REVIEW

CME CREDIT

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Fecal-based DNA assays: A new, noninvasive approach to colorectal cancer screening

ABSTRACT

Stool-based DNA testing is a new, noninvasive method of colorectal cancer screening. Because it is easier to use and more sensitive than fecal occult blood testing, physicians may be more likely to recommend it, and patients may be more apt to comply. Although it is expensive, initial assessments show it to be cost-effective.

KEY POINTS

A commercially available assay detects common mutations in cells shed into the stool from the surface of colorectal adenomas and carcinomas.

Fecal DNA testing is more accurate in detecting colorectal cancer than is fecal occult blood testing, but it is less sensitive than colonoscopy.

Patients with a positive fecal DNA test should undergo colonoscopy. The best way to manage patients with a positive fecal DNA test and a negative colonoscopic examination has yet to be determined.

Whether stool-based DNA testing will be widely used may depend on the outcomes of two ongoing multicenter trials and on subsequent evaluation of cost-effectiveness.

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FECAL-BASED DNA testing is a new, noninvasive screening tool that detects genetic mutations characteristic of colorectal cancer in cells that are shed in the stool. Although it is more accurate than fecal occult blood testing, it is not as sensitive as colonoscopy.

Screening for colorectal cancer in people over age 50 is a known lifesaver. However, even though a variety of options are available, most Americans forgo screening.[1] Thus, fecal-based DNA testing may be an alternative for patients who otherwise would not be screened.

This article focuses on this new test, how it works, how it has fared in initial clinical trials, and how cost-effective it is.

CANCER DEATHS CAN BE PREVENTED

Colorectal cancer is the second leading cause of cancer-related deaths in the United States. An estimated 146,940 Americans will be diagnosed with colorectal cancer in 2004, and a staggering 56,730 people will die of it.[2]

Most colorectal cancer deaths can be prevented by early detection through screening, which is universally endorsed by guideline groups as both beneficial and cost-effective.[3–6]

SCREENING IS UNDERUSED

According to current recommendations, people of average risk who are 50 years or older should choose a screening method. The conventional options (which vary considerably in invasiveness, effectiveness, and cost) are:

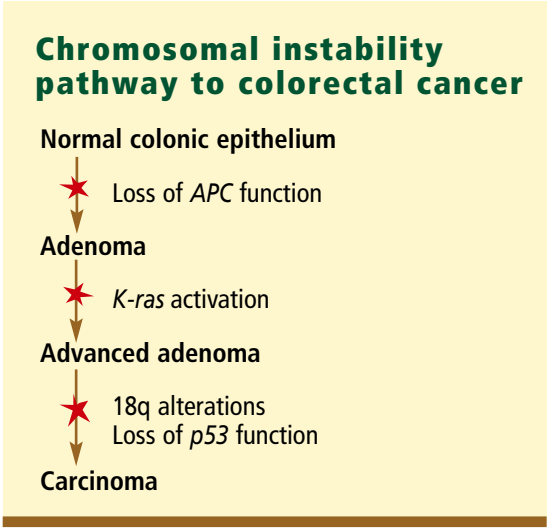

FIGURE 1

ADAPTED FROM FEARON ER, VOGELSTEIN B. A GENETIC MODEL FOR COLORECTAL TUMORIGENESIS. CELL 1990; 61:759–767.

- Fecal occult blood testing every year
- Flexible sigmoidoscopy every 5 years
- Fecal occult blood testing every year plus flexible sigmoidoscopy every 5 years
- Colonoscopy every 10 years
- Double-contrast barium enema every 5 years.[5]

Fewer than half of adults undergo colorectal cancer screening

Adherence rates are low. In a recent study of adults older than 50 years,[7] only 44.6% reported ever having been tested for fecal occult blood, and 47.3% reported ever having undergone lower endoscopy (either sigmoidoscopy or colonoscopy).[7] Only 53.1% had undergone lower endoscopy within the past 10 years or fecal occult blood testing within the past 12 months. Of interest, although colorectal cancer screening has substantial evidence to show that it can reduce colorectal cancer mortality, it is used less often than prostate cancer screening with the prostate-specific antigen test, which has not been proven to reduce mortality.[8]

In view of these low adherence rates, it is critical for primary care physicians to try to understand patients' preferences and attitudes toward screening and to help them make informed decisions.[9]

Leard et al[10] explained the advantages and disadvantages of the various testing methods to 100 patients and then asked which one they would chose as their primary method of screening. Their preferences were:

- Colonoscopy 38% (higher for people who had already undergone colonoscopy than for those who had undergone fecal occult blood testing or no prior testing)
- Barium enema 14%
- Flexible sigmoidoscopy 13%
- Fecal occult blood testing 31% (refusing all forms of invasive testing).

