

Colorectal Cancer Biomarkers -A New Trend in Early Diagnosis

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ABSTRACT: Colorectal cancer (CRC) is one of the most widespread malignancy, posing as a great challenge due to its high incidence and mortality in both genders. Yet, it also stands as one of the most preventable diseases because of its known malignant transformation mostly from tubular adenomas or serrated polyps, therefore offering a strong incentive to the screening programs that are being developed for this disease. Current diagnosis of CRC has surely evolved along with the evolutionary step in gastrointestinal technology of flexible endoscopy. These innovations have promoted colonoscopy as a primary choice for screening programs of colonic lesions, proving to be of great benefit for patient's well-being. In this review, we present the current status of CRC screening methods from the non-invasive options to the long developed colonoscopic and imaging techniques. We search through PubMed and Medline databases and chose relevant articles on CRC with focus on blood based biomarkers and stool based tests. Additional relevant publications were also according to the reference lists of firstly identified articles.

KEYWORDS: colorectal cancer screening, Blood-Based Biomarkers, Stool Based Tests

Introduction

Despite all advances in diagnosis and management, Colorectal Cancer (CRC) still represents a major health burden worldwide, especially in developing countries with westernized lifestyle and growing aging population [1]. Regardless the implemented screening programs, or major efforts for developing new tools for early diagnostic, CRC still require a better strategy as it still is the fourth most common cause of cancer deaths worldwide [2].

The most important factor that clearly influences the survival rate is considered the time of diagnosis. CRC is a slow evolving disease which becomes symptomatic when it progresses to advanced stages, thus, more diagnostic opportunities should be tested for an early diagnostic. Improvements have been made on treatment procedures, reaching a survival rate of 85 to 90 % in some types of colon tumors. However when distant metastases are presented the overall survival rate decreases to less than 12.5% [3,4].

Therefore, there is an ongoing strategy and general focus on providing a better and affordable method so that CRC can be predicted and diagnosed. Most of the colonic tumors are known to evolve from adenomatous polyps in multi-step process with genetic and epigenetic

alterations which finally evolves from adenoma to carcinoma. Early detection enhances the chances for a proper treatment and high survival rate [5]. While colonoscopy to the cecum represents the standard method for diagnosis of CRC it still represents a challenge when considering it as a screening method in every country. Thus the need of other diagnostic procedures has been discussed and tested [6]. Fecal Occult blood test (FOBT), carbohydrate antigen (CA 19-9) and carcinoembryonic antigen (CEA) have been suggested as a diagnostic option, however their low sensitivity and specificity in this situations did not allow them to be integrated in a screening method [7-9].

Many biomarkers have been suggested for different types of malignancies, including CRC so that a less invasive screening method may be used. In this review we tried to synthesize and provide a future perspective of available and ready to use blood-based and stool based biomarkers for early detection and prognosis in CRC. We search through PubMed and Medline databases and chose relevant articles on CRC with focus on blood based biomarkers and stool based tests and selected only the relevant records according to the keywords: colorectal cancer screening, blood based biomarkers, stool-based tests. (Fig.1)

