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Brief Overview about Biomarkers of Colorectal Cancer

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Abstract: Background: Colorectal cancer (CRC), which comprises colon and/or rectum cancer, represents a significant health problem as the world's third most diagnosed and second fatal cancer globally. Approximately 9.4% of cancer-related deaths were due to CRC in 2020. Biomarkers for detecting CRC must allow detection of the disease at earlier stages. Such tests using biomarkers should have high sensitivity and specificity to prevent subjecting healthy individuals to unnecessary colonoscopies. Carcinoembryonic antigen (CEA) has been the most extensively investigated tumor marker for colon cancer. CEA is present in normal adult tissues in addition to malignant tissues, but very low levels normally are seen in the blood from healthy individuals with normal concentrations of 2.5 to 5.0 ng/ml. Although 80% or more of patients with advanced colonic adenocarcinoma have circulating CEA, the CEA assay should not be used as the sole diagnostic test for suspected carcinoma. The combined assays of CEA and Carbohydrate antigen 19-9 (CA 19.9) may increase diagnostic sensitivity in colorectal cancer detection. Moreover, the determination of both markers is used as a postoperative prognostic factor in the evaluation of the stage of the disease and survival rate. A high level of tissue polypeptide specific antigen (TPS) occurs in about 60–80% of patients with colorectal cancer. Serum concentrations of Tumor-associated glycoprotein-72 (TAG-72) were elevated in 43% of patients with colorectal cancer. It is advisable to determine TAG-72 together with other markers, primarily CEA. Sixty-one percent of patients had at least one marker with elevated levels when measuring these three markers. Other studies showed elevated levels of several proinflammatory cytokines, such as interleukin-6 (IL 6), IL 8, tumor necrosis factor-α (TNF-α) and acute-phase proteins in patients with colorectal carcinoma and other malignancies. Mroczko et al showed a potential role for stem cell factor and IL 3 as tumor markers for colorectal cancer, especially in combination with CEA and CA19-9.

Keywords: *Biomarkers, Colorectal Cancer*

Introduction

Colorectal cancer (CRC), which comprises colon and/or rectum cancer, represents a significant health problem as the world's third most diagnosed and second fatal cancer globally. Approximately 9.4% of cancer-related deaths were due to CRC in 2020. However, considering the significant increase in the number of identified cases in the older population, it is estimated that the global incidence of CRC will more than double by 2035, with the most significant increase occurring in less developed nations **(1)**.

CRC is the third most popular occurring cancer in men and the second most commonly occurring cancer in women. There were over 1.9 million new cases in 2020. CRC is the second most common cause of death from

cancer, estimated to be responsible for almost 935,000 cancer deaths. Globally it is one of the cancers whose incidence is increasing comprising 11% of all cancer diagnoses. CRC is the fourth most common cancer overall worldwide contributing to 9.7% of global cancer burden. It affects 746,000 men (10% of all cancer cases) and 614,000 women (9.2% of all cancer cases) with most cases (55%) occurring in developed countries. Moreover, the burden of CRC is expected to increase with 2.2 million new cases and 1.1 million deaths expected globally by 2030. In addition, significant challenges remain in managing disease burden. **(2)**.

It has been recognized that the most significant increase in CRC incidence and mortality occurs in medium and high human development index countries that are adopting the "western" way of life. Developed countries are at the highest risk of colon cancer. Obesity, sedentary lifestyle, red meat consumption, alcohol and tobacco are considered the driving factors behind the growth of CRC. Therefore, colorectal cancer is a disease of developed countries with a western lifestyle. In recent years, the global burden of CRC will increase by 60%, to over 2.2 million new cases and 1.1 million deaths by 2030 **(3)**.