These findings suggest that many patients (nearly a third) will decline any form of invasive testing, but would be willing to undergo noninvasive testing.

THE MOLECULAR BASIS OF FECAL-BASED TESTING

Cancers develop and grow as a result of disturbed function of oncogenes or tumor suppressor genes or both. If a gene that normally stimulates cell growth undergoes a mutation that increases its function, it can turn into an oncogene, and the result can be abnormal and accelerated cell growth.

Conversely, tumor suppressor genes regulate and "brake" cell growth. Potentially dangerous mutations in this type of genes are those that reduce their function.

Colorectal adenomas and cancers arise from at least three different genetic pathways (which may not be completely independent of one another): chromosomal instability, microsatellite instability, and CpG island methylation.

Chromosomal instability: Loss or rearrangement of genetic material

Chromosomal instability, the loss of whole chromosomes during cell division or the loss of parts of chromosomes through structural rearrangements, accounts for about 85% of sporadic colorectal cancers and essentially all tumors arising in the inherited syndrome of familial adenomatous polyposis.

Vogelstein et al[11,12] described the association between the accumulation of mutated tumor suppressor genes and oncogenes with the development of colonic adenomas and their eventual transformation into colorectal cancer. The process in which chromosomal instability causes cancer is generally slow: tumors tend to accumulate mutations over 1 to 2 decades (FIGURE 1).[13]

Although many genes can mutate, ones that are often implicated in colorectal cancer include:

APC (adenomatous polyposis coli), a tumor suppressor gene on chromosome 5q. Mutations in this gene tend to appear first, or at least early, in the development of an adenoma.

K-ras (an oncogene). Mutations of this gene often occur second in the development of colorectal cancer, after the APC gene has mutated.

p53 (a tumor suppressor gene on chromosome 17p). Mutations of this gene often occur later in the process and are associated with larger adenomas containing more severe grades of dysplasia.

Microsatellite instability: A marker for defective mismatch repair genes

Microsatellite instability is responsible for fewer cases of colorectal cancer than chromosomal instability.[14–16] It is implicated in about 20% of right-sided colorectal cancers but in only 1% to 2% of left-sided cancers.[17–22] It is, however, found in more than 90% of colorectal cancers arising in patients with hereditary nonpolyposis syndrome, who inherit one defective copy of a mismatch repair gene.

Mismatch repair genes are the genome's spell checkers: they produce proteins that detect and repair errors in DNA. Loss of function of any of these genes (there are at least five of them) may lead to failure to repair mutations, which can then accumulate.[17,23] Particularly vulnerable are microsatellites, which are short, repeating sequences of DNA.

Eventually, if enough mutations accumulate, the gene will malfunction or fail. If the mutated gene controls cell growth or regulates tumor suppression, loss of function may lead to cancer.

Genes commonly affected are those with microsatellites in their coding regions; these include transforming growth factor (TGF) beta-1 receptor II, insulin-like growth factor II receptor, *BAX*, *hMSH3*, *hMSH6*, *TCF 4*, caspases, beta-catenin, *WISP-3*, and *MBD4*.

Typical assays for microsatellite instability test for mutations in at least five microsatellite loci. Tumors with mutations at two or more loci are regarded as having high-frequency microsatellite instability, a strong indication of a failure of a mismatch repair gene. One microsatellite—BAT-26, a single locus of 26 consecutive adenine nucleotides—is strongly associated with failure of a mismatch gene. Thus, testing for mutations in BAT-26 is almost as effective as screening all five microsatellite loci.[18–21]

CpG island methylation: Promoter regions are inactivated

Tumor suppressor genes can also be inactivated by a third pathway for colorectal cancer development: hypermethylation of CpG islands in their promoter regions.

