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Multitarget Stool DNA Testing for Colorectal-Cancer Screening

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ABSTRACT

BACKGROUND

An accurate, noninvasive test could improve the effectiveness of colorectal-cancer screening.

METHODS

We compared a noninvasive, multitarget stool DNA test with a fecal immunochemical test (FIT) in persons at average risk for colorectal cancer. The DNA test includes quantitative molecular assays for *KRAS* mutations, aberrant *NDRG4* and *BMP3* methylation, and β-actin, plus a hemoglobin immunoassay. Results were generated with the use of a logistic-regression algorithm, with values of 183 or more considered to be positive. FIT values of more than 100 ng of hemoglobin per milliliter of buffer were considered to be positive. Tests were processed independently of colonoscopic findings.

RESULTS

Of the 9989 participants who could be evaluated, 65 (0.7%) had colorectal cancer and 757 (7.6%) had advanced precancerous lesions (advanced adenomas or sessile serrated polyps measuring ≥1 cm in the greatest dimension) on colonoscopy. The sensitivity for detecting colorectal cancer was 92.3% with DNA testing and 73.8% with FIT (P=0.002). The sensitivity for detecting advanced precancerous lesions was 42.4% with DNA testing and 23.8% with FIT (P<0.001). The rate of detection of polyps with high-grade dysplasia was 69.2% with DNA testing and 46.2% with FIT (P=0.004); the rates of detection of serrated sessile polyps measuring 1 cm or more were 42.4% and 5.1%, respectively (P<0.001). Specificities with DNA testing and FIT were 86.6% and 94.9%, respectively, among participants with nonadvanced or negative findings (P<0.001) and 89.8% and 96.4%, respectively, among those with negative results on colonoscopy (P<0.001). The numbers of persons who would need to be screened to detect one cancer were 154 with colonoscopy, 166 with DNA testing, and 208 with FIT.

CONCLUSIONS

In asymptomatic persons at average risk for colorectal cancer, multitarget stool DNA testing detected significantly more cancers than did FIT but had more false positive results. (Funded by Exact Sciences; ClinicalTrials.gov number, NCT01397747.)

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Colorectal cancer is a major cause of death and disease among men and women in the United States.[1] The underlying neoplastic processes of colorectal carcinogenesis lend themselves to screening.[2] Evidence supports and guidelines endorse several tests and strategies,[3-5] and screening for colorectal cancer has been found to be cost-effective.[5-7]

Despite the supporting evidence, recommendations, and availability of several screening tests, a substantial proportion of the U.S. population is not up to date with screening.[8] A simple, noninvasive test with high sensitivity for both colorectal cancer and advanced precancerous lesions might increase uptake and adherence rates, which could improve clinical outcomes.

Colorectal cancer arises from accumulated genetic and epigenetic alterations, which provide a basis for the analysis of stool to identify tumor-specific changes.[9] Large-scale screening studies of previously available stool-based DNA tests showed only fair sensitivity for the detection of colorectal cancer (i.e., the capacity to detect cancers, or true positive tests [see Glossary]) and low sensitivity for the detection of advanced adenomas.[10,11] Important advances have since been incorporated, including the use of a stabilizing buffer,[12,13] more discriminating markers,[14,15] more sensitive analytic methods,[14,16,17] automation,[16] and an overall determination of results with the use of a logistic-regression algorithm, which together result in higher sensitivity for the detection of both cancer and advanced precancerous lesions.[14,16] However, evaluation of the more recent tests was based largely on analyses of archived specimens, including those collected from patients after the diagnosis but before the resection of colorectal cancer or advanced precancerous polyps.

In this study, we evaluate the multitarget stool DNA test as a tool for screening. The primary aim was to determine the performance characteristics of the DNA test in the detection of colorectal cancer. The secondary aims were to determine the performance of the DNA test in the detection of advanced precancerous lesions and to compare it with a commercially available fecal immunochemical test (FIT) for human hemoglobin in the detection of both colorectal cancer and advanced precancerous lesions.

METHODS

STUDY DESIGN

From June 2011 through November 2012, we enrolled participants in this cross-sectional study at 90 sites throughout the United States and Canada, including private-practice and academic settings. The study was approved by the institutional review board at each site, and all participants provided written informed consent.

