The Role of Stool DNA Testing in Colorectal Cancer Screening

Screening all individuals 50 years and over for colorectal cancer could substantially decrease mortality associated with this disease. Despite the availability of a number of options, the majority of the population remains unscreened, and there is a need for simple, non-invasive alternatives with which patients will comply. Stool DNA testing is based on an improved understanding of the molecular biology underlying colorectal cancer and recent technological advances. Results from multiple studies with a panel of markers indicate a sensitivity of 60%–70% for invasive cancer, with a specificity of about 96%. The commercially available stool DNA test may be particularly useful in individuals who are unwilling to be screened endoscopically, and may offer an improved option for individuals seeking more reassurance than that afforded by fecal occult blood testing. The stool DNA panel should not be substituted for colonoscopy in individuals with symptoms or in whom colonoscopy is indicated because of underlying conditions.

Colorectal cancer is the second leading cause of cancer mortality, with almost 150,000 new cases and 56,000 deaths per year in the United States. The orderly progression from adenoma through early invasive cancer into the more advanced stages provides an excellent opportunity both to prevent colorectal cancer, through the detection and removal of adenomatous polyps, and to decrease colorectal cancer mortality, through its detection while still surgically curable.

Screening: Current Options and Issues

The understanding of the progression of colorectal neoplasia, along with rapid advances in endoscopy, has led to the recommendation that screening for colorectal cancer become an integral part of primary health maintenance, for average risk individuals, from age 50 and up. The American Cancer Society guidelines for screening are summarized in Table 1 and include several options (1). Until recently, the only available non-invasive option for screening has been testing stool for evidence of occult blood (FOBT). When cancer is present, bleeding is often intermittent; thus the recom-
Table 1
Guidelines for Colorectal Cancer Screening (1)

<table>
<thead>
<tr>
<th>Test</th>
<th>Frequency (starting at age 50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fecal occult blood test (2 samples from three consecutive stools)</td>
<td>Annual</td>
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<tr>
<td>Flexible sigmoidoscopy</td>
<td>Every five years</td>
</tr>
<tr>
<td>Fecal occult blood test plus flexible sigmoidoscopy</td>
<td>Annual fecal occult blood test and flexible sigmoidoscopy every five years</td>
</tr>
<tr>
<td>Double contrast barium enema</td>
<td>Every five years</td>
</tr>
<tr>
<td>Colonoscopy</td>
<td>Every ten years</td>
</tr>
</tbody>
</table>

mendation is that FOBT be repeated on two samples from each of three consecutive bowel movements. Because bleeding may be less common in early stage lesions, FOBT needs to be repeated on an annual basis. In the largest study demonstrating the efficacy of FOBT, annual testing was demonstrated to result in a decrease of colorectal cancer morality of approximately 30% (2).

Alternatives to FOBT utilize invasive procedures to structurally examine the colon. The two most widely used are flexible sigmoidoscopy and colonoscopy. Flexible sigmoidoscopy, recommended every five years, is a simple, office-based procedure, which can examine the distal colon, where the majority of colorectal cancers arise. To overcome the potential for missing more proximal lesions, flexible sigmoidoscopy can be combined with annual FOBT. Colonoscopy, recommended every ten years, has gained favor as an alternative to flexible sigmoidoscopy, because of its ability to thoroughly examine the entire colon. In addition colonoscopy provides the opportunity to biopsy and potentially resect lesions that are identified. Virtual colonoscopy can provide radiographic imaging of the colon; technical improvements in radiographic equipment and computer software may make this a feasible option as well. Double contrast barium enema also examines the entire colon, and remains in the screening guidelines, although its use has decreased substantially with advances in colonoscopy.

Despite the availability of multiple screening options, more than half the population has not been screened at all, and compliance is often incomplete among those who have been screened. There are a variety of reasons for this. With FOBT there is the need to modify both diet and medications for up to week, there is a need to manipulate stool, and there is a perception by many physicians that the FOBT screening process is so ineffective as to not be worth the effort. For flexible sigmoidoscopy, there is the need for a limited bowel preparation, and there is the discomfort and potential embarrassment associated with the procedure. For colonoscopy, there is a much more intensive bowel preparation, anesthesia, time lost from work, and the risks associated with potential perforation. In addition, resource limitations, in terms of both number of gastroenterologists and geographic accessibility, have limited access to colonoscopy. Virtual colonoscopy, although sounding simpler, requires the same intensive bowel prep as colonoscopy. Accordingly, another simple, non-invasive option, with improved performance characteristics compared with FOBT, could be valuable in inducing more people to be screened.

