CRC IN FOCUS

Current Developments in the Management of Colorectal Cancer

Section Editor: Axel Grothey, MD

Stool DNA Testing: A New, Noninvasive Option for Colorectal Cancer Screening

David A. Ahlquist
Professor of Medicine
Mayo Clinic
Rochester, Minnesota

H&O What are the limitations of the existing methods of colorectal cancer (CRC) screening?

DA Although mortality rates have fallen with screening, CRC remains the second leading cause of cancer death in the United States. Conventional screening approaches are hampered by a number of factors. First, accuracy is less than optimal—although colonoscopy is highly accurate when done well, its quality is variable and operator-dependent. Second, participation rates are relatively low—only about half of the population is regularly screened. Many people are unable to take the typically uncompensated 1 to 2 days off from work or do not have convenient local access, or else they are unwilling to undergo this procedure because it is invasive and requires bowel preparation. Finally, the recommended screening frequency of every 10 years may result in missed aggressive interval lesions, particularly in the proximal colon.

An ideal test for population screening would noninvasively and accurately detect early-stage cancer and the subset of polyps most likely to progress; would not require bowel preparation, dietary changes, medication restrictions, or time away from work; and would be widely available.

H&O How can the noninvasive multitarget stool DNA test you helped develop make a difference?

DA The Cologuard multitarget stool DNA test has been approved for population screening by the US Food and Drug Administration (FDA) and is now available clinically. It is fully covered by Medicare and Medicaid at a frequency of every 3 years. This test has potential to improve the effectiveness of CRC screening in several ways. Its high accuracy rate means better detection of neoplasms, the fact that it is user-friendly should enhance patient adherence, and the ability to distribute via mail improves access to screening.

H&O How does the stool DNA test work?

DA The test is based on the detection of acquired DNA changes in cells exfoliated into stool from colorectal neoplasms—both cancers and large polyps. In contrast to the intermittent or absent occult bleeding from neoplasms, exfoliation occurs continuously from all neoplasms. The amount of exfoliated DNA is proportional to the surface area of the neoplasms, which accounts for the high detection accuracy of cancers and large polyps from a single stool sample.

Testing is performed on samples sent to a central laboratory. To minimize sampling error, an aliquot is tested from the whole stool homogenates. Analytically sensitive assays target a panel of highly informative markers comprising mutant *KRAS*, aberrant methylation on *BMP3* and *NDGR4* genes, and human hemoglobin. A software algorithm converts quantitative data into a positive or negative result. Automation allows high-throughput capacity and high reproducibility; quality is uniformly high and is not operator-dependent.

H&O How are sampling and delivery of results conducted?

DA The multitarget stool DNA test involves a simple stool collection at home using a device mounted to the toilet seat, followed by mailing the sample to a central laboratory. No preparations or restrictions are necessary.

At this time, the test must be ordered by a health care provider. The collection kit is mailed to the patient's home, and test results are electronically communicated to the patient's designated health care provider.

H&O What are the advantages of this method?

DA This new screening test was designed to deliver the important elements desired in an ideal screening tool—it accurately detects the critical neoplasms, is easy to use, and is accessible by mail. A test with these performance characteristics broadly applied to the general population could have a major impact on reducing both the incidence of and mortality from CRC.

The stool DNA test achieves the same high sensitivity for early-stage CRC as has been reported for colonoscopy, and detects the majority of large polyps at greatest risk to progress. But, it does so noninvasively without a burdensome bowel preparation, diet or medication restrictions, or disruption of work or daily routines.

H&O What are the limitations of this method?

DA Because the test has just become available commercially, patient and provider awareness and familiarity with clinical use algorithms will need to evolve through educational efforts. Although Medicare coverage is in place, coverage of new technologies by private insurance companies typically lags behind. Decisions should occur over the coming year.

Some have expressed early concerns over the specificity of the stool DNA test. Based on recent multicenter case-control and cross-sectional studies, the point-in-time specificity is about 90%, compared with approximately 95% by a fecal immunochemical test (FIT) for occult blood. However, what is most important from an applied standpoint is the rate of false positives per year within a longitudinal program. In addition, testing frequency must be considered. The stool DNA test applied at the recommended frequency of every 3 years would generate false positives at a rate of about 3% per year, whereas FIT done at the recommended annual frequency would generate false positives at a rate of approximately 5% year. So, with programmatic application, the stool DNA test is potentially more specific than FIT.

H&O What did the DEEP-C trial show?

DA Results from this study, which involved 10,000 patients from 90 centers across North America, were published in the *New England Journal of Medicine* last year. The multitarget stool DNA test was compared with FIT using prescheduled screening colonoscopies as the reference standard. The stool DNA test detected 94% of stage I and II cancers, 92% of cancers overall, 69% of polyps with high-grade dysplasia, 66% of adenomas 2 cm or larger, 42% of adenomas 1 cm or larger, and 42% of sessile serrated polyps 1 cm or larger. In contrast, FIT detected significantly fewer lesions in all categories, with sensitivities of 70%, 74%, 42%, 24%, and 5%, respectively. Cancer detection by stool DNA testing was unaffected by tumor stage or location within the colon. Results of this large cross-sectional study were corroborated in a separate and similarly designed study on Alaska Native people, who have one of the world's highest rates of CRC (see the figure).

