

## Biomarkers in Colorectal Cancer Screening

Vijeta Pamudurthy<sup>1</sup>, Marc Bissonnette<sup>2</sup> and Vani Konda<sup>2\*</sup>

<sup>1</sup>Department of Medicine, Riverside Medical Center, Kankakee, Illinois, USA

<sup>2</sup>Department of Medicine, Section of Gastroenterology, University of Chicago Medical Center, Chicago, Illinois, USA

\*Corresponding author: Vani Konda, Department of Medicine, Section of Gastroenterology, University of Chicago Medical Center, Chicago, Illinois, USA, Tel: 773-702-1000; E-mail: [vkonda@medicine.bsd.uchicago.edu](mailto:vkonda@medicine.bsd.uchicago.edu)

Rec date: Dec 30, 2015, Acc date: Feb 3, 2016, Pub date: Feb 10, 2016

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### Abstract

Colorectal cancer is the second most frequent cause of death by cancer. Current gold standard screening tools are invasive, expensive, and require a lot of preparation, which result in decreased patient compliance. Screening modalities that are less invasive, have high sensitivity and specificity, inexpensive, and more accepted by the general population than the current gold standard screening tools may potentially prevent the deaths caused by colorectal cancer. Noninvasive stool tests like fecal occult blood test and fecal immunohistochemical test have become widely used in detecting hemoglobin in stool. Some of these biomarkers have shown favorable results to detecting adenomas and early stage colorectal cancer diagnosis. Furthermore, the evolution of biomarkers is becoming more promising for the early diagnosis of colorectal cancer. This article will discuss the advances of biomarkers in stool, serum, and urine assays that enable early detection of colorectal cancer.

The recent advances in stool DNA testing have allowed for high detection rates of colorectal cancer and advanced adenomas. Stool nucleic acids include the genes that are involved in chromosomal instability, which make up 50-80% of those involved in non invasive testing and microsatellite instability, which make up 10-15%. The different categories of stool DNA testing that will be discussed in this article include nucleic acid testing including multitarget stool DNA testing, epigenetic biomarkers like DNA methylation, hypermethylation, and hypomethylation markers, genetic biomarkers, long DNA, microRNA, fecal protein assay biomarkers. Serum markers that will be discussed include nonenzymatic tumor markers: CEA, CA 19-9, TPS, TAG72 and enzymatic tumor marker, which include neutrophil elastase, cathepsin D and lysosomal exoglycosidases, N methyltransferase, TIMP1, among others. Several of the urinary markers are also introduced. We also review other noninvasive technologies that are being investigated that may allow for highly accurate methodology for cancer screening. Currently, the following noninvasive biomarkers are commercially available: plasma septin 9, CEA, CA19-9, TPS, TAG72, and stool Cologuard test which consists of KRAS mutations, aberrant NDRG4 and BMP3 methylation, and  $\beta$ -actin, plus a hemoglobin immunoassay.

Noninvasive screening of colorectal cancer allows for increased patient acceptance and compliance, and is making a lot of progress since its inception. Although there have been promising advances in the use of biomarkers for colorectal cancer detection, large, long term prospective-multicenter clinical studies are necessary to determine if these tests have high enough sensitivity and specificity to be first line tests to diagnose colorectal cancer.