The study, which was funded by Exact Sciences, was designed by the authors; Health Decisions, a contract research organization, gathered and monitored the data. The first author wrote the first draft of the manuscript, incorporating the other authors' contributions; one of the authors, who is a statistician, analyzed the data and, along

Glossary of Screening Terms

Sensitivity (true positive rate): The proportion of persons with disease who have a positive test (positive test results among persons with disease).

Specificity (true negative rate): The proportion of persons without disease who have a negative test (negative test results among persons without disease).

False negative rate (1 minus sensitivity): The proportion of persons with disease who have a negative test (negative test results among persons with disease).

False positive rate (1 minus specificity): The proportion of persons without disease who have a positive test (positive test results among persons without disease).

Positive predictive value: The proportion of persons with disease among those with a positive test (disease present among those with positive test results).

Negative predictive value: The proportion of persons without disease among those with a negative test (disease absent among those with negative test results).

Number needed to screen: The number of persons who would need to be screened to identify one person with the disease.

with the last author, vouches for the data and adherence to the study protocol, which is available with the full text of this article at NEJM.org. All the authors signed confidentiality agreements with Exact Sciences.

STUDY POPULATION

The target population was asymptomatic persons between the ages of 50 and 84 years who were considered to be at average risk for colorectal cancer and who were scheduled to undergo screening colonoscopy. Enrollment was weighted toward persons 65 years of age or older in order to increase the prevalence of cancer. We excluded participants who had a personal history of colorectal neoplasia, digestive cancer, or inflammatory bowel disease; had undergone colonoscopy within the previous 9 years or a barium enema, computed tomographic colonography, or sigmoidoscopy within the previous 5 years; had positive results on fecal blood testing within the previous 6 months; had undergone colorectal resection for any reason other than sigmoid diverticula; had overt rectal bleeding within the previous 30 days; had a personal or family history of colorectal cancer; had participated in any interventional clinical study within the previous 30 days; or were unable or unwilling to provide written informed consent.

CLINICAL PROCEDURES

All participants were required to provide a stool specimen and undergo screening colonoscopy within 90 days after providing informed consent. Stool was collected before routine bowel preparation. No dietary or medication restrictions were required. Colonoscopists were required to describe the extent of the examination, document cecal visualization, rate the quality of preparation (on a modified Aronchick scale),[18] and record the size and location of lesions.

Although colonoscopists reported the location and size of all lesions, only the most advanced colorectal epithelial lesion (the index lesion) and its location (proximal or distal) were used to categorize participants for the analysis. If two similarly advanced lesions were present, the larger of the two was designated as the index lesion. The proximal colon was considered to include the splenic flexure and all segments proximal to it, an insertion depth of more than 60 cm, or any part described by the phrase "right colon"; the distal colon was considered to include all other segments, an insertion depth of 60 cm or less, or any part described by the phrase "left colon."

The biopsy and surgical specimens underwent histopathological analysis at the laboratory typically used by each study site. Polyps with high-grade dysplasia or 25% or more villous elements in adenomas measuring less than 1 cm, as well as sessile serrated or hyperplastic polyps measuring 1 cm or larger, were re-reviewed centrally by a gastrointestinal pathologist for confirmation, with diagnostic disagreements resolved by consensus of at least two central pathologists.

PRIMARY AND SECONDARY OUTCOMES

The primary outcome was the ability of the DNA test to detect colorectal cancer (i.e., adenocarcinoma), with disease stage determined with the use of the American Joint Committee on Cancer (AJCC) staging system.[19] The secondary outcome was the performance of the DNA test for the detection of advanced precancerous lesions, including advanced adenomas (high-grade dysplasia or with ≥25% villous histologic features or measuring ≥1 cm in the greatest dimension) and sessile serrated polyps measuring 1 cm or more in diameter.

LABORATORY PROCEDURES

A central biorepository received all stool specimens. Laboratory testing was performed without knowledge of the results of either the comparator FIT or clinical findings. (Details of stool collection and processing for DNA testing are shown in Fig. S1 in the Supplementary Appendix, available at NEJM.org.) Buffered stool samples were homogenized, separated into aliquots, and frozen at –80°C on receipt. Stool aliquots were subsequently sent in batches to one of three laboratories: Exact Sciences (Madison, WI), Mayo Medical Laboratory (Rochester, MN), and Molecular Pathology Laboratory Network (Knoxville, TN). Each laboratory received, in a blinded fashion, a similar distribution of specimens on the basis of colonoscopic findings.