The CRC usually begins with the non-cancerous proliferation of mucosal epithelial cells. These growths are known as polyps and can grow gradually for 10–20 years before becoming cancerous. The most common form is an adenoma or polyp that originated from granular cells, whose function is to produce the mucus that lines the large intestine. Only about 10% of all adenomas progress to invasive cancer, although the risk of cancer increases as the polyp grows larger. Invasive cancer arising from such polyps is known as adenocarcinoma and accounts for 96% of all CRCs **(4)**

The CRCs that grow into the wall of the colon or rectum can penetrate blood or lymphatic vessels, allowing metastasis to distant organs via the blood or to nearby lymph nodes. The extent of invasion determines the staging, and thus the prognosis, of a CRC diagnosis. *In situ* cancers are polyps that have not yet invaded the colon or rectum wall and are thus not reported as CRCs. Local cancers are cancers that have grown into the wall but have not yet extended past it. Regional cancers are those that have invaded nearby lymph nodes or tissues, while distant cancers are those that have metastasized, via the bloodstream, to distant organs with capillary beds where they have taken root, such as in the lungs or liver **(5)**.

Certain dietary and lifestyle choices can promote intestinal inflammation and modify the intestinal microflora to promote an immune response, both of which can facilitate polyp growth and conversion to cancer. Likewise, hereditary or spontaneous mutations in oncogenes and tumour-suppressor genes can provide certain mucosal cells with a selective advantage and encourage hyper-proliferation and ultimately carcinogenesis. Lifestyle modification, early colorectal screening, and genetic testing hold promise in preventing CRC **(4)**.

The transformation of the normal colonic epithelium to a precancerous lesion (adenoma) and ultimately to invasive carcinoma requires an accumulation of genetic mutations either somatic (acquired) and/or germline (inherited). The theory of colonic carcinogenesis features a clonal mutation evolution that gives a cell survival-immortality advantage and allows to develop more mutations providing other cancer hallmarks as proliferation, invasion, metastasis, and others. Clinical evidence has shown that CRCs frequently arise from adenomatous polyps that typically acquire dysplastic changes in a 10 to 15-year period before developing invasive carcinoma, and the early detection-removal of polyps will reduce the incidence of CRC. New evidence has exposed that hamartomatous, and serrated polyps could lead to CRC **(6)**.

There are three major molecular pathways linked to CRC, chromosomal instability, mismatch repair, and hypermethylation. The chromosomal instability pathway is a gain of mutations unbalancing oncogene and tumor suppressors equilibrium as seen with mutations in the adenomatous polyposis coli (APC), a hallmark of familial adenomatous polyposis. Cells with deficiency of DNA mismatch repair (dMMR), commonly mutL homologue 1 (MLH1) or mutS homologue 2 (MSH2), accumulate errors within the genome that further will be repeated causing high levels of microsatellite instability (MSI-H), a hallmark of Lynch syndrome. CpG hypermethylation of DNA could activate or silence the expression of certain genes, BRAF and MLH1 respectively. Sporadic oncogenes somatic mutations (RAS, SRC, MYC) have been implicated in CRC, being RAS the most clinical relevance **(7).**

RAS mutations variants (HRAS, KRAS, NRAS) are found in 50% of CRC sporadic cases, currently being exploited on CRC screening by stool-DNA testing, the absence of epidermal growth factor receptors (EGFR) targeted therapy response and potential direct targeted agents **(7).**

In the other hand, tumor suppressors genes require bi-allelic loss ("two-hit model") and are described in loss of APC 5q21 gene (80% sporadic), TP53 17p gene (50-70% sporadic), and DCC/SMAD2-4 18q gene (73% sporadic). MutY homolog *Escherichia coli*, homolog of *MYH*, *hMYH* (MUTYH) defects have a recessive inheritance pattern at a time requiring bi-allelic second hit or in conjunction with APC gene mutation. Cyclooxygenase (COX-2) and peroxisome proliferator–activating receptor (PPAR) genes have been implicated in CRC tumorigenesis currently under investigation for chemo-protection **(8)**.

Persons with CRC can present in several ways:

- First, cancer can be detected because of screening. Colonoscopy is undertaken as a diagnostic test to evaluate those with an abnormal screening test. During the colonoscopy, polyps are removed, and masses or other suspicious lesions are either removed or biopsied to establish a pathological diagnosis.
- Second, cancer can be detected when an individual undergoes colonoscopy to evaluate large bowel symptoms, such as rectal bleeding, anemia, or a change in bowel habits.
- Third, some individuals may present as an emergency, such as a large bowel obstruction, in which case the cancer may be diagnosed at surgery without prior diagnostic evaluation **(9)**.

