ORIGINAL ARTICLE

Next-Generation Multitarget Stool DNA Test for Colorectal Cancer Screening

Thomas F. Imperiale, M.D., Kyle Porter, M.A.S., Julia Zella, Ph.D., Zubin D. Gagrat, B.S., Marilyn C. Olson, Ph.D., Sandi Statz, M.S., Jorge Garces, Ph.D., Philip T. Lavin, Ph.D., Humberto Aguilar, M.D., Don Brinberg, M.D., Charles Berkelhammer, M.D., John B. Kisiel, M.D., and Paul J. Limburg, M.D., for the BLUE-C Study Investigators*

From the Indiana University School of Medicine, Indiana University Melvin and Bren Simon Comprehensive Cancer Center, and Regenstrief Institute, Indianapolis (T.F.I.); Exact Sciences, Madison, WI (K.P., J.Z., Z.D.G., M.C.O., S.S., J.G., P.J.L.); Boston Biostatistics Research Foundation, Framingham, MA (P.T.L.); Louisiana Research Center, Shreveport (H.A.); Great Lakes Gastroenterology Research, Mentor, OH (D.B.); Southwest Gastroenterology, Oak Lawn, IL (C.B.); and the Division of Gastroenterology and Hepatology, Mayo Clinic, Rochester, MN (J.B.K.). Dr. Imperiale can be contacted at timperia@iu.edu or at Regenstrief Institute, 1101 W. 10th St., Indianapolis, IN 46202.

*A list of the investigators in the BLUE-C Study is provided in the Supplementary Appendix, available at NEJM.org.

N Engl J Med 2024;390:984-93.
DOI: 10.1056/NEJMoa2310336

ABSTRACT

BACKGROUND

A next-generation multitarget stool DNA test, including assessments of DNA molecular markers and hemoglobin level, was developed to improve the performance of colorectal cancer screening, primarily with regard to specificity.

METHODS

In a prospective study, we evaluated a next-generation multitarget stool DNA test in asymptomatic adults 40 years of age or older who were undergoing screening colonoscopy. The primary outcomes were sensitivity of the test for colorectal cancer and specificity for advanced neoplasia (colorectal cancer or advanced precancerous lesions). Advanced precancerous lesions included one or more adenomas or sessile serrated lesions measuring at least 1 cm in the longest dimension, lesions with villous histologic features, and high-grade dysplasia. Secondary objectives included the quantification of sensitivity for advanced precancerous lesions and specificity for nonneoplastic findings or negative colonoscopy and comparison of sensitivities for colorectal cancer and advanced precancerous lesions between the multitarget stool DNA test and a commercially available fecal immunochemical test (FIT).

RESULTS

Of 20,176 participants, 98 had colorectal cancer, 2144 had advanced precancerous lesions, 6973 had nonadvanced adenomas, and 10,961 had nonneoplastic findings or negative colonoscopy. With the next-generation test, sensitivity for colorectal cancer was 93.9% (95% confidence interval [CI], 87.1 to 97.7), and specificity for advanced neoplasia was 90.6% (95% CI, 90.1 to 91.0). Sensitivity for advanced precancerous lesions was 43.4% (95% CI, 41.3 to 45.6), and specificity for nonneoplastic findings or negative colonoscopy was 92.7% (95% CI, 92.2 to 93.1). With the FIT, sensitivity was 67.3% (95% CI, 57.1 to 76.5) for colorectal cancer and 23.3% (95% CI, 21.5 to 25.2) for advanced precancerous lesions; specificity was 94.8% (95% CI, 94.4 to 95.1) for advanced neoplasia and 95.7% (95% CI, 95.3 to 96.1) for nonneoplastic findings or negative colonoscopy. As compared with FIT, the next-generation test had superior sensitivity for colorectal cancer ($P<0.001$) and for advanced precancerous lesions ($P<0.001$) but had lower specificity for advanced neoplasia ($P<0.001$). No adverse events occurred.

CONCLUSIONS

The next-generation multitarget stool DNA test showed higher sensitivity for colorectal cancer and advanced precancerous lesions than FIT but also showed lower specificity. (Funded by Exact Sciences; BLUE-C ClinicalTrials.gov number, NCT04144738.)