The multitarget stool DNA test consists of molecular assays for aberrantly methylated *BMP3* and *NDRG4* promoter regions, mutant *KRAS*, and *β*-actin (a reference gene for human DNA quantity), as well as an immunochemical assay for

human hemoglobin. Quantitative measurements of each marker were incorporated into a validated, prespecified logistic-regression algorithm, with a value of 183 or more indicating that the test result was positive (for details, see the Supplementary Appendix). Analytic results were transferred to the study's biostatistician.

FIT (OC FIT-CHEK, Polymedco) was performed according to the manufacturer's instructions with the use of the same stool sample used for the DNA test.[20] Samples were refrigerated on receipt and sent in batches to a separate single laboratory for blinded analysis. Stool samples with more than 100 ng of hemoglobin per milliliter of buffer were considered to be positive.[20]

STATISTICAL ANALYSIS

The study was designed to have a power of 90% to test the prespecified hypothesis that the DNA test would have a sensitivity of 65% or more for the detection of colorectal cancer (AJCC stages I through IV) under the null hypothesis, at a one-sided type I error rate of 0.05. A secondary hypothesis was to rule out a 5% noninferiority margin for sensitivity for the detection of colorectal cancer with the DNA test as compared with FIT, at a one-sided type I error rate of 0.05. Testing of the two hypotheses with a power of at least 80% required the diagnosis of 49 and 56 adjudicated colorectal cancers, respectively, which required the enrollment of 10,500 to 12,000 participants, under the assumption of a colorectal-cancer prevalence of 4.5 cases per 1000 population.

We conducted prespecified analyses to determine the sensitivity of the multitarget DNA test, as compared with FIT, for the detection of screening-relevant colorectal cancer (AJCC stages I through III); the specificity of the multitarget

