative margins in liver resection^[221,222]. Furthermore, although the presence of extrahepatic disease is a poor prognostic factor, the possibility of extrahepatic disease resection in selected patients may increase the DFS median^[219]. Resection of lung metastases in punctual cases could have an impact on survival^[223].

The surgical approach to peritoneal carcinomatosis along with hyperthermic intraperitoneal chemotherapy and perioperative systemic chemotherapy are considered palliative, although with encouraging results in terms of survival^[224].

Local liver treatments: In daily clinical practice, other techniques (radiofrequency ablation^[225], hepatic arterial chemotherapy^[226] administered directly to the liver, radioembolization with yttrium-90 microspheres^[227] and stereotactic radiotherapy^[228]) have been incorporated in the treatment of selected patients with few liver metastases or as a complement to surgery to achieve resectability. The role of such treatments is not fully defined in the absence, in most cases, of randomized trials. Stereotactic radiotherapy and radiofrequency are also being evaluated for the treatment of pulmonary metastases in selected

patients^[229].

Chemotherapy: In metastatic CRC treatment, chemotherapy can be used as a complement to metastases potentially curative by surgery as neoadjuvant treatment to achieve resectability of initially unresectable disease or as palliative therapy.

As adjuvant treatment, the administration of chemotherapy with 5-FU for 6 mo (Mayo scheme, which to date is considered suboptimal) has resulted in improvements in 5-year DFS^[230]. Another study has compared the use of perioperative FOLFOX for 6 mo with surgical treatment alone in patients with up to 4 resectable liver metastases, demonstrating a better 3-year PFS. This scheme is currently considered first choice as adjuvant to surgery for liver metastases^[231].

Regarding neoadjuvant treatment for potentially resectable liver disease, FOLFOX and FOLFIRI have exhibited similar response rates of approximately 30%^[232], whereas the combination of oxaliplatin, irinotecan and 5-FU (FOLFOXIRI) exhibit higher reported response rates (66%), achieving a higher rate of resectability than FOLFIRI^[233].

Palliative therapy with chemotherapy increases survival, can reduce the symptomatology and can improve quality of life. The treatment length may be 6 mo or until disease progression^[234], depending mainly on toxicity. The choice of both first-line and subsequent treatments depends on previous treatments, the administration and toxicity profiles as well as the objectives to be achieved with the treatment. The use of 5-FU results in approximately 12-mo survival^[235], being equivalent to Capecitabine treatment^[236]. FOLFOX and FOLFIRI exhibit similar survival outcomes in first-line metastatic disease^[232], with 15 mo OS, being also equivalent in oxaliplatin schemes the substitution of 5-FU by capecitabine^[237].

New drugs: The addition of new drugs to the traditional chemotherapy schemes has not demonstrated efficacy as a complementary treatment in the resection of liver metastases.

Bevacizumab is a partially humanized monoclonal antibody against VEGF. Several studies have demonstrated the benefit of adding bevacizumab to oxaliplatin and irinotecan chemotherapy schemes. In first line therapy, the addition of bevacizumab to IFL scheme with irinotecan resulted in a statistically significant increase of both, PFS and OS^[238]. Its combination with oxaliplatin schemes has resulted in an increased PFS but not OS^[239,240].

The effectiveness of adding bevacizumab to chemotherapy is independent of the *KRAS* status^[241]. Although the response rate with bevacizumab is approximately 50%^[239], its role as neoadjuvant treatment with resectability intention is not clearly defined.

Cetuximab is a partially humanized monoclonal antibody against EGFR (epidermal growth factor receptor). Mutations in *KRAS* or *BRAF* in the signaling pathway downstream EGFR prevent tumors with mutated *KRAS*

or *BRAF* from being sensitive to cetuximab treatment, being effective only in tumors with unmutated *KRAS*. Cetuximab and FOLFIRI combination increases PFS and OS^[242]. In contrast, in first-line therapy, there is no benefit in statistically and clinically significant survival after the addition of cetuximab to FOLFOX^[243]. Cetuximab has been shown to reverse resistance to irinotecan after progression to schemes containing the drug^[244].