CpG islands are clusters of cytosine-guanosine residues that are abundant in the promoter region of several genes; the promoter region instructs the gene to turn on its transcription.[24]

CLINICAL TRIALS OF STOOL-BASED DNA ASSAYS

Colorectal cancer arises from at least three pathways

Armed with an understanding of how colorectal neoplasms arise at the molecular level, workers have developed assays that can detect asymptomatic colorectal cancer by detecting altered DNA in cells shed into the stool from the surface of colorectal adenomas and cancers.

In 1992, Sidransky et al[25] first reported detecting colorectal cancer by testing for mutant *K-ras* in stool. In early studies, tests for single mutations (mainly in *K-ras*) were approximately 40% sensitive for detecting cancer.[26]

Newer assays look for more than one mutation and are significantly more sensitive. Dong et al[19] developed a stool DNA assay with three genetic targets: *p53*, BAT-26, and *K-ras*. These markers together detected the cancer in 36 (71%; 95% CI 56%–83%) of 51 patients with colorectal cancer; these 36 constituted 92% (95% CI 79%–98%) of the 39 patients whose tumors had one or more of these genetic alterations.

Ahlquist et al[20] analyzed stool samples in a blinded fashion from 22 patients with colorectal cancer, 11 patients with adenomas at least 1 cm in size, and 28 patients with endoscopically normal colons. The assay targeted

TABLE 1

Sensitivity of screening tests for colorectal cancer

TEST	SENSITIVITY*
Colonoscopy	95%
Stool-based DNA assay	50%–75%
Fecal occult blood test	13%–35%

*When used a single time

point mutations at any of 15 sites on *K-ras*, *p53*, *APC*, and the microsatellite instability marker BAT-26. The assay also tested for "highly amplifiable DNA," using a DNA integrity assay, which identifies redundant DNA often present in cells that are no longer undergoing normal apoptosis (programmed cell death). The sensitivity was 91% (95% CI 71%–99%) for cancer and 82% (95% CI 48%–98%) for adenomas 1 cm or larger; the specificity was 93% (95% CI 76–99%).

Results are better with multitarget vs single-target assays

Syngal et al[27] used a fecal-based assay (PreGen-Plus, EXACT Sciences Corporation, Maynard, Mass) to detect 23 DNA markers, including 21 point mutations in *K-ras*, *APC*, and *p53*; the microsatellite instability marker BAT-26; and highly amplifiable DNA. In patients with known lesions, the sensitivity was 68% (95% CI 56%–80%) for detecting invasive colorectal carcinoma, 40% for adenomas with high-grade dysplasia, and 20% for adenomas with low-grade dysplasia.

Results with assays capable of detecting more mutations on the *APC* gene were better than with assays that could detect fewer mutations on this gene. Traverso et al[28] developed a protein truncation assay that detected *APC* alterations in 17 (61%) of 28 cancers and in 9 (50%) of 18 large adenomas, with no detectable alterations in 28 control subjects.

Traverso et al[18] also developed a digital polymerase chain reaction assay for BAT-26. The assay was positive in 18 of 46 patients with cancers overall, and in 17 of the 18 patients with cancers bearing BAT-26 mutations.

Overall, the sensitivity of multitarget DNA stool assays has ranged from 68% to 91% for colorectal cancer and from 40% to 82% for advanced adenomas.[3,19,29–31] The specificity of the PreGen-Plus assay has been approximately 95%.[18,20,31] Early studies with a prototype multitarget stool-based DNA assay have approached a specificity of 100% by excluding *K-ras* markers, without compromising the test's sensitivity.[17,19,27]

Two large prospective studies are under way in the United States to compare multitarget stool-based DNA testing (PreGen-Plus), fecal occult blood testing, and colonoscopy. All patients, who are of average risk and without symptoms, undergo all three tests. According to initial findings presented by T. Imperiale, MD, at the meeting of the American College of Gastroenterology in 2003, in more than 4,000 screened subjects, the sensitivity of the DNA-based assay for colorectal cancer was 52%, vs 13% for single-time fecal occult blood testing.

COMMERCIALLY AVAILABLE ASSAYS

The first commercially available fecal-based DNA test for colorectal cancer (PreGen-26, EXACT Sciences Corporation, Maynard, Mass) has been marketed for patients with hereditary nonpolyposis colorectal cancer who refuse to undergo colonoscopy. It tests for only one DNA abnormality—mutations in BAT-26.[21] Awareness of the assay has been limited, and it is only minimally used in clinical practice.

