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Cancer Epidemiology, Biomarkers & Prevention

Review

Biomarkers for Early Detection of Colorectal Cancer and Polyps: Systematic Review

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Abstract

There is growing interest in early detection of colorectal cancer as current screening modalities lack compliance and specificity. This study systematically reviewed the literature to identify biomarkers for early detection of colorectal cancer and polyps. Literature searches were conducted for relevant papers since 2007. Human studies reporting on early detection of colorectal cancer and polyps using biomarkers were included. Methodologic quality was evaluated, and sensitivity, specificity, and the positive predictive value (PPV) were reported. The search strategy identified 3,348 abstracts. A total of 44 papers, examining 67 different tumor markers, were included. Overall sensitivities for colorectal cancer detection by fecal DNA markers ranged from 53% to 87%. Combining fecal DNA markers increased the sensitivity of colorectal cancer and adenoma detection. Canine scent detection had a sensitivity of detecting colorectal cancer of 99% and specificity of 97%. The PPV of immunochemical fecal occult blood test (iFOBT) is 1.26%, compared with 0.31% for the current screening method of guaiac fecal occult blood test (gFOBT). A panel of serum protein biomarkers provides a sensitivity and specificity above 85% for all stages of colorectal cancer, and a PPV of 0.72%. Combinations of fecal and serum biomarkers produce higher sensitivities, specificities, and PPVs for early detection of colorectal cancer and adenomas. Further research is required to validate these biomarkers in a well-structured population-based study. *Cancer Epidemiol Biomarkers Prev; 23(9); 1712–28. ©2014 AACR.*

Introduction

Colorectal cancer is the third most commonly diagnosed cancer in the world. It is estimated that worldwide 1.23 million new cases of colorectal cancer are diagnosed annually, and around 608,000 deaths are due to colorectal cancer a year (1). Colorectal cancer is known as a "silent" disease, as many people do not develop indicators, such as bleeding or abdominal pain until the cancer is difficult to cure (2). Most colon cancers start as noncancerous growths called polyps. If the polyps are removed, then the cancer may be prevented. Survival from colorectal cancer is significantly affected by the stage of the disease at presentation. Those presenting with early cancers Dukes A ($T^{1/2}N^0M^0$) have a 93.2% 5-year survival rate, in contrast to those presenting with a Dukes C ($T^{3/4}N^{1/2}M^0$) cancer in which the 5-year survival rate drops to 47.7% (3). Therefore, early detection of precancerous colorectal lesions plays a key role in improving the 5-year survival rate.

Therefore, screening for colorectal cancer has the potential to reduce both the incidence and mortality from this disease (4). The key strategy for these screening programs is detecting and eliminating colonic lesions before they become cancerous or symptomatic, to remove them at an earlier stage of disease (4).

There are several screening modalities in practice for colorectal cancer, including fecal occult blood testing (FOBT), flexible sigmoidoscopy, and colonoscopy. Each one has its own merits and disadvantages. Pooled meta-analyses of randomized trials show that FOBT screening reduces colorectal cancer mortality by 16% and flexible sigmoidoscopy screening reduces colorectal cancer mortality by 30% (5). Despite certain degrees of success with these modalities, there are still overwhelming limitations.

Patient adherence to FOBT program is low at 40% to 50% (6). Other limitations of FOBT screening include its low sensitivity for polyps and detecting cancers located in the distal colon. In addition, the test has a relatively low specificity, and thus there are many false-positive screens, which, as can be seen from our cost analysis, have a significant cost implication. For it to be most effective, repetitive screening is necessary (7).

Flexible sigmoidoscopy is a fairly quick and safe test, which does not usually require the need for full bowel preparation and can be performed without sedation. There is also a lower risk of serious complications compared with colonoscopy, such as perforation or bleeding.

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However, compliance issues are still likely to be a problem, with pilot studies showing a likely uptake of only 50% (8). In addition, the quality of the prep can be very variable, which can limit its usefulness.

Colonoscopy is the gold-standard screening test, and is used in Germany, with a sensitivity and specificity for identifying polyps and cancers in excess of 98% (9). However, it is an invasive test, needs repeating frequently (3–5 years), and is expensive to implement, has poor compliance rates, and there is risk of perforation of between 1 in 1,000 and 10,000 colonoscopies. Therefore, these limitations render this test unsuccessful as a screening tool in terms of cost to implement in many countries. Computed tomography (CT) colonography is another alternative to colonoscopy, but has the same limitations as the latter, and radiation concerns limit its use in the general population (10).

There is anecdotal evidence that individuals who do not comply with the current screening programs are usually those with the highest risk of having a cancer (6). Therefore, a drive to identify simpler, less invasive tests to improve compliance has stimulated considerable interest in researching potential biomarkers.

A biomarker is a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention. Hundt and colleagues (11) in 2007 reviewed a wide variety of potential blood markers in their systematic review of colorectal cancer. In the last decade, there has been substantial experimental work in cancer research with significant improvements in our understanding of cancer biology and thereby identifying new potential targets. Several areas of interest in recent reviews have been the search for new epigenetic biomarkers (12), proteomic markers (13), and fecal DNA markers (14), in an attempt to develop a novel screening modality that can overcome the inherited limitations of the current screening modalities.

The aim of this study was to systematically review the recent literature to identify all published biomarkers for early detection of colorectal cancer and polyps, to summarize performance characteristics of each biomarker, to assess these characteristics within the context of disease prevalence, and evaluate their suitability to be used for designing new screening tests for colorectal cancer.

Materials and Methods

Search strategy

A comprehensive systematic review of published work was conducted according to the preferred reporting items of systematic review and meta-analysis (PRISMA) guidelines. Literature searches were performed in the Ovid SP versions of Medline, EMBASE, and PubMed using MeSH terms, search terms, and Boolean operators with synonyms and plurals in addition to keywords. The search strategy was designed by 3 reviewers (J.A. Conti, E. Jones, and N.K. Francis) and conducted by E. Jones and R. Shah. The search terms presented in Table 1 were used as keywords in several combinations to conduct the search strategy.

Two reviewers (J.A. Conti and N.K. Francis) independently assessed titles and abstracts of all abstracts as part of the primary screen. A secondary screen of titles and abstracts was then conducted by a further 3 reviewers (P.J.K. Kuppen, V. Vidart, and E. Jones). Following the second screen full text articles were obtained and reviewed by J.A. Conti, N.K. Francis, and R. Shah. The search results were supplemented with hand searching of the reference lists. The results were analyzed by R. Shah, J.A. Conti, and N.K. Francis. All authors contributed to drafting the manuscript.