Colorectal cancer is diagnosed in 153,000 persons annually in the United States and is the second most common cause of cancer-related death.[1] The U.S. Preventive Services Task Force and the American Cancer Society recommend colorectal cancer screening for adults 45 to 75 years of age who are at average risk.[2,3] Despite the effectiveness of colorectal cancer screening in reducing the incidence of colorectal cancer and related mortality,[4-11] screening adherence was just under 60% in 2021,[12] which is below the 80% target established by the National Colorectal Cancer Roundtable.[13]

A noninvasive multitarget stool DNA test that includes assessment of DNA molecular markers and hemoglobin level was approved by the Food and Drug Administration in 2014[14,15] and is included in the colorectal cancer screening guidelines for persons at average risk.[2,3,16,17] In the initial trial of the multitarget stool DNA test, the detection of colorectal cancers and advanced precancerous lesions was significantly higher than with a comparator fecal immunochemical test (FIT), but specificity was lower.[15] In an effort to improve specificity and reduce the occurrence of false positive results while maintaining or improving sensitivity, a next-generation multitarget stool DNA test was developed.

In the current BLUE-C study, we evaluated the performance characteristics of this next-generation test. The primary objective was to determine the sensitivity of the test for colorectal cancer and the specificity for advanced neoplasia. Secondary objectives included the quantification of sensitivity for advanced precancerous lesions and specificity for nonneoplastic findings or negative colonoscopy and a comparison of the test results with those of a commercially available FIT.

METHODS

STUDY DESIGN AND OVERSIGHT

We conducted this study at 186 sites across the United States. The study protocol (available with the full text of this article at NEJM.org) was approved by either a central (Advarra) or internal (local) institutional review board at each site, as appropriate. All the participants provided written informed consent. The study was conducted according to the principles of the International Ethical Guidelines for Biomedical Research Involving Human Subjects, the principles of the Declaration of Helsinki, and Good Clinical Practice guidelines.

This study was funded by Exact Sciences (the sponsor) and was designed by the sponsor and authors. Data collection and monitoring were conducted by ICON, an independent clinical research organization, and the second author analyzed the data. All the authors had access to and participated in the interpretation of the data, reviewed and revised the manuscript, and approved the manuscript for submission for publication. Medical writing and editorial assistance was funded by the sponsor. The authors vouch for the completeness and accuracy of the data and for the fidelity of the study to the protocol.

STUDY POPULATION

The study population included asymptomatic persons 40 years of age or older who were scheduled to or planned to undergo screening colonoscopy. We excluded persons who had a history of colorectal cancer or advanced precancerous lesions; had a medical or family history of familial adenomatous polyposis, hereditary nonpolyposis colorectal cancer syndrome, or other hereditary cancer syndromes; had inflammatory bowel disease or Cronkhite–Canada syndrome; had had positive results on a first-generation multitarget stool DNA test within the previous 2 years or on a FIT or fecal occult blood test within the previous 6 months; had undergone colonoscopy within the previous 9 years; or had had rectal bleeding within the previous 30 days. We evaluated the representativeness of the trial population with regard to age, sex, and race and ethnic group by comparing the demographic characteristics of the study population with those of the U.S. population according to the 2020 Census.

CLINICAL PROCEDURES

Stool specimens for the next-generation multitarget stool DNA test and FIT were obtained before the colonoscopy preparation, mailed for processing, and inspected for acceptability on receipt. Adverse events were recorded for events that occurred during the specimen-collection procedure. Possible events included wrist sprain; minor cuts or injuries incurred while opening the kit, obtaining the specimen, or preparing the stool specimen for shipment; and accidental

exposure to the preservative buffer solution. At each study site, screening colonoscopy was performed according to the standard of care.

All submitted tissue specimens, including all colorectal cancers and advanced precancerous lesions, and information about colonoscopy reports, histopathological reports, and relevant postcolonoscopy follow-up procedures or cancer-related imaging reports were reviewed centrally by at least one independent pathologist and were considered to be the reference standard. Endoscopists and pathologists at the central laboratory were unaware of the results of the multitarget stool DNA test and FIT.