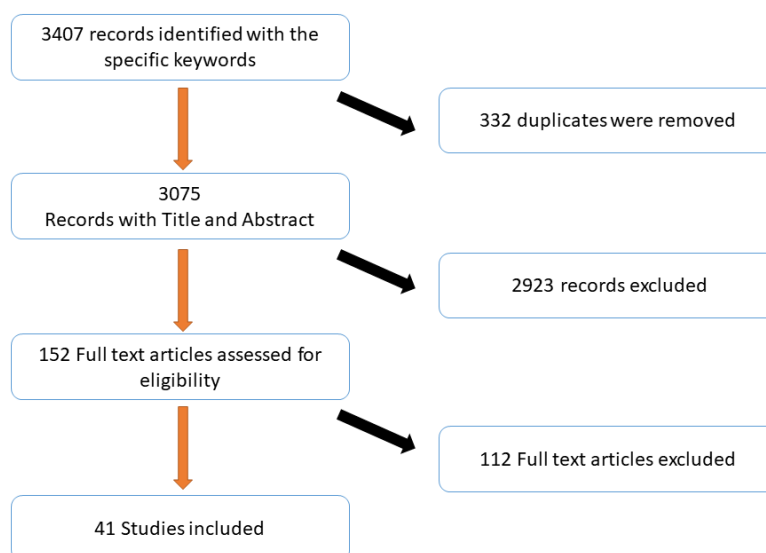


Fig. 1. Information flow from articles selected

Blood-Based Biomarkers

Carcinoembryogenic Antigen. Currently there are only two markers which have confirmed as an important option for CRC blood markers for CRC patients monitoring and prognosis. CEA was discovered in 1965 and is used all over the world with high levels suggesting disease recurrence and a grim prognosis. Apparently this biomarker is directly related with the disease evolution as its' sensitivity increases with CRC progression [10]. This high molecular weight glycoprotein is not specific for CRC only, as high levels may be encountered in many other malignancies or other pathological conditions. CEA for CRC has not been embedded as a potential screening method since elevated levels may be encountered in some of the patients or in advanced disease. In contrast CA 19-9 is more specific and less sensitive in CRC, while its focus is more in pancreatobiliary malignancies [10]. A recent study [11] which compared CEA, CA 19-9, CA 72-4 CA 125 and serum ferritin with preoperative levels as well as with pathological parameters of 279 CRC diagnosed patients, have shown that their use together is more efficient than using a single marker. Even more, this combination may be used for CRC predictment of vascular invasion, tumor differentiation and pTNM staging, lymph node dissemination, neural invasion. Other biomarkers that have been assessed and related to metastatic disease are tissue polypeptide-specific antigen (TPS) and tissue polypeptide antigen (TPA) which are

correlated with cytokeratins 8, 18 and 19 and in combination with CEA have a greater sensitivity in CRC patients recurrence disease [12-15].

Other biomarker proteins

While CEA and CA19-9 have limited possibilities in CRC more proteins have been tested as diagnostic tools. Recently, 43 proteins have been tested for distinguishing between CRC and healthy individuals and some had promising results. MAPKAPK3 and ACVR2B had a sensitivity of 83.3% and a specificity of 73.9% which made these biomarkers as the most reliable so far. Also, TIMP-1 as a single marker protein was tested with a sensitivity of 42-65% and a specificity of 95% [16,17].

Circulating DNA-Based Biomarkers

Usually cell-free DNA (cfDNA) comes from cellular apoptosis process and it may be found in serum or plasma. This tool has been tested on CRC patients and high level were observed on disease progression. Normally, along with oncological treatment their levels should decrease, even though reports have been made that there may be some fluctuating levels during chemotherapy. cfDNA assessment might be a window of opportunity to monitor disease evolution, even though there are some flaws in their use, beginning from their unstable situations as their circulating life ranges from 15 to several hours to the fact that it was observed that increased levels are found more in serum than plasma.

cfDNA varies from 185 to 200 bp long fragments when released from apoptotic tumor cells, which have led to the definition of a new potential biomarker following the short fragments of cfDNA. This was confirmed in a recent study on 4,105 volunteers that underwent colonoscopy for various reasons. Blood samples were harvested from each individual and cell-free circulating nucleosomes which contained a variety of epigenetic signals were tested and concluded with a promising predictor models for CRC early diagnosis when comparing patients found with colon tumors and naive [19-20].

Stool based tests

Guaiac Based Fecal Occult Blood Test

Guaiac based fecal occult blood test (gFOBT) was firstly recognized in 1996 as a valid screening method for CRC detection, by indirectly detecting hemoglobin in feces through a peroxidase reaction [21]. As a new introduced option that may succeed in detecting CRC, several studies have shown that performing an annual gFOBT morbidity and mortality was reduced by 33% on a 13 year follow up [22].

