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REVIEW

Current state and future direction of screening tool for colorectal cancer

Ji Taek Hong, Eun Ran Kim

ORCID number: JiTaek hong (0000-0002-6310-2958); Eun Ran Kim (0000-0002-0495-2565).

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Ji Taek Hong, Department of Internal Medicine, Hallym University College of Medicine, Chuncheon 24253, South Korea

Eun Ran Kim, Division of Gastroenterology, Department of Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul 06351, South Korea

Corresponding author: Eun Ran Kim, MD, Staff Physician, Division of Gastroenterology, Department of Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, 81 Irwon-ro, Gangnam-gu, Seoul 06351, South Korea. er.kim@samsung.com
Telephone: +82-2-34103409
Fax: +82-2-34106983

Abstract

As the second-most-common cause of cancer death, colorectal cancer (CRC) has been recognized as one of the biggest health concerns in advanced countries. The 5-year survival rate for patients with early-stage CRC is significantly better than that for patients with CRC detected at a late stage. The primary target for CRC screening and prevention is advanced neoplasia, which includes both CRC itself, as well as benign but histologically advanced adenomas that are at increased risk for progression to malignancy. Prevention of CRC through detection of advanced adenomas is important. It is, therefore, necessary to develop more efficient detection methods to enable earlier detection and therefore better prognosis. Although a number of CRC diagnostic methods are currently used for early detection, including stool-based tests, traditional colonoscopy, etc., they have not shown optimal results due to several limitations. Hence, development of more reliable screening methods is required in order to detect the disease at an early stage. New screening tools also need to be able to accurately diagnose CRC and advanced adenoma, help guide treatment, and predict the prognosis along with being relatively simple and non-invasive. As part of such efforts, many proposals for the early detection of colorectal neoplasms have been introduced. For example, metabolomics, referring to the scientific study of the metabolism of living organisms, has been shown to be a possible approach for discovering CRC-related biomarkers. In addition, a growing number of high-performance screening methodologies could facilitate biomarker identification. In the present, evidence-based review, the authors summarize the current state as recognized by the recent guideline recommendation from the American Cancer Society, US Preventive Services Task Force and the United States Multi-Society Task Force and discuss future direction of screening tools for colorectal cancer. Further, we highlight the most interesting publications on new screening tools, like molecular biomarkers and metabolomics, and discuss these in detail.

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Key words: Colorectal cancer; Screening tool; Early detection; Biomarkers; Metabolomics

Core tip: A large proportion of colorectal cancer (CRC) cases and deaths could be prevented by screening with early detection and removal of colorectal adenomas or early stage CRC. Reliable and non-invasive screening tools for early stage CRC and precancerous lesions, such as adenoma is indispensable. However, current screening methods have limitations. Therefore, it is important to review the current literature on new screening tools such as molecular biomarkers and metabolomics for the development of new diagnostic tools.

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INTRODUCTION

Colorectal cancer (CRC) remains a global health problem and currently is considered one of the leading causes of death in the world[1]. The patient's survival is predicted by the tumor stage at the time of diagnosis. Early CRC diagnosis maximizes the benefit of treatment. Typically, it takes 7-10 years for an adenoma to become a carcinoma, which provides a timeframe allowing for early detection of CRC[2]. At present, the best available option for early detection and elimination of premalignant lesions is colonoscopy. However, it is invasive, expensive, and inconvenient for patients. Therefore, non-invasive and reliable methods for diagnosing CRC are valuable due to colonoscopy risks: Puncture of the colon, intraperitoneal bleeding, post-polypectomy, and infection. In particular, with regards to the detection of CRC precursor lesions, such as adenoma, the lack of sensitivity and specificity or an unacceptably wide range of the FOBT has hampered the clinical application in CRC screening[3]. Therefore, newer, non-invasive screening methods and biomarkers to permit identification of CRC and its precursors in easily accessible biospecimens are needed. Consequently, current screening methods have limitations, and it is necessary to find new screening methods that can detect CRC in the early phase to improve survival and quality of life for patients with CRC.

The following qualities are what an ideal screening method would possess: It should show high sensitivity and specificity, it must be safe and cost-effective to be widely used, it must be simple to measure, and readings must be consistent among patients of all genders and races. Acceptability of screening method is also important in target population[4]. In this review, we summarize the current status of screening tools for colorectal cancer and discuss the future direction of colon cancer screening, including metabolism and proteomics.

CURRENT STATE OF SCREENING TOOLS FOR CRC

The American Cancer Society (ACS), US Preventive Services Task Force (USPSTF) and the United States Multi-Society Task Force (USMSTF) Guideline recommends stool-based tests and structural examinations as options for colorectal cancer screening[5-7]. Stool-based tests consist of guaiac-based tests, immunochemical tests, and mt-sDNA tests. Structural examinations consist of colonoscopy, computed tomography colonography, and flexible sigmoidoscopy (FS). These screening tests are currently in use, and we will first discuss the screening value and limitations of currently-used tests. The characteristics, advantages and disadvantages of colorectal cancer screening tests currently in use are shown in Table 1.

Stool-based CRC screening tests

Stool-based tests are conventionally known as fecal occult blood tests (FOBTs) because they aim to discover the presence of occult blood in stool, which may derive from colorectal cancer or lager polyps of at least 2 cm in size. FOBT are divided into

Table 1 Characteristics of colorectal cancer screening tests currently in use in the United States

Screening test	Interval	Evidence	Advantages	Disadvantages	Other considerations
Stool-based screening tests					
FIT with high sensitivity[123]	Every year	Improved performance compared with high-sensitivity gFOBT Mortality reduction: indirect evidence from RCTs of guaiac-based stool tests	Can be performed at home Requires only a single specimen No diet or medication restrictions Does not require bowel preparation or anesthesia Inexpensive compared with structural examinations and mt-sDNA	High nonadherence to yearly testing (especially without reminder systems) Less effective for advanced adenoma detection Few accessible tests have published peer-reviewed performance data	Varies in test performance due to brand and version Follow-up colonoscopy for positive test may charge extra costs
gFOBT with high sensitivity[12] (HSgFOBT)	Every year	Good RCT evidence for incidence and mortality reduction[112-116] Varies in test performance characteristics by version of the test	Inexpensive compared with structural examinations and mt-sDNA Can be done at home Does not require bowel preparation or anesthesia	High nonadherence to yearly testing (especially without reminder system) Less effective for advanced adenoma detection Difficulty in determining test performance among the many FDA-cleared tests Requires multiple samples Requires dietary and medication restriction Higher false-positive rate than FIT leads to more colonoscopies	Follow-up colonoscopy for positive test may charge extra costs
mt-sDNA[1]	Every 3 yr	Mortality reduction: indirect evidence from RCTs of guaiac-based stool tests Improved sensitivity for cancer and AA and poorer specificity compared with FIT	Can be done at home Does not require bowel preparation or anesthesia	More expensive than other stool-based tests Higher false-positive rate than FIT	Follow-up colonoscopy for positive test may charge extra costs A new test with limited data on screening outcomes. Uncertainty in management of positive results followed by a negative colonoscopy
FIT-DNA[23]	Every 1 or 3 yr	Test characteristic studies	Improved sensitivity compared with FIT per single screening test Does not require bowel preparation or anesthesia Can be done at home	Higher false-positive rate than FIT	Uncertainty in management of positive results followed by a negative colonoscopy
Direct visualization screening tests					