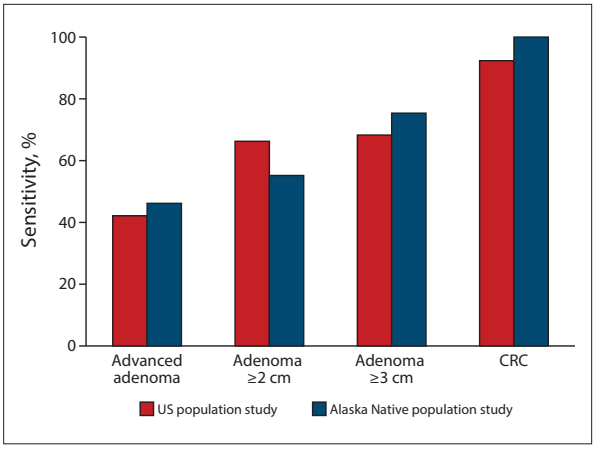

Figure. Screen detection of colorectal adenomas and cancers by a multitarget stool DNA test in a representative US population study and an Alaska Native population study.

CRC, colorectal cancer.

Data from Imperiale TF et al. *N Engl J Med.* 2014;370(14):1287-1297 and Redwood D et al [AGA abstract Su1213]. *Gastroenterology.* 2014;146(5):S-403.

It is important to give context to the polyp detection rates observed with the stool DNA test. Because most polyps grow slowly, there is an opportunity to detect them on repeat testing in subsequent screening cycles. Applying the observed point-in-time sensitivities for polyps, we have estimated that compounded detection rates of polyps within a cohort that are at greatest risk for progression (ie, those with high-grade dysplasia or those 2 cm or larger) would exceed 90% by the second screen.

H&O Is there a potential role for this screening method in CRC diagnosis, or for determining prognosis?

DA The FDA approved the multitarget stool DNA test for use in average-risk screening; that is, in those 50 to 80 years of age with no risk factors for CRC. With appropriate evidence and modeling, other uses for this test may evolve. For example, it might be used as an interval test

between screening colonoscopies, as a follow-up test for incomplete colonoscopies, or as an alternative approach in high-risk persons who are averse to or refuse colonoscopy. Another potential use might be for persons aged 40 to 49 years, which is a subset that currently is not screened but is witnessing a marked increase in CRC rates.

A stool DNA test would not be considered diagnostic, and a positive test result would be followed by a diagnostic colonoscopy. Molecular markers assayed in tissue have been used to predict CRC outcomes and to individualize treatment, and such markers could potentially be determined from stool in the future.

H&O Is there a role for similar technology in detecting other types of cancer?

DA There is a potential role. Our group has demonstrated the feasibility of detecting cancer and dysplasia in people with inflammatory bowel disease using a differently configured stool DNA test, and a multicenter study is under way (NCT01819766). Our preliminary data also suggest that stool DNA testing could be used to detect gastrointestinal cancers above the colon, including esophageal, gastric, pancreatic, and hepatobiliary cancer. We do not screen for any of these cancers now, but collectively they account for more deaths than does CRC. Optimization and further clinical testing are needed to assess the value of these extended applications.

H&O What other new methods of CRC screening are being developed?

DA New testing approaches for CRC screening that have been studied recently include molecular blood tests and capsule endoscopy. To date, plasma-based DNA tests have failed to achieve high detection rates for early-stage CRC and generally have been unable to detect polyps. In a direct comparison with a commercially available plasma DNA test that we published in *Clinical Gastroenterology and Hepatology* in 2012, we found that a prototype of the multitarget stool DNA test detected 91% of stage I to III CRCs vs 50% by plasma *SEPT9* testing. For very large adenomas, detection rates were 82% and 14%, respectively. It is unclear at this time whether there are fundamental biological limitations to blood test screening for CRC, or whether technical advances can overcome these. Swallowed video capsule endoscopy may detect both CRC and polyps with moderately high to high sensitivity based on recent observations, but larger population-based studies are needed. It remains to be determined whether the extensive bowel preparations required will be a disincentive to this approach.

Pilot studies suggest that CRC may be detectable by molecular testing in urine or even by breath testing. However, further examination and corroboration of these interesting concepts are needed.

Dr Ahlquist's employer, Mayo Clinic, has licensed technology to Exact Sciences that was used in the development of Cologuard. As an inventor on this licensed technology, Dr Ahlquist shares in royalties from Exact Sciences to Mayo Clinic. Dr Ahlquist is also a scientific advisor to and research collaborator with Exact Sciences.

Suggested References

Ahlquist DA, Taylor WR, Mahoney DW, et al. The stool DNA test is more accurate than the plasma septin 9 test in detecting colorectal neoplasia. *Clin Gastroenterol Hepatol*. 2012;10(3):272-7.e1.

Ahlquist DA, Zou H, Domanico M, et al. Next-generation stool DNA test accurately detects colorectal cancer and large adenomas. *Gastroenterology*. 2012;142(2):248-256.

Ahlquist DA. Multi-target stool DNA test: a new high bar for noninvasive screening. *Dig Dis Sci*. 2015;60(3):623-633.

Heigh RI, Yab TC, Taylor WR, et al. Detection of colorectal serrated polyps by stool DNA testing: comparison with fecal immunochemical testing for occult blood (FIT). *PLoS One*. 2014;9(1):e85659.

Imperiale TF, Ransohoff DF, Itzkowitz SH, et al. Multitarget stool DNA testing for colorectal-cancer screening. *N Engl J Med*. 2014;370(14):1287-1297.

Kisiel JB, Yab TC, Nazer Hussain FT, et al. Stool DNA testing for the detection of colorectal neoplasia in patients with inflammatory bowel disease. *Aliment Pharmacol Ther*. 2013;37:546-554.

Lidgard GP, Domanico MJ, Bruinsma JJ, et al. Clinical performance of an automated stool DNA assay for detection of colorectal neoplasia. *Clin Gastroenterol Hepatol*. 2013;11(10):1313-1318.

Redwood D, Asay E, Sacco P, et al. Stool DNA testing for screen-detection of colorectal neoplasia in Alaska Native people [AGA abstract Su1213]. *Gastroenterology*. 2014;146(5):S-403.