Eligibility criteria

Studies published between January 1, 2007, and June 30, 2013, were included to ensure that all new published evidence on potential markers for colorectal cancer screening since the last large systematic review were encompassed. All study designs were included as well as validated and unvalidated measures. The review was limited to studies on humans published in English that addressed early detection of colorectal cancer and/or colorectal polyps using biomarkers.

Exclusion criteria

Reasons for exclusion included studies with less than 10 participants, those conducted on cell lines and not in part on human subjects. In addition, studies that were designed for prognostic purposes and/or to assess advanced cancer (defined as stage III or IV) or its response to chemotherapy were excluded. In addition, the study was limited to biomarkers; hence, all other conventional tumor blood markers such as carcinoembryonic antigen were excluded. Finally, abstracts and conference proceedings were excluded because of the probability of incomplete data for a thorough review.

Data extraction

The studies, which satisfied the inclusion criteria, were categorized into fecal assessment, blood or serum assessment, tissue assessment, and a combination of tissue and blood assessment. These were then further subdivided depending on the category of marker being examined: (i) DNA biomarkers, (ii) RNA biomarkers, (iii) protein biomarkers, or (iv) other. Information about the number of cases and controls was obtained from each article. The cases were separated into those with colorectal cancer or those with adenomas, and where data were available, these were further partitioned by tumor stage or by adenoma size.

Outcome measures

The sensitivity and specificity, alongside their 95% confidence interval (CI) ranges, of each tumor marker was sought in order to describe the tumor markers performance characteristics and usefulness of each diagnostic test in their ability to detect a person with colorectal

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Table 1. Search strategy

1	(CSC or "Cancer Stem Cell" or "molecular marker*" or biomarker* or "free cancer cells" or "stem cell*" or fecal or fecal).ti,ab.
2	Limit 1 to y = "2006 -Current"
3	("early diagnosis" or "early detection" or diagnos* or detect*).ti,ab.
4	Limit 3 to y = "2006 -Current"
5	(Blood or serum or plasma or protein or DNA or RNA or tissue or assay).ti,ab.
6	Limit 5 to y = "2006 -Current"
7	("colorectal cancer" or colorectal or colon* or colorectal or polyp or adenoma).ti,ab.
8	Limit 7 to y = "2006 -Current"
9	prognos*.ti,ab.
10	Limit 9 to y = "2006–Current"
11	2 and 4 and 6 and 8
12	11 not 9

cancer or exclude a patient without colorectal cancer. The sensitivity of a test was defined as the probability that an individual with the disease would screen positive, and the specificity of the test was defined as the probability that an individual without the disease would screen negative. Combining the sensitivity and specificity alone could not be performed to estimate the probability of disease in a patient, or the usefulness of the test as a screening tool. However, when used in conjunction with disease prevalence, a positive predictive value (PPV) and negative predictive value were obtained.

Positive and negative predictive values

Disease prevalence for colorectal cancer or adenoma was sought from the literature and applied to one nation for demonstration (the UK population size is 63,230,000; ref. 15). Out of this population, approximately 8,852,200 would fall between the ages 60 to 74 years (16), for inclusion in the colorectal cancer screening program. The number of new cases of colorectal cancer diagnosed per year in the UK is approximately 40,695 (17), which leads to a disease prevalence of 1 in 1,500 people in the United Kingdom with colorectal cancer. Approximately 20% of the screening population have adenomas (18) but relatively few of these long-term will become cancers. Combining these values with the biomarker sensitivity and specificity in this review is aimed to enable calculation of the predictive values. A PPV illustrated the probability that an individual with a positive screening result has the disease; where as the negative predictive value illustrated the probability of a disease-free individual being given a negative result. This level of analysis enables an accurate evaluation of the diagnostic utility of biomarkers for detecting colorectal cancer or adenomas.

Methodologic quality

Methodologic quality of the 44 included studies was assessed. Points relevant to laboratory studies taken from the Cochrane collaboration's tool for assessing risk of bias (19) included assessment of detection bias (blinding of outcome assessment) and selection bias (randomization can prevent selecting interventions to participants). Data were also extracted on age and gender matching and whether or not the test was repeated, as these are also known indicators of quality in laboratory studies. Studies were graded under 1 of 3 categories [A = adequate (yes); B = unclear (not specified); C = not used (no); Fig. 1].

Results

A PRISMA diagram of studies selected for this systematic review is summarized in Fig. 2. The search strategy identified 3,348 suitable abstracts, from which 3,125 were excluded by review of the title and abstract during the primary and secondary screens, as they did not meet the eligibility criteria. Full text articles were obtained for 223 studies. A total of 179 of these articles were excluded for differing reasons, including not being original research articles (32 articles); written in a foreign language without an English translation (18 articles); research conducted on animals or cell lines, not humans (14 articles); reported inappropriate outcomes (19 articles); were not specific to colorectal cancer detection (19 articles); or did not have enough participants (15 articles).

A total of 44 papers, examining 67 different tumor markers were included in this review for data extraction and analysis. Included studies were conducted in-Germany, UK, USA, Australia, China, Japan, Spain, India, Italy, Poland, Sweden, Netherlands, Denmark, Canada, and Greece. They described a total of 9,908 participants: 3,393 in fecal testing, 4,628 in blood testing, 1,665 in tissue testing, and 222 in combined blood and tissue testing.

Fecal biomarkers

A total of 16 papers (20–35) evaluated 17 different fecal tumor markers. The results of all papers on fecal biomarkers are summarized in Table 2. These were further subdivided into fecal DNA biomarkers, RNA markers, combined DNA and RNA markers, protein assay markers, and other markers.

