Non-invasive colorectal cancer screening

Past, present and future

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ABSTRACT

Colorectal cancer (CRC) is a major cause of morbidity and mortality throughout the world and is the second most common cause of Canadian cancer-related deaths in men and the third most common in women. Most CRC appears to arise from the gradual development and advancement of colonic adenomatous polyps to cancerous tissue. This developmental process of CRC is the rationale for screening programs which aim to reduce CRC-related morbidity and mortality by early detection and removal of adenomatous polyps, specifically advanced adenomas. Although both the gFOBT and FIT function to detect occult bleeding in asymptomatic patients at average risk for CRC development, the mechanisms of these screening tests are distinct. gFOBT works by detecting the peroxidase activity of heme whereas FIT selectively detects human hemoglobin. The sensitivity in detecting CRC is higher for the FIT, with sensitivity of 0.79 compared to gFOBT with sensitivity of 0.36, they have similar specificities of 0.94 and 0.96, respectively. Currently, both the gFOBT and FIT are strongly recommended across Canada, with all provinces using the FIT, apart from Ontario and Manitoba which currently use the gFOBT to screen asymptomatic patients for CRC. A newer test, the sDNA test, identifies mutations in DNA that are shed by both adenomatous polyps and CRC cells. The sDNA test is more sensitive (0.92 95% CI 0.83-0.98) than both the gFOBT and FIT, however, is less specific and more expensive. Further data surrounding the sDNA test will be required prior to its implementation and recommendation for population based CRC screening in Canada.

BACKGROUND

Colorectal cancer (CRC) is a major cause of morbidity and mortality throughout the world. CRC is the second most common cause of Canadian cancer-related deaths in men, and the third most common in women.¹ Risk factors associated with the development of CRC include increasing age, male gender, previously known adenomatous polyps or CRC, history of inflammatory bowel disease (IBD), Lynch syndrome, and familial adenomatous polyposis.² Additionally, although not involved in the stratification of high-risk populations, certain lifestyle habits may increase the risk of CRC development. Lifestyle habits that may increase the risk of CRC development include smoking, sedentary lifestyle, obesity, and diets containing inadequate fibre or excessive intake of fats and red meats.²

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advancement of colonic adenomatous polyps to cancerous tissue.³ This developmental process of CRC is the rationale for screening programs which aim to reduce CRC-related morbidity and mortality by early detection and removal of adenomatous polyps, specifically advanced adenomas. Currently, all Canadian provincial CRC screening programs recommend screening with fecal occult blood testing (FOBT), either guaiac FOBT (gFOBT) or fecal immunochemical testing (FIT). A recent guideline published in the CMAJ on CRC screening presented current recommendations for screening, and updates the previous Canadian Task Force on Pre-

Table 1. Summary of recommendations.

- Screening is recommended for adults 50 - 59 years of age with FOBT (either gFOBT or FIT) every two years or flexible sigmoidoscopy every 10 years.
- Screening is recommended for adults 60 - 74 years of age with FOBT (either gFOBT or FIT) every two years or flexible sigmoidoscopy every 10 years.
- Screening is not recommended for adults 75 years of age and older.
- Colonoscopy is not recommended as a screening test.
- These recommendations apply to asymptomatic adults 50 years of age and older without a history of CRC in one or more first-degree relatives, previous adenomatous polyps, previous CRC, IBD, lynch syndrome, or familial adenomatous polyposis.

ventive Health Care recommendations from 2001 (Table 1).^{4,5}

A notable new, and distinct, suggestion within this recent guideline by the Canadian Task Force on Preventive Health Care is the recommendation against colonoscopy as a screening test for CRC.⁴ This is in part due to the lack of evidence demonstrating efficacy in comparison to alternative screening modalities, increased procedural risks, and increased costs as a screening test. This recommendation challenges previously established screening guidelines, along with other large academic bodies such as the American Gastrointestinal Association, the American College of Gastroenterology, the British Society of Gastroenterology, the National Cancer Institute, and the Canadian Association of Gastroenterology which all support colonoscopy as a tool for CRC along with non-invasive fecal testing.⁶⁻¹⁰ Controversy has arisen as a result of the new CMAJ guidelines for CRC in regards to recommending against colonoscopy as a screening test for asymptomatic patients.¹¹ Due to page limitations we will not provide a position paper but rather we will review current non-invasive testing, with focus on three forms of fecal testing.