1. Clinical Symptoms:

Symptoms are common and prominent late in colon cancer when the prognosis is poor but are less common and less obvious early in the disease. The classic warning signs include loss of appetite, loss of weight, worsening constipation, alternating bowel habits, blood in the stool, decrease in stool caliber, and nausea or vomiting**.** Partial obstruction occasionally paradoxically produces intermittent diarrhea as stool moves beyond the obstruction. proximal cancers rarely produce bleeding because the blood becomes mixed with stool and chemically degraded during colonic transit **(10).**

2. Signs:

A palpable abdominal mass is a rare finding that suggests advanced disease. Hypoactive or high-pitched bowel sounds suggest gastrointestinal obstruction. Rectal examination, including fecal occult blood testing (FOBT), is important in the evaluation of possible colon cancer. Other physical findings, including peripheral lymphadenopathy, especially a Virchow's node in the left supraclavicular space; hepatomegaly from hepatic metastases; and temporal or intercostal muscle wasting from cancer cachexia. Very rare findings with colon cancer include a Sister Mary Joseph node caused by metastases to a periumbilical node, and a Blumer's shelf caused by perirectal extension of the primary tumor **(10).**

3. Laboratory abnormalities:

Routine tests:

Anemia, however, is very common, so that only a small minority of patients with anemia have colon cancer. Iron deficiency anemia of undetermined etiology, however, warrants evaluation for colon cancer, particularly in the elderly. In general, cancer patients have a high prevalence of hypoalbuminemia. Studies suggest that albumin is a negative acute phase reactant rather than a nutritional marker **(11).**

Tumor markers:

Biomarkers for detecting CRC must allow detection of the disease at earlier stages. Such tests using biomarkers should have high sensitivity and specificity to prevent subjecting healthy individuals to unnecessary colonoscopies (Figure 1 and table 1**) (12).**

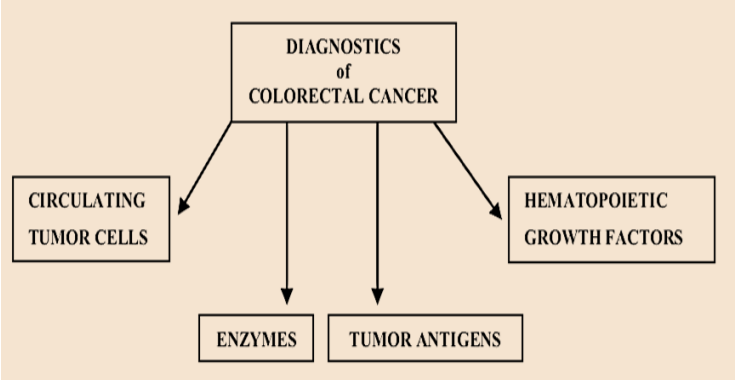

Figure (1): Division of colorectal cancer marker **(12).**

Table (1): Diagnostic Criteria for Markers of Colorectal Cancer

Group/Markers	Diagnostic Sensitivity (%)	Diagnostic Specificity (%)	Area under ROC Curve
Tumor antigens			
Carcinoembryonic antigen CEA[10,22]	64	90	0.7940
Carbohydrate antigen CA 19–9[15]	34	55	0.6520
Tissue polypeptide specific antigen TPS[16]	95	83	0.8020
Tumor-associated glycoprotein-72 TAG-72[19]	40	77	No data
Hematopoietic growth factors (HGFs)			
Stem cell factor (SCF)[24]	89	17	0.7232
Granulocyte-colony stimulating factor (G-CSF)[21]	31	95	0.6900
Macrophage-colony stimulating factor (M-CSF)[21]	65	95	0.8300
Interleukin6[22]	72	96	0.8960
Interleukin3[24]	55	80	0.6840
Enzymes			
Alcohol dehydrogenase (ADH)[27]	60	70	0.6538
Isoenzyme class I of ADH[27]	76	82	0.7231
N-acetyl-β-D-hexosaminidase (HEX) in serum[28]	90	95	0.9326
N-acetyl-β-D-hexosaminidase (HEX) in urine[28]	86	81	0.8739
CathepsinD[31]	91	93	0.9137
Ornithine decarboxylase (ODC)[35]	82	85	No data
Circulating tumor cells			
Cytokeratin 20 (CK20)[39]	No data	No data 56	No data
Multidrug resistance-related proteins (MRPs)[41]	No data	No data	No data