**Keywords:** Cancer screening; Colon cancer

### Abbreviations:

PRoBE: Prospective Specimen Collection Retrospective Blinded Evaluation; FOBT: Fecal Occult Blood Test; FIT: Fecal Immunochemical Test; gFOBT: Guaiac-based Fecal Occult Blood Test; CRC: Colorectal Cancer; sDNA: Stool DNA; SEPT9: Septin 9; PCR: Polymerase Chain Reaction; VSX2: Visual System Homeobox 2; BEND4: BEN Domain Containing 4; NPTX1: Neuronal Pentraxin I; ALX3: ALX Homeobox 3; GLP1R: Glucagon-like Peptide 1 Receptor; HOMER2: Homer Homolog 2; ZNF583: Zinc Finger Protein 583; DOCK8: Deducator of Cytokinesis 8; GJC1: Gap Junction Protein, Gamma 1; APC: Adenomatous Polyposis Coli; miRNAs: MicroRNAs; TuM2-PK: Enzyme Tumor Type M2; MMR: Mismatch Repair Genes; TGF- $\beta$ : Transforming Growth Factor Beta; TCF4: Transcription Factor 4; CEA: Carcinoembryonic Antigen; CA 19-9: Carbohydrate Antigen 19-9; NMCD: Non-Malignant Colorectal Disease; AFP: Alpha-

Fetoprotein; TPS: Tissue Polypeptide Specific Antigen; TAG 72: Tumor Associated Glycoprotein 72; SEPT9: Septin 9 Gene; DK: Dermokine; TIMP-1: Tissue Inhibitor of Metalloproteinases-1; HEX: N-acetyl- $\beta$ -D-Hexosaminidase; GAL:  $\beta$ -D-Galactosidase; FUC:  $\alpha$ -Fucosidase; MAN:  $\alpha$ -Mannosidase; NEUROG1: Neuro D3/Neurogenin 1/NGN1; PGE 2: Prostaglandin E2; PGE M: Prostaglandin M; PWS: Partial Wave Spectroscopy

### Introduction

Colorectal cancer is the third most frequent cancer in men, the second most frequent cancer in women, and the second most frequent cause of death by cancer when both genders are considered together [1]. The United States Preventative Services Task Force recommends screening for colorectal cancer beginning at age 50 years and continuing until age 75 years, using fecal occult blood testing, sigmoidoscopy, or colonoscopy [2]. Colonoscopy is currently considered the preferred method of evaluation because it is the best

technique for visualizing the entire colonic mucosa and provides the ability to remove colon polyps to potentially prevent colorectal cancer [3]. However, the procedure is relatively invasive, expensive, requires considerable expertise and equipment [4], and can cause abdominal discomfort and pain [5]. Moreover, the quality of the colonoscopy depends on the bowel preparation, which many patients find unpleasant resulting in decreased patient compliance [6]. An ideal screening technique should be able to detect disease at a curable stage, should be highly sensitive and specific, and acceptable to elicit high participation rates. The screening method should also be affordable, safe for patient and physician and easy to perform with benefits outweighing adverse effects [4]. There is much room for improvement to accomplish all of these goals in assessing currently recommended screening techniques.

To emphasize, there is a need for more convenient, cost-effective, non-invasive, simple-to-use methods with better sensitivities and specificities that allow for earlier detection of colorectal cancer, which ultimately lead to better survival rates and decreases the incidence and mortality of this disease [4]. In lieu of detecting the cancer itself, some tests are designed to look for surrogate marker or biomarker. A biomarker is a cellular, biochemical or molecular alteration that is an indicator of a biological process, pathogenic process, or pharmacological responses to a therapeutic intervention [7]. It might be either a molecule secreted by the tumor or a specific response of the body to the presence of the tumor such as an antibody. It is advantageous for these biomarkers to be measurable in serum, urine, or sputum, which maximizes its usefulness and minimizes the cost of screening [8]. Validation of biomarkers is a regulatory requirement involving several different criteria to ensure reproducible and accurate data validated by multicenter studies [9]. Early Detection Research Network has established clear milestones for reaching a decision of “go” or “no go” during the biomarker development process, which are established on the basis of statistical criteria, performance characteristics of biomarkers, and anticipated clinical use [10]. The system consists of (a) establishing a reference set of specimens collected under PROBE (Prospective Specimen Collection Retrospective Blinded Evaluation) design criteria, (b) using the reference set to prevalidate candidate biomarkers before committing to full-scale validation, (c) performing full-scale validation for those markers that pass prevalidation testing, and (d) ensuring that the reference set is sufficiently large in numbers and volumes of sample that it can also be used to study future candidate biomarkers [11]. This review will explore non invasive biomarker methods including stool, serum, and urine assays that have potential for colorectal cancer screening and detection.