GUAIIAC FECAL OCCULT BLOOD TEST

As previously mentioned, FOBT's, both the gFOBT and FIT, are strongly recommended options for CRC screening. Although both the gFOBT and FIT function to detect occult bleeding in asymp-

tomatic patients at average risk for CRC development, the mechanisms of these screening tests are distinct. The mechanism of the gFOBT is based on the oxidation of guaiac, a compound impregnated directly onto the gFOBT screening card. To complete a gFOBT, 6 fecal samples (2 samples from each of 3 consecutive spontaneous stools) are applied onto test areas of a gFOBT screening card. Hydrogen peroxide is then applied to the individual test areas and, in the presence of occult blood; the peroxidase activity of heme will catalyze the oxidation of guaiac resulting in a rapid blue colour change visually interpreted by a trained laboratory technician.¹²

The gFOBT is a commonly recommended CRC screening method and has been shown to be a cost-effective method, which has demonstrated a reduction in mortality from colorectal cancer with repeated screening.^{13,14} Despite its utility, the gFOBT does contain some disadvantages. Firstly, for most accurate results, manufacturers of these gFOBT kits recommend dietary restrictions prior to, and during, the screening process. To decrease false positive results, it is recommended to avoid the ingestion of non-steroidal anti-inflammatories, which may cause gastrointestinal bleeding, red meats, which may cause animal heme cross-reactivity, and peroxidase containing fruits and vegetables, which may mimic the peroxidase activity of heme. Peroxidase-rich fruits and vegetables recommended to avoid include parsnips, turnips, broccoli, cauliflower, radishes, horseradish, and cantaloupe. To decrease false positive results, it is recommended to avoid the ingestion of vitamin C in excess of 250 mg per day for 2 days prior to, and during, the screening process which may inhibit the peroxidase activity of heme. Despite these dietary recommendations, a systematic review by Pignone et al. suggests these dietary restrictions do not appear to affect positivity rates and physicians may not be required to advise dietary changes prior to, and during, gFOBT screening.¹⁵ Compared to other forms of stool testing the sensitivity of the gFOBT for detecting CRC is quite low at 36%, with a specificity of 96% (Table 2).¹⁶ Its sensitivity for detection of advanced adenomas is 19.8% with a specificity of 97.4%.¹⁷ It was also found that although the gFOBT shows a reduction in mortality from CRC, no change was seen in all-cause mortality.⁴

FECAL IMMUNOCHEMICAL TEST

Mechanistically, FIT is different from the gFOBT in that it selectively detects human hemoglobin. FIT uses monoclonal or polyclonal antibodies directed against the globin chain of human hemoglobin. By using labeled antibodies, the bound antibody-hemoglobin complex can be detected.¹⁸ Two different types of FIT exist, qualitative and quantitative. Qualitative uses a lateral-flow immunochromatographic assay that results in a binary signal (positive/negative). The quantitative FIT directly measures the hemoglobin concentration using immunoturbidimetry, which detects the amount of analyte present in a sample using a photometer.¹⁹

The gFOBT detects heme in stool. Heme is slowly degraded along the GI tract, therefore all sources of bleeding in the GI tract (eg peptic ulcers) can produce a positive result.¹⁸ FIT detects the globin portion of hemoglobin which is degraded rapidly along the