A second assay (PreGen-Plus) has been available commercially since August 2003. It has multiple DNA targets and is designed for screening patients who are at average risk. The assay's single-use sensitivity is substantially less than that of colonoscopy but much higher than that of fecal occult blood testing (TABLE 1), so it should be considered only for patients unwilling to undergo colonoscopy. The relative sensitivity of fecal DNA testing and fecal occult blood testing in a programmatic fashion, in either clinical trials or clinical practice, remains unknown.

How tests are used clinically

The patient receives a kit that includes a fecal collection container and ice packs for shipping. Approximately 30 g of stool is needed for

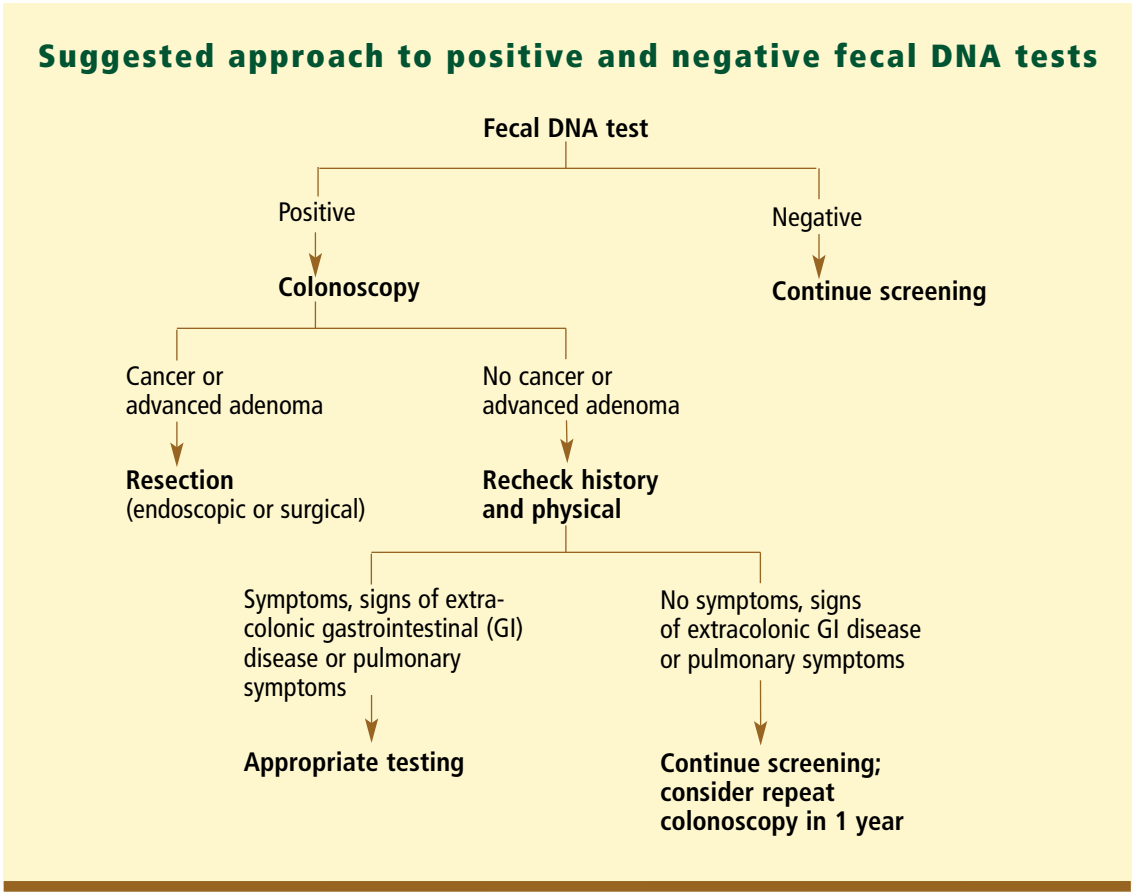

FIGURE 2

Patients with a positive fecal DNA test should undergo colonoscopy

the assay. After passage of the bowel movement, the patient closes the container, packs it with the ice pack that comes with the kit, and returns it to the laboratory. At the laboratory, the sample is homogenized, and abnormal DNA is separated from bacterial and normal human DNA. The abnormal DNA is then amplified and tested for specific abnormalities, and a report is sent to the physician.

