Stool DNA Testing for Colorectal Cancer Shows Promise

Ahlquist DA, Sargent DJ, Loprinzi CL, et al. Stool DNA and occult blood testing for screen detection of colorectal neoplasia. Ann Intern Med 2008;149:441–50.

Study Overview

Objective. To compare fecal occult blood testing and stool DNA testing for detection of screening-relevant neoplasia.

Design. Prospective, blinded, cross-sectional, multicenter study.

Setting and participants. National Institutes of Health–supported trial with industry involvement that included 4482 adults in communities surrounding 22 participating regional and academic health systems in the United States. Participants were asymptomatic, aged 50 to 80 years, and deemed to be at average risk for colorectal cancer. Exclusion criteria included structural colorectal evaluation within 10 years, high risk for or previous colorectal cancer, fecal occult blood testing within the past year, previous colorectal resection, aerodigestive cancer within 5 years, overt rectal bleeding, inability to stop use of nonsteroidal anti-inflammatory drugs or anticoagulants, recent chemotherapy, coagulopathy, inflammatory bowel disease, > 2 first-degree relatives with colorectal cancer, and contraindication to colonoscopy. All participants underwent colonoscopy after stool testing. Participants were given 2 types of fecal occult blood stool cards to use (Hemoccult and HemoccultSensa, Beckman Coulter, Fullerton, CA) and were also asked to collect 3 whole stools that were express-shipped to the main study center on ice. Stool cards were analyzed using rigorous standardized protocols, and whole stools were sent to an industry laboratory for stool DNA analysis. Two stool DNA tests were performed. The first test (SDT-1) included a panel of 21 tumor-specific point mutations, the microsatellite instability marker BAT-26, and long DNA (marker for delayed apoptosis). Because the manufacturer of SDT-1 altered the assay midway through the study, an unplanned interim analysis was conducted in the first 2497 patients who received the test. On the basis of these results, the investigators switched to another stool DNA test (SDT-2), which consisted of 3 tumor-specific markers (*APC*, *K-ras*, and vimentin methylation) broadly associated with colorectal cancers and adenomas.

Main outcome measures. Sensitivity and specificity of 2 fecal occult blood tests and 2 stool DNA tests for detecting screening-relevant neoplasia, using the findings of screening colonoscopy as a reference standard. Screening-relevant neoplasia was defined as curable-stage cancer, high-grade dysplasia, or adenomas ≥ 1 cm. SDT-2 was performed in a subset of patients, including all patients with colorectal cancer, high-grade dysplasia, and adenomas > 2 cm; 50 randomly selected patients with adenomas of 1 to 2 cm; and 75 patients with normal colonoscopy results. Subgroup analysis was performed for both SDT-1 and SDT-2 on neoplasm site (proximal vs. distal), adenoma size, hyperplastic polyp presence, and age.

Main results. A total of 3764 participants were evaluated with fecal occult blood tests and colonoscopy. 545 participants were excluded because of cancellations or ineligibility, 171 due to incomplete colonoscopies, and 2 due to distant metastases. Screening-relevant neoplasms were found in 290 (7.7%) patients; 39 were nonmetastatic cancer or high-grade dysplasia and 251 were adenomas ≥ 1 cm. Only 4 patients had major colonoscopy-related complications, and no procedure-related deaths were reported. The detection sensitivity for the 290 screening-relevant neoplasms using Hemoccult was 10% (95% confidence interval [CI], 7%–13%) as compared with 18% for HemoccultSensa (95% CI, 13%–22%; $P < 0.001$). The corresponding specificity for Hemoccult was 98% (95% CI, 96%–97%) compared with 97% for HemoccultSensa (95% CI, 96%–97%). Based on the interim analysis of the first 2497 eligible patients who received the SDT-1 test, the sensitivity of SDT-1 for screening-relevant neoplasia was 20% (95% CI, 14%–26%), higher than Hemoccult (11% [95% CI, 6%–16%]; $P = 0.020$) but not different than HemoccultSensa (21% [95% CI, 15%–27%]; $P = 0.80$). The specificity of SDT-1 was 96% (95% CI, 95%–97%), comparable with that of Hemoccult (98% [95% CI, 98%–99%]) and HemoccultSensa (97% [95% CI, 96%–97%]). SDT-2 had a weighted sensitivity of 40% (95% CI, 32%–49%) for screening-related neoplasms in a subset of 217 patients. For adenomas ≥ 1 cm, SDT-2 had a true positivity rate of 46% (95% CI, 35%–54%) compared with 10% (95% CI, 4%–15%; $P < 0.001$) for Hemoccult and 17% (95% CI, 9%–24%; $P < 0.001$) for HemoccultSensa. Neoplasm site did not affect true positivity rates of SDT-2 but did for Hemoccult and HemoccultSensa. Both Hemoccult and HemoccultSensa had detection rates that were significantly lower for lesions proximal to the splenic

flexure as compared with distal lesions (P = 0.060 and P = 0.010, respectively). Restriction of red meat in the diet and nonsteroidal anti-inflammatory drug use for 3 days prior to the stool specimen collection affected the detection results of HemoccultSensa but not those of Hemoccult or the stool DNA tests. Age was a factor in false-positive rates for SDT-2 (overall rate, 16%). The false-positive rate increased from 6% in participants aged < 65 years to 26% in participants aged > 65 years (P = 0.020).