### Fecal Occult Blood Test (FOBT)

FOBT is an easy, noninvasive, and simple diagnostic test for colorectal cancer [12]. It detects hemoglobin in feces which indicates bleeding from the gastrointestinal tract [13]. Currently, there are two main types of FOBTs: guaiac-based fecal occult blood test and fecal immunochemical test (FIT).

Guaiac-based fecal occult blood test (gFOBT) is very widely used; it is simple and has a proven benefit [14]. Several large randomized control trials showed that a screening program based on a gFOBT repeated every 1 or 2 years reduces colorectal cancer [CRC] specific mortality by about 16% [15-20], and rehydrating the sample before development will increase its sensitivity and decrease CRC incidence [12,21]. It has a low sensitivity [25%-38%] for detecting CRC, however,

and it is even lower for advanced adenomas (16%-31%) [12,21]. It has a specificity of 87%-98% [22]. Repeating the test increases its sensitivity up to 90% [13]. There are several disadvantages of gFOBT besides its low sensitivity. It is not specific for human hemoglobin, and it requires three consecutive stools to get a result. These factors can limit patient compliance [21]. In addition, this test is a nonspecific indicator of CRC because a positive test may occur with polyps >1-2 cm [13]. Another disadvantage is that the laboratory quality control opportunities are limited, and they have a fixed hemoglobin concentration cutoff determining positivity, which means they are not capable of quantification [14].

Fecal immunochemical test uses antibodies specific for the globin moiety of human hemoglobin. It is not interfered by other fecal contents including medication and dietary products [14], and it is insensitive to upper GI bleeding [21]. The qualitative FIT test devices have a pre-set sensitivity for detecting globin that determines whether a test is positive or negative. These devices are primarily used at the point-of-care. The quantitative FIT test allows the user to set a cutoff concentration to a desired sensitivity or specificity, which enables clinical interpretation of the result and its significance associated with the risk of colorectal cancer [14,21]. The diagnostic performance of FITs depends on the cutoff value for a positive test result; various studies have defined different optimal cut off values [23,24].

A meta-analysis of nineteen studies evaluating diagnostic accuracy of FITs for CRC in asymptomatic, average-risk adults showed a pooled sensitivity of 79% and specificity of 94% [23]. Increasing the number of FIT samples did not affect the pooled sensitivities or specificities [23,24]. A Japanese study showed that the risk of CRC was 63% lower in repeated testing than in initial screening group [25]. In another study, randomly selected asymptomatic patients that underwent colonoscopy were also asked to undergo one sample FIT, and the authors found that the rates of false-positive and false-negative results were 65% and 64%, respectively [26]. Participants older than 60 years and smokers had a significantly higher risk of a false negative FIT result. Males, smokers, and regular NSAID users were at increased risk of a false positive result [27]. Although previous studies have concluded that antiplatelet medications can cause false positive fecal test [28], a more recent study demonstrated that the positive predictive value of FIT was not affected by ongoing antithrombotic therapy [29]. Some previous studies concluded that FIT is unstable at temperatures greater than 20 degrees Celsius [14,21] which could cause a decrease in positivity rate in the summer, but a recent study concluded that seasonal variations do not have an effect on the superiority of FIT [30].

Guaiac-based FOBTs, although widely used, have significant deficiencies, and FIT assays for hemoglobin have been established as the better, easier tests that are more acceptable by patients [14]. Future studies may involve identifying an optimal cutoff value for defining a positive result in FIT, comparing the performance of different commercial FIT brands, and assessing FIT's impact on CRC specific mortality.