Compliance may limit the use of gFOBT as it has important characteristics: three stools samples should be collected, and a specific diet should be taken into account, even though this aspect was dropped due to limited clinical significance. Moreover, gFOBT cannot detect hemoglobin concentrations less than 600 μ g/g in feces which proves that even if it has a high specificity, its sensitivity is limited, thus this may affect the detection of adenomatous polyps or advanced adenomas. Low sensitivity and adherence rates of gFOBT screening is very often associated with long interval cancers. For example, a Scottish survey showed that the proportion of interval cancers increased from 31.2 to 58.9 % after the first, respectively, third screening round. Even more gFOBT may also detect bleeding from the upper gastrointestinal tract [24-26].

Fecal Immunochemical Test

Fecal Immunochemical Test (FIT) detects human hemoglobin by using globin-specific antibodies [27]. Compared to gFOBT, FIT has some benefits including, not only the fact that no dietary restrictions are needed, but also that it requires only one stool sample instead of three. Even more, it offers the possibility of obtaining both qualitative and quantitative results. With a sensitivity for CRC ranging from 69 to 100% and a specificity of 92-96 % [28,29,30], FIT

proves to have a higher adherence rate and allows a better detection for advanced adenomas [31].

Findings regarding sensitivity and sensibility are contrasting. For example, a study performed by Lee et al shows that CRC detection accuracy was 95% with a 79% sensitivity and 94% specificity [32]. On the other hand a meta-analysis which used colonoscopy as the reference diagnostic method pointed out that FIT's CRC sensitivity and specificity is between 71% and 94% [33]. According to recent studies, it was shown that FIT sensitivity is variable with cancer location. Thus, FIT has a higher sensitivity for lesions located on the left colon than the right one [30,34]. Also, people taking aspirin on a regular basis have higher sensitivity rates than nonusers [35]. Due to the fact that the prevalence of advanced neoplasia is higher in men than women, FIT is more frequently positive in men [36,37].

In order to evaluate the impact that FIT tests have on reducing mortality in CRC, researchers compared results given by FIT with colonoscopy results. In a large cohort study of 4,045 patients, samples were obtained just the day before colonoscopy was performed and the lesions found were compared with positive FIT results. The study resulted in FIT sensitivity for non-advanced adenoma, advanced adenoma, and CRC of 10.6%, 28.0%, and 78.6% [38]. Similar studies are in progress in Spain and the United States [39,40].

However, several limitations should be noted when comparing with gFOBT, such as higher costs such and the fact that its sensitivity decreases along with the delay in mailing and processing the sample while FIT results decline from 8.7% at 1-4 days to 6% at ≥ 5 days, and 4.1% at ≥ 7 days [41].

DNA and RNA based stool tests

DNA and RNA based stool test is a non-invasive, easy to perform test which aims to detect markers of aberrant DNA or RNA from neoplastic cells. The principle which stands at the basis of this test is the fact that CRC sheds neoplastic surface cells in stool. The DNA or RNA of these cells is analyzed in order to discover different mutations that might have appeared during carcinogenesis. [42,43].

Combining DNA markers with FIT resulted in a higher sensitivity for advanced adenomas (42%) as well as CRC (92%), than FIT alone, therefore increasing the demand for colonoscopy. Even though DNA stool test is safe and poses no patient risk, its limitations are

rather important as they include expensive costs and its inability of detecting all types of cancer. Also multiple tumor markers have to be used on the grounds that there are various colorectal cancer subtypes, each with its specific features [44,45].

MicroRNAs are small non-coding RNA molecule that regulate gene expression and thus being involved in carcinogenesis, angiogenesis and metastasis [46]. It can be identified in stool by using polymerization chain reaction and considered to be disease-specific. A Japanese study revealed that adding fecal miRNA-106a to FIT testing improves the sensitivity, but decreases the specificity of detecting FIT [47].

Protein-based stool markers

Protein-based stool markers aim to detect either cancer-specific proteins or proteins released from inflamed or bleeding tissue. Two of the most studied fecal protein markers for CRC screening are fecal calprotectin and M2 pyruvate kinase.