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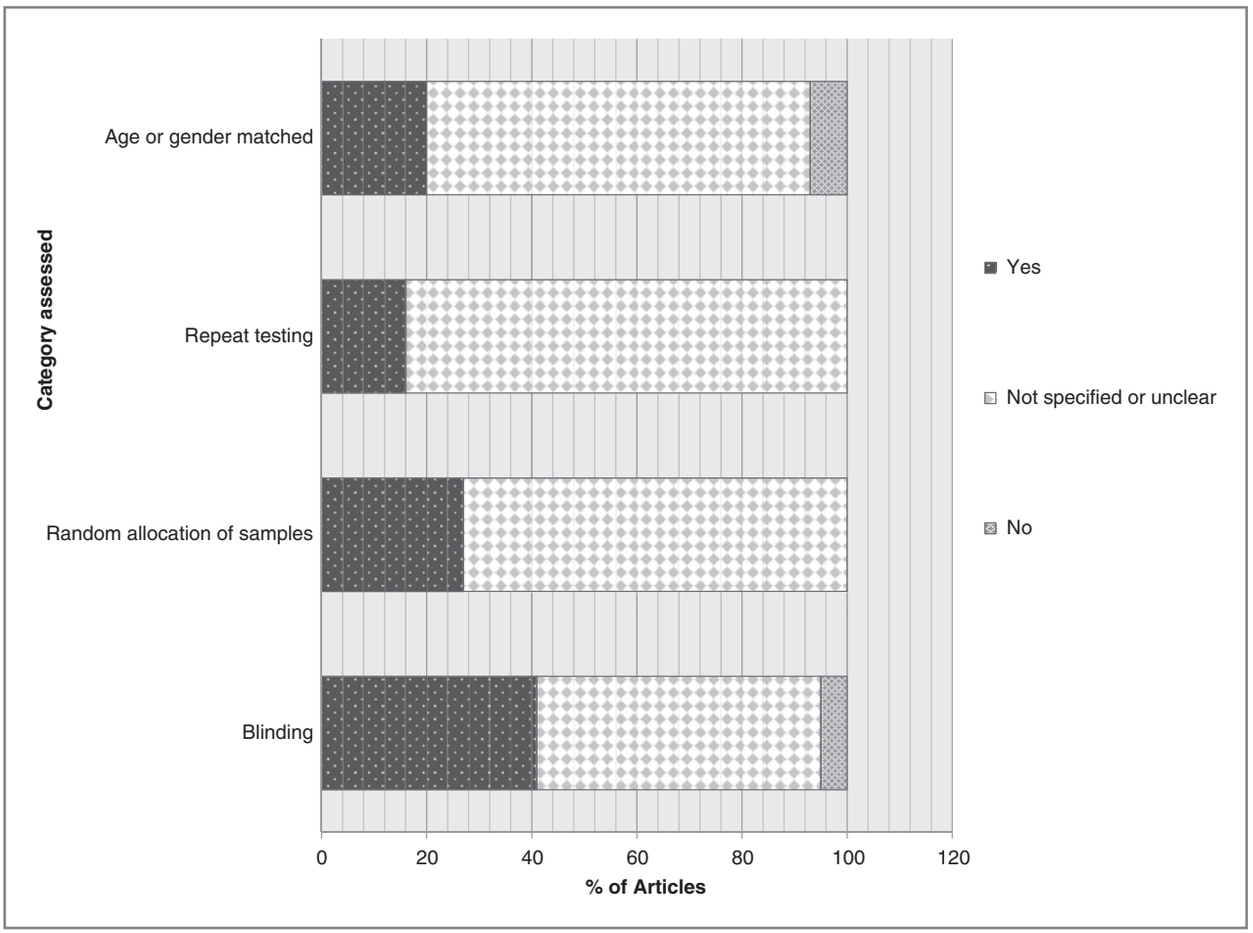

Figure 1. Risk of bias assessment.

Seven studies investigated fecal DNA markers, looking at DNA hypermethylation of a single gene, or of a panel of genes. Sample sizes for all 7 studies were relatively small with Kalimutho and colleagues (22) having the biggest sample size of 192 participants. Four of these studies (28, 30, 32, 34) assessed the tumor marker sensitivity according to colorectal cancer staging and 9 studies looked at the sensitivities for adenoma detection. Overall sensitivities for colorectal cancer detection by fecal DNA markers ranged from 53% to 87% with varying specificities, however, all above 76%. Adenoma detection sensitivity ranged from 17% to 61%.

Two studies (21, 25) examined the same tumor marker TFP12, obtaining similar results. Zhang and colleagues (21) however combined TFP12 with another marker, long DNA, to increase the sensitivity of colorectal cancer detection to 86%. Wang and colleagues (26), who evaluated SFRP2 expression, seemed to have very promising results with high sensitivities for both colorectal cancer and adenoma detection, however they have obtained these results with a significantly lower specificity (76%) than the other included studies. The fecal DNA markers, which obtained the highest sensitivities alongside high specificities, are SP20 (20) and long DNA, especially when long DNA is used in conjunction with another marker (TFP12 or iFOBT).

Two of the 16 papers evaluating fecal biomarkers examined mRNA markers. Takai and colleagues (28) looked at COX2 mRNA and MMP7 mRNA, whereas Leung and colleagues (27) solely looked at COX2 mRNA alongside a panel of DNA markers. Takai and colleagues (28) assessed different stages of colorectal cancer and Leung and colleagues (27) examined adenoma detection as well as colorectal cancer detection. Overall sensitivities for colorectal cancer detection with mRNA ranged from 38% to 96%, with Dukes B cancers having the higher sensitivity values.

Adenoma detection with COX2 mRNA only had a sensitivity of 4%. However, when COX2 mRNA and MMP7 mRNA where used as a combined marker, their sensitivity increased to 90% with a small 95% CI range. Leung and colleagues (27) assessed a 6-gene panel of DNA markers, which obtained a high sensitivity for adenoma detection (68%). This panel included SFRP2, which was also evaluated by Wang and colleagues (26) for adenoma detection, and showed a great improvement in specificity

