ORIGINAL ARTICLE

Fecal DNA versus Fecal Occult Blood for Colorectal-Cancer Screening in an Average-Risk Population

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ABSTRACT

BACKGROUND

Although fecal occult-blood testing is the only available noninvasive screening method that reduces the risk of death from colorectal cancer, it has limited sensitivity. We compared an approach that identifies abnormal DNA in stool samples with the Hemoccult II fecal occult-blood test in average-risk, asymptomatic persons 50 years of age or older.

METHODS

Eligible subjects submitted one stool specimen for DNA analysis, underwent standard Hemoccult II testing, and then underwent colonoscopy. Of 5486 subjects enrolled, 4404 completed all aspects of the study. A subgroup of 2507 subjects was analyzed, including all those with a diagnosis of invasive adenocarcinoma or advanced adenoma plus randomly chosen subjects with no polyps or minor polyps. The fecal DNA panel consisted of 21 mutations.

RESULTS

The fecal DNA panel detected 16 of 31 invasive cancers, whereas Hemoccult II identified 4 of 31 (51.6 percent vs. 12.9 percent, P=0.003). The DNA panel detected 29 of 71 invasive cancers plus adenomas with high-grade dysplasia, whereas Hemoccult II identified 10 of 71 (40.8 percent vs. 14.1 percent, P<0.001). Among 418 subjects with advanced neoplasia (defined as a tubular adenoma at least 1 cm in diameter, a polyp with a villous histologic appearance, a polyp with high-grade dysplasia, or cancer), the DNA panel was positive in 76 (18.2 percent), whereas Hemoccult II was positive in 45 (10.8 percent). Specificity in subjects with negative findings on colonoscopy was 94.4 percent for the fecal DNA panel and 95.2 percent for Hemoccult II.

CONCLUSIONS

Although the majority of neoplastic lesions identified by colonoscopy were not detected by either noninvasive test, the multitarget analysis of fecal DNA detected a greater proportion of important colorectal neoplasia than did Hemoccult II without compromising specificity.

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Colorectal cancer is the second leading cause of death from cancer among adults.[1,2] Despite recommendations endorsing screening, less than 40 percent of people 50 years of age or older undergo screening for colorectal cancer.[3] Guaiac-based chemical detection of fecal occult blood is the only noninvasive screening method with proven effectiveness, reducing both the incidence[4] and the risk of death from colorectal cancer[5-7] when used programmatically. However, the sensitivity of fecal occult-blood testing for colorectal cancer and especially for colorectal adenomas is low because neoplasms may not bleed and thus cannot be detected in this way.[2] The availability of a simple, noninvasive test that detects tumor-specific products with reasonable sensitivity and specificity might overcome barriers to screening among patients who are not willing to undergo more sensitive but more invasive tests, such as colonoscopy.

The molecular genetics of colorectal cancer provides the basis for the analysis of fecal DNA.[8,9] Eighty-five percent of colorectal cancers result from chromosomal instability, with mutations progressively accumulating in the adenomatous polyposis coli (*APC*) gene, the *p53* tumor-suppressor gene, and the K-*ras* oncogene.[10] The other 15 percent arise from a loss of genes involved in DNA-mismatch repair, manifested by microsatellite instability.[11] Colorectal cancer may also be detectable through the use of DNA markers associated with disordered apoptosis.[12]

Previous studies using fecal-based DNA testing have reported a sensitivity of 62 to 91 percent for cancer and 27 to 82 percent for advanced adenomas, with a specificity of 93 to 96 percent in persons with normal findings on colonoscopy.[13-17] However, those studies assessed persons with advanced, symptomatic lesions. We made a head-to-head comparison of a fecal-based, multitarget DNA panel with Hemoccult II in asymptomatic adults, 50 years of age or older, who were at average risk for colorectal cancer. The primary objective was to compare detection rates for colorectal cancer and for colorectal cancer plus adenomas with high-grade dysplasia.