### Stool DNA testing

Stool DNA [sDNA] testing relies on the concept that there is continuous and abundant exfoliation of dysplastic cells into the lumen as the stool passes through the colon [31]. Colorectal neoplasms are more prone to exfoliation because of increased proliferation, decreased apoptosis, and decreased cell to cell adhesion inherent in the neoplastic transformation process [32]. The vast majority of tumors (about 50% to 80%) are caused by chromosomal instability with more abundant

DNA per cell compared to normal cells, while a smaller fraction (10% to 15%) are characterized by microsatellite instability [33].

### Stool nucleic acids

In earlier blinded screening studies, only about half of the screen-relevant neoplasms were detected by sDNA testing [34]. Their performance was compromised by various technical limitations [35]. Several key technical advances have led to increasingly accurate approaches for stool DNA testing including use of a DNA preservative buffer with stool collection in order to stabilize the analyte [31,36,37], efficient target capture and amplification methods [31,38], broadly informative marker panels [31,37,39], and sensitive, automated assay methods [31,40]. In a large clinical assessment of a next-generation sDNA test that incorporates the technical advances, Ahlquist et al. concluded that sDNA screening showed high detection rates for neoplasm or adenoma ( $\geq 1$  cm), the detection rates were not affected by lesion site [proximal colon vs. distal colon], and detection of the neoplasm increased in proportion to adenoma size [35].

### Multitarget stool DNA test

Studies have shown that combinations of molecular markers in stool DNA testing produce high detection rates for both colorectal cancer and advanced adenomas. Imperiale et al. compared a multitarget stool DNA test to FIT. The multitarget stool DNA test included quantitative molecular assays for KRAS mutations, aberrant NDRG4 and BMP3 methylation, and  $\beta$ -actin, plus a hemoglobin immunoassay, which is almost identical to the assay used in FITs. The sensitivity of the DNA test for the detection of both colorectal cancer (92.3%) and advanced precancerous lesions—which included advanced adenomas or sessile serrated polyps  $\geq 1$  cm in greatest dimension (42.4%) exceeded that of FIT by an absolute difference of nearly 20 percentage points. However, FIT was more specific for the detection of both colorectal cancer and advanced precancerous lesions, by absolute differences of 6.6% to 8.3% because of fewer false positive results [41]. These results were supported by another study which showed one-time screening with a new stool DNA test (Cologuard) detected 92% of cases of colorectal cancer in asymptomatic average-risk persons, but detected less than half of advanced precancerous lesions and produced a substantial number of false-positive results. This multitarget stool DNA test is now approved by Food and Drug Administration to screen average-risk adults  $\geq 50$  years for CRC, and costs \$599 [42]. In another recent study, Lidgard et al. developed an automated, multitarget stool DNA test with the same combination of markers. They found that the sensitivity of sDNA for detecting CRC is 98% and specificity is 90%. The sensitivity for advanced adenomas increased with increasing size, and the test was able to detect advanced adenomas and high grade dysplasias with a sensitivity of 83% [40].

Another study compared the sensitivities of a multimarker test for stool DNA and a plasma test for methylated septin 9 (SEPT9) in identifying patients with large adenomas or CRC. They found that the sDNA test detected adenomas (1 cm to 5 cm) with 82% sensitivity and CRC with 87% sensitivity, whereas plasma test detected adenomas with 14% sensitivity and CRC with 60% sensitivity [43]. In a multicenter, case-control study, Ahlquist et al. utilized 4 methylated genes (NDRG4, BMP3, vimentin and TFP12), a mutant form of KRAS,  $\beta$ -actin gene, and hemoglobin to create a stool DNA test based on a quantitative allele-specific real-time target and signal amplification assay to assess colorectal neoplasm by a next-generation sDNA test. The sDNA identified 85% of patients with CRC and 54% of patients with

adenomas  $\geq 1$  cm with 90% specificity [35]. Overall, these studies show very promising results and larger studies are needed to further improve the stool DNA test [44,45].