Colonoscopy[123]	Every 10 yr	Non-RCT evidence of incidence and mortality reduction Prospective cohort study with mortality end point	Requires less frequent screening Screening, diagnosis, treatment and prevention through polypectomy can be done at the same-session. Gross visualization of the entire colon	Pain and discomfort Lower tolerability and compliance than FS[117] Possibility of bowel perforation / bleeding and cardiopulmonary complications from anesthesia Requires full bowel cleansing Performance varies upon adequacy of bowel preparation, the cecal intubation rate, withdrawal time, and adenoma detection rate Lower sensitivity for neoplasia in the proximal than the distal colon	Polypectomy and anesthesia may charge extra costs Most expensive test, but currently reimbursable with insurance Requires day-off (if sedation is used)
CTC[123]	Every 5 yr	Test characteristic studies Extrapolation from RCTs of sigmoidoscopy demonstrating mortality reduction	Rapid, non-invasive imaging method Well-tolerated by patients Does not require anesthesia Better tolerability and acceptance than colonoscopy and FS[118]	Exposure to low-dose radiation Requires full bowel cleansing A second bowel cleansing will be required before Follow-up colonoscopy for positive test	Follow-up colonoscopy for positive test may charge extra costs Insufficient evidence about the benefit-burden balance of additional tests on incidental extracolonic findings Relatively expensive and may not be covered by insurance
FS[123]	Every 5 yr	RCTs with mortality end points:	Does not require anesthesia Requires more limited bowel cleansing Better acceptance than colonoscopy[117]	Pain and discomfort Does not examine the proximal Colon Requires enema prior to procedure Abnormal findings require second colonoscopy	Follow-up colonoscopy for positive test may charge extra costs Concerns about lack of quality standards, limited availability, failure to achieve a complete examination
FS with FIT[2]	FS every 10 yr plus FIT every year	RCT with mortality end point (subgroup analysis)	More benefits than when combined with FIT or compared with other strategies It may be an potentially option for patients who want endoscopy screening but do not want colonoscopy		Test declined in the US

[1]The American Cancer Society Guidelines recommend.
[2]US Preventive Services Task Force Guidelines recommend.
[3]The U.S. Multi-Society Task Force Guidelines recommend [Tier 1: Colonoscopy every 10 yr, annual fecal immunochemical test; Tier 2: CT colonography every 5 yr, FIT-fecal DNA every 3 yr, flexible sigmoidoscopy every 10 years (or every 5 yr); Tier 3: Capsule colonoscopy every 5 yr]. CRC: Colorectal cancer; CTC: Computed tomographic colonography; FDA: US Food and Drug Administration; FIT: Fecal immunochemical test; FS: Flexible sigmoidoscopy; gFOBT: Guaiac-based fecal occult blood test; mtsDNA: Multitarget stool DNA; RCT: Randomized controlled trial.

two primary categories according to the detected analyte: Guaiac-based fecal occult blood tests (gFOBT) and fecal immunochemical tests (FITs). gFOBT, which detects the peroxidase activity of hemoglobin, was first recognized as an effective screening for CRC. The use of this stool testing for CRC screening has been supported by multiple consistent randomized clinical trials[8]. However, the use of gFOBT is complicated by its poor sensitivity and specificity as the test shows false negatives when a patient uses antioxidants, like vitamin C, whereas false positives occur when a patient has upper GI bleeding from NSAIDS intake, or consumes red meat or dietary peroxidase from certain vegetables and fruits[9]. On the other hand, FITs specifically detect antibodies against human globin. Thus, it is not influenced by upper GI bleeding since globin is degraded in the upper GI tract, and the test result is well protected from the

influence of medications, red meat, or peroxidases from foods, eliminating the need for pre-testing food restrictions[9].

Individual gFOBT and FIT versions show various performance characteristics. Although non-rehydrated, low-sensitivity gFOBT variants are still commercially available, it is not recommended or used for CRC screening test due to the poor performance of these gFOBT. Though there may be other tests having higher sensitivity, at the time of publication, only Hemoccult II Sensa (Beckman Coulter Inc., Brea, CA) performed as a high-sensitivity gFOBT (HSgFOBT) among the many guaiac-based tests evaluated in population-based studies. The ranges of sensitivity and specificity of HSgFOBT are from 62% to 79% and 87% to 96%, respectively[8,10,11]. The fact that gFOBT testing is often carried out in the physician's office in the form of a single-panel test after a digital rectal exam is a potential limitation of this testing. According to Collins *et al*[12], for advanced neoplasia, the gFOBT testing sensitivity was merely 4.9%, and for cancer, it was just 9%. The accuracy of this method is so low that it cannot, under any circumstances or rationale of convenience, be endorsed as a method for CRC screening. The sensitivity of many individual tests requires optimum situations, and this is another limitation of gFOBT, which can be even more compromised by insufficient and imperfect specimen collection together with absent or inappropriate processing and interpretation. Meanwhile, FITs present higher sensitivity and slightly lower specificity for cancer and advanced neoplasia when compared with low-sensitivity gFOBTs, while demonstrating similar or higher sensitivity and specificity than HSgFOBTs. The ranges of sensitivity and specificity of single-sample FIT are 73% to 92% and 91% to 97%, respectively[9,13-16]. FIT is a non-invasive test. Moreover, in one meta-analysis it showed a one-time sensitivity for cancer of 79% and also had a reasonable sensitivity for advanced adenomas (about 30%), and it is very inexpensive (about $20)[17]. Despite these numbers and advantages, the majority of the brands of FIT do not have sufficient data to show the accuracy of the test in identifying the presence of CRC, as Daly *et al*[18] were only able to review validation data for less than half of the versions of FIT available in the United States. The FITs were not tested in randomized studies and most of the evidence for the effectiveness of FIT test was based on indirect evidence of reduced mortality due to randomized controlled trials (RCTs) of the guaiac-based stool tests. Studies also used different versions of FITs tests to analyse their outcomes. As the FIT is intended to be repeated, the results for single-sample sensitivity and specificity alone are not sufficient. Although some evidence has started being published recently, the data for the long-term performance of FIT is still lacking[19]; thus, these studies should not be the basis for determining the performance qualities of FITs because they do not yet have adequate data.

Based on the general-population MISCAN modeling analysis that was conducted in 2018, annual FIT from 45–75 year-old adults became a recommendable strategy by providing 94% of the life-year gains (LYGs) compared with that of the standard screening test, a colonoscopy every 10 years for 45–75 year-old adults[20]. Compared to annual FIT, annual HSgFOBT from adults 45 to 75 years of age presented a higher rate of false positives, requiring more colonoscopies; thus, it was not considered to be a model-recommendable strategy, though LYGs of HSgFOBT were same as that of FIT (403 LYGs)[20]. Despite such limitations, HSgFOBT (*i.e.*, Hemoccult II Sensa) is considered to be an option for CRC screening in the updated ACS guidelines because of its high sensitivity and low cost. These benefits can be advantageous when FIT is not available.

No direct injury is caused by HSgFOBT or FIT screening. However, special care must be taken to avoid physical injury when a practitioner performs a colonoscopy to confirm a positive HSgFOBT[8]. Lately, in screening programs for CRC, the original, low-sensitivity guaiac test has been used over HSgFOBT or FIT, with the Unites States similarly changing their screening programs in accordance with this trend.

A third stool test is the multi-targeted stool DNA (mt-sDNA) test that uses an immunochemical assay for human hemoglobin and assays for aberrantly methylated BMP3 and NDRG4, mutated K-ras, and β-Actin from exfoliated cells from colonic neoplasms[21]. Based on a manufacturer-supported, multi-center comparative study of mt-sDNA and FIT testing in average-risk individuals, the sensitivity of mt-sDNA and FIT were 92.3% and 73.8%, respectively[21]. Although the sensitivity of FIT could improve to 77% when the specificity of FIT was as high as that of mt-sDNA (86.6%), the sensitivity of FIT is significantly lower than that of mt-sDNA and did not show sufficient specificity for screening program. Compared with FIT, mt-sDNA was superior in detecting advanced adenomas, especially sessile serrated polyps that were larger than 1 cm. The sensitivity for serrated sessile polyps was 42.4% for mt-sDNA but 5.1% for FIT. However, mt-sDNA had a higher false positive rate, indicating significantly lower specificity (89.8%) compared with that of FIT (96.4%).