For each participant, the findings on colonoscopy were categorized according to the histopathological diagnosis of the most clinically significant lesion detected (Table S1 in the Supplementary Appendix, available at NEJM.org). Bowel preparation for colonoscopy was rated as excellent, good, fair, or poor. The colonoscopy was considered to be complete and acceptable for study purposes if cecal intubation was documented and the quality of bowel preparation was rated as fair or better. A colonoscopy that identified colorectal cancer or an advanced precancerous lesion was considered to be complete, regardless of any limiting factors. An evaluable colonoscopy was defined as a complete colonoscopy that was performed within 180 days after the stool sample was obtained. Histopathological information was collected for tissue that had been removed during the colonoscopy and was used to determine the most advanced finding.

PRIMARY AND SECONDARY OUTCOMES

The primary outcomes were sensitivity of the multitarget stool DNA test for colorectal cancer, with sensitivity defined as the proportion of participants with colorectal cancer who have positive test results, and specificity for advanced neoplasia (defined as colorectal cancer or advanced precancerous lesions), with specificity defined as the proportion of negative test results among participants without advanced neoplasia. Advanced precancerous lesions included adenomas and sessile serrated lesions (including large, hyperplastic polyps) that were at least 1 cm in the longest dimension, lesions with villous histologic features, and high-grade dysplasia. Secondary outcomes were sensitivity for advanced precancerous lesions, specificity for nonneoplastic findings or negative colonoscopy, and comparison of sensitivity for colorectal cancer and advanced precancerous lesions between the multitarget stool DNA test and the commercial FIT.

Additional prespecified outcomes included the following: sensitivity according to cancer stage (with stages of I to IV assigned on the basis of the American Joint Committee on Cancer staging system[18]); lesion location (proximal or distal colon or rectum; see below); lesion size; subgroup analysis according to type of advanced precancerous lesion; specificity according to participant age; specificity according to subgroups of participants with nonneoplastic findings or negative colonoscopy; specificity among participants with negative colonoscopy; receiver operating characteristic (ROC) curves for the next-generation multitarget stool DNA test and FIT; and comparison of the next-generation multitarget stool DNA test with FIT at fixed specificity. The proximal colon included the cecum, ascending colon, hepatic flexure, transverse colon, splenic flexure, any part described by the phrase "right colon," or areas to an insertion depth of more than 60 cm. The distal colon included the descending colon, sigmoid colon, rectosigmoid colon, any part described by the phrase "left colon," or areas to an insertion depth of 16 to 60 cm. The rectum included areas to an insertion depth of 0 to 15 cm.

LABORATORY PROCEDURES

The next-generation multitarget stool DNA test was conducted at the sponsor's laboratories (Exact Sciences Laboratories), and the FIT was conducted by a separate central laboratory (Molecular Pathology Laboratory Network). In brief, the next-generation test incorporated a new molecular panel (including the methylated DNA markers ceramide synthase 4 gene [*LASS4*], leucine-rich repeat-containing protein 4 gene [*LRRC4*], serine–threonine protein phosphatase 2A 56-kDa regulatory subunit gamma isoform gene [*PPP2R5C*], and the reference marker zinc finger DHHC-type containing 1 gene [*ZDHHC1*], while retaining fecal hemoglobin). Additional details of the new biomarker panel, the next-generation algorithm, and stool collection and processing for DNA testing are shown in Figure S1. Technicians were unaware of the findings on colonoscopy and the alternate test.

The commercial FIT (OC-AUTO FIT, Polymedco) was processed according to the manufacturer

instructions, and stool samples with a hemoglobin level of more than 100 ng per milliliter of buffer (>20 μg per gram of feces) were considered to be positive on FIT.[19] The sponsor was unaware of the results until after the algorithm and clinical database lock.

STATISTICAL ANALYSIS

The study was designed to have at least 90% power to assess all the primary and secondary analyses. We calculated that the enrollment of at least 71 participants with colorectal cancer would provide the study with at least 90% power to evaluate the hypothesis regarding sensitivity for colorectal cancer.

The primary and secondary analyses were based on all available data without imputation for missing data. The primary effectiveness population included all the enrolled participants who met the inclusion criteria and had a valid next-generation multitarget stool DNA test and an evaluable colonoscopy. The comparative-effectiveness population included all the participants in the primary effectiveness population who also had a valid FIT. Because only 32 participants in the primary effectiveness population were excluded from the comparative-effectiveness population, results are presented only for the comparative-effectiveness population. Multiple imputation analysis that included all the study participants was conducted to evaluate potential bias from missing data.