### Epigenetic biomarkers: DNA methylation, hypermethylation, and hypomethylation markers

Colorectal cancer is driven by the accumulation of genetic abnormalities and epigenetic alterations [44]. Epigenetic alterations, particularly aberrant DNA methylation [including hypomethylation and hypermethylation] are now considered to be one of the earliest abnormalities in the progression of adenoma to carcinoma [39]. Epigenetic alterations continue to evolve and contribute to tumor progression [32], influencing key transformation steps in CRC formation [38]. Changes in DNA methylation promote progression of adenomatous precursor lesions into malignant tumors [33]. Epigenetic markers are actively being investigated to assess their utility in screening and detection of colorectal cancer. Many of the advances in methylation biomarker development are mainly based on real-time polymerase chain reaction (PCR) approaches. The current gold standard for genome-wide identification of differentially methylated regions at single nucleotide resolution involves whole-genome sequencing of bisulphite treated DNA [44].

A study by Mori et al. found that the following methylated genes achieved the highest discriminative accuracy in identifying CRC from normal mucosa: Visual system homeobox 2 (VSX2) (most accurate), BEN domain containing 4 (BEND4), neuronal pentraxin I [NPTX1], ALX homeobox 3 (ALX3), miR-34b, glucagon-like peptide 1 receptor (GLP1R), BTG4, homer homolog 2 (HOMER2), zinc finger protein 583 (ZNF583), dedicator of cytokinesis 8 (DOCK8), and gap junction protein, gamma 1 (GJC1). Adenomas were discriminated from control by hypermethylation of the following markers: of VSX2, BEND4, NPTX1, miR-34b, and HOMER2, indicating that these might constitute ideal markers for early-stage disease detection [38]. In a meta-analysis, 30 clinical studies were analyzed for the diagnostic value of CRC detection from hypermethylated DNA in stool [46]. The results indicated a great diagnostic potential for stool DNA hypermethylation as a reliable marker for CRC.

### Genetic biomarkers

The most common genetic biomarkers investigated to diagnose colorectal cancer include Adenomatous polyposis coli (APC), P53, KRAS, and BAT 26 [44]. *APC* and *P53* are important tumor suppressor genes mutated in CRC [47]. KRAS and BRAF are oncogenes mutated in CRC [48,49]. The earliest of these studies focused on detecting isolated KRAS and APC mutations, but due to the heterogeneous mutational spectrum of cancers, multiple assays to detect multiple genes were developed to improve sensitivity [44].

### Long DNA

Long DNA is derived from cancerous or precancerous cells shed from dysplastic mucosa which have not undergone apoptosis. The latter is the physiological mechanism that eliminates most normal colonic epithelial cells and results in DNA being fragmented into small sizes. Thus, shed cancer cells contain longer, intact DNA as transforming cells are resistant to apoptotic processes [49]. Studies have shown that the sensitivity of the long DNA assay using a combination of four genes was increased when compared to that of single genes, and the sensitivity was higher in distal CRC (64.4%) than

in proximal CRC (37.5%). The effective detection of distal colon neoplasms is important, as >70% of CRCs occur in the distal colon [49].

### MicroRNA

MicroRNAs [miRNAs] are short, non-coding RNA that regulate gene expression post-transcriptionally by controlling coding mRNA stability or mRNA translation. MicroRNAs control diverse cellular processes including developmental transitions, organ morphology, apoptosis, and cell proliferation [50]. They are commonly dysregulated in neoplasia [32] and are now well established as contributing to drive tumorigenesis, either by their downregulations (tumor suppressor miRNAs) or by their up-regulations (oncogenic miRNAs) [51]. Recent advances suggest that genetic variations or polymorphisms that are present in miRNAs play roles in hereditary cancer-susceptibility and can be useful for cancer diagnosis. The detection of polymorphisms may potentially improve cancer diagnosis [51].