In the case of mt-sDNA testing for detecting large adenomas and CRC, the fact that

the sensitivity of the test is based on a panel of markers, which seem to identify only a subset of CRC, presents an obvious drawback of mt-sDNA testing. Another possible drawback is the high expense per unit of the currently used test compared to other stool tests. It is also unclear how often the test should be performed. A benefit, though, is the lack of direct harm associated with mt-sDNA, but practitioners should, again, be careful in performing the necessary colonoscopy once a patient's stool test is positive. Unlike other stool-based tests, mt-sDNA has issues with interpretation of false positive results because the reported results from the mt-sDNA test currently available in the United States cannot distinguish between a positive result originating from FIT versus mt-sDNA testing. A false positive from the mt-sDNA test could result from a failure to detect a visible lesion, invisible neoplastic changes, or from the presence of a non-colonic digestive tract neoplasm. Patients may proceed to more aggressive short-term surveillance to confirm a false positive result from mt-sDNA. Some follow-up studies that observed patients with false positive results from mt-sDNA for approximately 4 years showed that no patient developed CRC or aero-digestive malignancies[22,23]. According to the follow-up study of Cooper *et al*[24], only three out of 12 patients with previous false positive results on mt-sDNA had positive colonoscopy results upon follow-up. The study also emphasizes the importance of long-term follow-up, and high-quality colonoscopy, especially in the proximal colon, for patients presenting with positive mt-sDNA test results.

In the 2018 MISCAN modeling analysis, mt-sDNA was not a recommended test because it requires higher numbers of colonoscopies per LYGs[20]. Mt-sDNA done every 3 years resulted in 93% of the LYGs compared with annual FIT testing, but when compared with the LYGs of colonoscopy every 10 years, the percentage was 2% less than the *a priori* criterion of 90%[20].

The ACS's 2018 guidelines decided to include mt-sDNA as one of the testing options for CRC screening at 3-year intervals, as mt-sDNA is superior in detecting advanced adenomas and serrated sessile polyps, and some adults prefer mt-sDNA over other screening tests. The USMSTF 2017 guidelines recommend that the combined FIT-fecal mt-sDNA test be performed at three-year intervals as a second-tier test (Table 1)[6].

Options for CRC structural (visual) examinations

Structural (visual) examinations, such as endoscopic and radiologic examinations [CT colonography (CTC)] are preferred options for CRC screening when bowel visualization is necessary. Since it directly visualizes the bowel, the screening interval is longer than for stool tests. Structural CRC screening tests require bowel preparation prior to implementing the test, which requires the patient's active participation for self-administering an enema for FS or ingesting polyethylene glycol oral laxative for CTC. For CTC, patients are also restricted to a liquid diet for one day prior to CTC for bowel cleansing. Unlike FS and CTC, colonoscopy is often performed with anesthesia; hence, the patient must be accompanied by a caretaker[25].

Colonoscopy: Colonoscopy is a widely performed screening for CRC. Patients who are found to be positive for other CRC screening tests undergo colonoscopy for additional assessment. Colonoscopy has a high sensitivity to detect cancer and all classes of precancerous lesions, it allows single-session diagnosis and treatment, and the intervals between examinations are usually long (10 years) in subjects with normal findings. According to a large, prospective, observational cohort study by Nishihara *et al*[26], the CRC mortality hazard ratio was 0.32 [95% confidence interval (95%CI): 0.24–0.45], when the comparison was made between colonoscopy done at least once and none done at all over 24 years. In addition, distal cancers had a lower hazard ratio of 0.18 (95%CI: 0.10–0.31) compared to proximal cancers, which had a hazard ratio of 0.47 (95%CI: 0.29–0.76). Furthermore, a decrease in incidence was shown in participants who were found to have negative results for colonoscopy (hazard ratio: 0.53, 95%CI: 0.40–0.71)[26]. According to the study by Lin *et al*[8], the sensitivity and specificity of colonoscopy for identification of adenomas of at least 6 mm in size were 75%–93% and 94%, respectively, whereas those for identification of adenomas at least 1 cm were 89%–98% and 89%, respectively.

According to the three Cancer Intervention and Surveillance Modeling Network models that informed the USPSTF's 2016 CRC screening guide, CRC incidence and mortality would decrease by 62%–88% and 79%–90%, respectively, if colonoscopy was done every ten years from 50 through 75 years of age[27]. According to the study by Knudsen *et al*[28], the median LYG for colonoscopy every 10 years was 270, which was higher than those of other exams. Based on the 2018 general-population MISCAN modeling, a large decrease in the incidence of CRC and the number of deaths from CRC along with an increase in LYG compared to other recommendable strategies were observed, although the frequency of lifetime colonoscopy is more than twice that

of stool-based testing[20].

However, there are some drawbacks of colonoscopy. Although colonoscopy proved to be more effective as a screening tool than other models, its disadvantages include excessive detection and removal of minute low cancer risk polyps, increasing the risks associated with polypectomy as well as the possibility which could result in unnecessary follow-up evaluations. Thorough bowel cleansing is unavoidable, risk of bowel perforation is higher than for other screening methods, there is a greater risk of pneumonitis due to aspiration (especially when deep sedation is involved in the procedure), a slight risk of splenic injury necessitating splenectomy, and greater occurrence of bleeding after the procedure compared to other screening tests. Major complications of screening through colonoscopy are perforation and bleeding, amounting to approximately four cases per 10,000 screenings and eight cases per 10000 screenings, respectively, according to USPSTF[29]. The frequency of these dangers is increased when polypectomy is performed. There was a significantly greater rate of complications from performing colonoscopy after other positive non-colonoscopy screening tests than when performing an initial colonoscopy[8,30]. Injuries that result from undergoing colonoscopy increase significantly and nonlinearly with the comorbidity burden and age of the patients[31]. Colonoscopy has a greater probability of not being able to detect serrated polyps compared to typical adenomas[32]. Colonoscopy's performance is also operator-dependent. The skill of the operator affects the detection of cancer, adenomas, and serrated lesions, as well as the selection of appropriate screening and surveillance intervals after colonoscopy[33-42]. Despite these risks, colonoscopy is still the preferred approach to allow gross visualization of the entire colon and same-session detection, biopsy, and removal of polyps.

CTC: CTC, or virtual colonoscopy, produces multiple thin-slice CT images that can be printed on 2D film or compiled into 3D images, enabling examiners to observe internal organs without the use of colonoscopy. CRC detection rates with CTC were similar to that with colonoscopy based on two extensive studies[43,44]. A systemic review and meta-analysis of CTC and colonoscopy based on 49 studies calculated the sensitivity and specificity of CTC. The sensitivity of CTC for detecting CRC was 96.1%, and the sensitivity for detecting adenomas larger than 6 mm was 73%–98% with a specificity of 89%–91%[45]. In CTC, the chances of perforation are less than colonoscopy, and the 82%–92% sensitivity achieved for detecting adenomas larger than 1 cm is also an advantage of CTC[46-49]. Nevertheless, the sensitivity for detecting polyps less than 1 cm is inferior to colonoscopy, and a major deficiency of CTC is the difficulty in detecting flat and serrated lesions[50,51]. Patient who undergo CTC may experience some undesirable symptoms, such as abdominal pain resulting from bowel preparation, pain related to the examination, neuro-cardiogenic syncope and pre-syncope, and very rare worst adverse effects (*i.e.*, GI perforation and radiation exposure-induced cancer). The detection of incidental extracolonic findings is also an unresolved problem. There is the limited evidence about the cost-benefit balance for additional tests necessary for these incidental extracolonic findings[8]. There is insufficient proof that CTC reduces the mortality or incidence of CRC. Both ACS and USPSTF (2016) guideline agreed that CTC every 5 years beginning from 45 to 50 years of age and continuing through 75 years of age is a recommended strategy for CRC screening.