METHODS

STUDY DESIGN AND RATIONALE

The rationale for the study was based on screening guidelines indicating that newer screening tests need not demonstrate a reduction in cost-specific mortality but should be at least as sensitive, specific, and safe, among other features, as current screening tests.[18] Hemoccult II (Beckman Coulter, formerly SmithKline Diagnostics) was chosen for the comparison with the DNA panel because it is the only fecal occult-blood test proven to reduce the incidence and risk of death from colorectal cancer and is the most widely used guaiac-based test.[2] The study was designed by the authors, with advice from national experts on colorectal cancer, cancer screening and prevention, and study design.*

The study was conducted at 81 sites, including private-practice and university-based settings. Subjects were enrolled between August 2001 and March 2003. All subjects first provided a fecal sample for DNA testing and then completed three Hemoccult II cards before undergoing screening colonoscopy. All tests were conducted in a blinded fashion. Stool samples were analyzed for DNA abnormalities without knowledge of Hemoccult II or colonoscopy results; colonoscopy was performed without knowledge of the results of fecal DNA testing. Since Hemoccult II testing was conducted at the study sites, the results were potentially available to the colonoscopists. A clinical research organization (Parexel) received the results of Hemoccult II tests and colonoscopy directly from the clinical sites and received the results of fecal DNA analyses from the clinical laboratory (Exact Sciences).

Parexel conducted the data analyses according to a prespecified plan and provided the results to the investigators after completion of the study. Only Parexel had access to the data until the blinding was removed, at which time the information was shared with the authors. The authors wrote the article; Exact Sciences guaranteed the first author the right to publish the results of the study regardless of the outcome. Parexel, CareStat (the company that provided biostatistical support), and the authors each independently vouch for the veracity of the data and data analysis.

STUDY POPULATION

The target population consisted of asymptomatic persons at average risk for colorectal cancer. The appropriate institutional review board at each site approved the study. Written informed consent was obtained from all participants. Study sites recruited persons from local practices and undertook ac-

*See NAPS document no. PC0001 for 112 pages of supplementary material regarding the study protocol. To order, contact NAPS, c/o Burrows Systems, P.O. Box 3976, New Hyde Park, NY 11040.

tivities to enhance the public's awareness of colorectal cancer and the availability of screening. The costs of colonoscopy were not covered by the study; Hemoccult II and fecal DNA testing was provided without charge. Participants were compensated in a manner approved by each site's institutional review board.

All participants were at least 50 years old. Enrollment was stratified according to age, with a minimum of three quarters of subjects 65 years of age or older. Exclusion criteria included gastrointestinal bleeding within the preceding month, a change in bowel habits or a recent onset of abdominal pain, previous colorectal cancer or polyps, prior resection of any part of the colon, iron-deficiency anemia, or other coexisting visceral cancer. Persons who had undergone colonoscopy, sigmoidoscopy, or double-contrast barium enema within the preceding 10 years or who had had a positive fecal occult-blood test within the preceding 6 months were excluded, as were those with inflammatory bowel disease, familial adenomatous polyposis or hereditary nonpolyposis colon cancer, more than one first-degree relative with colorectal cancer, or any first-degree relative with colorectal cancer before the age of 50 years. Persons unwilling or unable to undergo colonoscopy were also excluded.

PROCEDURES

Subjects were given detailed instructions for stool collection; no dietary or medication modifications were required. Specimens were shipped directly to the clinical laboratory in a bar-coded container, chilled to between 0 and 4°C. Specimens were required to arrive within 72 hours after collection; a minimal 30-g sample was required. If a sample failed to meet these requirements, another sample was sought before colonoscopy was performed. Samples were stored at −80°C until analysis.

Subjects were given three Hemoccult II cards and instructions regarding dietary and medication modifications to comply with current recommendations.[1,19,20] Cards were returned to physicians' offices for non-rehydrated analysis by the physician or a designee, consistent with the manufacturer's instructions and current guidelines. If all three cards (six panels) were not completed, additional cards were provided.

