Colorectal cancer is often considered the most preventable, yet least prevented, cancer. What makes it so preventable is the existence of a precancerous phase of the disease, the adenomatous polyp, which has a rather long dwell time and is usually readily detectable by structural examinations of the colon, such as colonoscopy, sigmoidoscopy, and more recently, computed tomography colonography. Indeed, the removal of adenomas markedly reduces the subsequent development of colorectal cancer. Thus, screening for colorectal cancer, which has been endorsed by all major medical societies, received a grade A recommendation by the United States Preventive Services Task Force in its most recent guidelines (1). Over the last decade, colonoscopy has become the preferred screening test in the United States because it not only detects cancers and polyps with high accuracy but is also therapeutic by virtue of its ability to remove polyps and even early cancers.

Despite its considerable strengths, colonoscopy is still associated with organizational, logistical, and patient-related barriers that limit its full acceptance as a screening method. For this reason, stool-based screening tests are an attractive alternative for many screen-eligible people because the tests are noninvasive, can be done in the privacy of the home, and do not require a cleansing bowel preparation or the need to miss work. Moreover, in parts of the world where colonoscopy resources are limited, stool-based tests, which to date have been based on detecting occult blood, remain the cornerstone of colorectal cancer screening. Indeed, for decades, stool-based tests relied on detecting occult blood with the chemical guaiac reagent (eg, Hemoccult). Whereas this approach reduces colorectal cancer mortality by one-third when the test is performed annually, guaiac-based tests, including the more sensitive versions (eg, Hemoccult SENSA), have limited sensitivity and specificity for colorectal cancer, are quite poor at detecting adenomas, and rely on patients complying with annual, if not biennial, testing to demonstrate efficacy. Newer fecal immunochemical tests have better sensitivity and specificity than guaiac fecal occult blood tests, but blood is a rather nonspecific screening target, with the potential for false positives and false negatives.

Because so much is known about the molecular pathogenesis of gastrointestinal neoplasia, DNA is becoming a more relevant and promising analyte in stool. In the last decade, the feasibility of detecting mutant human-derived DNA among the morass of bacterial DNA in stool became a reality. This soon led to the development of the first-generation fecal DNA tests (2). These tests analyzed what is now considered to be a somewhat cumbersome marker panel of multiple mutations in genes known to be involved in microsatellite stable colorectal cancers (APC, TP53, KRAS), a marker of microsatellite instability (BAT-26), and an assay that detected aberrant colonocyte apoptosis (DNA integrity assay). After several preliminary studies demonstrated an approximate 60%–65% sensitivity for the multimarker fecal DNA test (PreGen-Plus; Exact Sciences Corporation, Marlborough, MA) to detect colorectal cancer (3), two large prospective multicenter studies of average-risk individuals were performed. The first study by Imperiale et al. (4) found that the sensitivity for detecting colorectal cancer was 52% with fecal DNA compared with 13% with Hemoccult, with the two tests having comparable 94%–95% specificity. The sensitivity of fecal DNA for adenomas

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with high-grade dysplasia and for villous adenomas was 33% and 18%, respectively, double the sensitivity rates with Hemoccult. In hindsight, the lower than expected sensitivity for colorectal cancer was due in large part to the degradation of stool during transport to the laboratory, despite precautions taken to chill the sample and ship it overnight. Parenthetically, because all subjects in that study performed fecal DNA, Hemoccult, and colonoscopy, it is intriguing to note that the fecal DNA test was the most preferred of the three screening tests (5). The second study by Ahlquist et al. (6) found that a three-marker panel detected substantially more screen-relevant neoplasia than either Hemoccult or Hemoccult SENSA. The detection of adenomas 1 cm or larger substantially more screen-relevant neoplasia than either Hemoccult or Hemoccult SENSA. The detection of adenomas 1 cm or larger in diameter was 46%, 10%, and 17% by fecal DNA, Hemoccult, or Hemoccult SENSA. The detection of adenomas 1 cm or larger in diameter was 46%, 10%, and 17% by fecal DNA, Hemoccult, and Hemoccult SENSA, respectively.

The data from these first-generation fecal DNA tests formed the basis for including fecal DNA among colorectal cancer screening options by the American Cancer Society, the US Multi-Society Task Force, and the American College of Radiology (7). This served as an important acknowledgment of the potential for fecal DNA screening tests. However, the United States Preventive Services Task Force found that there was insufficient evidence to recommend fecal DNA testing for routine screening (1). In part, this is because first-generation fecal DNA tests, despite their superiority over guaiac tests, still demonstrated modest sensitivity for cancer (in the 55% range), were complex assays (thereby making them expensive), and were not very good at detecting adenomas. It is important to realize, however, that the original molecular markers were purposely chosen to detect cancers more than adenomas.

Can we do better? Yes, we can. In the past 2 years, advances to fecal DNA tests have been rapidly emerging with a goal toward improving the performance characteristics. One important advance was the addition of a DNA-stabilizing buffer to the stool to inhibit DNA degradation by fecal flora during specimen transport and avoid the need to chill the sample (8). This alone was found to markedly improve the sensitivity of the first-generation fecal DNA test from 52% to 73% (9). Other advances have been made with respect to the technological analysis of fecal DNA by methods such as BEAMing (10) and digital melt curve analysis (11). In a recent pilot study of 26 adenomas with tissue-proven KRAS mutations, the digital melt curve assay detected more than 70% of adenomas, which was superior to Hemoccult, Hemoccult SENSA, and PreGen-Plus, without compromising specificity (2,11).