STOOL DNA TESTING: RATIONALE AND RESULTS

An increase in our knowledge of the molecular changes associated with colorectal carcinogenesis has laid the foundation for detecting colorectal neoplasia using DNA found in stool. Sporadic colorectal cancer has been divided into two main groups—those with chromosomal instability and those with an impaired mismatch repair mechanism. As originally described (3), colorectal cancers with chromosomal instability are characterized by the progressive accumulation of mutations and changes in several key genes, including
Table 2
Reported Sensitivity and Specificity of Stool DNA

<table>
<thead>
<tr>
<th>Study</th>
<th>Panel Components</th>
<th>Sensitivity for CRC Positive/Total (%)</th>
<th>Specificity (Controls) Negative/Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Studies Using Exact Sciences Technology</strong></td>
<td></td>
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</tr>
<tr>
<td>Ahlquist et al (4)</td>
<td>APC, k-ras, p53, BAT-26, long DNA</td>
<td>20/22 (91%)</td>
<td>26/28 (93%)</td>
</tr>
<tr>
<td>Brand et al (5)</td>
<td>APC, k-ras, p53, BAT-26, long DNA</td>
<td>11/16 (60%)</td>
<td>—</td>
</tr>
<tr>
<td>Tagore et al (6)</td>
<td>APC, k-ras, p53, BAT-26, long DNA</td>
<td>33/52 (63%)</td>
<td>204/212 (96%)</td>
</tr>
<tr>
<td>Syngal et al (7)</td>
<td>APC, k-ras, p53, BAT-26, long DNA</td>
<td>35/56 (62%)</td>
<td>—</td>
</tr>
<tr>
<td>Overall</td>
<td>APC, k-ras, p53, BAT-26, long DNA</td>
<td>99/146 (67.8%)</td>
<td>230/240 (95.8%)</td>
</tr>
<tr>
<td><strong>Other Studies</strong></td>
<td></td>
<td>(95% C.I. 59.6–75.3)</td>
<td>(95% C.I. 92.5–98.0)</td>
</tr>
<tr>
<td>Dong et al. (8)</td>
<td>p53, k-ras, BAT-26</td>
<td>36/51 (71%)</td>
<td>—</td>
</tr>
<tr>
<td>Traverso et al (9)</td>
<td>APC</td>
<td>26/46 (57%)</td>
<td>28/28 (100%)</td>
</tr>
<tr>
<td>Traverso et al (10)</td>
<td>BAT-26</td>
<td>17/46 (37%)</td>
<td>69/69 (100%)</td>
</tr>
<tr>
<td>Calisti et al (11)</td>
<td>p53, k-ras, APC, 5 MSI markers, long DNA</td>
<td>33/53 (62%)</td>
<td>37/38 (97%)</td>
</tr>
</tbody>
</table>

the tumor suppressors genes, *APC* and *k-ras*, and the *p53* oncogene. These constitute about 85% of sporadic colorectal cancers. Defects in mismatch repair characterize most of the remaining cancers, and are found most commonly in proximal (right-sided) lesions. Both of these pathways are associated with characteristic DNA alterations, which may be detected in stool.

The fecal stream comes in contact with the entire colonic mucosal surface, and colonocytes, and their DNA, are constantly being shed into the lumen of the bowel. Thus the DNA in stool should provide a good representation of any molecular changes throughout the colon. This sampling of the colonic mucosa, combined with the understanding of the pathogenesis of colorectal cancer at the molecular level and improved biochemical techniques for DNA isolation and analysis, have made possible the detection of cancer-related changes in stool to detect the presence of neoplasia.

Each cancer may accumulate a distinct series of molecular alterations in the key genes involved in cancer development. The heterogeneity in these molecular changes among different cancers has necessitated the utilization of panels of markers, encompassing the changes found most commonly. The commercially available panel, PreGen-Plus™, is based on technology developed by EXACT Sciences. This panel includes 21 specific point mutations, 10 in *APC*, 3 in *k-ras*, and 8 in *p53*, plus BAT-26, a marker of microsatellite instability, and long DNA, a marker of disordered apoptosis. A positive result in any component of the panel is sufficient to categorize the overall result as positive.