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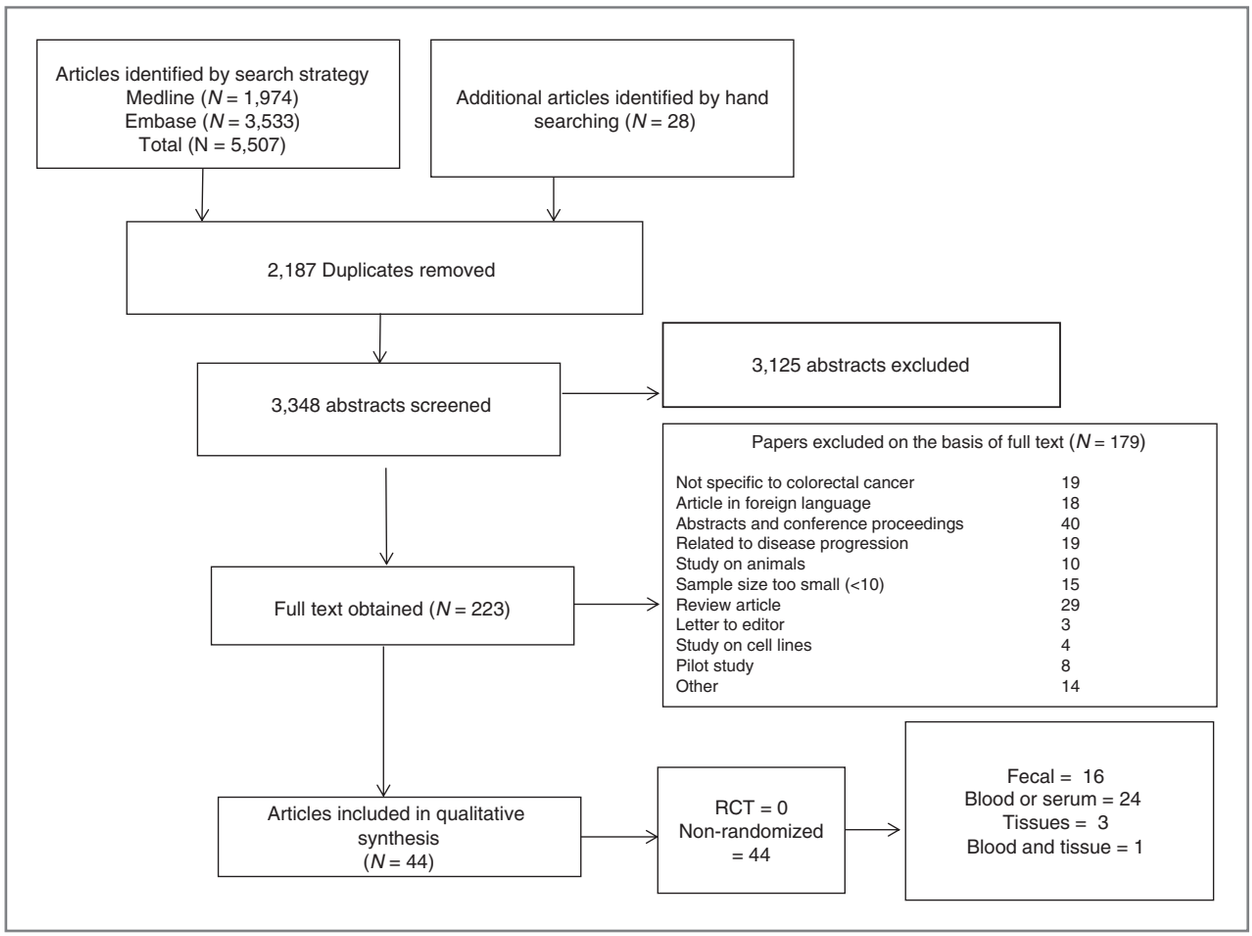

Figure 2. PRISMA diagram of studies searched and selected.

(90%) alongside a minor improvement in sensitivity when in combined use with other genes.

Six papers looking at fecal biomarkers assessed the same enzyme TuM2-PK as a potential biomarker in colorectal cancer detection, as this is derived from neoplastic colonocytes. These studies used a sandwich ELISA to measure TuM2-PK activity, obtaining overall sensitivities ranging from 68% to 91%. In the 2 studies by Shastri and colleagues (29, 33), they compared tumor M2-PK activity and guaiac based FOBT in the first study and subsequently immunologic FOBT in the second study. They found that although measuring tumor M2-PK activity was more sensitive than FOBT screening, when compared with iFOBT, the latter was more sensitive, cheaper, and faster than tumor M2-PK activity assays. Koss and colleagues (31) showed the tumor M2-PK assay could also be utilized to detect adenomas with a sensitivity of 60%. However, results were obtained on a sample size of 5 patients. When Shastri and colleagues (29) conducted sensitivities of M2-PK for adenoma detection for a larger sample size, sensitivities obtained were much lower at 37%.

The study by Sonoda and colleagues (35) looked at canine scent detection to determine whether odor material can become an effective tool in colorectal cancer screening. This test utilizes the olfactory ability of dogs to detect very low concentrations of the alkanes and aromatic compounds generated by tumors (volatile organic compounds, VOC). Canine scent detection had a sensitivity of detecting colorectal cancer of 99% and a specificity of 97% on a study of nearly 300 patients.

In summary, overall sensitivities for colorectal cancer detection by fecal DNA markers ranged from 53% to 87%, with varying specificities. Combining DNA markers increased the sensitivity of colorectal cancer detection and the use of a panel of fecal DNA biomarkers, as well as VOCs detection, seem promising options for future screening tools.

Blood/serum biomarkers

Table 3 lists the 24 (36–59) studies evaluating potential blood/plasma biomarkers in colorectal cancer detection. Overall sensitivity ranges from 30% to 94% with specificity greater than 46%. Eight papers assessed plasma DNA markers. Blood samples were analyzed for epigenetic changes of genes involved in the tumor progression sequence. Four of these papers (38, 40, 41, 43) evaluated

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Table 2. Summary of fecal biomarkers

Author + date	Journal	Method	Marker	Mechanism	Number of participants included: NC	Adenoma	Colorectal cancer	Sensitivity (%)	95% CI	Specificity (%)	95% CI
1. Fecal DNA Biomarkers											
Zhang 2013 (20)	Intern J Med Sci	MS-PCR	SP20	DNA hyper-methylation	30	0	96	CRC 80.2	NS	100	NS
Zhang 2012 (21)	Intern J Cancer Epidemiol	Fluorescent quantitative Alu PCR	TFPI2	DNA promoter methylation	30	20	60	CRC 68.3		100	
			Long DNA					CRC 53.3	NS	83.3	NS
			TFP12 + Long DNA					CRC 86.7		83.3	
			Long DNA					CRC 79	59–92	92	84–96
								Ad 17	9–28		
Kalimutho 2010 (22)	Int J Colorectal Dis	QdHPLC	iFOBT	DNA integrity	95	69	28	CRC 52	32–71	98	93–100
						34 Ad >1 cm		Ad 21	12–33		
						18 Ad <1 cm					
						17 HP					
			Calprotectin					CRC 72	51–88	75.5	66–84
								Ad 28	18–41		
			Long DNA + iFOBT					CRC 89.3	72–98	94.7	88–98
								Ad 33	22–46		
Azuara 2010 (23)	Clin Colorectal Cancer	MS-PCR	4 gene panel – RARB2, $P16^{INK4A}$, MGMT, APC	DNA hyper-methylation	20	20	26	CRC 62	41–83	NS	NS
								Ad 40	19–62		
Melotte 2009 (24)	J Natl Cancer Inst	MS-PCR	NDRG4	DNA promoter methylation	75	0	75	CRC 61	43–79	93	90–97
Glockner 2009 (25)	Cancer Res	MS-PCR	TFPI2	DNA promoter methylation	30	19	47	CRC 76	60–88	93	77–99
								Ad 21	6–46	93	78–99
Wang 2008 (26)	World J Gastroenterol	Methylight	SFRP2	DNA hyper-methylation	30	60:	69	CRC 87			
						34 Ad > 1 cm		Ad 61.8	NS	76.8	NS
						26 HP		HP 42.3			

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Table 2. Summary of fecal biomarkers (Cont'd)