The study had two prespecified primary hypotheses and four prespecified secondary hypotheses. The primary hypotheses tested the sensitivity of the next-generation multitarget stool DNA test for colorectal cancer against a 75% null hypothesis and tested the specificity for advanced neoplasia against an 85.9% null hypothesis. Secondary hypotheses tested the sensitivity of the next-generation multitarget stool DNA test for advanced precancerous lesions against a 38.9% null hypothesis, the superiority of the next-generation test to the commercial FIT for the sensitivity for colorectal cancer and advanced precancerous lesions, and the specificity for nonneoplastic findings or negative colonoscopy against an 87.5% null hypothesis.

To preserve the overall type I error at a one-sided alpha level of 2.5%, the two primary null hypotheses were required to be rejected in order to declare study success and proceed to the secondary hypothesis tests. For the secondary hypotheses, the following hierarchical testing strategy with a prespecified order of testing was used to control the type I error: sensitivity for colorectal cancer had to be greater with the next-generation multitarget stool test than with FIT, sensitivity for advanced precancerous lesions had to be greater with the next-generation test than with FIT, sensitivity for advanced precancerous lesions with the next-generation test had to be greater than 38.9%, and specificity for nonneoplastic findings or negative colonoscopy of the next-generation test had to be greater than 87.5%. The comparisons of sensitivity for colorectal cancer and advanced precancerous lesions between the next-generation test and FIT were conducted by exact McNemar tests at a one-sided significance level of 2.5%. Specificity for advanced neoplasia was also formally compared between the next-generation test and FIT, given that the development of the next-generation test was motivated by improvement with respect to specificity. Confidence intervals for outcomes other than the primary and secondary outcomes were not adjusted for multiplicity and cannot be used to infer effects.

Full details of the statistical analysis plan are available with the protocol. Details of primary and secondary hypothesis testing and imputation analyses are provided in the Supplementary Appendix. All the statistical analyses were conducted with the use of SAS software, version 9.4 (SAS Institute).

RESULTS

STUDY PARTICIPANTS

The study was conducted between November 15, 2019, and January 5, 2023. Of 26,758 enrolled participants, 20,176 (75.4%) had results that were valid for full evaluation (comparative-effectiveness population) (Fig. S2). The most common reasons for exclusion were incomplete screening colonoscopy (in 2218 participants [8.3%]), stool sample not usable per the study protocol (in 851 [3.2%]), and nonreceipt of stool sample (in 832 [3.1%]). The mean age of the participants with evaluable samples was 63.0 years; 53.2% of the participants were women, and 60.1% were White (Table S2). The study population was generally representative of the racial and ethnic group distribution of the United States among screening-eligible persons (Table S3).

Among the 20,176 participants with results that were valid for full evaluation, colorectal cancer was detected in 98 participants (0.5%), of whom 82 (84%) had stage I, II, or III disease (Table 1 and Table S4). The most advanced findings were advanced precancerous lesions in 2144 participants (10.6%), nonadvanced adenomas in 6973 (34.6%), nonneoplastic findings in 3451 (17.1%), and negative results on colonoscopy in 7510 (37.2%). No adverse events were reported with the stool-collection process for either the multitarget stool DNA test or FIT.

CHARACTERISTICS OF THE NEXT-GENERATION TEST

All the primary and secondary null hypotheses were rejected under the prespecified hypothesis testing strategy that accounted for multiplicity (Table S5). The results here are presented according to clinical categories (sensitivity, then specificity) rather than according to the order of statistical testing. A valid result on the next-generation test was obtained for 99.5% of the usable samples (24,354 of 24,477).

The next-generation multitarget stool DNA test identified 92 of 98 participants with colorectal cancer and 76 of 82 participants with screening-relevant cancers (stage I, II, or III), for test sensitivities of 93.9% (95% confidence interval [CI], 87.1 to 97.7) and 92.7% (95% CI, 84.8 to 97.3), respectively (Table 1). Sensitivity did not vary substantially according to disease stage or location (Fig. 1A and 1B). Sensitivities in subgroups that were defined according to precancerous-lesion subtype and lesion size are shown in Figure 1C and 1D.