(12).

a) Tumor antigens:

- Carcinoembryonic antigen (CEA):

Since its initial description by **Gold and Freedman (13),** CEA has been the most extensively investigated tumor marker for colon cancer. CEA is present in normal adult tissues in addition to malignant tissues, but very low levels normally are seen in the blood from healthy individuals with normal concentrations of 2.5 to 5.0 ng/ml.

Although 80% or more of patients with advanced colonic adenocarcinoma have circulating CEA, the CEA assay should not be used as the sole diagnostic test for suspected carcinoma.

Preoperative serum CEA levels in diagnosed colorectal carcinoma are elevated in 40% to 70% of patients. Preoperative serum CEA concentrations correlate inversely with the grade of the carcinoma and directly with the pathologic stage and is an independent prognostic factor for recurrence. CEA is elevated in 95% of patients with well-differentiated lesions, while it is elevated in as few as 30% of those with poorly differentiated adenocarcinomas**.** The higher the preoperative CEA level, the higher the stage and the greater the likelihood of a postoperative recurrence. Currently the CEA level is assessed preoperatively as a prognostic factor and predictor of recurrence and postoperatively for follow up and detection of recurrence. More over its level is used for assessment of the response to chemotherapy for metastatic colon cancer patients **(14).**

Carbohydrate antigen 19-9 (CA 19.9)

CA 19.9 is a glycoprotein characterized by a high molecular weight which may be released to the blood. This marker is used in the diagnostics of pancreatic, colorectal and gastric cancers. Like CEA, it is not specific to a particular histological type of carcinoma and the organ which it comes from. **Vukobrat- Bijedic et al (15)** showed that CA 19.9 is less sensitive than CEA. The combined assays of CEA and CA 19.9 may increase diagnostic sensitivity in colorectal cancer detection. Moreover, the determination of both markers is used as a postoperative prognostic factor in the evaluation of the stage of the disease and survival rate**.** Both CA 19.9 concentration and sensitivity increase with higher stage of disease, but do not correlate with the tumor location and number of positive lymph nodes **(12).**

Tissue polypeptide specific antigen (TPS)

TPS has been described as a useful tumor marker in many malignant cancers and as a response factor in monitoring chemotherapy in different advanced gastrointestinal carcinomas. It is a singular conjugated chain of polypeptide, which is produced in different phases of the molecular cycle (S or G2) and subsequently released to tissue after mitotic division **(16).**

TPS is a soluble fragment derived from the carboxy-terminal end of cytokeratin. High TPS concentration is a marker of tumor activity, but not necessarily mass of tumor. The level of TPS in blood, strongly associated with proliferation of cancer cells. Estimation of tissue polypeptide specific antigen may be applicable in the early stages of cancers. A high level of tissue polypeptide specific antigen occurs in about 60–80% of patients with colorectal cancer. The survival rate was significantly lower in patients with initially higher concentrations of TPS. Repeated determination of TPS concentration during therapy may be of clinical importance, especially as a marker of non-response. Therefore, TPS is superior to the commonly used CEA **(17).**

Tumor-associated glycoprotein-72 (TAG-72)

TAG-72 is a glycoprotein formed in bile duct endothelial cells, gastric epithelium, or renal pelvis cells. It is a mucin-like molecule with a molar mass of over 1000 kDa. TAG-72 is found on the surface of many cancer cells, including colon, ovary, breast, and pancreatic cells. Serum concentrations of TAG-72 were elevated in 43% of patients with colorectal cancer. It is advisable to determine TAG-72 together with other markers, primarily CEA and CA 19-9. Sixty-one percent of patients had at least one marker with elevated levels when measuring these three markers (**17**).