Author + date	Journal	Method	Marker	Mechanism	Number of participants included: NC	Adenoma	Colorectal cancer	Sensitivity (%)	95% CI	Specificity (%)	95% CI
2. Combined fecal DNA + RNA biomarkers											
Leung 2007 (27)	Am J Gastroenterol	MS-PCR	COX2 MRNA	Over-expression of mRNA + DNA hyper-methylation	30	30 8 Ad > 1 cm 17 Ad <1 cm 5 HP	20	CRC 50 Ad 4	27–73 0–20	93	77–92
			6 Gene panel —APC, ATM, hMLH1, sFRP2, HLTF, MGMT					CRC 75 Ad 68	51–91 47–85	90	74–98
3. Fecal RNA biomarkers											
Takai 2009 (28)	Cancer Epidemiol Biomarker	RT-PCR	COX2 mRNA	Over expression of mRNA	29	0	62	CRC 87 A/B/C/D 77/96/82/82	76–94	100	90–100
			MMP7 mRNA				Dukes A/B/C/D 13/27/11/11	CRC 65 A/B/C/D 38/78/73/55	51–76	100	90–100
			iFOBT					CRC 73 A/B/C/D 38/81/91/73	60–83	90	73–98
			COX2 mRNA + MMP7 mRNA					CRC 90	80–96		
4. Fecal protein assay biomarkers											
Shastri 2008 (29)	Am J Gastroenterol	ELISA	TuM2-PK	Isoenzyme expression in proliferating cells	516	69 21 Ad > 1 cm 48 Ad < 1 cm	55	CRC 78.2 Ad 37.7	65–88 26–50	73.8	67–77
			iFOBT					CRC 70.9 Ad 30.4	57–84 20–43	96.3	94–98
Mulder 2007 (30)	Eur J Gastro & Hepatology	ELISA	TuM2-PK	Isoenzyme expression in proliferating cells	63	47	52	CRC 85 A/B 67 C/D 89 Ad 28	NS	90	NS
			iFOBT					CRC 92 A/B 100 C/D 89 Ad 40	NS	97	NS

(Continued on the following page)

Table 2. Summary of fecal biomarkers (Cont'd)

Author + date	Journal	Method	Marker	Mechanism	Number of participants included			Sensitivity (%)	95% CI	Specificity (%)	95% CI
					NC	Adenoma	Colorectal cancer				
Koss 2007 (31)	Int J Colorectal Dis	ELISA	TuM2-PK	Isoenzyme expression in proliferating cells	13	10 5 Ad >1 cm 5 Ad <1 cm	32 Dukes A/B/C 3/17/12	CRC 91 Ad >1 cm 60 Ad <1 cm 20	76–97 23–88 4–62	92	67–99
			gFOBT					CRC 21 Ad >1 cm 20 Ad <1 cm 0	9–41 4–62 0–43	100	76–100
Haug 2007 (32)	Br J Cancer	ELISA	TuM2-PK	Isoenzyme expression in proliferating cells	917	0	65 Dukes A/B/C/D 12/18/12/6	CRC 68 A/B/C/D 67/61/67/100	55–79	79	NS
Shastri 2006 (33)	Int J Cancer	ELISA	TuM2-PK	Isoenzyme expression in proliferating cells	128	31 10 Ad > 1 cm 21 Ad < 1 cm	74	CRC 81 Ad 25.8	70–89 12–45	71.1	62–79
			gFOBT					CRC 36.5 Ad 16.1	26–49 5–34	92.2	86–96
Tonus 2006 (34)	World J Gastroenterol	ELISA	TuM2-PK	Isoenzyme expression in proliferating cells	42	0	54	CRC 78 A/B/C/D 60/76/89/90	NS	93	NS
5. Other fecal biomarkers											
Sonoda 2011 (35)	Gut	Canine scent detection	Volatile organic compounds	Scent detection	148	0	37	CRC 97	NS	99	NS

Abbreviations: Ad, adenoma; CRC, colorectal cancer.

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a panel of 2 or more DNA markers. Only 3 of the 8 studies (40–42) reported results with a specificity >90%. Sample sizes ranged from 76 to 583 participants. All papers looked at differing tumor markers.

From the studies assessing DNA hypermethylation of a single gene, Warren and colleagues (39) had the most promising results. Evaluating SEPT9 expression in 144 participants achieved a sensitivity of 90% for all tumor–node–metastasis (TNM) stages of colorectal cancer, at 88% specificity, with 86% sensitivity for stage I + II detection. Lee and colleagues (41) reported performance characteristics for a panel of 4-gene expression. This study had the largest cohort of patients of 583 patients, and reported a sensitivity of 86% for colorectal cancer detection alongside sensitivity of 74% for adenoma detection with both specificities being above 91%.

Four studies (44–47) applied quantitative real-time polymerase chain reaction to detect microRNA (miRNA) expressed in circulating tumor cells. The miRNAs with most interest were miR601, miR760, miR21, miR29a, and miR92a. Huang and colleagues (46) reported performance characteristics for miR29a and miR92a. Combined use of these assays produced a sensitivity of 83% and specificity of 84% for colorectal cancer detection. Wang and colleagues (44) combined this panel of assays with a further miRNA, miR760, to maintain the sensitivity but improve the specificity to 93%. Kanaan and colleagues (45) investigated miR21 as a potential screening assay and obtained results with high sensitivities and specificities (90%); however, these were conducted on a small sample size of just 40.

Immune responses in patients with cancer may be initiated by alterations in the tumor itself that result in increased immunogenicity of self-antigens. Humoral immunity, or the development of autoantibodies against tumor-associated proteins, may be used as a marker for cancer exposure. A total of 11 papers reviewed protein assays, including autoantibodies. The overall sensitivities and specificities were lower in this group than those in the serum DNA and RNA assays. Liu and colleagues (49) demonstrated that protein finger printing could be used to screen critical proteins with differential expression in the serum of patients with colorectal cancer. They determined a panel of 4 proteins of different molecular weights, which were able to differentiate colorectal cancer from healthy controls with a sensitivity of 92% and specificity of 91%.

In summary, using panels of DNA or miRNAs seems to offer the most likely candidate serum markers, as a panel of protein markers maintains sensitivity, however increases specificity of all tumor stages.

Tissue and combined assessment biomarkers

The results of tissue, taken from biopsy samples, and combined tissue and serum biomarkers are summarized in Tables 4 and 5. Three articles (60–62) evaluated tissue biomarkers and only 1 paper (63) examined combined use of tissue and serum biomarkers. These papers looked at 10 potential biomarkers. Methylation loci, looking at a panel of 10 (60) in a study of approximately 100 patients, found that the VSX2 gene was most specific at identifying those at risk of colorectal cancer with a sensitivity of 83% and a specificity of 92%. The other papers did not mention their specificity values, however Magnusson and colleagues (62) combined 2 protein markers SATB2 and CK20 to achieve a sensitivity of 97% when tested on a large cohort of 1,074 carcinomas. Kanojia and colleagues (63) systematically investigated the sperm-associated antigen 9 gene (SPAG9) mRNA and protein expression in patients with colorectal cancer and their role in the tumorigenicity of colon cancer. SPAG9 was expressed in 74% of patients with colorectal cancer and demonstrated a sensitivity of 100% in blood and 88% in tissue samples in stages I + II of colorectal cancer development.