Among 2144 participants with advanced precancerous lesions, the next-generation multitarget stool DNA test was positive in 931, for a sensitivity of 43.4% (95% CI, 41.3 to 45.6). Sensitivity for colorectal cancer was 93.3% (95% CI, 81.7 to 98.6) among participants younger than 65 years of age

Table 1. Sensitivity and Specificity of the Next-Generation Multitarget Stool DNA Test and the Commercial FIT.*

Variable	Colonoscopy (N=20,176)	Next-Generation Multitarget Stool DNA Test (N=20,176)		FIT (N=20,176)	
	No. of Participants	No. of Results	Assessment (95% CI)	No. of Results	Assessment (95% CI)
			%		%
Sensitivity					
Colorectal cancer					
Any	98	92	93.9 (87.1–97.7)†	66	67.3 (57.1–76.5)
Stage I, II, or III‡	82	76	92.7 (84.8–97.3)	53	64.6 (53.3–74.9)
Advanced precancerous lesions	2,144	931	43.4 (41.3–45.6)†	500	23.3 (21.5–25.2)
High-grade dysplasia	114	85	74.6 (65.6–82.3)	54	47.4 (37.9–56.9)
Specificity					
Advanced neoplasia§	17,934	16,245	90.6 (90.1–91.0)	16,997	94.8 (94.4–95.1)¶
Nonneoplastic findings or negative colonoscopy‖	10,961	10,156	92.7 (92.2–93.1)	10,492	95.7 (95.3–96.1)
Negative colonoscopy**	7,510	7,012	93.4 (92.8–93.9)	7,207	96.0 (95.5–96.4)

* In evaluations of sensitivity, numbers of positive results are shown, and in evaluations of specificity, numbers of negative results are shown. Statistical analyses are presented only for comparisons of the sensitivity for colorectal cancer and advanced precancerous lesions and of the specificity for advanced neoplasia between the next-generation multitarget stool DNA test and the fecal immunochemical test (FIT). CI denotes confidence interval.
† P<0.001 for the comparison of the next-generation multitarget stool DNA test with FIT.
‡ Disease stage was defined according to the American Joint Committee on Cancer staging system.[18]
§ Specificity for advanced neoplasia included all participants who did not have advanced neoplasia. Absence of advanced neoplasia was defined as all nonadvanced adenomas, nonneoplastic findings, and negative colonoscopy (categories 3 through 6 in the study-specific category scheme).
¶ P<0.001 for the comparison of FIT with the next-generation multitarget stool DNA test.
‖ Nonneoplastic findings or negative colonoscopy included category 6 (6.1 or 6.2).
** Negative colonoscopy was defined as no findings on colonoscopy (category 6.2).

and 94.3% (95% CI, 84.3 to 98.8) among those 65 years of age or older; sensitivity for advanced precancerous lesions in these subgroups was 39.6% (95% CI, 36.6 to 42.6) and 47.0% (95% CI, 44.1 to 50.0), respectively. Sensitivities of the next-generation test for colorectal cancer and advanced precancerous lesions were consistent across age groups (Table S6). Sensitivities for colorectal cancer and advanced precancerous lesions were similar among participants with a first-degree relative with colorectal cancer and those without such a relative (Tables S7 and S8).