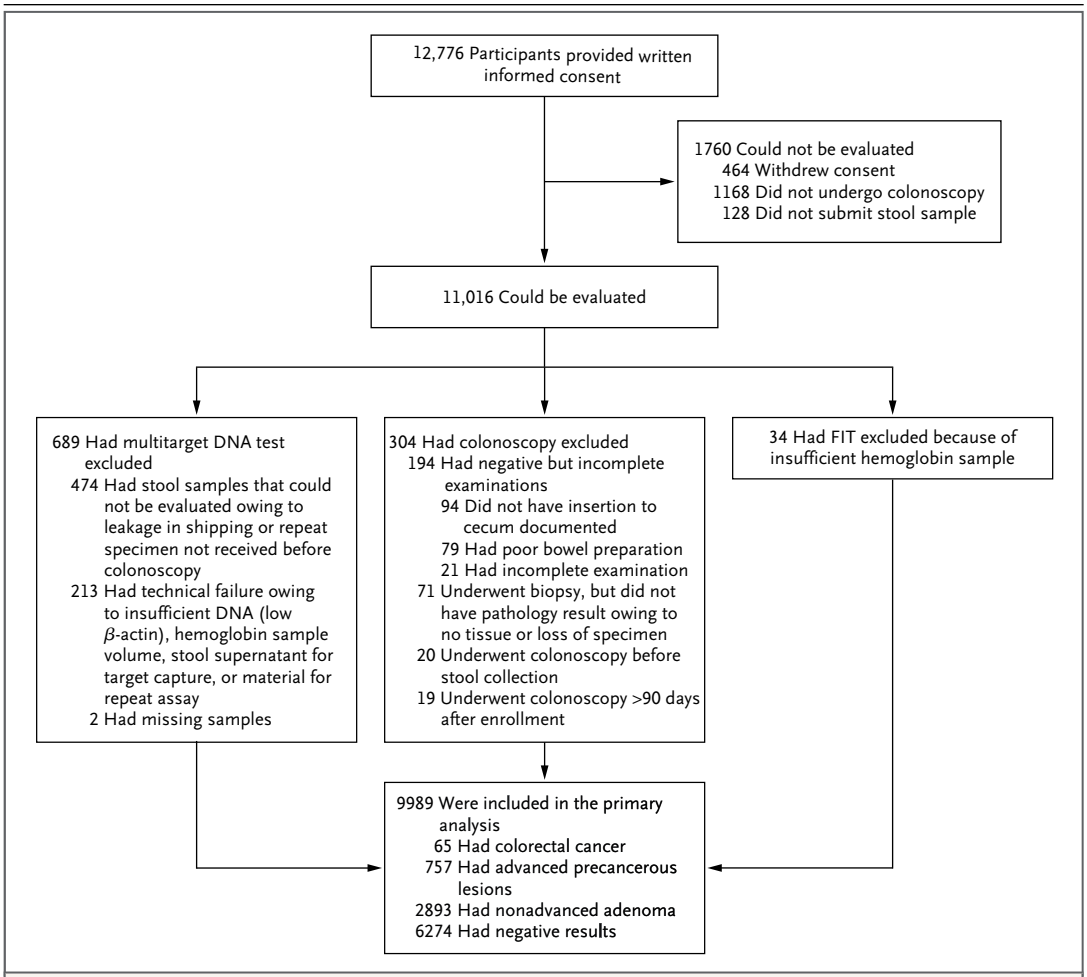

Figure 1. Enrollment and Outcomes.

DNA stool test (i.e., true negative rate), with advanced precancerous lesions on colonoscopy excluded and only nonadvanced adenomas and negative results included (the primary measure of specificity) and with only negative results included (the secondary measure of specificity); and the sensitivity of the multitarget stool DNA test, as compared with FIT, for the detection of advanced precancerous lesions. The analyses were based on data from all participants who had valid results on multitarget stool DNA testing, FIT, and colonoscopy; all reported subgroup analyses were prespecified.

For test characteristics, 95% lower boundaries were computed with the use of an exact binomial test. Lower 95% confidence limits for comparative analyses were computed with the use of a one-sided McNemar paired-comparisons test for the observed difference in sensitivity between the DNA test and FIT. The Hanley–McNeil method was used to calculate P values for the analysis of the receiver operating characteristic (ROC) curve.[21] There were no interim analyses of the data. All analyses were conducted with the use of SAS software, version 9.1, and StatXact software, version 7.

RESULTS

STUDY POPULATION

A total of 12,776 participants were enrolled at 90 sites; 9989 of these participants (78.2%) had results that could be fully evaluated (Fig. 1). The participants whose results could be fully evaluated and those whose results could not be fully evaluated differed significantly with respect to mean age and race, although the magnitudes of the differences were small (Table S1 in the Supplementary Appendix).

A total of 65 participants who could be evaluated were found to have colorectal cancer on colonoscopy (prevalence, 0.7%). Of these participants, 60 had screening-relevant (stage I to III) cancers. A total of 757 participants who could be evaluated had advanced precancerous lesions (prevalence, 7.6%).

DNA TEST CHARACTERISTICS

Multitarget stool DNA testing identified 60 of 65 participants with cancer, including 56 of the 60 participants with screening-relevant cancers, for respective sensitivities of 92.3% (95% confidence interval [CI], 83.0 to 97.5) and 93.3% (95% CI,

Table 1. Sensitivity and Specificity of the Multitarget Stool DNA Test and the Fecal Immunochemical Test (FIT) for the Most Advanced Findings on Colonoscopy.

Most Advanced Finding	Colonoscopy (N=9989)	Multitarget DNA Test (N=9989)		FIT (N=9989)	
		Positive Results	Sensitivity (95% CI)	Positive Results	Sensitivity (95% CI)
	no.	*no.*	%	*no.*	%
Colorectal cancer					
Any	65	60	92.3 (83.0–97.5)	48	73.8 (61.5–84.0)
Stage I to III*	60	56	93.3 (83.8–98.2)	44	73.3 (60.3–83.9)
Colorectal cancer and high-grade dysplasia	104	87	83.7 (75.1–90.2)	66	63.5 (53.5–72.7)
Advanced precancerous lesions†	757	321	42.4 (38.9–46.0)	180	23.8 (20.8–27.0)
Nonadvanced adenoma	2893	498	17.2 (15.9–18.6)	220	7.6 (6.7–8.6)
			Specificity (95% CI)		Specificity (95% CI)
All nonadvanced adenomas, non-neoplastic findings, and negative results on colonoscopy	9167	1231	86.6 (85.9–87.2)	472	94.9 (94.4–95.3)
Negative results on colonoscopy	4457	455	89.8 (88.9–90.7)	162	96.4 (95.8–96.9)

* These stages of colorectal cancer, as defined by the system recommended by the American Joint Committee on Cancer, are associated with an increased rate of cure.
† Advanced precancerous lesions include advanced adenomas and sessile serrated polyps measuring 1 cm or more.

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