There are different, even conflicting results in publications concerning the diagnostic accuracy of miRNAs. Ahmed et al. found that levels of twelve miRNAs (miR-7, miR-17, miR-20a, miR-21, miR-92a, miR-96, miR-106a, miR-134, miR-183, miR-196a, miR-199a-3p and miR214) were increased in the stool of patients with colon cancer whereas levels of eight other miRNAs (miR-9, miR-29b, miR-127-5p, miR-138, miR-143, miR-146a, miR-222 and miR-938) were decreased in the stool of patients with colon cancer. All these miRNA changes were accentuated as cancers progressed [52]. Other studies have found that the levels of stool miR-221 [53] and miR-135b [54] were higher in patients with CRC. In both of these studies, there were no significant differences in stool biomarker levels found between patients with proximal and distal CRCs [53,54]. Wu et al. showed that stool miR-92a could be used for early detection of CRC. In contrast to previous studies on other miRNA stool biomarkers which indicate the same sensitivity for distal and proximal CRC, stool miR-92a level was reported as having higher detection sensitivity for distal CRC compared to proximal CRC [55]. Other studies have found that expression levels of tumor suppressor miR-143 and miR-145 [56] and miR-4478 and miR-1295b-3p [57] were lower in the stool of the CRC patients compared to healthy controls.

A meta-analysis of 80 studies (55 involving single-miRNA assays and 25 involving multiple-miRNA assays) aimed at evaluating the diagnostic value of miRNAs in CRC detection suggests that multiple-miRNA assays show a greater diagnostic accuracy compared to single-miRNA assays. In addition, blood-based miRNA assays were more accurate than stool-based miRNA assays, and results were more accurate in Asians compared to Caucasians [58].

Genomic and proteomic technologies can be used to measure expression levels of transcripts and proteins and have the potential to be used to develop biomarkers. Studies have shown the advantage of integrating proteomics and emerging methylomic and miRNAomic strategies in the identification and validation of colorectal cancer (CRC) biomarkers [59]. All these studies suggest that stable miRNAs maybe used as potential non-invasive molecular biomarkers for CRC diagnosis. Larger, randomized, prospective studies are necessary, however, to analyze the ability of miRNA to detect different stages of colon cancer in order to determine the true sensitivity and specificity of stool miRNA for early detection of CRC.

In this regard, an interesting proposed technological advance includes developing a multiplex miRNA chip to enhance molecular screening for colon cancer [52].

### Fecal protein assay biomarkers

Enzyme Tumor type M2 pyruvate kinase (TuM2-PK) is expressed by neoplastic colonocytes and is a potential biomarker for CRC detection. Studies have found that tumor TuM2-PK activity was more sensitive than FOBT [60,61], but the assay was less specific, more expensive, and slower when compared to immunologic FOBT [62,63].

### Microsatellite Instability

A microsatellite is a non-coding stretch of DNA in which short sequences of mono, di, or tri-nucleotides are repeated many times. With loss of function of mismatch repair genes (MMR), the microsatellites are not replicated faithfully as base insertions or deletions are not corrected and new microsatellites of different lengths are created [47]. MMR genes play a key role in maintaining genomic stability [48]. Defective DNA mismatch repair is present in approximately 15% of sporadic colorectal cancers [64] and also in the Lynch syndrome, a hereditary type of colon cancer. Several key genes are mutated in tumors with MMR defects such as BAX, Caspase 5, transforming growth factor beta (TGF- $\beta$ ) type II receptor, and transcription factor 4 (TCF4). These tumors have distinct histological and molecular features that include poorly differentiated histology, mucinous phenotype, marked lymphocytic infiltration, and diploid status [65]. Shemirani et al. used a panel of five mononucleotide microsatellite markers (NR-21, BAT-26, BAT-25, NR-27 and NR-24), and found that NR-21 was the most useful marker for diagnosis of MMR defective CRC, followed by BAT-25 and NR-24 in tumor tissues [66].

### Serological biomarkers

Diagnostic blood tests are currently available for nonenzymatic tumor markers and these include CEA, CA19-9, TPS, TAG72, and SEPT9.