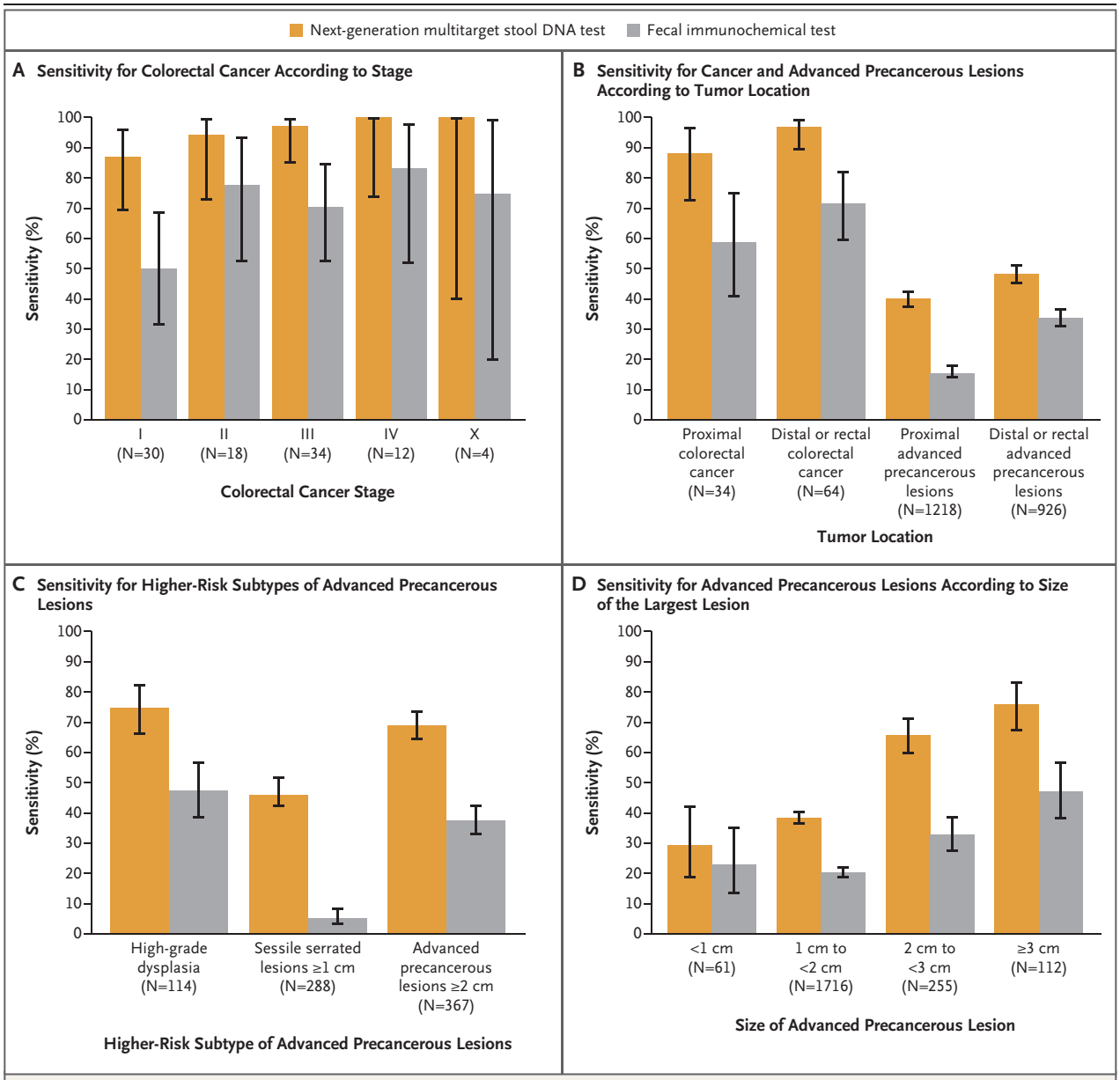

Figure 1. Sensitivity of the Next-Generation Multitarget Stool DNA Test and the Commercial Fecal Immunochemical Test.

Shown are sensitivities for colorectal cancer according to disease stage (Panel A), for cancer and advanced precancerous lesions according to tumor location (Panel B), for higher-risk types among advanced precancerous lesions (Panel C), and for advanced precancerous lesions according to the size of the largest lesion (Panel D). Disease stage was defined according to the American Joint Committee on Cancer staging system.[18] Tumor location was determined as proximal or distal to the cecum or in the rectum. Lesion size was determined according to the longest dimension. I bars indicate 95% confidence intervals.

Among 17,934 participants without colorectal cancer or advanced precancerous lesions (i.e., no advanced neoplasia), specificity of the next-generation multitarget stool DNA test was 90.6% (95% CI, 90.1 to 91.0). Among 10,961 participants with nonneoplastic findings or negative colonoscopy, specificity was 92.7% (95% CI, 92.2 to 93.1), and among the 7510 participants with a negative colonoscopy, specificity was 93.4% (95% CI, 92.8 to 93.9) (Table 1). Specificity for advanced neoplasia was 92.7% (95% CI, 92.1 to 93.2) among participants younger than 65 years of age and 88.2% (95% CI, 87.5 to 88.9) among those 65 years of age or older. In analyses conducted according to 5-year age intervals, specificity was 97.3% (95 CI, 94.8 to 98.8) among participants 45 to 49 years of age, 95.9% (95% CI, 94.8 to 96.9) among those 50 to 54 years of age, 87.0% (95% CI, 85.7 to 88.3) among those 70 to 75 years of age, and 84.6% (95 % CI, 81.7 to 87.2) among those 76 years of age or older (Table S9). Specificities were similar among participants with a first-degree relative with colorectal cancer and those without such a relative.