b) Hematopoietic Growth Factors

Colorectal cancer cells are capable of producing hematopoietic growth factors (HGFs). Stem cell factor, macrophagecolony stimulating factor and granulocyte macrophage- colony stimulating factor are members of glycoprotein cytokines called colony-stimulating factors or HGFs. Hematopoietic growth factors are involved in the regulation of growth and spread of cancer **(12).** Other studies showed elevated levels of several proinflammatory cytokines, such as interleukin-6 (IL 6), IL 8, tumor necrosis factor-α (TNF-α) and acute-phase proteins in patients with colorectal carcinoma and other malignancies. **(18).**

c) Enzymes:

Newly conducted research by **Jelski et al. (12)** on the use of enzymes as markers for colorectal cancer, including alcohol dehydrogenase, cathepsin D and lysosomal exoglycosidases reported that the activity of alcohol dehydrogenase is significantly higher in cancerous cells than that in healthy tissue.

Development of colorectal cancer and its metastases can be supported by exoglycosidases released by macrophages. **Szajda et al. (19)** showed a marked increase of N-acetyl-β-D-hexosaminidase, its isoenzymes A and B activity in the blood and urine of CRC patients.

Ornithine decarboxylase (ODC) activity is higher in colorectal cancer and increases gradually from normal, through adenomatous, to cancerous. It has been shown that ODC activity in microscopically normal colon tissue from patients with CRC is higher than in the normal colon of patients without CRC **(20).**

d) Circulating tumor cells (CTC)

CTC have been reported in patients with metastatic CRC as an independent predictor of overall and progression-free survival. There are at least three advantages to CTCs. The first is the monitoring of the treatment efficacy of CRC patients. The second is the molecular characterization of captured CTCs for targeted treatment, and the third is the cultivation of captured CTCs for drug sensitivity testing **(21).**

e) Genomic biomarkers

First, chromosomal instability (CIN), which is mainly manifested in structural chromosome and aneuploidy structural abnormalities, as well as chromosomal deletion and rearrangement. Carcinogenesis through the chromosomal instability pathway involves chromosomal aberrations and various gene mutations, such as adenomatous polyposis coli (APC), Kirsten rat sarcoma viral oncogene, Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha, loss of heterozygosity of the long arm of chromosome 18, and tumor protein p53 (Table 2) **(22).**

Table (2): Summary of mutated genes involved in the colorectal cancer.

Gene	Types of cancer gene	Chromosomal location	Pathway	Function
APC	tumor suppressor	5q22.2	Wnt/β-catenin	Inhibits cell growth
KRAS	oncogene	12p12.1	Ras-RAF-MAPK	Induces cell invasion, metastasis
BRAF	oncogene	7q34	Ras-RAF-MAPK	Induces cell proliferation
PIK3CA	oncogene	3q26.3	PI3K/Akt/mTOR	Induces cell proliferation
TP53	tumor suppressor	17p13.1	p53 pathway	Inhibits cell cycle
SMAD4	tumor suppressor	18q21	TGF-β pathway	Inhibits tumor metastasis, prognosis, overall survival

(23)

f) Epigenetic biomarkers

DNA methylation is an epigenetic biomarker which was involved in the diagnosis of many diseases. It has been demonstrated that three DNA methylation indicators (NDRG4, BMP3, and SEPT9) can be employed for the early detection of CRC. Moreover, the expression of Syndecan-2 methylation was higher in the early stage of CRC than that of in normal tissues, suggesting that this protein may serve as a potential biomarker for the early diagnosis of CRC. Also, the methylation of other genes, including wif-1, IMPA2, and SFRP2, shown good sensitivity and specificity for the diagnosis of CRC **(24).**

g) Transcriptomic biomarkers

Approximately over 90% of the human genome is actively transcribed, but less than 2% of these transcripts−most of which are noncoding RNAs (ncRNAs)−are protein-coding genes that create translational messenger RNA (mRNA) transcripts. More and more research evidence show that microRNA (miRNA) can be used as diagnostic markers for various cancers and diseases. Moreover, miRNAs have significant impacts on the

evolution and occurrence of cancers as proto-oncogenes or tumor suppressor genes. Many miRNAs have been implicated with CRC, with miR-21 receiving the most attention among all of them (Table 3) **(23).**