Coincidental with these methodological advances, attention is now being paid to identifying new markers of colorectal neoplasia. One important advance has been the recognition that gene hypermethylation is a more common pathway in colorectal cancer than previously thought. Somewhat surprisingly, Chen et al. (12) demonstrated that hypermethylation of the vimentin gene, a gene not considered to be part of the usual molecular alterations associated with the adenoma–carcinoma sequence, was only rarely found in normal colonic tissue, but was quite common in colonic adenomas and carcinomas. This was subsequently validated in two independent sets of patients with colon cancer vs normal colonoscopy (9,13). What emerged from these studies was the observation that a single methylation marker 1) can have greater than 75% sensitivity for colorectal cancer, 2) can detect cancer of the proximal colon just as well as the distal colon, 3) can identify colorectal cancer regardless of stage, and 4) can make fecal DNA testing much easier to perform, thereby enabling distribution to local clinical laboratories and at a lower cost. It has been suggested that fecal DNA testing be considered as an interval test between colonoscopies in an effort to detect neoplasms, particularly those in the proximal colon, that are sometimes missed by colonoscopy (14).

Subsequent exploratory studies have identified other promising genetic targets for fecal DNA testing. Glöckner et al. (15) reported that tissue factor pathway inhibitor 2 (TFPI2), a potential tumor suppressor gene, was aberrantly methylated in the tissue of almost all colorectal adenomas (97%) and cancers (99%). When analyzed in the stool of patients with stage I–III colorectal cancer and controls with normal colonoscopy, results of training and validation sets revealed that TFPI2 methylation demonstrated sensitivities ranging from 76% to 89% for cancer, 21% for adenomas, and specificity of approximately 93%. A more recent study published in the Journal, reported that methylation of the promoter region of another tumor suppressor gene, N-myc downstream-regulated gene 4 (NDRG4), occurred in 70%–86% of colorectal cancer tissues compared with 4% in noncancerous colonic mucosa (16). In fecal DNA, methylated NDRG4 had a sensitivity of 61% and 53% for detecting colorectal cancer in training and validation sets of patients, respectively, with corresponding specificities of 93% and 100%.

In the current issue of the Journal, Nagasaka et al. (17) contributed important new findings to the field of noninvasive detection of gastrointestinal neoplasia. Unlike most previous studies that have focused on the colon, these investigators analyzed methylation patterns of two genes, RASSF2 and SFRP2, known to be methylated in colorectal and gastric cancers (18–20). They analyzed methylation in two promoter regions of each gene and reported their findings in a large set of gastric and colorectal tissues representing noncancerous, precancerous, and malignant states, as well as a sizable set of stool samples. For the fecal DNA methylation assay, they employed a high-sensitivity assay for bisulfite DNA, which had a greater sensitivity than the more standard Combined Bisulfite Restriction Analysis. Curiously, patients collected a relatively small amount of stool without stabilizing buffer, then chilled or froze the specimen, and delivered it to the laboratory within 24 hours. In tissues, methylation increased with progression from normal to adenoma to colon cancer, being lowest in normal colonic mucosa (4.5% for RASSF2, 17% for SFRP2), intermediate in colorectal adenomas (41% for RASSF2, 40% for SFRP2), and highest in colorectal cancer (68.3% for RASSF2, 86% for SFRP2). Likewise, in gastric tissues, RASSF2 methylation occurred in 53% of cancers compared with 6% of normal gastric mucosa, whereas SFRP2 was methylated in almost all (97%) gastric cancers, but also in 63% of normal gastric mucosa. Extensive methylation, as distinguished from partial methylation, was a hallmark of neoplastic colonic and gastric tissues, being found only rarely in noncancerous counterparts. In fecal DNA, one or more methylated marker was detected in 57% of patients with gastric cancer, 75% with colorectal cancer, 44% with advanced colorectal adenomas, but only 10.6% without neoplastic or inflammatory gastric or colorectal diseases. Other investigators have reported that SFRP2 methylation in fecal DNA has an approximate 77%–90% sensitivity and 77% specificity for colorectal cancer (21).
Thus, the findings of this study confirm that even one or two markers (in this case, using methylated genes) may be useful for fecal DNA testing and that with the appropriate markers, fecal DNA testing can detect neoplasia of both upper and lower gastrointestinal tract. Ahlquist (2) has also reported that fecal DNA can be used to detect not only cancers of the colorectum and stomach but also those of the oropharynx, esophagus, pancreas, and bile duct and/or gallbladder. Collectively, these cancers result in more than 130,000 deaths each year in the United States alone. Because some of these cancers (eg, pancreatic and biliary cancer) are hard to screen with current imaging techniques, further development of fecal DNA as a pan-detection assay for gastrointestinal tract cancers represents an intriguing and exciting new frontier.

References


Note

The author reports no conflict of interests.