The analysis of stool for DNA changes involves the collection of a whole bowel movement; using the whole bowel movement minimizes chances for sampling error and maximizes the likelihood that abnormal DNA from lesions throughout the colon can be detected. After shipment to the clinical laboratory, the sample is stored at −80°C until processing. The stool is homogenized with EDTA buffer, to prevent enzymatic degradation of DNA prior to analysis. Next, the crude DNA is separated from fecal matter and cellular debris, and the relevant areas of the human genome are isolated by hybridization with sequence-specific probes. These DNA fragments are amplified using PCR, and the DNA is analyzed for the presence of cancer-related changes.

Four studies have been reported using the commercially available technology (Table 2). The initial
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study was carried out on a series of archived stool specimens from patients at the Mayo Clinic (4). In this series, 20 of 22 (91%) patients with colorectal cancer were successfully detected by stool DNA analysis. A concurrent series of patients without colonscopic abnormalities revealed that mutations were also identified in 2 of 28 cases, for a specificity of 93%.

Another key question around stool DNA testing was whether additional sensitivity could be derived from analyzing multiple samples, as is the case with FOBT. This question was addressed in a series of patients diagnosed with colorectal cancer at colonoscopy, who provided three consecutive bowel movements for stool DNA analysis (5). Although minor differences were seen in the specific mutations identified, there was no advantage, in terms of overall detection, in analyzing more than one sample per patient. This study thus confirmed that only one stool was required for the stool DNA assay.

The encouraging results from these two preliminary studies clearly suggested that studies with a much larger numbers of patients were warranted. Two such prospective studies have been conducted. In the first, stools were collected prior to surgical resection from a large series of patients whose cancers had been detected at flexible sigmoidoscopy (6). Two hundred controls, 100 with negative colonoscopy and 100 with minor polyps only, were analyzed concomitantly. This study reported a sensitivity of 63% for invasive cancer, and a specificity of 96%. The other major study reported the results of stools analyzed from 56 patients whose cancers had been diagnosed at colonoscopy (7). The results of this study are similar, with a sensitivity of 62% for invasive cancer. In this study, stools were also obtained following surgical resection of the primary lesion. By six months after surgery, evidence of DNA abnormalities in stool generally was not detectable. These results suggest that in addition to screening, stool DNA analysis may ultimately have a role in monitoring for colorectal neoplasia after cancer resection.

The results in these studies in patients with advanced adenomas have been quite variable, ranging from as high as 73% (4) to as low as 27% (7). The criteria for including patients with advanced adenomas varied widely in these studies, and the size of adenomatous polyps may have played an important role.

Overall, results with this stool DNA panel have now been reported for 146 patients with invasive colorectal cancer. The combined sensitivity has been 67.8%, with a specificity of 95.8%. Factors limiting the sensitivity for the detection of cancer include the possibility that some cancers do not contain markers that are a component of the panel, limited DNA shedding into the stool and limited technological sensitivity. However, stool DNA testing has the potential to improve further in the future. Improved ability to isolate more DNA from stool and improved analytic techniques for specific markers may further increase sensitivity. The addition of new markers to the panel, either from the genes currently represented or from other genes, may also result in a substantial improvement in the panel’s sensitivity. The specificity of the panel is approximately 96%; the biological meaning of a positive result in the face of negative colonoscopy has not yet been clarified.

At least four other studies have been carried out, using related but alternative technologies (8–11). The results from these four studies are also included in Table 2. The sensitivity in these studies ranged from 37% using a single marker to 71% using multiple markers, but differences in the panels of markers make direct comparisons between studies impossible.

All of these studies were conducted in patients with known colorectal cancer. Because these were not asymptomatic patients, this may not fully represent the results in an asymptomatic, average-risk population. Two large studies are currently underway, comparing the stool DNA panel with FOBT in this target population. The results of these studies may clarify the role of stool DNA testing compared with other modalities for colorectal cancer screening; however, due to rapid improvements in technologies, the results of these trials may not fully reflect results currently obtainable.

CLINICAL IMPLICATIONS FOR STOOL DNA TESTING

With a stool DNA panel now commercially available, the key questions that need to be addressed include the appropriate population in which this technology can be used, how results should be interpreted and how frequently the test should be repeated.
The available data clearly indicate that stool DNA testing is not as sensitive as colonoscopy, but it does appear to be more sensitive than FOBT. Stool DNA testing offers the patient simplicity in its application, and is totally non-invasive, characteristics that may help overcome compliance barriers. This combination of performance and ease of use defines a number of situations where the test may help address our current shortfall for those individuals who are not being screened at all or are being screened inadequately.