Table (3): List of MiRNAs used as biomarkers for early diagnosis of colorectal cancer. **(23)**

MiRNA	Abundance	Target	Impact on colorectal cancer
miR-21	High	MEG2	Promoting cell proliferation and inhibit apoptosis
miR-485-3p	Low	TPX2	Inhibiting cell proliferation
miR-4728-5p	Low	MST4	Inhibiting cell proliferation
miR-3937	High	BCL2L12	Promoting cell invasion and migration
miR-31	High	RAS p21 GTPase	Promoting cell proliferation
miR-22-3p	Low	KDM3A	Inhibiting the proliferation, migration and invasion of CRC cells
miR-20a	High	Smad4, Ecadherin	Promoting the invasion and migration of CRC cells
miR-145	Low	Fasin-1, MYC	Inhibiting cell proliferation and metastasis
miR-223	High	FBXW7	Promoting CRC cell proliferation, invasion and migration
miR-182	High	MTDH	Enhancing CRC cell survival, invasion, and drug resistance,
miR-92a	High	Bim	Promoting CRC cell proliferation, invasion and migration
miR-18a	Low	CDC42	Making cell cycle arrest, promote apoptosis

circRNA is a new type of single-stranded RNA. In contrast to the conventional RNA structure, a circular RNA with improved stability is created when its 3' and 5' ends are combined. Furthermore, circRNAs have been employed in clinical practices as diagnostic markers for numerous diseases. hsa_circ_001978, hsa_circ_105039, hsa_circ_103627, and circ_0124554 could be used as biomarkers for the diagnosis of colorectal cancer. However, only a small number of them, such as CircRNACIRC_0124554, have been proved to promote phosphorylation of AKT and block AKT ubiquitination, then prevent colorectal cancer metastasis **(25).**

Long non-coding RNAs (lncRNAs) are linear ncRNAs that have a length of more than 200 nucleotides. Abnormal expression of lncRNA is associated with the tumorigenesis, malignant transformation, and progression of CRC **(26).**

h) Proteomics biomarkers

Proteomics is a study of the whole protein from any cell or tissue of an organism. It represents an abroad range of approaches for identification, measurement, characterization, and analysis of proteins. With further study of proteomics, the pan-cancer proteomic map of 949 human cell lines provides a vast array of protein-level cancer data beyond existing omics data. A total of 189 kinds of biomarkers for drug sensitivity prediction were developed, which has far-reaching significance for cancer scientific research and drug application and provides a comprehensive resource for proteomics analysis **(27).**

Screening

Methods that are used to detect colon cancer are recently being introduced to screen for colon cancer either by one or a combination of tests. The Stool-based tests are at-home tests that are simple and inexpensive ways to detect hidden and invisible blood in a people's stool. There are two types of stool-based tests, one is stool tests, and another one is stool DNA test. Fecal immunochemical test (FIT) and FOBT were the two types of stool test. Both FIT and FOBT tests are used to detect the presence of blood in the stool. The FOBT can supply quick results while is relatively less sensitive and specific than the FIT. A stool DNA test is a tool for the early identification of colorectal cancer that checks for abnormal DNA and hidden blood in the stool. In contrast to the FIT, there are no dietary or medication restrictions prior to the stool DNA test. It can be taken at home without any bowel preparation or hospital visit (Figure 2) **(28).**