Conclusion. A first-generation stool DNA test was no better than the fecal occult blood test HemoccultSensa for detecting screening-relevant neoplasia. A second-generation stool DNA test detected significantly more neoplasms than either fecal occult blood test, especially for adenomas. However, many positive results were seen in colonoscopically normal patients, especially among older participants.

Commentary

Colorectal cancer is the second leading cause of cancer death in the United States [1]. Although mortality reductions have been achieved through the use of colonoscopy to identify adenomas and low-grade carcinomas, screening rates remain relatively low and unequally distributed in the population. Beyond fecal occult blood tests, an important need exists for noninvasive tests that have proven efficacy for the detection of adenomas, dysplasia, and curable carcinoma [1].

Basic science models of the progression from adenoma to invasive colorectal carcinoma have now been translated into testable stool DNA assays that search for specific genetic mutations in cells that exfoliate from the colonic lumen into stool. Imperiale et al [2] conducted the first major trial involving stool DNA tests and found that the stool DNA test had a sensitivity of 52% for invasive cancer and 15% for advanced adenomas among more than 5000 average-risk patients, using colonoscopy as a reference standard [2]. The assay used in the study by Imperiale and colleagues was similar to the SDT-1 assay evaluated in this study by Ahlquist et al.

The purpose of the study by Ahlquist et al was to compare stool DNA testing with 2 types of fecal occult blood testing for screening-relevant neoplasia. Because the company that made the stool DNA assay changed their assay mid-study, an unplanned interim analysis was conducted on the first 2497 eligible participants, and the effectiveness of the SDT-2 test was then analyzed in a subset of the overall study group that included all neoplasms, dysplastic polyps, and adenomas. Although the SDT-1 test did not improve detection of screening-relevant neoplasms compared with fecal occult blood testing, the SDT-2 detected significantly more neoplasms and large adenomas, which are an important part of the early causal pathway. However, the SDT-2 had a high false-positive rate, especially among older patients. Many of these older patients had evidence of age-related changes to luminal cells that can give false-positive results.

This study was a multicenter, triple-blinded trial with a low participant exclusion rate and a number of other strengths. All of the testing was done in 1 standardized laboratory setting using stringent methods that likely led to higher sensitivity rates for the fecal occult blood tests. Nonetheless, this study adds further to the literature suggesting the limited sensitivity of fecal occult blood testing. Pathologic evaluation was performed in a central location, and neoplastic tissue was evaluated for confirmation of DNA mutations tested for in the assays. The outcome measure was tailored to finding lesions that could lead to cancer prevention and early cure. Its external validity is bolstered by the inclusion of a representative sample of 22 health care systems across the United States.

Some limitations deserve mention. Because the stool DNA assay was changed halfway through the trial, the apparently more efficacious SDT-2 assay was not tested on all participants. It was tested on only 217 of the 3764 patients in the trial, including all cancer-positive samples, most patients with adenomas, and a small subset of normal participants. Therefore, the sensitivity calculation is not for the entire sample but rather is weighted according to the number of participants tested in each category. Furthermore, this limited subsample did not allow the authors to calculate an overall specificity estimate for the SDT-2 test, an important limitation given the 16% false-positive rate for the test (even higher among older patients).

Applications for Clinical Practice

A second-generation stool DNA test focused on 3 broad categories of mutations found in the adenoma-carcinoma pathway had a significantly higher sensitivity than fecal occult blood testing for detecting screening-relevant neoplasms. However, this stool DNA test still had a sensitivity of less than 50% and a notable false-positive rate. Although stool DNA testing is not yet ready for widespread use, this study provides encouraging evidence that further test refinement can lead to a powerful noninvasive molecular screening option for detecting early colorectal cancer in the next decade.

—Review by Asaf Bitton, MD

References

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2. Imperiale TF, Ransohoff DF, Itzkowitz SH, et al; Colorectal Cancer Study Group. Fecal DNA versus fecal occult blood for colorectal-cancer screening in an average-risk population. N Engl J Med 2004;351:2704–14.