Colonoscopy was performed with the preparation and sedation customarily used at each site. The colonoscopist documented the extent of the colon that was visualized and the quality of the bowel preparation. Adequate colonoscopy required visualization of the cecum and a minimum of 90 percent of the mucosa. The size and location of any lesions were recorded. Biopsy and surgical resection specimens were examined histopathologically at each site; no centralized pathological review was performed.

Subjects could be evaluated only if the specimen for fecal DNA analysis was adequate, all six Hemoccult II panels had been completed, and colonoscopy was adequate. Subjects were classified according to the most advanced lesion identified. Advanced adenoma was defined as any lesion containing high-grade dysplasia, a polyp containing clinically significant villous architecture, or a tubular adenoma that was at least 1 cm in diameter. Minor polyps included tubular adenomas less than 1 cm in diameter and hyperplastic polyps.

Parexel provided the clinical laboratory with a coded list of stool specimens to be analyzed for DNA abnormalities on the basis of colonoscopy and pathological reports. The prespecified analytic plan was designed to maximize the study's efficiency without compromising measures of sensitivity, specificity, and adherence to the protocol. DNA analysis was performed on stool samples from all subjects with an invasive cancer or advanced adenoma who could be evaluated and on randomly selected subgroups of 600 subjects with minor polyps and 1400 subjects with no polyps; these groups comprised the analyzed subgroup.

FECAL DNA ANALYSIS

All samples analyzed for fecal DNA were processed in a single laboratory. The fecal DNA panel consisted of 21 mutations: 3 in the K-*ras* gene, 10 in the *APC* gene, and 8 in the *p53* gene; the microsatellite-instability marker BAT-26; and a marker of long DNA thought to reflect disordered apoptosis of cancer cells sloughed into the colonic lumen.[13,14,16] The plan for DNA analyses has been described previously[13,16] and is shown in Figure 1. Laboratory handling of all samples was fully automated, and quantitative analysis of the area under the curve, a measure of signal intensity of the labeled nucleotides, was compared with that for control DNA fragments with a known mutation. Each marker was assessed independently; a positive result for any component of the panel constituted a positive fecal DNA test. Laboratory technicians were unaware of both the clinical data associated with each sample and the sampling protocol.