FS: FS, a procedure to evaluate the lower half of the colon, is the first visual examination used for CRC screening. Advantages of FS are that it is very cheap, has significantly lower risks than that of colonoscopy, does not require thorough bowel preparation, and sedation is unnecessary. A disadvantage of FS is that it provides less benefit in protecting against right-sided colon malignancy when compared with the amount of protection achieved by colonoscopy in case-control and cohort studies. In addition, since the procedure does not involve sedation, the patient might experience discomfort, could be dissatisfied by the procedure, and be more hesitant to repeat the examination compared to colonoscopy[52].

By analyzing four RCTs of FS with one or two screening examinations at intervals of every 3-5 years, there was significant decrease in CRC incidence and mortality[53-56]. According to pooled analysis conducted for the USPSTF, patients who had regular follow-up over 11 or 12 years generally decreased their CRC mortality by 27% (relative risk: 0.73, 95%CI: 0.66–0.82), which is especially significant for distal CRC but not proximal CRC[8,27]. There was also a decrease in CRC incidence of 21%. According to the US-based Prostate, Lung, Colon, and Ovarian Cancer (PLCO) Screening Trial, proximal and distal CRC incidence were both significantly reduced with FS screening[27]. In a study by Atkin *et al*[57], though, there was significant reduction in the incidence of CRC by 26% and mortality by 30%. The study concluded that the result derived from the detection of distal CRC because there was no significant reduction in

incidence or mortality of proximal CRC after FS screening. Overall, FS screening showed a 21% reduction in incidence and a 27% reduction in mortality for CRC when analyzing the pooled data from the three studies (PLCO, SCORE, and NORCCAP) that had an average follow-up of 10 to 12 years[58]. Since FS is not effective in screening proximal CRC, which disproportionately affects older women, the incidence and mortality of CRC was not reduced in women aged 60 years or older.

According to MISCAN modeling analysis adjusted for increased incidence, FS screening is recommended beginning at age 45 and repeated every 5 years until the age of 75 years; whereas, assuming stable incidence, USPSTF (2016) is not recommending FS alone for CRC screening[28]. Because the effectiveness of FS screening is mostly restricted to the rectum and distal colon, its use has been replaced by colonoscopy in the United States[20]. A recent report found that only 2.5% of adults who are recommended for CRC screening test underwent FS screening, while 60% of them received colonoscopy[59]. Even if there is solid evidence that FS is an effective CRC screening test, it is questionable whether community-based clinicians are receiving regular training or performing an adequate number of FSs to maintain their skill because it is less frequently used in the United States. This assumption is also supported by proposed FS screening standards that do not provide information about strict quality standards[60]. The reasons mentioned above are leading the ACS Guideline Development Group to remove FS from the list of recommended CRC screening tests. However, it is still considered to be one of the recommended tests for CRC screening in some countries where colonoscopy is not yet commonly performed due to its efficacy in reducing CRC mortality and its availability as a primary visual examination tool.

Emerging technologies not currently recommended for routine screening

Blood screening for methylated SEPT9 DNA (mSEPT9) and capsule endoscopy are not recommended procedures but are FDA approved for certain situations.

Blood screening test for methylated SEPT9 DNA (mSEPT9): The FDA recently cleared a blood test that identifies a CRC biomarker, mSEPT9[61]. This blood screening test is performed on patients with average CRC risk who have declined other screening tests listed in the USPSTF CRC guidelines.

An advantage of the mSEPT9 test is that, as a serum assay, it is more convenient for patients. A disadvantage of the mSEPT9 test is that the performance characteristics are inferior to FIT, that is, sensitivity for cancer is lower than that of FIT, detection of advanced adenoma is impossible, and the cost is more than for other screening tests[62,63]. The test seems to be more sensitive for later stage compared to earlier stage cancer[64]. Patients who receive a positive result from this blood screening test should be ready to have follow-up tests, such as colonoscopy, which they have refused to undergo previously. Whether patients positive for mSEPT9 would be willing to undergo colonoscopy is questionable. There is also limited evidence in asymptomatic populations who are the targeted candidates for screening. Furthermore, no microsimulation modeling for the newer version of the test was done to evaluate the benefit and benefit-to-harm ratio or to determine the optimal timing for screening. Because of these limitations, most guidelines discourage the use of mSEPT9 for screening.

Capsule endoscopy: Initially, capsule endoscopy was predominantly used for gross assessment of the small bowel, but later, there were attempts to use it as a tool to screen the large bowel for CRC. In a systematic review that studied patients with a high risk or who presented with signs or symptoms of CRC, the pooled sensitivity was 87% (95%CI: 77%–93%), and the pooled specificity was 76% (95%CI: 60%–87%) for capsule endoscopy in identification of colorectal polyps at least 6 mm in size[65]. Increased pooled sensitivity and specificity were observed (89%, 95%CI: 77%–95% and 91%, 95% CI: 86%–95%, respectively) in tests on lager colorectal polyps that were at least 10 mm in size[65]. However, the use of capsule endoscopy as a screening tool is limited due to its side effects. Adverse effects were found to include predominantly gastrointestinal problems such as nausea, vomiting, abdominal pain, and fatigue from the required bowel preparation, and these were found in less than 4% of patients[65]. The most severe problem was capsule retention (0.8% of patients with 95%CI: 0.2%–2.4%). Capsule endoscopy also necessitates sufficient colon preparation and further evaluation with colonoscopy if polyps are detected. Capsule endoscopy is not presently approved by the FDA for use in CRC screening.

FUTURE DIRECTION OF SCREENING TOOLS FOR CRC

Currently, finding a CRC-specific tumor marker for the development of a new, non-invasive screening method is a primary focus among researchers. CRC is a disease of a highly heterogeneous nature. To interpret the heterogeneous mechanisms that bring about tumorigenesis, "-omics" data derived from genomic, transcriptomic, epigenetic, and proteomic analysis through multi-omics is required. With a single "-omics" approach, the degree of internal and individual variability related to tumor composition and oncogenic signals may be misinterpreted. Therefore, in order to understand the occurrence of tumor, various approaches are required and being studied. We will review the molecular biomarker studies that have been carried out so far and identify new approaches and studies, including metabolomics for the discovery of new CRC biomarkers.

Molecular biomarkers

CRC is a multifactorial disease caused by genetic and epigenetic changes in oncogenes, mismatch repair genes, tumor suppressor genes, and cell cycle regulating genes of the colon mucosal cells. As these molecular changes provide indications for diagnosis, prognosis, and information on treatment response, they were considered possible CRC biomarkers. The three main molecular pathways contributing to the genetic alterations responsible for carcinogenesis are microsatellite instability (MSI), chromosomal instability (CIN), and the CpG island methylator phenotype. Recently, new methods of molecular detection are being evaluated. Nonetheless, the majority of these methods have not yet been validated in larger, preclinical research using randomized study designs. Studies characteristics, patients characteristics, major markers and diagnostic performance of various molecular biomarkers studies are shown in Table 2.