CEA [carcinoembryonic antigen] is an oncofetal protein [67] that is significantly increased in the serum of some patients with adenocarcinoma of the colon [68]. It is fairly specific for CRC but its sensitivity and validity are not sufficient for early cancer detection [69]. Several studies have supported the use of a combination of CEA with another biomarker for early CRC detection [70]. Diagnostic blood tests based on the detection of CEA are currently widely available, although the sensitivity of this marker in early-stage cancer is only 5–10% [14]. The major role for CEA is to detect recurrences in CEA positive tumors following tumor resection.

CA19-9 (carbohydrate antigen 19-9) is a tumor antigen that is elevated in the serum in some colorectal [67] cancers as well as other GI malignancies [69]. It is not specific for a particular tumor type and it is less sensitive than CEA [69]. A recent retrospective-prospective study which assessed CEA and CA 19-9 levels in serum of 91 patients with histologically confirmed diagnosis of colorectal adenocarcinoma concluded that CEA and CA 19-9 are late markers for the detection of colon cancer, and are significantly elevated in many patients with metastatic but not early colorectal cancer [67]. In a retrospective study involving 46 patients with pathological or cytological confirmation of CRC and 36 cases with non-malignant colorectal disease [NMCD], the diagnostic CRC sensitivity for detection for CEA, alpha-fetoprotein

(AFP) and CA19-9 were 80.43%, 73.91% and 69.57%; the diagnostic specificity of CRC for CEA, AFP and CA19-9 were 75.00%, 69.44% and 61.11%. The authors suggested that serum CEA, AFP and CA19-9 may be a useful biomarker panel for diagnosis of colorectal carcinoma against NMCD [71].

TPS [Tissue polypeptide specific antigen] is a single conjugated polypeptide chain that is released from cells after mitosis. TPS serum concentration is increased with underlying tumors and reflects the rate of cell division and may therefore be a marker of early tumor stage [69].

TAG 72 (Tumor associated glycoprotein 72) has a diagnostic sensitivity of 28 to 67% as a CRC marker, and has been recommended to be included along with other biomarkers [69]. In addition, a blood-based assay that detects methylated septin 9 gene (SEPT9) has been commercialized by Epigenomics AG [Berlin, Germany] under the name Epi proColon® and is currently being marketed in several countries [44].

Other serum markers that are currently being investigated and have promising potential include cytokeratins [72], Dermokine (DK) [73], Melanotransferrin [74], N methyltransferase and Tissue inhibitor of metalloproteinases-1 (TIMP-1) [70,75-77]. Other enzymatic tumor markers include neutrophil elastase [78], cathepsin D and lysosomal exoglycosidases such as N-acetyl-β-D-hexosaminidase (HEX), its isoenzymes A (HEX A) and B (HEX B), β-D-galactosidase (GAL), α-fucosidase (FUC), and α-mannosidase [MAN], and cathepsin D [68,69,79,80]. Other potential biomarkers under investigation include methylation abnormalities of Neuro D3/Neurogenin 1/NGN1 [NEUROG1] [81], TAC1, EYA4 [33], RUNX3 [82], S100P promoter [83], p16 [84] and THBD [85], volatile organic compounds and several microRNAs [86-88].

## Urine Biomarkers

Urine contains tumor markers including tumor-derived DNA released into the circulation [89]. The levels of urinary markers need to be high enough in the plasma to exceed renal reabsorption [90]. Recent studies have suggested many urine DNA markers for potential early detection of CRC including DNA for arylsulfatase [91], lysosomal exoglycosidases and cathepsin D [68,80]; prostanoids metabolites, Prostaglandin E2 and M (PGE2, PGEM) [90,92]; (N(1),N(12)-diacetylspermine) and DiAcSpd (N(1),N(8)-diacetylspermidine [93,94], changes in DNA methylation in several genes including vimentin [95], Wif-1 and ALX-4 [96]; detection of mutant genes such as KRAS [89]; the presence of volatile organic compounds [97], panel of metabolite biomarkers including citrate, hippurate, p-cresol, 2-aminobutyrate, myristate, putrescine, and kynurenate [98], and nucleosides including adenosine, cytidine, N(2),N(2)-dimethylguanine, 8-hydroxy-2'-deoxyguanosine and uridine [99]. Urinary PGE-M and volatile organic compounds seem to be promising biomarkers for detection of advanced adenomas or multiple adenomas [92] and CRC detection [97], respectively.