Assuming that the screening population has a prevalence of cancers of 0.5% and advanced adenomas of 5%, that the test's specificity is 95%, that its sensitivity for cancer is 50%, and that its sensitivity for advanced adenomas is 15%, the positive predictive value of PreGen-Plus should be about 15%.

Managing patients with a positive result

Patients with a positive test should undergo colonoscopy (FIGURE 2). However, if no cancer or advanced adenoma is found by colonoscopy, the further management of these patients is unclear. In an ongoing trial, patients in this situation undergo abdominopelvic computed tomography (CT), upper endoscopy, and small bowel follow-through radiography to detect whether the abnormal DNA originated outside the colon. Chest CT is also done in case abnormal DNA from a lung cancer was coughed up and swallowed.[19]

In clinical practice, it may be reasonable to perform a physical examination and pursue extracolonic testing if evidence of a specific abnormality is found (FIGURE 2). Repeat colonoscopy after a year, in case a lesion was missed by the initial colonoscopy, may also be justified.

ADVANTAGES OF FECAL-BASED DNA TESTING

Stool-based DNA testing is noninvasive, and it is more sensitive than fecal occult blood testing. Only a single stool sample is needed,

and the patient and physician do not need to handle it as much. The test does not require diet or medication restrictions, it evaluates the whole colon and rectum, and it is now generally available. Initial data from an ongoing trial suggest that patients generally prefer the stool-based DNA test and would be more likely to use it again compared with fecal occult blood testing and colonoscopy.[32]

DISADVANTAGES

Stool-based DNA testing is expensive ($795 per kit). It is less sensitive than colonoscopy, and if the stool-based test is positive, colonoscopy needs to be done anyway. The positive predictive value is low, and there is uncertainty regarding how to manage patients with a positive test and a normal colonoscopic test. It is unclear whether screening for extracolonic malignancies will prove to be an advantage of stool-based DNA testing.

COST-EFFECTIVENESS

Charge for fecal DNA testing: $795

Although data are limited, evidence suggests that stool-based DNA testing has acceptable cost-effectiveness.[33,34]

Vanness and Ahlquist[33] used a discrete event simulation model to assess the incremental cost-effectiveness of stool DNA testing every 3 years compared with fecal occult blood testing, flexible sigmoidoscopy, and double-contrast barium enema (plus colonoscopy, polypectomy, and other treatments as needed in the case of positive findings). They concluded that DNA testing holds promise; the cost per quality-adjusted life year was estimated at $674 to $9,120, a range considered cost-effective for new technologies.

Ness et al[34] compared a variety of screening strategies: one-time colonoscopy, annual fecal occult blood testing, fecal DNA testing every 3 years, sigmoidoscopy every 5 years, annual fecal occult blood testing plus sigmoidoscopy every 5 years, one-time colonoscopy followed by fecal DNA testing every 5 years, colonoscopy every 10 years, colonoscopy alternating with fecal DNA testing every 5 years, and no screening. The most effective strategy was colonoscopy alternating with fecal DNA testing at 5-year intervals, which decreased the incidence of cancer by 59% and mortality by 60%. In addition, compared with no screening, this strategy was associated with a quality-adjusted life-year savings of $14,528 for men and $17,095 for women.

Song and Ladabaum[35] adapted their previously published Markov model to evaluate the cost-effectiveness of stool-based DNA testing vs colonoscopy every 10 years. They assumed that fecal DNA testing would cost $300 and colonoscopy $623 ($900 with polypectomy), colonoscopy would be performed for all positive fecal DNA tests, the sensitivity of DNA testing for cancer is 70%, and its sensitivity for large adenomas is 50%. Under these assumptions, they estimated that fecal DNA testing every 4 years is less effective and more costly than colonoscopy every 10 years.

Assuming that the sensitivity of DNA testing for cancer is 90% and for large adenomas 70%, however, fecal DNA testing every 4 years would be more effective and less costly than colonoscopy every 10 years. Conversely, at the 52% sensitivity level found in the recent multicenter study, fecal DNA screening for colorectal cancer would clearly be less cost-effective than colonoscopy in this model.

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