Adenomatous polyposis coli mutation: Multifunctional proteins that control Wnt signaling, cell cycle regulation, cytoskeleton stabilization, intracellular adhesion, and apoptosis are encoded by the adenomatous polyposis coli (*APC*) gene. The *APC* gene mutation qualifies as a molecular biomarker for CRC diagnosis because approximately 90% of patients with CRC show APC gene mutation[66]. Liang *et al*[67] have performed meta-analysis study between 1997 and 2010 to correlate APC polymorphisms and CRC risk. It was found that while E1317Q significantly increased the risk of adenoma, I1307K was linked to a high risk of CRC.

MSI: Commonly, MSI is diagnosed by estimating missing MMR gene products, amplification via polymerase chain reaction (PCR), or immunohistochemistry (IHC)[68]. Through meta-analysis and prospective studies, it was shown that MSI serves as an exclusive marker with significant prognostic value in early-stage CRC[69]. The prognosis for MSI CRC was found to be superior to that of microsatellite stable (MSS) CRC[70]. The Bethesda panel consists of five microsatellite loci (BAT25, BAT26, D17S250, D5S346, and D2S123)[71]. At present, most clinical laboratories use a panel of five mononucleotide markers (Bat-25, Bat-26, NR-21, NR-24, and mono-27) to detect MSI[72]. MSI is found to be highly prevalent in stage II CRC with an approximate 20% incidence and rare in stage IV CRC with about a 4% prevalence. Hence, MSI screening may aid in early detection of CRC[72,73].

Detection of CRC-specific RNA Markers in stool: While the fecal occult blood test (FOBts) is commonly used as a screening tool, it still has poor sensitivity and specificity. Many tools using protein, DNA and RNA to detect various markers in stool were recently developed[74]. The idea is such that miRNA not only regulates specific mRNAs and serves a fundamental role in oncogenesis, but also plays critical role in normal development or in tumor cell multiplication, division, and death[75,76]. To diagnose CRC early, several miRNAs were recently assessed. Wu *et al*[77] obtained 424 stool specimens from adenoma, CRC, and control patients to investigate miRNA. They found out that when compared with the control, expression of miRNA-135b was significantly increased in advanced adenoma and CRC stool specimens. According to a study performed by Kalimutho *et al*[78], investigating hypermethylated miR-148a in stool specimens may be capable of early CRC detection. Also, following examination of 648 miRNAs from stool specimens of CRC, Kalimutho *et al*[78] have determined that fecal miR-144 may be used as a tool for CRC diagnosis. With 74% sensitivity and 87% specificity, miR-144 expression was found to be highly significant in CRC stool specimens. Additionally, PTGS2, a transcript of a specific colorectal tumor gene, expression is extremely specific for early diagnosis of CRC[79]. Koga *et al*[80] acquired stool specimens from 206 patients with CRC and 134 normal individuals and performed a study on miRNA expression in desquamated colonocytes from stool specimens. The result showed a sensitivity of 74.1% and specificity of 74.1%. Although it did not show sufficient specificity to be used as a screening test, they proposed that the profile of miRNA expression may be useful as a CRC screening test

Table 2 Summary of the current and potential biomarkers for early diagnosis of colorectal cancer

Characteristics of the studies				Training set [test set] (if applicable)			Diagnostic performance (if applicable)			
Ref.	**Study type, country**	**Study group**	**Population (*n*)**	**Male (%)**	**Age (mean / SD)**	**Stage (0) / I/ II/ III/ IV/ (?)**	**Sample**	**Marker**	**Sn / Sp**	**AUC / *P*-value**
Microsatellites loci										
Piñol *et al*[119], 2005	Prospective, multicenter, nation-wide study/Spain	CRC	1222	59.8	70/11	161/510/337/214	Blood	Bethesda panel	81.8/98	N/A
Umar *et al*[71], 2004	Guidelines	N/A	N/A	N/A	N/A	N/A	Blood	Bethesda panel	81.8/98	N/A
Berg *et al*[120], 2009	Recommendations	N/A	N/A	N/A	N/A	N/A	Blood	Microsatellites instability (MSI)	55-90/90	N/A
Liang *et al*[67], 2013	Meta analysis/China	N/A	N/A	N/A	N/A	N/A	Blood	APC Polymorphisms	N/A	N/A
CRC-specific RNA markers										
Wu *et al*[77], 2014	Case-control China	Normal	109	45.9	60.4/7.0	I + II/III + IV/(?) 24/76/4	Stool	MiRNA-135b	78 (CRC) 73(Advanced adenoma) 65(any adenoma) /68	0.79 (CRC) 0.71 (adenoma) / <0.0001
		Adenoma < 1cm	110	53.6						
		Advanced adenoma	59	50.7						
		CRC	104	57.7						
		IBD	42	61.9						
Kalimutho *et al*[78], 2011	Case-control, Italy	CRC	28	46	66	(5)/2/6/3/0/(NA:12)	Stool	miRNA-148	74/87	N/A
		HGD	12	67	62					
		Cn	39	28	58					
Koga *et al*[74], 2010	Case-control, Japan	CRC	206	67	63	23/46/133/4	Stool	PTGS2	74.1/74.1	N/A, <0.0001
		Cn	134	44	60					
Methylation biomarkers										
Luo *et al*[86], 2011	Meta Analysis/China	N/A	N/A	N/A	N/A	N/A	Stool	VIM	80/80	N/A
Guo *et al*[88], 2013	Case-control, China	CRC	75	61	58.5 (12.5)	12/30/30/3	Stool	FBNI	72/93.3	N/A, < 0.001
		Cn	30	67	58.4 (12.9)					
Glockner *et al*[89], 2009	Case-control, United States	CRC	26 [47]	52 [45]	69.33 [71.1]	Stage I to III	Stool	TFP12	89/93	N/A
		Adenoma	[19]		[61.4]					
		Cn	45 [30]	46 [54]	55 [52.3]					
Oh *et al*[90], 2013	Case-control, South Korea	CRC	131	69	58.4	26/57/36/12	Blood	SDC2	87/95	0.927, < 0.0001
		Cn	125	64	51					
Grützmann *et al*[121], 2008	Case-control, Germany	CRC	252[126]	57 [60]	61 [67]	63/83/59/29/(NA:19)	Blood	Septin 9	48/93	N/A
		Cn	102[183]	35 [41]	59 [56]	[22/37/54/11/(NA:3)]			[58/90]	
Warren *et al*[91], 2011	Case-control, United States	CRC	50	54	62	I + II/III + IV	Blood/Stool	Septin 9	90/88	N/A
		Cn	94	45	58	38/12				
Tóth *et al*[92], 2012	Case-control, Hungary	CRC	93	52	67.8 (9.8)	25/14/36/18	Stool	Septin9 (gFOBT)	100/100	N/A
		Cn	94	38	62.6 (9.9)					

SD: Standard deviation; Sn: Sensitivity; Sp: Specificity; AUC: Area under the curve; CRC: Colorectal cancer; N/A: Not available; Cn: control; IBD: Inflammatory bowel disease; HGD: High grade dysplasia; VIM: Vimentin; TFP12: Tissue factor pathway inhibitor 2.

from stool specimens.