For the prevalence of observed colorectal cancer of 0.5%, the positive predictive value for colorectal cancer was 3.4% (95% CI, 2.7 to 4.1), and the negative predictive value was 99.97% (95% CI, 99.93 to 99.99). For the prevalence of advanced neoplasia of 11.1%, the positive predictive value for advanced neoplasia was 37.7% (95% CI, 35.9 to 39.6), and the negative predictive value was 93.0% (95% CI, 92.6 to 93.4) (Table S10). Among participants with a positive result on the multitarget stool DNA test, colorectal cancer was detected in 3.4%, advanced precancerous lesions in 34.3%, and nonadvanced adenomas in 32.6%; no colorectal neoplasia was detected in 29.7% of the participants (Table S11). The positive likelihood ratio for colorectal cancer was 7.19 (95% CI, 6.75 to 7.64), and the negative likelihood ratio was 0.07 (95% CI, 0.02 to 0.13). The positive likelihood ratio for advanced neoplasia was 4.84 (95% CI, 4.53 to 5.16), and the negative likelihood ratio was 0.60 (95% CI, 0.58 to 0.62) (Table S12).

COMPARISON OF THE NEXT-GENERATION TEST WITH THE FIT

The next-generation multitarget stool DNA test had higher sensitivity than FIT with regard to the detection of colorectal cancer and advanced precancerous lesions (P<0.001 for both comparisons). FIT detected 66 of 98 colorectal cancers (67.3%; 95% CI, 57.1 to 76.5) and 500 of 2144 advanced precancerous lesions (23.3%; 95% CI, 21.5 to 25.2) (Table 1). At a fixed 90.6% specificity for the next-generation test, the sensitivity of FIT was 75.5% (95% CI, 65.8 to 83.6) for colorectal cancer and 31.8% (95% CI, 29.8 to 33.8) for advanced precancerous lesions; both estimates were lower than those of the next-generation multitarget stool DNA test. Sensitivities appeared to be higher with the next-generation test than with FIT for the detection of colorectal cancer of stage I to III (92.7% vs. 64.6%), proximal colorectal cancers (88.2% vs. 58.8%), and distal colorectal cancers (96.9% vs. 71.9%), as well as for higher-risk subtypes of advanced precancerous lesions, including sessile serrated lesions (45.8% vs. 5.2%) and advanced precancerous lesions ranging in size from 1 cm to 3 cm or longer in the longest dimension (Table 1 and Fig. 1). Test positivity according to adenoma size is shown in Table S13.

The next-generation multitarget stool DNA test was positive for 26 of the 32 colorectal cancers (81%) that were undetected by FIT, including for 23 of 29 screening-relevant colorectal cancers (79%) and for 555 of 1644 advanced precancerous lesions (33.8%). FIT was not positive for any colorectal cancers that were undetected by the next-generation test and was positive for 124 of 1213 advanced precancerous lesions (10.2%) that were undetected by the next-generation test.

The specificity of FIT for advanced neoplasia was 94.8% (95% CI, 94.4 to 95.1), which was superior to the results with the next-generation multitarget stool DNA test (P<0.001) (Table 1). The specificity of FIT was 95.7% (95% CI, 95.3 to 96.1) for nonneoplastic findings or negative colonoscopy and 96.0% (95% CI, 95.5 to 96.4) for negative colonoscopy (Table 1). FIT specificity was consistently high across age groups. The area under the ROC curve was greater for the next-generation multitarget stool DNA test than for FIT with regard to the sensitivity for colorectal cancer as compared with the specificity for advanced neoplasia (0.98 vs. 0.85) and with regard to the sensitivity for advanced colorectal neoplasia as compared with the specificity for advanced neoplasia (0.76 vs. 0.65) (Fig. S3).

Multiple imputation analyses that accounted for all the participants yielded results that were consistent with results for the population of

participants with evaluable samples (Table S14). In these analyses, sensitivity for colorectal cancer was 93.7% (95% CI, 89.4 to 97.9) with the next-generation multitarget stool DNA test and 65.2% (95% CI, 56.6 to 73.9) with FIT. Sensitivity for advanced precancerous lesions was 43.3% (95% CI, 41.2 to 45.5) with the next-generation test and 23.9% (95% CI, 21.1 to 25.8) with FIT. Specificity for advanced neoplasia was 90.6% (95% CI, 90.2 to 91.0) with the next-generation test and 94.6% (95% CI, 94.3 to 94.9) with FIT.