Calprotectin is a calcium-binding protein in granulocytes, macrophages, and epithelial cells. Calprotectin is also a non-cancer-specific protein marker whose level may rise during intestinal inflammation, with proven results for inflammatory bowel diseases.

Thus its performance does not reaches the FIT levels with lower sensitivity (67% vs. 75%) and specificity (76% vs. 90%) for both CRC and precancerous lesions, as shown in a Norwegian CRC screening trial involving 2,321 asymptomatic subjects [48].

Studies on Fecal tumor M2 pyruvate kinase (M2-PK) have shown contradictory results

regarding the sensitivity in CRC which is ranging from 68% to 85% for a cutoff value of 4U/mL [45-48]. The possibility of combining more protein markers in a biochip in order to detect CRC is being studied by a Chinese group since no protein stool marker has proved to be entirely accurate for CRC screening [49].

Human fecal microbiome-based biomarkers

A welcome development, was the use of gut microbiome, due to the fact it has the ability to detect non bleeding lesions. On the assumptions that patients with CRC have a different gut microbiome than healthy subjects, several bacteria have been related with CRC, *Streptococcus bovis* being one of the most common bacteria found at these subjects [50].

Other bacteria such as *Fusobacterium nucleatum* and *Helicobacter pylori*, which apart from their already proven role in gastric carcinogenesis also seem to be associated with CRC [51-53].

Fusobacterium nucleatum contributes to CRC not only by invading the colonic mucosa and through a proinflammatory mechanism with increased expression of cytokines but also by recruiting tumor-infiltrating immune cells and generating an oncogenic/proinflammatory microenvironment [54-56]. These findings are supported by Zackular et al, which found high levels of *fusobacterium nucleatum* CRC samples when compared to adenoma [57].

Even more, this bacteria seems to have a high sensitivity for detecting serrated polyps [58-60].

Table 1. Stool tests screening characteristics. gFOBT - Guaiac based fecal occult blood test; FIT - Fecal Immunochemical test. Low +; Intermediate ++; High +++

Screening test	Sensitivity	Specificity	Interval
gFOBT	++	++	1 y
FIT	++	++	1 y
DNA-based test	++	+++	-
Protein tests	+	+	-

Screening strategies

Currently there is no worldwide consensus on which non-invasive technique should be used for CRC screening. There is still a high variability between continents and countries in screening strategies for several parameters such as premalignant lesion detection, test performance, screening involvement, use of stool or blood methods, costs-effectiveness or over-diagnosis.

There is no doubt that a non-invasive test would be more effective on large population cohort and might enhance the spectrum of patients that require a colonoscopy and therefore reduce CRC incidence. Regardless the provided test, some aspects should be taken into considerations and should be discussed along with patient's characteristics. From risk stratification and targeted population based on their or parent's medical history to selecting

specific surveillance intervals suggesting a worldwide non-invasive screening method for CRC still represents a challenge.

Till now the use of stool tests have the main stage for non-invasive options in CRC, even though blood biomarkers also have a sustainable basis with the fact that they might be more easily be included as a screening method in a random blood testing.

Screening strategies are the main objective for CRC treatment, and their results have been substantial especially in the US and some European Countries. The objective of a screening CRC test should put together a series of features with the main focus on detecting curable tumors. This emphasize the fact that from current available methods, more efforts should be made to develop methods for adenoma detection, which should provide both the physician and patient more time in providing the current window for therapy (Table 1).

Conclusion

Based on the perceived balance of providing a better screening method to select patients for colonoscopy, a rigorous evaluation of the scientific evidence needs to be carefully tested in randomized controlled maner to assess it's efficacy on the long-term. Ensuring reliable measures that might raise some concerns on preventing CRC development might be more worthy of further appraisal. While colonoscopy will surely remain the golden standard in the diagnosis and non-invasive paliative treatment of CRC, other non-invasive options might suggest possible new methods that might allow an early diagnosis. A screening population non-invasive test, either blood-based or stool samples could provide a better guidance for patients to colonoscopy, however all methods should be tested before implemented to fulfill the role that colonoscopy has today.

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