Methylation biomarkers: A number of factors, including one's lifestyle, diet, aging,

reduction of folate levels, exposure to arsenic, and health problems (such as colitis) can lead to colorectal mucosa's abnormal DNA methylation[81-84]. One can detect patterns of aberrant DNA methylation from CRC cells in the DNA derived from blood or stool specimens from patients with colorectal cancer[85]. Along with the various levels of specificity and sensitivity, several abnormally methylated genes that have been identified in either blood or stool can be used as diagnostic biomarkers in CRC patients. In the United States, for example, vimentin (VIM) gene methylation analysis in a stool-based test is readily available, with about 80% specificity and sensitivity[86]. These abnormally methylated genes are also AIX4, SEPT9, FBNI, WiF-1, P53, PGR, MGMT, TIMP3, and GATA4[81,87]. Guo *et al*[88] used PCR to study hypermethylation of FBNI in patients with CRC. The study involved tissues and stool specimens from 75 patients with CRC and 30 normal individuals. FBNI hypermethylation was found in 78.7% of CRC tissue specimens and 72% in stool specimens compared to 6.7% of controls, showing a specificity of 93.3% and a sensitivity of 72%. According to Guo *et al*., estimating hypermethylated FBNI in stool specimen can be a useful non-invasive biomarker for identification of CRC. One of the genes, tissue factor pathway inhibitor 2 (TFPI2), was methylated in almost all patients with CRC of all stages with 97% in adenoma and 99% in CRC[89]. TFPI2 gene methylation in CRC patient's stool specimens yielded up to a 93% specificity and a 89% sensitivity. Oh *et al*[90] conducted a study to measure methylation of the SDC2 gene in blood specimens. This study included 131 patients with CRC representing all stages and 125 normal individuals. The results showed a high level of specificity, 95.2%, and an 87.0% level of sensitivity. Also, the sensitivity for early-stage was 92.3%. Therefore, SDC2 methylation in blood was suggested to be a non-invasive, highly sensitive, and specific biomarker for CRC screening[90]. There are a number of CRC screening tests available on the market detecting aberrant gene methylation from either blood or stool. As described previously, the mSEPT9 assay is an example of these available tests. Warren *et al*[91] conducted a study on the efficacy of the blood-based mSEPT9 assay for CRC detection using blood specimens from 50 CRC patients and 94 healthy individuals. The results showed 90% sensitivity and 88% specificity for all stages. Accordingly, Tóth *et al*[92] studied the efficiency of detection of mSEPT9, gFOBT, and CEA from CRC and normal plasma. As mSEPT9 achieved high sensitivity and specificity levels of 100%, it is considered to be a superior screening test for CRC detection over CEA and gFOBT.

Despite the wide variety of molecular techniques, More research is needed to produce a new molecular biomarker or biomarker panel that could be used for a broad range of screening. In the future, studies should provide solutions to resolve the predictive and prognostic problems of the proposed and presently used molecular biomarkers. Developing effective molecular screening for CRC capable of detecting early-stage colorectal malignancies would be an innovation. In considering the molecular background of the tumor, molecular markers ensure that the field develops a more personalized approach. Identifying clinically-related, cost-effective and easily tested biomarkers to facilitate patient management decisions and provide direct benefits to the patient is, after all, the goal.

Metabolomics

One option for non-invasive screening is metabolomics, which is a potential tumor marker for CRC. It is important to have a comprehensive understanding of all small-molecule marker metabolites of CRC to accurately understand the tumor metabolic pathway that will assist diagnosis and become the basis for novel preventive and therapeutic methods.

Published studies that attracted large amounts of publicity have recently peaked interest in the possibilities of metabolomic analysis to identify biomarkers for advanced identification of disease progression from easily obtainable biofluids. Therefore, metabolomics analysis had only just started to join the conventional practices of cancer diagnosis and treatment.

One of newly rising "omics" studies, metabolomics investigates global, or system-wide, metabolic profiles, offering a dynamic portrait of the metabolic status of living systems. Being highly potent for diagnosing various cancers using advanced analytic techniques and biometric tools, this approach has been used for therapeutic monitoring and drug development. There are some metabolic markers always found in CRC; however, metabolic profiles of patients with early-stage CRC, including precancerous lesions, are not clearly understood. Due to the non-invasive nature of the approach, it warrants further investigation.

Characteristics of Colorectal Cancer Screening By Biofluid Sample Type (Blood, Urine, Stool): Novel diagnostics can be subdivided based on the type of biofluid sample to be analyzed, primarily blood, urine, or stool specimens. The pros and cons of each specimen are shown in Table 3.

Table 3 Characteristics of colorectal cancer screening of bio fluidic sample types (blood, urine, stool)

Sample types	Evidence of efficacy	Advantage	Disadvantage
Blood-based biomarkers (serum, plasma, and dried blood spot)	A combination of 8 metabolites (99.3% sensitivity, 93.8% specificity, and AUC 0.996)[94] Gastrointestinal tract acid 446 (83.3% sensitivity, 84.8% specificity, 85.7%, and 52.1% , respectively)[96,97] Decanoic acid (87.87% sensitivity, 80.0% specificity, 71.0%, and 75.0%, respectively)[98,99]	Easily accessible Less affected by diet than urine Less diurnal variation and Less inter- and intra-subject variability than urine Stable over a 4-mo period frozen at -80 °C except at room temperature	Affected by smoking status More invasive than urine and stool Analysis can be more complex than urine
Urine	Cross-validated panel of seven metabolites (97.5% sensitivity, 100% specificity, and AUC 0.998)[104] 10 different metabolites (100% sensitivity, 80% specificity but small sample size)[103] N1, N12-Diacetylspermine[105,106]	Easily accessible Less invasive than blood	More affected by diet than serum samples More diurnal variation and More inter- and intra-subject variability than serum A full day storing at room temperature or on cool packs altered metabolite concentration More than 2 freeze and thaw cycles affected the metabolic profile significantly
Stool	A three metabolite panel (AUC 1.0 but very small sample size)[107] A metabolomics panel (AUC 0.94)[108]	Easily accessible Less invasive than blood	Inconvenient to collect of stool samples Low compliance

(1) Blood-based biomarkers: Blood-based markers can be found in either plasma or serum samples, as well as in dried blood spots, which only requires minimal amounts of blood. Moreover, blood-based markers from dried blood spots have particular advantages, such as easy transportation, convenient storage, and ability to delay processing[93].

In a study of blood-based biomarkers, a dried blood spot biomarker that was composed of four amino acids and four acylcarnitines resulted a quite reasonable sensitivity (81.2%) and specificity (84.0%)[93]. One issue of this study, however, was that the 62% of participants were already in a later stage (III or IV) of CRC. Among the available blood-based panels, the most effective biomarker was introduced by Nishiumi *et al*[94], who combined eight metabolites to detect early-stage CRC. The panel showed 99.3% sensitivity, 93.8% specificity, and an area under the curve (AUC) of 0.996. The highest sensitivity and specificity were reported for a single marker, but the study involved limitations, such as a small study population and relatively young age (18–22 years) of healthy controls[95]. Most of all, the study was not validated. Gastrointestinal tract acid 446 (GTA-446) is a rising biomarker that has been newly introduced by Hata *et al*[96] (83.3% sensitivity, 84.8% specificity) and Ritchie *et al*[97] (85.7% sensitivity, 52.1% specificity). In addition, two independent studies found that decanoic acid could be a promising biomarker candidate (87.87% and 71.0% sensitivity, 80.0% and 75.0% specificity)[98,99].

(2) Urine: Most studies of biomarkers found in urine have discovered that a panel is more suitable than solitary metabolites. The outcomes of three Canadian studies were based on identical study settings[100-102]. Among the studies, the assay with the highest sensitivity used ten distinct metabolites. However, no additional categorization was done for the latter[103]. The study showed 100% sensitivity and a specificity of 80%. However, it had a small sample size. A cross-validated panel that included seven metabolites had a sensitivity of 97.5% (AUC: 0.998) and a specificity of 100%, the highest percentage[104]. Two studies, one by Deng, Deng *et al*[101] and another by H. Wang *et al*[102] reported similarly high sensitivities. In addition, two separate studies detected N1, N12-diacetylspermine as a distinct biomarker that could be used for a future screening test[105,106].