Panitumumab is a completely humanized monoclonal antibody against EGFR that is effective as monotherapy in patients in chemotherapy progression^[245] or in combination. Its mechanism of action is similar to that of cetuximab; thus, the presence of *KRAS* mutations should also be considered. The addition of panitumumab to chemotherapy has demonstrated efficacy in PFS in combination with FOLFOX^[246] in first-line therapy and in combination with FOLFIRI^[247] in second-line therapy.

Aflibercept is a new molecule targeting against VEGF. The addition of aflibercept to FOLFIRI in second-line therapy increases PFS and OS^[248] compared with chemotherapy alone.

The combination of chemotherapy with more than one biological agent (anti-EGFR and anti-VEGF) does not provide benefits but increases toxicity; therefore, it is contraindicated^[249,250].

In Table 7, a summary of the different systematic treatments available for CRC is presented.

CONCLUSION

CRC is one of the most common cancers worldwide. Today, various techniques are available to detect CRC in its early stages or as precursor lesions, thereby preventing aggressive treatment.

Screening programs have helped make these techniques more accessible to the population, with FOBT, sigmoidoscopy and colonoscopy representing the most used. FIT is emerging as the best test for screening due to its better acceptance among populations. However, its sensitivity, yet distant from the gold standard (colonoscopy), requires further research.

The enormous work of basic and translational research in recent times has identified a large number of potential biomarkers. Although there is not sufficient evidence yet to recommend the analysis of biomarkers such as DNA, RNA or proteins in the blood or stool, it is likely that given the quick progression of technological tools in molecular biology, increasingly sensitive and less expensive, they will gradually be implemented in clinical practice and will most likely be developed in mass.

Chemotherapy remains the cornerstone of systemic treatment today, but several new targeted drugs have emerged in this field in the last decade, improving the management of metastatic disease. The recent advances in molecular biology and the genetic classification of CRC are essential to individualize these therapies and will be basic for improving the treatment in the next years.

Table 7 Systemic treatment of colon cancer

Adjuvant therapy (stage III and stage II with high-risk features for systemic recurrence)
FOLFOX
CapeOx
If oxaliplatin is contraindicated or elderly patients
Capecitabine
5-FU/Leucovorin
Metastatic disease (stage IV)
Resectable disease (lung, hepatic or peritoneal metastasis)
Consider surgery and/or locoregional treatment (radiofrequency, stereotactic radiotherapy) and 6 mo of perioperative chemotherapy (FOLFOX, CapeOx preferred)
Potentially resectable
FOLFOX
FOLFIRI
FOLFOXIRI
Cetuximab + FOLFIRI (only <i>KRAS</i> wild type)
Panitumumab + FOLFOX (only <i>KRAS</i> wild type)
Bevacizumab + FOLFOX
Unresectable (palliative)
FOLFOX
CapeOx
FOLFIRI
FOLFOX + Bevacizumab
CapeOx + Bevacizumab
FOLFOX + Panitumumab (only <i>KRAS</i> wild type)
FOLFIRI + Panitumumab (only <i>KRAS</i> wild type)
FOLFIRI + Cetuximab (only <i>KRAS</i> wild type)
FOLFIRI + Afibercept
Capecitabine
5-FU/Leucovorin
Cetuximab + Irinotecan (only <i>KRAS</i> wild type)
Cetuximab monotherapy (only <i>KRAS</i> wild type)
Panitumumab monotherapy (only <i>KRAS</i> wild type)
Regorafenib

FOLFOX: 5-fluorouracil (5-FU), leucovorin, and oxaliplatin; FOLFIRI: 5-FU, folinic acid, irinotecan; FOLFOXIRI: 5-FU, folinic acid oxaliplatin irinotecan; CapeOx: Capecitabine together with oxaliplatin.

ACKNOWLEDGMENTS

The authors greatly thank Olga Lopez for her unconditional technical and emotional support.

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ISSN 1007-9327