In summary, it is difficult to evaluate the true accuracy of the results obtained from tissue assessment of biomarkers, as only 1 study commented of specificity. However, VSX2 seems to be the most promising potential biomarker from this group.

Assessment of methodologic quality

Analysis revealed that overall methodologic quality, when judged against the criterions from the Cochrane collaboration's tool for assessing risk of bias, was poor. Blinding was the most well-reported methodologic standard with 41% of papers giving a clear description of samples being collected and prepared by independent blinded individuals (commonly both endoscopists and stool testing technicians) rendering risk of detection bias low. However, 54% of papers did not refer to blinding at all with 2 papers stipulating samples were conducted unblinded.

Twenty-seven percent of papers reported on the use of a random number table or random coding of samples before processing and testing. Repeated testing and age/gender matching was poorly reported with in-adequate description of the type of repeat testing and matching between normative and diseased groups. Data on attrition bias were not formally extracted. However, data on withdrawals were not identified in the initial screen and all participant data were included within analysis.

In laboratory studies it is important that assay techniques are quality assured and standardized. However, across the 44 full text papers identified there was a huge variation in the techniques used, with varying use of control populations. It is widely recognized that assay complexity, cost, and time factors play an important part in the choice of assay. Therefore, quality assurance and validation of techniques were not considered within this review.

Positive and negative predictive values of biomarkers within the context of disease prevalence

The current UK colorectal colorectal cancer program of gFOBT has a sensitivity of 36.5% and specificity of 92.2% (33). Using the screening population and disease prevalence figures calculated in the methodology section, we

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Table 3. Summary of blood/serum biomarkers

Author + date	Journal	Method	Marker	Mechanism	Number of participants included: NC	Adenoma	Colorectal cancer	Sensitivity (%)	95% CI	Specificity (%)	95% CI
1. Serum/plasma DNA biomarkers											
Pack 2013 (36)	Int J Colorectal Dis	MSP-SSCP	E cadherin	DNA hyper-methylation	60	40	60 Stage I/II/III/IV	CRC 60 Stage I 48	NS	84 87	NS
			APC				14/14/28/4	CRC 57 Stage I 57	NS	86 89	NS
			SMAD4					CRC 52 Stage I 47	NS	64 87	NS
			DAPK1					CRC 50 Stage I 43	NS	74 70	NS
			FHIT					CRC 50 Stage I 29	NS	84 67	NS
Lange 2012 (37)	PLos ONE	Methylight	THBD-M	DNA hyper-methylation	98	0	106 Stage I/II/III/IV	CRC 71 Stage I/II 74	NS	80	NS
			C9orf50-M				28/30/45/3	NS		NS	
Cassinotti 2012 (38)	Int J Cancer	MS-PCR	6 Gene panel - CYCD2, HIC1, PAX 5, RASSF1A, RB1, SRBC	DNA hyper-methylation	30	30 18 Ad >1 cm	30 Stage I/II 11/19	CRC 83.7 Ad 54.6	71–97 37–72	67.9 64.5	51–85 47–82
Warren 2011 (39)	BMC Med	QRT-PCR	SEPT9	DNA hyper-methylation	94	0	50	CRC 90 Stage I/II/III/IV 71/90/100/100 Stage I + II 86.8	77–96 71–95	88	80–94
Tanzer 2010 (40)	PLoS ONE	QRT-PCR	ALX4 +SEPT9	DNA hyper-methylation	22	49 36 ad 13 HP	5 Stage I/III 4/1	Precancerous Colorectal lesion 71	30–95	95	75–99
Lee 2009 (41)	Clin Cancer Res	MS-PCR	4 gene panel - APC, MGMT, RASSF2A, Wif-1	DNA hyper-methylation	276	64	243 Stage I/II 44/199	CRC 86.5 Ad 74.7	82–91 63–85	92.1 91.3	88–95 86–95
Lofton-Day 2008 (42)	Clin Chem	QRT-PCR	TMEFF2	DNA hyper-methylation	185	0	135	CRC 30		95	
			NGFR					CRC 33	NS	95	NS
			SEPT9					CRC 52		95	

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Table 3. Summary of blood/serum biomarkers (Cont'd)

					Number of participants included						
Author + date	Journal	Method	Marker	Mechanism	NC	Adenoma	Colorectal cancer	Sensitivity (%)	95% CI	Specificity (%)	95% CI
Han 2008 (43)	Clin Cancer Res	QRT-PCR	5 gene panel - CDA, MGC20553, BANK1, BCNP1, MS4A1	DNA hyper-methylation	57	0	58	94	NS	77	NS
2. Blood/serum RNA biomarkers											
Wang 2012 (44)	PLoS ONE	QRT-PCR	miR601	RNA expression	58	43	90 Stage I/II/III/IV	CRC 69.2 Ad 72.1	NS	72.4 51.7	67–83
			miR760				26/25/29/10	CRC 80 Ad 69.8	NS	72.4 62.1	71–86
			miR760 + miR 29a + miR92a					CRC 83.3	NS	93.1	91–98
Kanaan 2012 (45)	Ann Surg	QRT-PCR	miR21	Tumor-associated RNA expression	20	0	20	CRC 90	NS	90	NS
Huang 2010 (46)	Int J Cancer	QRT-PCR	miR29a	Tumor-associated RNA expression	59	37	100 Stage I/II/III/IV	CRC 69 Ad 62.2		89.1 84.7	
			miR92a				27/25/38/10	CRC 84 Ad 64.9	NS	71.2 81.4	NS
			miR29a + miR92a					CRC 83 Ad 73		84.7 79.7	
Ng 2009 (47)	Gut	QRT-PCR	miR92	Tumor-associated RNA expression	69	0	90 Stage I/II/III/IV 6/34/23/27	CRC 89	NS	70	NS
			miR17-3p					CRC 64		70	
3. Blood/serum protein assay biomarkers											
Wilson 2012 (48)	British J Cancer	ELISA	MMP9	Over expression of proteolytic enzymes	525	125	46	CRC 79	NS	70	NS
Liu 2011 (49)	Int J Med Sci	MALDI-TOFMS	4 molecular weights 2870.7, 3084, 9180.5, 13748.8	Protein finger-printing	120	0	144 Dukes A/B/C/D 28/6/23/27	CRC 92.85	NS	91.25	NS
Chen 2011 (50)	Clinic Chim Acta	Western Blot	RPH3AL auto-antibodies	Auto-antibodies targeting tumor-associated antigens	63	0	84 Dukes A+B/C+D 34/50	CRC 72.6 Duke A+B 64.7 Dukes C+D 78	NS	84.1	NS