(3) Stool: In a systematic review of studies on early identification of abnormal colorectal growths using biomarker detection, one study reported an AUC of 1.0 based on a three-metabolite panel[107]. However, the research only had a small population size. Participants from true screening study showed another metabolomics panel to identify advanced colorectal neoplasms. The panel demonstrated good performance (AUC: 0.94)[108].

Sample type, analytical techniques, major metabolites, outcomes, sensitivity, specificity and significant findings of various metabolomic studies are shown in Table 4. It seems that a panel of metabolites is superior to a single marker for advanced colorectal neoplasms. As for amino acids in blood specimens and nucleosides in urine samples, the findings were consistent.

Table 4 High-throughput metabolomic studies of potential biomarkers in CRC screening

Sample type		Ref.	Analytical technique(s)	Major metabolites	Out-comes	Sn / Sp	Significant finding(s)
Dried blood		Jing *et al*[93], 2017	Direct infusion MS	AA (4) FA (4)	CRC	81.2/84	Establishing a reasonable diagnostic regression model with eight blood parameters
SERUM	BP	Zhang *et al*[122], 2018	UPLC-MS/MS	FA(2): Eicosanoids	CRC	N/A	Identification of eicosanoids as potential biomarkers for identifying among health, enteritis and CRC
		Guo *et al*[123], 2017	FTICR MS	FA(5): Male FA(2): Female	CRC	77.3/92.4 80.8/85.9	Presenting the relationship between the change trends of six phospholipids and cancer stages
		Farshidfar *et al*[124], 2016	GC-MS	AA (9) FA(7) CH (12) Others (13)	CRC	85.0/86.0	Discovery of a suite of CRC biomarkers that provide early detection, prognostication and preliminary staging information
		Zhang *et al*[125], 2016	FTICR MS	FA (6)	CRC	93.8/92.2	Identification of Free Fatty Acids as diagnostic indicators of early-stage CRC patients
		Gu *et al*[126], 2015	LC-MS/MS	AA (8)	CRC	65.0/95.0	Performing a combined analysis of amino acids in three different domains: FAAs, FSPAAs, and IPAAs
		Zhu *et al*[127], 2014	LC-MS	AA (7) FA (3) CH (3)	CRC	96.0/80.0	Establishing Partial least-squares-discriminant analysis (PLS-DA) models for distinguishing CRC patients
		Li *et al*[128], 2013	DI-ESI (±) -FTICR MS	FA (9)	CRC	86.5/96.2	Emphasize that the facile loss of methyl chloride from the [M + Cl] (-) form of LPC (16:0) in its tandem mass spectrum
		Tan *et al*[129], 2013	UPLC-QTOFMS	AA (6) FA (1) CH (3)	CRC	83.7/91.7	Identification of serum metabolite markers as diagnostic indicators for the detection of CRC
		Ma *et al*[130], 2012	GC-MS	AA (3) CH (3)	CRC	93.3[1]/96.7[1]	Emphasize integrated network connectivity analysis for the diagnosis

	Nishiumi *et al*[131], 2012	GC-MS	AA (3) CH (1)	CRC	83.1/81.0	Establishing potential predictive model for early detection of colorectal cancer
	Ritchie *et al*[132], 2010	FTICR MS	FA (3)	CRC	75.0/90.0	IdentifIcation of a systemic metabolic dysregulation comprising previously unknown hydroxylated polyunsaturated ultra-long chain fatty acid metabolites in CRC patients
	Ludwig *et al*[133], 2009	Hadamard-encoded TOCSY spectra	FA (1) CH (4)	CRC	70.0/95.0	Showing the potential of fast Hadamard-encoded TOCSY spectra for improved classification of serum samples from colorectal cancer patients using a metabolomics approach
S	Hata *et al*[96], 2017	FIA–MS/MS	FA (1: GTA-446)	CRC	83.3/84.8	Identification of GTA-446 as promising tool for primary colorectal cancer screening
	Uchiyama *et al*[98], 2017	CE-TOFMS	FA (1): Benzoic FA (1): Octanoic FA (1): Decanoic AA (1): Histidine	CRC	89.0/82.0 76.0/71.0 71.0/75.0 63.0/82.0	The first report to determine the correlation between serum metabolites and CRC stage using CE-TOFMS Identification of benzoic acid as diagnostic indicators
	Ritchie *et al*[97], 2013	TQ-MS	FA (1)	CRC	85.7/~52.1[2]	Identification of low-serum GTA-446 as significant risk factor for CRC and sensitive predictor of early-stage disease
	Ikeda *et al*[134], 2012	GC-MS	AA (1): Alanine CH (1): GluL AA(1): Glutamine	CRC	54.5/91.6 75.0/75.0 81.8/66.7	Showing the potential of metabolomics as an early diagnostic tool for cancer
	Leichtle *et al*[135], 2012	TIS-MS	AA (1)	CRC	N/A	Showing serum glycine and tyrosine in combination with CEA are superior to CEA for the discrimination

PLASMA	BP	Nishiumi *et al*[94], 2017	GC/QqQMS	AA (3) FA (3) CH (2)	Stage 0/I/II	99.3/93.8	Establishing potential predictive model of colorectal cancer that do not involve lymph node or distant metastasis
		Li *et al*[136], 2013	Lipid extraction MS	FA (3)	CRC	88.3/80.0	Identification of the plasma choline-containing phospholipid levels as potential biomarkers to distinguish between healthy controls, AP and CRC cases, implying their clinical usage in CRC and/or AP-CRC progression detection
		Miyagi *et al*[137], 2011	HLPC-ESI-MS	AA (10)	CRC	N/A	Showing the potential of plasma free amino acids profiling for improving cancer screening and diagnosis and understanding disease pathogenesis
		Okamoto *et al*[138], 2009	HLPC-ESI-MS	AA (6)	CRC	N/A	Presenting the possibility of plasma free amino acids profiling
		Zhao *et al*[139], 2007	LC- MS	FA (4)	CRC	82.0/93.0	Identification of percentage of 18:1-LPC or 18:2-LPC plasma levels compared with total saturated LPC levels, either individually or in combination as potential biomarkers for CRC
	S	Liu *et al*[140], 2018	N/A	AA(1) :Homocysteine	CRC/A	43.5/98.8	Presenting the possibility of using homocysteine with CEA in screening of early rectal cancer
		Shen *et al*[95], 2017	2D LC-QToF/MS	FA (1): PG FA (1): SM	CRC	1.00/1.00 1.00/1.00	Presenting the possibility of 2D LC-QToF/MS-based lipidomics profiling
		Crotti *et al*[99], 2016	GC-MS	FA (1)	CRC	87.8/80.0	Identification of the C10 fatty acid as valuable early diagnostic biomarker of CRC
		Cavia-Saiz *et al*[141], 2014	high pressure-LC	AA (1)	CRC	85.2/100	Identification of the plasma levels of l-kynurenine as a potential biomarkers of CRC