## Other Technologies

Imaging technologies also show promise for early CRC detection. Partial wave spectroscopic [PWS] microscopy allows for the detection of nanoarchitectural changes in normal appearing colon that harbors remote neoplasia. PWS microscopy measures the disorder strength, which is a statistical parameter proportional to the size and density of macromolecular structures in patient's cells [100]. This technique can

be used to screen for adenomas in the colon by brushing the endoscopically normal rectum. In individuals harboring adenomas, increased disorder strength has been reported [101]. The quantified nanoarchitectural changes are believed to be driven by intracellular process, such as chromatin clumping, which may reflect cancer risk and derangements in the genetic and microenvironmental milieu [102-104]. PWS microscopy is currently not clinically useful because of low sample throughput associated with the slow manual process required to analyze signals [100]. In the future with improvements in throughput analysis, however, PWS might provide a minimally invasive, highly accurate methodology for cancer screening [101].

An interesting proposed technological advancement for the future includes developing a chip to enhance molecular screening for colon cancer, as it has been accomplished for the detection of genetically-modified organisms in foods [52].

## Cancer detection Vs. Polyp detection

Detecting colon cancer early in patients allows for better survival rates, longer survival, and a better quality of life [32]. Although these non-invasive tests have shown some promise for diagnosis of established colorectal cancer, they do not have the sensitivity to detect pre-cancerous lesions. Noninvasive approaches such as fecal testing and plasma-based DNA tests tend not to detect earlier-stage CRC compared to their improved ability to detect later-stage CRC [35]. Detection rates of tumor DNA in stool progressively increase with increased adenoma size beyond 1cm which if further optimized could allow for detection of precursor lesions [35]. Some studies, however, have shown changes in certain miRNAs and volatile organic metabolites offer high sensitivities for early detection of polyps [102]. Stool mutant KRAS detection may be useful for early dysplasia and hyperplastic polyps [47], whereas VSX2, BEND4, NPTX1, miR-34b, HOMER2 [38], serum melanotransferrin [60], serum DK-β/γ [73], urine lysosomal exoglycosidases, cathepsin D [68,80], and PGE-M [92], are promising biomarkers for early-stage CRC diagnosis.

## Adherence and patient factors

Stool-based DNA tests are easy to perform, requiring only one sample, and no bowel preparation [33]. With mail-in next-day delivery, geographic availability and access are no longer barriers. Samples can be collected in privacy without inconvenience. These advantages also lead to increased patient compliance [32]. Serologic biomarkers can be analyzed relatively noninvasively and economically compared to other diagnostic procedures [73]. Blood and urine testing could be used as an option in individuals that decline colonoscopy and stool testing. Advantages to urine studies compared to serum or plasma studies are that the urine testing can be done in remote geographic areas and requires no special facility or equipment apart from sterile collection containers, as compared to more expensive and more demanding biosafety requirements for serum or plasma collection [89].

## Conclusion

Colonoscopy continues to be the gold standard for screening and detection of colorectal cancer. In contrast to endoscopy based screening such as colonoscopy or radiology based tests, biomarker assays are more cost effective, less invasive, and more convenient. Research studies have shown that many of the biomarkers have high sensitivity and specificity and are promising for CRC screening and diagnosis. In particular, many studies support the use of combination

tests as opposed to single biomarker to screen for cancer. Although these non-invasive methods allow for diagnosis of colorectal cancer, they do not offer an immediate therapeutic solution like colonoscopy with polypectomy. Long term prospective-multicenter clinical studies with large sample sizes are needed to determine the sensitivity and specificity of these biomarkers and to assess whether some combination of these assays could serve as first line screening tests for colorectal cancer.

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