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Table 3. Summary of blood/serum biomarkers (Cont'd)

					Number of participants included						
Author + date	Journal	Method	Marker	Mechanism	NC	Adenoma	Colorectal cancer	Sensitivity (%)	95% CI	Specificity (%)	95% CI
Mead 2011 (51)	Br J Cancer	ELISA	Linel 79bp, Alu 247bp, Alu 115bp, Mitochondrail DNA	Mitochondrial and small DNA fragments	35	26	24	CRC 83	NS	72	NS
Babel 2011 (52)	Mol Cell Proteomics	ELISA	SULF1	Auto-antibodies targeting tumor-associated antigens	103	0	50	CRC 73.9		50	
			NHSL1					CRC 52.2		52	
			MST1					CRC 71.7		46	
			GTF2i					CRC 52.2	NS	58	NS
			SREBF2					CRC 60.9		48	
			GRN					CRC 58.7		58	
			6 Combined					CRC 73.9		72	
Pederson 2011 (53)	Int J Cancer	ELISA	MUC1 + MUC4	Auto-antibodies with altered glycosylation and expression	53	0	58	CRC 79	NS	92	NS
Tagi 2010 (54)	J Gastroeneterol	ELISA	4 protein panel - DK-BLY, CEA, Ca 19-9, S-p53	Aberrantly expressed protein isoforms	25	0	130	CRC 60.6	NS	80.0	NS
Mroczko 2010 (55)	Int J Colorectal Dis	ELISA	MMP9	Proteolytic enzyme degradation	70	35	75 Duke	CRC 55			
			TIMP-1				A/B/C/D	CRC 61			
							0/28/27/20		NS	100	
			MMP9+TIMP-1					CRC 75			
			MMP9 + CEA					CRC 75			
De Chiara 2010 (56)	BMC Cancer	ELISA	sCD26	Diminished protein expression	68	108	33	CRC 81.8	65–93	79.4	68–88
						48 Ad >1 cm	Duke	CRC+ Ad 58	47–69	75.5	69–81
						40 Ad <1 cm	A/B/C/D				
						18 HP	1/12/15/4				
Kim 2009 (57)	J Proteome Res	Western Blot	S100A8	Over expression of proteins in cancer tissue	21	11	77	CRC 41		95	
							Stage	Ad+ Stage I		95	
							I/II/III/IV	CRC 32			
			S100A9				14/23/21/19	CRC 44		95	
								Ad + Stage I	NS	95	NS
								CRC 40			
			CEA					CRC 22		100	
								Ad + Stage I		100	
								CRC 21			

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Table 3. Summary of blood/serum biomarkers (Cont'd)

Author + date	Journal	Method	Marker	Mechanism	Number of participants included			Sensitivity (%)	95% CI	Specificity (%)	95% CI
					NC	Adenoma	Colorectal cancer				
Fentz 2007 (58)	Proteomics Clin Appl	SELDI-TOF-MS	Transthyretin	Auto-antibodies targeting	58	58	54	CRC 60.7		100	
							All stage III	Ad 85.7		67.8	
			C3a-desArg	tumor-associated antigens				CRC 60.7	NS	92.5	NS
								Ad 78.5		77.5	
			Transthyretin + C3a-desArg					CRC 60.7		100	
								Ad 96.4		70.3	
4. Other blood/serum biomarkers											
Bellows 2011 (59)	Canc Epidemiol Biomarkers Prev	Flow cytometry	MSCs	Circulating progenitor cells	26	0	45	64		73	
			CPCs				Stage I/II/III/IV	51		58	
			LCs					71	NS	81	NS
			ECs				2/8/11/24	38		70	
			CD34 bright cells					77		66	

Abbreviations: Ad, adenoma; CRC, colorectal cancer.

can estimate that from the bowel cancer screening population, 692,165 patients would have a positive screening test and be referred for further investigation with colonoscopy, from which only 2,154 patients would be truly positive for the disease. However, 3,746 patients would achieve a false-negative test, leading to a PPV for gFOBT of 0.31% (Supplementary Table S1).

If a different screening tool was implemented with a higher sensitivity and specificity for colorectal cancer detection, for example iFOBT, with a sensitivity of 70.9% and specificity of 96.3% (29), the number of patients undergoing further investigation for false-positive results would reduce to 327,313 and patients with colorectal cancer being missed through the screening program with a false-negative result would fall to 1,717. This increases the PPV to 1.26%, while maintaining a high-negative predictive value of 99.98% (Supplementary Table S2).

Applying prevalence to the detection of adenomas. Lee and colleagues (41) reported an adenoma detection sensitivity of 74.7% and specificity of 91.3% for a 4 gene panel—APC, MGMT, RASSF2A, and Wifi1. Looking at disease prevalence this would lend to a PPV of 68.22% and negative predictive value of 93.52%, with 1,938,632 patients in the screening population undergoing further investigation with colonoscopy, from which only 616 113 patients would be negative. However, approximately half a million patients from the screening population would have an adenoma missed by this screening tool. (Supplementary Table S3).

These calculations demonstrate that a small difference in the biomarkers performance characteristics has much larger consequences in terms of a potential screening tool as colorectal cancer has a relatively low prevalence.

Discussion

The effectiveness of a screening program depends on the accuracy and the acceptance of the screening test used to detect the condition. An ideal screening test should have high compliance, sensitivity, and specificity, be minimally invasive and remain cost effective. Because of the limitations of the current screening modalities in colorectal cancer, there has been an increasing body of evidence researching on the role of biomarkers, as an alternative screening tool.

This systematic review, to our knowledge, is the first to report on all biomarkers across different mediums, including feces, blood, and tissue, that can detect colorectal cancer and adenomas. This appraisal also provides updated evidence on early detection of colorectal cancer using biomarkers since the last review on blood biomarkers by Hundt and colleagues (11) in 2007. In addition, this article also explores the performance characteristic of biomarkers within the context of disease prevalence of colorectal cancer and polyps.