As an alternative to colonoscopy, screening with stool DNA may be useful in at least two situations. With the rapid increase in utilization of colonoscopy, and with mandated coverage of colonoscopy by Medicare, adequate resources to perform colonoscopy on the entire asymptomatic, average-risk population may not be feasible, particularly in geographically remote or underserved regions. Under these circumstances, stool DNA testing may provide an important alternative to bring more patients into the system, and to triage the high risk individuals to limited colonoscopy resources. Second, colonoscopy and flexible sigmoidoscopy are invasive procedures; although their value is clear, many individuals are unwilling to be screened, whether because of embarrassment, discomfort, or fear of significant complications. The use of the non-invasive stool DNA panel may provide an option that these individuals are willing to consider; if the results are positive, the physician may then be able to encourage the patient to accept colonoscopy. For patients willing to undergo screening colonoscopy and for whom adequate colonoscopy resources are available, the stool DNA panel should not be substituted for colonoscopy for routine screening.

Stool DNA testing may represent an important alternative for patients who insist on a non-invasive screen, and for whom the only current option is FOBT. Despite its proven value in decreasing mortality, the perception that FOBT detects only late stage disease, the need for dietary and medication restrictions, and the need for annual testing all limit the clinical utility of FOBT. Stool DNA may offer these individuals a test with which it is easier to comply, requiring less frequent repetition. The available results suggest that stool DNA testing offers a higher sensitivity than FOBT, and this could provide extra incentive for the patient to comply with a screening program.

There are several situations in which the stool DNA panel does not represent the preferred option. In the symptomatic patient, there is a clear indication for a structural examination of the colon. Stool DNA testing has demonstrated a sensitivity of approximately 65%: as such, a negative result does not provide the same level of reassurance as colonoscopy that colorectal neoplasia is not present. For example, patients with iron deficiency anemia, change in bowel habits, or similar indications cannot rely on a negative stool DNA panel result to avoid a structural examination of the colon. Second, there are high-risk situations where colonoscopy is clearly indicated. For example, in patients with hereditary non-polypsis colorectal cancer, the high incidence for the development of colorectal cancer, combined with the possibility that these cancers may progress more rapidly, all indicate that stool DNA testing should not be substituted for colonoscopy. Patients with long-standing inflammatory bowel disease are at high risk for the development of colorectal neoplasia and the indication for colonoscopy surveillance is clear. Furthermore, the molecular changes found in colorectal neoplasia associated with inflammatory bowel disease may differ from sporadic cancer, raising questions about the utility of the current stool DNA panel in this population. Ongoing clinical studies in patients with inflammatory bowel disease are evaluating this question.

If the stool DNA panel is positive, there is a definitive need to conduct a thorough colonoscopy examination of the entire colon. Specificity for the panel is estimated to be 96%, and the presence of an abnormality does not mean that colorectal neoplasia is necessarily present. Given the low prevalence of colorectal cancer in the asymptomatic population, many patients with a positive test result will not have colorectal cancer. One of the key issues that has not been resolved is how to respond if colonoscopy does not reveal significant pathology. Colonoscopy is known not to be 100% sensitive for colorectal neoplasia, and the possibility that a lesion may have been missed could explain such a result and must be considered. Alternatively, pathology in the proximal gastrointestinal tract could be responsible for this. A careful consideration of the overall status of the patient should be considered in determining whether any further investi-
gations are indicated, as well as the frequency for reevaluation of the colon.

No studies have specifically addressed the question of the appropriate interval to repeat testing if the stool DNA panel is initially negative. Given the ability of the stool DNA panel to detect early stage disease, an interval of three to five years may be appropriate; consideration should also be given to initially repeating the test after one year, and then to repeating the test every three to five years. Any symptomatology suggesting colorectal pathology should be fully evaluated even in the presence of a negative stool DNA panel.

SUMMARY

There is general acceptance of the value and need for screening the asymptomatic population for colorectal cancer, in an effort to decrease the morbidity and mortality associated with this disease. Currently, compliance with this goal is unsatisfactory. Although not as sensitive as colonoscopy, testing with a stool DNA offers an important option for patients seeking a non-invasive screening method, and as such may enable physicians to increase the total number of patients being screened.

References