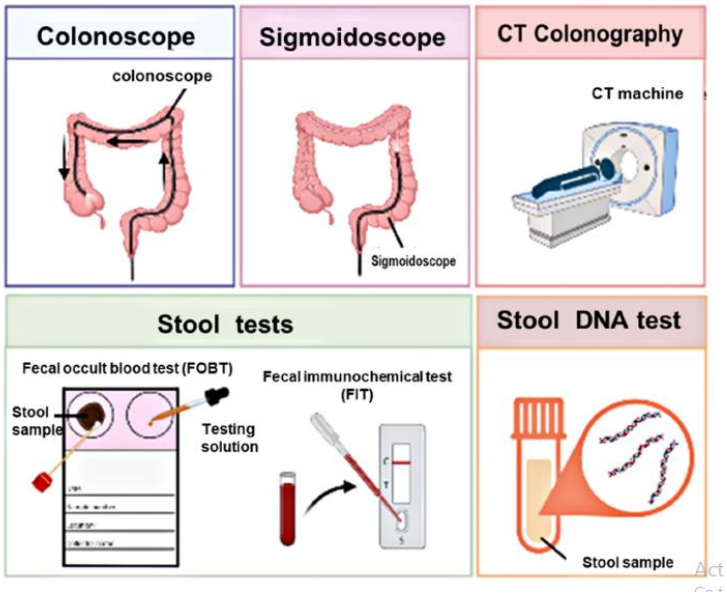

Figure (2): Colorectal cancer screening and surveillance **(23).**

Tests that detect adenomatous polyps and cancer: **(29).**

- Flexible sigmoidoscopy every 5 years.
- Colonoscopy every 10 years.
- Double-contrast barium enema every 5 years, or
- Computed tomographic colonography every 5 years.

Tests that primarily detect cancer: **(29).**

- Annual guaiac-based FOBT with high test sensitivity for cancer, or
- Annual FIT with high test sensitivity for cancer, or
- Stool DNA (sDNA) test with high sensitivity for cancer, interval uncertain.

Declining mortality due to improvements has been shown with early detection through screening and effective treatment. The CRC screening program relying on FOBT and colonoscopy has led to 16% decline in overall mortality rate without affecting incidence. However, FOBT has reduced sensitivity for advanced adenomas and CRC which may improve with newly implemented immunochemical testing (FIT). This screening test is offered in the UK every 2 years between 60–74 years with a one-off test aged 55 years. These tests are precursors for more invasive colonoscopy to identify pre-malignant or malignant lesions **(30)**.

Furthermore, studies randomized trials have shown a reduction in CRC incidence up to 23% and CRC-related mortality by 31% using flexible sigmoidoscopy as a primary screening tool. However, this remains an invasive and resource intensive technique. There is no universally agreed screening protocol for early disease stages, and significant variation remains. In addition, up to 70% of cancers presenting with symptoms are at an advanced stage. This emphasizes the value of screening programs with early detection of pre-malignant or early stage (I-II) CRC leading to improved CRC survival, quality of life and disease-free outcomes. Moreover, screening for biomarkers at all stages, including diagnostic, prognostic, and predictive, may provide opportunities for targeted intervention to improve outcomes whilst reducing the risk of treatment toxicity **(30)**.

Evaluation

Initial evaluation may involve barium enema or CT colonography, but ultimately a colonoscopy is required for tissue biopsy. Colonoscopy sensitivity is about 94.7% (95% CI 90% to 97%) and may be missed from 2% to 6% of cases, mostly right sided, pending on preparation quality and hands experience. Flexible sigmoidoscopy is no replacement for a complete diagnostic colonoscopy, still is a screening modality that reduces CRC mortality. The Federal Drug Administration (FDA) has approved PILLCAM 2 for those non-obstructed patients with incomplete colonoscopy, and not for routine screening **(31)**.

Routine laboratory workup with complete blood count, iron studies, basic metabolic panel, liver function test and coagulation tests are not diagnostic but often useful. CEA greater than 5 ng/mL has a poor prognostic value when present but lacks diagnostic sensitivity 46% (95% CI 0.45 to 0.47) and has limited specificity 89% (95% CI 0.88 to 0.92). Pre-operative CEA is indicated on all newly diagnosed Cca, normalization after surgical resection is expected and serial assays to be monitored on follow up visits **(31)**.

Baseline CT of the chest, abdomen, and pelvis with IV and oral contrast is the preferred cost-effective staging imaging study before Cca surgical resection. CT abdomen and pelvis provides a moderate strength initial assessment of accurately staging T (50%) and N (73%) but rather provide immediate higher sensitivity for distant metastasis (87%). CT chest remains controversial, as 9% will show indeterminate lesions, of that 11% represented metastatic lesions. MRI and CT triple phase imaging have improved the detection of liver metastasis. Positron emission tomography (PET) is not routinely indicated on the preoperative staging. Biopsy of the suspicious metastatic site should be performed to confirm the diagnosis **(32).**

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