The main finding of this review is supporting the use of combined tests to maximize the benefits of various systems of biomarkers for detection of colorectal cancer and

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Table 4. Summary of tissue biomarkers

Author + date	Journal	Method	Marker	Mechanism	Number of participants included: NC	Adenoma	Colorectal cancer	Sensitivity (%)	95% CI	Specificity (%)	95% CI
1. Tissue DNA biomarker											
Mori 2011 (60)	Endocr Relat Cancer	QRT-PCR	VSX2, BEND4, NPTX1, miR34b, GLP1R, HOMER2	DNA hypermethylation	34	9	51	VSX2 83.3	NS	VSX2 92.3	NS
Lind 2011 (61)	Oncogene	MS-PCR	SPG20	DNA hypermethylation	59	51	105	CRC 88 Ad 82	NS	NS	NS
2. Tissue protein biomarkers											
Magnusson 2011 (62)	Am J Surg Pathol	Immunohistochemistry Western Blot	SATB2 CK20	Antibody expression	194	88	1074 Stage I/II/III 119/440/515	SATB2 85 SATB2 + CK20 97	NS	NS	NS

Abbreviations: Ad, adenoma; CRC, colorectal cancer.

Table 5. Summary of combined serum ± tissue biomarkers

Author + date	Journal	Method	Marker	Mechanism	Number of participants included: NC	Adenoma	Colorectal cancer	Sensitivity (%)	95% CI	Specificity (%)	95% CI
Kanoja 2011 (63)	Am J Pathol	RT-PCR HIS ELISA	SPAG9	Auto-antibody expression	Tissue 40		78 Stage I + II/ III + IV 26/52	Tissue stage I + II 88 III + IV 67	NS	NS	NS
					Blood 50		54 Stage I + II/III + IV 12/42	Blood stage I + II 100 III + IV 62	NS	NS	NS

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polyps. This is likely to maximize the benefits of various biomarker systems, minimize the number of false positive tests, and the number of patients undergoing invasive investigations with the potential of complications. However, the difficulty at present is using these tests in a mass-screening program to produce reliable and reproducible results while remaining cost-effective. Further research is required.

This evaluation has identified that DNA markers are most likely to be of promise in the future as will detection of volatile organic compounds. Using panels of DNA (41) or miRNAs (46) seems to offer the most likely candidate serum or fecal markers, but further validation studies are required before considering them as a screening tool. Tissue markers are potentially useful when combined with endoscopy to help stratifying patients into high-risk groups, however the current available biomarkers are not suitable for this at present, because of high false-negative results.

This study however has its limitations. First, it can only report on the published data of the various tests and this can be limited by incomplete reporting of data in the original articles. For example, in many studies, characterization of the study population was rather scarce and some studies did not report on specificity and/or sensitivity. Second, because of heterogeneity between studies, a meta-analysis with pooling of results of different studies could not be conducted. Furthermore, reported sensitivities and specificities may provide an overoptimistic perspective because of publication bias, which may have led to selected publication of more promising results. Hence, we analyzed some of the results within the context of prevalence to generate PPVs.

This review has shown that fecal screening has been the mainstay in many screening programs. This is consistent with a recent expert panel recommending the use of a multitarget stool DNA test as a screening tool (64). The disadvantage with all fecal screening modalities is compliance as many people find this method of screening unpalatable and thus those that may benefit the most from it do not perform the test. Indeed, patient adherence to the current FOBT program is low at around 40% and 50% (6). The most reliable screening method demonstrated in this systematic review is canine scent detection for volatile organic compounds in feces (35). However, this requires further research to identify the optimum mechanism(s) of identifying these particular compounds.

A simple blood test, which can be included in a patients annual health check-up, could be the most successful screening test. This test is minimally invasive and requires little special preparation. Looking for panels of DNA and RNA markers seems to be the most promising test for identifying cancers. However, these all have limitations when it comes to identifying adenomas, although several miRNA markers (ref. 44; e.g., miR601, miR760, and miR29a) offer high sensitivities for identifying polyps and using panels of markers have increased their specificity.

Tissue biomarkers can be combined with both flexible sigmoidoscopy and colonoscopy screening to potentially identify patients with normal colons who are at increased risk of cancer and thereby potentially reduce the need for repeated screening. Looking at DNA hypermethylation seems to be a useful test with VSX2 expression (60) the most likely to be of use, and SATB2 antibody expression with CK20 (62) another candidate. However, if used as a screening method, it relies on patient compliance to have an endoscopy, which we expect will be about 50%, based on pilots (8).

One of the major problems with any potential biomarker as a screening tool candidate is that, although colorectal cancer and polyps are common conditions, the current screening options in terms of biomarkers do not have the necessary sensitivity and specificity to serve as general screening without a massive increase in costs. The prevalence of colorectal cancer and adenomas in the general population means that, with the current screening biomarker options, there would be low PPVs with many patients undergoing further investigation of a positive screening test with a colonoscopy. This would have a considerable cost impact, with 690,011 patients undergoing colonoscopies for false-positive screening tests with the current screening method of gFOBT, at an estimated annual cost of £800,000,000. There would also be a fall in the negative predictive value, meaning that more patients with the disease/adenoma would be missed through the screening program.

The current stage of evidence supports a call for prospectively planned, systematic evaluations of both the most promising fecal, blood, and tissue tests in a well-defined, large-scale screening population, with standardized sample collection, processing, and storage. This can be linked to national screening programs for either sigmoidoscopy or colonoscopy to ensure the representation of both participants from a screening population and adenoma carriers. It would also allow direct comparison of performance characteristics and practicality of single and multiple tests. Longitudinal studies are also required to assess the potential of quantifying biomarkers over time to provide increased sensitivity for an emergent malignancy.

There are other emerging biomarkers that are not included in this review, including urinary biomarkers and gut microbiomes, with recent studies evaluating their efficacy. Urinary PGE-M seems to be a promising biomarker for adenoma detection with high PGE-M urinary levels being associated with an increased risk of advanced or multiple adenomas (65). Several studies have recently looked at microbial dysbiosis, a pathologic imbalance in the microbial community, in the etiology of colorectal adenomas and colorectal cancer. These, however, are in the early stages with additional studies required to define further the best sampling

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location, mucosal or luminal, and to elucidate the exact connections between the host gut microbiome and the onset of colorectal cancer (66).

This systematic review has demonstrated that volatile organic metabolites have a great potential in the early detection of colorectal cancer and polyps. A recent study highlighted the potential of VOC profiling as a noninvasive test to identify those with esophagogastric cancer (67). Selected ion flow mass spectrometry was applied for the quantification of VOCs in exhaled breath, identifying 4 VOCs that were statistically different between the esophagogastric cancer group and the control group. Chemical analytical research could lead to the development of a noninvasive VOC-based test that could significantly contribute in the early diagnosis of colorectal cancer.

Further work is required to investigate further the potential role of volatile biomarker metabolites and the optimum techniques for their detection in order to predict early detection of colorectal cancer and polyps.

Conclusion

This review has demonstrated that there are several fecal and serum biomarkers that can predict colorectal cancer and polyps. However, when combined into biomarker panels, higher sensitivity, specificities, and PPV for early detection of colorectal cancer and adenomas are achieved. Further research is required to validate these biomarkers in a well-structured population-based study.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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