URINE	BP	Nakajima *et al*[105], 2018	LC- MS	AA (2)	CRC	N/A	Presenting the potential of polyamines and a machine-learning method as a screening tool of CRC
		Deng,Fang *et al*[142], 2017	1-dimensional NMR	AA (7) FA (2) CH (8)	A	82.6/42.4	Presenting novel urine-based metabolomic diagnostic test for the detection of adenomatous polyps
		Deng *et al*[101], 2017	LC- MS	FA (1) CH (2)	A	$82.4^{3}/36.0^{3}$	Presenting a clinically scalable MS-based urine metabolomic test for the detection of adenomatous polyps
		Wang *et al*[143],2017	H-NMR	AA (3) CH (1)	Stage I/II	87.5/91.3	Supporting the utility of NMR-based urinary metabolomics fingerprinting in early diagnosis of CRC
		Rozalski *et al*[144], 2015	GC-MS	CH (3)	CRC	78.6/75.0	Identification of Urinary 5-hydroxymethyluracil and 8-oxo-7,8-dihydroguanine as potential biomarkers
		Wang *et al*[102], 2014	1-dimensional NMR	AA (7) FA (2) CH (8)	A	82.7/51.2	Presenting a proof-of-concept spot urine-based metabolomic diagnostic test
		Hsu *et al*[145], 2013	HPLC-MS/MS	CH (6)	CRC	69.0/98.0	Identification of a set of six targeted nucleosides as marker
		Eisner *et al*[100], 2013	H-NMR	AA (2) CH (2)	Polyps	64.0/65.0	Presenting a machine-learned predictor of colonic polyps based on urinary metabolomics
		Yue *et al*[103], 2013	RRLC-QTOF/MS	FA (9) Others (1)	CRC	100/80.0	Identification of CRC urinary metabolites as marker
		Cheng *et al*[104], 2012	GC/TOF-MS UPLC-QTOFMS	AA (4) FA (1) CH (2)	CRC	97.5/100	Reporting a second urinary metabonomic study on a larger cohort of CRC (n = 101) and healthy subjects (n = 103)
		Chen[146], 2012	CE-MS	AA (8) CH (4)	CRC	N/A	Presenting the usefulness of the technique of CE-MS based on moving reaction boundary
		Wang *et al*[147], 2010	UPLC-MS SPE-HPLC	AA(4) FA(5) / CH (7)	CRC	N/A	Identification of urinary metabolic biomarker based on UPLC-MS and SPE-HPLC

		Feng[148], 2005	RP-HPLC	CH (2)	CRC	71.2/93.3	Identification of Pseu and m1G as novel biomarkers for colorectal cancer diagnosis and surgery monitoring
		Zheng *et al*[149], 2005	Column switching HPLC	CH (14)	CRC	71.0/96.0	Identification of urinary nucleosides determined by column switching high performance liquid chromatography method
	S	Johnson *et al*[150], 2006	LC- MS	FA (1)	ACN	90.0/45.0	Identification of urinary PGE-M as a potential biomarker of ACN
		Hiramatsu *et al*[106], 2005	ELISA	AA (1)	CRC	75.8/96.0	Indicating that urinary N(1), N(12)-Diacetylspermine is a more sensitive marker than CEA, CA19-9, and CA15-3
FECES	BP	Amiot *et al*[108], 2015	H-NMR	AA (2) FA (4) CH (1)	ACN	N/A	Identification of (1)H NMR Spectroscopy of Fecal Extracts as biomarker
		Phua *et al*[107], 2014	GC/TOF-MS	FA (1) CH (2)	CRC	N/A	Establishing proof-of-principle for GC/TOFMS-based fecal metabonomic detection of CRC
		Bezabeh *et al*[151], 2009	(1)H-MRS	AA (6) FA (1) CH (3)	CRC	85.2/86.9	Detecting colorectal cancer by 1H magnetic resonance spectroscopy of fecal extracts
	S	Lin *et al*[152], 2016	H-NMR	FA (1): Acetate FA (1): Succinate	Early stage	94.7/92.3 91.2/93.5	Identification of the potential utility of NMR-based fecal metabolomics fingerprinting as predictors

[1]Sensitivity and specificity calculated from available data.
[2]Specificity was calculated for the intention to screening population (40–74 year-olds in the colonoscopy population).
[3]Additional results for different cut-off values can be read from the original article. BP: Biomarker panels; S: Single markers; MS: mass spectrometer; FTICR: Fourier transform ion cyclotron resonance; SM: sphingomyelins; PC: phosphatidylcholine; FIA-MS/MS: flow injection analysis–mass spectrometry; Arg: arginine; Val: valine; Phe: phenylalanine; Tyr: tyrosin; Ala: alanine; TQ-MS: triple-quadrupole tandem mass spectrometry; GluL: glucuronic lactone; TIS-MS: Turbo Ion Spray Source mass spectrometer; AP: adenomatous polyps; HLPC-ESI-MS: high-performance liquid chromatography-electrospray ionization-mass spectrometry; A: adenomas; GC/QqQMS: gas chromatography/triple-quadrupole mass spectrometry; 2D LC-QToF/MS: two dimensional liquid chromatography-quadrupole time-of-flight mass spectrometry; PG: phosphatidylglycerol(34:0); SM: sphingomyelin (38:8); CE-TOFMS: capillary electrophoresis-time-of-flight mass spectrometry; GC-MS: gas chromatography-mass spectrometry; LC-MS/MS: liquid chromatography tandem MS; FAAs: free amino acids; FSPAAs: free and soluble-proteome amino acids; IPAAs: insoluble-proteome amino acids; DI-ESI(±): Direct-infusion positive and negative ion electrospray ionization; ACN: advanced colorectal neoplasms; NMR: nuclear magnetic resonance spectra; H-NMR: proton nuclear magnetic resonance spectroscopy; RRLC: rapid resolution liquid chromatography; UPLC-QTOFMS: Ultra performance liquid chromatography quadrupole time-of-flight mass spectrometry; SPE, solid phase extraction; RP, reverse-phase; ELISA: Enzyme-linked immunosorbent assay; (1)H-MRS: (1)H magnetic resonance spectroscopy.

Limitations of current studies on metabolic biomarkers and influences on metabolomics profiles

Due to some drawbacks, interpreting and implementing metabolomics studies becomes complicated, in particular, poor standardization is a major concern. The sample to be analyzed also has advantages and disadvantages depending on the type,

and the results can be influenced by various situations (Table 3). For future, practical use, the Standard Metabolomics Reporting Structure Group attempted to standardize protocols for metabolomics studies beginning with the design of the study, collection and preparation of specimens[109]. Poor standardization could reduce the comparability of studies.

Another limitation is that there is insufficient individual validation of the biomarkers in controlled clinical settings or in a true screening setting for early detection of malignancy in a cohort of asymptomatic individuals[110]. The majority of studies report biomarker panels used in their studies that have not been validated. Insufficient validation could lead to overestimation of the performance of biomarker panels because of overfitting. There are concerns of generalization in the case of studies that only used internal validation. Also, the ability to detect valid biomarkers is limited because most of the studies were performed with comparatively small sample sizes[111]. In clinical practice, before using metabolomics for early detection, significant effort should be devoted to screening large cohorts under standardized circumstances. Also, since the majority of subjects in these studies were Asian, there may be limited generalization and transferability to other races.

CONCLUSION

Herein, we provide a review of the literature on the current state and future direction of screening tools for colorectal cancer. Generally, detecting cancer and its precursors at an early stage and initiating treating can prevent unnecessary deaths from colorectal cancer. However, because of the limitations of the screening tools currently in use, the development of new screening tools is required, and studies on metabolomics and proteomics are currently underway. It may be possible to develop a new non-invasive diagnostic test based on biomarkers, which is simple, cost-effective, and highly specific and sensitive. Yet, due to heterogeneity of the biomarkers, more research on this topic needs to be conducted before implementing these potential screening biomarkers in clinical settings. Especially important for achieving better efficacy in colorectal cancer screening are establishing standardized protocols in research for metabolomics and proteomics, carrying out larger studies in true screening settings, and external validation of the outcomes. For better diagnostic performance of non-invasive tests in detecting CRC or its precursors, combining various approaches, such as metabolomics and proteomics, should also be considered.

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