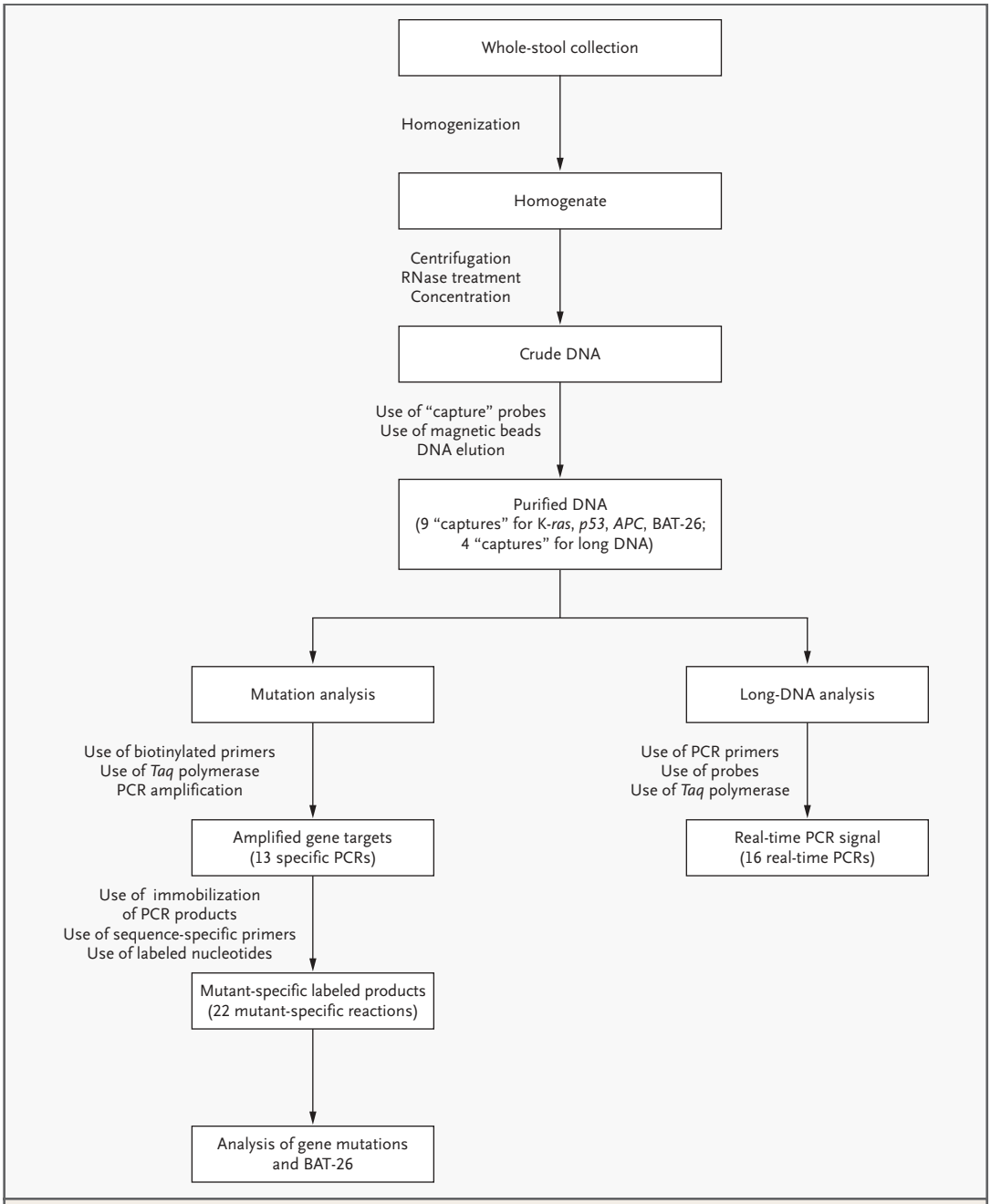

Figure 1. Approach to Extraction and Analysis of Fecal DNA.

Stool samples were thawed at room temperature and homogenized. Aliquots (each equivalent to 4 g of stool) were centrifuged to remove particulate matter. Crude DNA was precipitated and resuspended. Sequence-specific DNA fragments were then purified from the total nucleic acid preparations by performing oligonucleotide-based hybrid "captures." To analyze samples directly for the presence of long DNA, the purified DNA was amplified in a real-time polymerase chain reaction (PCR), targeting fragments approximately 1.3, 1.8, and 2.4 kb from the capture site. In parallel, for the mutation portions of the assay, PCR amplification reactions were conducted with the use of biotinylated primers for specific gene targets. Each of the PCR products was then bound to magnetic-bead supports, and a mutation-specific, solid-phase minisequencing protocol was used to identify point mutations. BAT-26 deletions (of 4 to 15 bp) were identified according to the size of the reaction products. All minisequencing reaction products were analyzed by capillary electrophoresis with laser-induced fluorescence detection. Each marker was assessed independently; a positive result for any component of the panel constituted a positive fecal DNA test.[17]

STATISTICAL ANALYSIS

The sample size was predetermined on the basis of the assumption that the fecal DNA panel and Hemoccult II had a sensitivity for the detection of colorectal cancer (i.e., tumor–node–metastasis [TNM] stage I through IV) of at least 65 percent and no more than 25 percent, respectively. Given this assumption, the enrollment of 32 subjects with colorectal cancer would provide the study with a statistical power of 90 percent to detect a significant difference at a two-sided alpha level of 0.05 with the use of McNemar's test.[21] A post hoc McNemar's test was performed to compare the ability of the fecal DNA panel and Hemoccult II to identify subjects with fully specified advanced neoplasia (advanced adenoma or cancer). No interim analyses were performed, and missing data were not imputed.