GI tract and thus is less likely to detect bleeds of a non-colonic cause.¹⁹ FIT also has many other advantages over gFOBT, first, it has higher sensitivity for detecting CRC, with a sensitivity of 79%, and specificity of 94%.²⁰ Secondly, it is an automated process allowing for high throughput analysis of large numbers of stool samples. As previously mentioned, a quantitative fit detects the amount of globin in a sample, so the cut off for positive test can be altered to better match available funding for colonoscopy in a region.^{19,21,22} Randomized control trials have shown that when compared, FIT had greater participation and public uptake versus gFOBT, as it removes the tedious dietary restrictions.^{22,23} The main disadvantage of FIT however, is the greater specimen instability for both time and temperature with specimens stable for 7 to 15 days and decreased in stability with increasing temperature.^{24,25} Considering the greater specimen instability with the use of FIT, the Cancer Care Ontario evidence based series currently recommends that a pilot study be conducted to assess proper implementation of FIT in Ontario.²⁴ Although sensitivity of the FIT for detecting of advanced adenomas is also slightly higher than the gFOBT, with sensitivity of 23.8%, it would be beneficial for the prevention of CRC to have a test with higher sensitivity for detection of advanced adenomas.¹⁹

STOOL DNA TEST

Both adenomatous polyps and CRC cells shed DNA into the stool. This DNA often contains mutations which is the basis of the stool DNA (sDNA) test. Rather than identifying blood in the stool, the sDNA test identifies DNA mutations in specific genes that may indicate the presence of CRC. The current sDNA test, Cologuard, is a molecular assay for DNA mutation and methylation, along with an immunochemistry assay for human hemoglobin similar to the FIT.²⁶ The Cologuard test looks for KRAS mutations, aberrant BMP3 and NDRG4 methylation, and beta-actin which is a reference for human DNA quantity.¹⁹ The results of these assays are combined and compared to a cut off value to determine a positive or negative test.²⁶ A large study conducted comparing the sDNA test and FIT found that the sDNA test had greater sensitivity for detecting both CRC and advanced precancerous lesions, however, there were greater false positive results, resulting in a lower specificity.²⁷ The sensitivity of the sDNA test for detection CRC is 92% while the sensitivity for detecting advanced adenomas is 42%.¹⁹ The number of patients who need screening in order to detect one CRC is 166 for the sDNA test and 208 for the FIT.¹⁹ Additional pitfalls include cost, with Cologuard reporting the test to cost \$599, and an unestablished frequency of screening with the sDNA test; currently Cologuard recommends every three years.²⁶

CONCLUSION

There are multiple available modalities for the screening of CRC in the asymptomatic adult population including both invasive and non-invasive options. As the gFOBT, FIT and sDNA test are all non-diagnostic, positive results require further diagnostic testing, which often involves colonoscopy in order to directly visualize the colon, remove adenomatous polyps, and look for both advanced ad-

Table 2. Sensitivity and specificity of gFOBT, FIT and sDNA in detecting colorectal cancer.

	Sensitivity	Specificity	PPV* (%)	NPV† (%)	NNS‡
gFOBT	0.36 (95% CI 0.25-0.47) ⁴	0.96 (95% CI 0.94-0.97) ⁴	13.6 (95% CI 6.4-25.5) ²²	99.6 (95% CI 97.4-100.0) ²²	377 ¹⁰
FIT	0.79 (95% CI 0.69-0.86) ⁵	0.94 (95% CI 0.92-0.95) ⁵	6.9 (95% CI 5.1-9.0) ²⁷	99.8 (95% CI 99.7-99.9) ²⁶	208 ⁸
sDNA	0.92 (95% CI 0.83-0.98) ²⁷	0.87 (95% CI 0.86-0.87) ²⁷	3.7 (95% CI 2.9-4.8) ²⁷	99.9 (95% CI 99.8-100) ²⁶	166 ⁸

* Positive predictive value. † Negative predictive value. ‡ Number needed to screen

enomas and CRC. Currently, both the gFOBT and FIT are strongly recommended across Canada, with some variances between provinces with regard to preference of gFOBT versus FIT. Ontario currently uses the gFOBT as the primary screening test for CRC.²⁴ The sDNA test is currently not available in Canada, however, it has currently been approved by the FDA for use in the United States.¹⁹ The sDNA test is more sensitive than both the gFOBT and FIT, however, is less specific and more expensive.^{16,19} Further data surrounding the sDNA test will be required prior to its implementation and recommendation for population based CRC screening in Canada.

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