RESULTS

STUDY POPULATION

A total of 5486 subjects were enrolled, of whom 4404 could be fully evaluated; 1082 (19.7 percent) could not be evaluated (Fig. 2). The demographic and clinical characteristics of the population that could be evaluated and the subgroup that was analyzed were similar (Table 1).

Colonoscopic findings are shown in Table 2. Invasive adenocarcinoma was identified in 31 subjects (a prevalence of 0.7 percent). The higher prevalence of pathological findings in the analyzed subgroup reflects the prespecified sampling strategy for stool processing in subjects with either no polyps or minor polyps. The only clinically significant complications were four colonoscopic perforations among 4404 subjects (0.09 percent).

FECAL DNA PANEL VERSUS HEMOCCULT II

The fecal DNA panel detected 16 of 31 invasive cancers (TNM stage I, II, or III), for a sensitivity of 51.6 percent; Hemoccult II detected 4 of 31 cancers, for a sensitivity of 12.9 percent (Table 2). The fecal DNA panel detected 13 cancers that were missed by Hemoccult II, whereas Hemoccult II detected 1 cancer that was missed by the panel. This difference in discordant test results was significant ($P=0.003$). In a post hoc analysis among subjects with node-negative disease (TNM stage I or II), the sensitivity of the fecal DNA panel was statistically superior to that of Hemoccult II (56.5 percent vs. 13.0 percent, $P=0.006$). Among persons with TNM stage 0, I, II, or III (TNM 0 is carcinoma in situ), the fecal DNA panel had a sensitivity of 40.8 percent, whereas Hemoccult II had a sensitivity of 14.1 percent. The fecal DNA panel detected 22 lesions that were missed by Hemoccult II, whereas Hemoccult II detected 3 lesions missed by the panel. This difference in discordant test results was significant ($P<0.001$).

Among the 40 subjects who had adenomas with high-grade dysplasia, the fecal DNA panel detected 13 of the adenomas (32.5 percent), whereas Hemoccult II detected 6 (15.0 percent). For the detection of other advanced adenomas (villous polyps and tubular adenomas 1 cm in diameter or larger) and for minor polyps, the sensitivities of both tests were consistently less than 20 percent (Table 2). Among 418 subjects with advanced neoplasia (defined as a tubular adenoma 1 cm in diameter or larger, a polyp with a villous histologic appearance, a polyp with high-grade dysplasia, or cancer), the DNA panel was positive in 76 subjects, whereas Hemoccult II was positive in 45 subjects (18.2 percent vs. 10.8 percent, $P=0.001$). There was no significant difference in sensitivity according to the size of the cancer or advanced adenoma for either test (data not shown).

Among 1423 subjects with negative findings on colonoscopy, 79 had a positive fecal DNA panel and 68 had a positive Hemoccult II test, for specificities of 94.4 percent and 95.2 percent, respectively (Table 2). Among subjects with minor polyps, specificities for the fecal DNA panel and Hemoccult II were 92.4 percent and 95.2 percent, respectively.

Table 3 shows the frequencies of abnormal components of the fecal DNA panel as they relate to the various histologic findings. All components of the panel contributed to the overall sensitivity of the test. Although no formal statistical analysis was performed because of the small size of the subgroups, the sensitivities of the point mutations in *APC*, *p53*, and K-*ras* were generally greater than those for the BAT-26 and long-DNA markers for clinically important lesions.

DISCUSSION

We compared a panel of fecal DNA markers and Hemoccult II as screening tests for colorectal cancer in an average-risk, asymptomatic population. The sensitivity of the fecal DNA panel was four times that of Hemoccult II for invasive cancer and more than twice as sensitive for adenomas con-

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