DISCUSSION

Adherence to colorectal cancer screening in the United States is well below the 80% national target,[12,13] and noninvasive screening tests could improve screening adherence.[20] In this prospective study involving more than 20,000 participants, we evaluated a next-generation multitarget stool DNA test and found that the sensitivity of the test for colorectal cancer was 93.9% and that the specificity for advanced neoplasia was 90.6%. Comparisons with FIT showed that the stool DNA test had higher sensitivity but lower specificity.

These results were obtained by means of a methodical process of identifying the most discriminating molecular markers for colorectal cancer and advanced precancerous lesions on the basis of case–control data sets,[21] followed by rigorous algorithm development.[22] We preliminarily tested the new markers with a locked algorithm on a large subset of samples from the study that established the test characteristics of the multitarget stool DNA test[15] to independently validate the algorithm[22] before initiating the current study analyses.

Improved specificity of the multitarget stool DNA test was the primary goal of designing and evaluating this next-generation test. In a retrospective analysis comparing the current and next-generation versions of the multitarget stool DNA test in an archived sample of 7662 prospectively collected specimens from the earlier clinical trial, specificity for advanced neoplasia was 88.5% with the next-generation test and 86.9% with the current test, sensitivity for colorectal cancer was 93.0% with each test, and sensitivity for advanced precancerous lesions was 48.4% and 41.2%, respectively.[22] Specificity is the main driver of positive tests in the context of screening showing low prevalence, and a noninvasive test with high specificity is desirable for reducing the number of unnecessary colonoscopies and the associated direct and indirect costs of screening.

As with the current version of the multitarget stool DNA test, there was an age-related decrease in specificity with the next-generation test that was not seen with FIT. The specificity of the next-generation test for advanced neoplasia was 87.0% among participants 70 to 75 years of age and 84.6% among those 76 years of age or older. Older persons who undergo colonoscopy because of false positive results are at higher-than-average risk for complications from colonoscopy.[23] Conversely, among younger participants, higher specificities were observed with the next-generation test (97.3% among those 45 to 49 years of age and 95.9% among those 50 to 54 years of age), and the results were similar to those observed with FIT.

The next-generation multitarget stool DNA test includes new biomarkers, which were designed to increase specificity without decreasing sensitivity. Sensitivity of the next-generation test was 93.9% for colorectal cancer and 43.4% for advanced precancerous lesions. Sensitivity for curable-stage (I, II, or III) colorectal cancer was 92.7%. In a previous study of the currently available version of the test, sensitivity was 92.3% (95% CI, 83.0 to 97.5) for colorectal cancer and 42.4% (95% CI, 38.9 to 46.0) for advanced precancerous lesions, with specificity for advanced neoplasia of 86.6% (95% CI, 85.9 to 87.2).[15] The present study did not directly compare the two tests, and direct comparisons between the two studies should not be made.

In a comparison of the sensitivities with FIT for colorectal cancer and advanced precancerous lesions (67.3% and 23.3%, respectively), the corresponding sensitivities with the next-generation multitarget stool DNA test were significantly higher (93.9% and 43.4%). Regarding the location of lesions within the colorectum, the sensitivities for proximal and distal colorectal cancer with the next-generation test (88.2% and 96.9%, respectively) also were higher than with FIT (58.8% and 71.9%, respectively). A similar trend was observed with regard to advanced precancerous lesions, with the next-generation test showing higher sensitivity than FIT for high-grade dysplasia, sessile serrated lesions, and other subtypes. The specificity for advanced neoplasia

was higher with FIT (94.8%) than with the next-generation multitarget stool DNA test (90.6%).

Among the strengths of this study is the large and diverse participant population. Although the study was enriched for older age for the purpose of identifying enough colorectal cancers, the population generally represented racial and ethnic groups of persons in the United States who are eligible for screening.[24] The sample size of the study provides reasonably precise estimates of sensitivity for colorectal cancer and of sensitivity in subgroups of advanced precancerous lesions according to size, histologic features, and location. The large sample size also enabled precise estimates of specificity for the most clinically relevant categories of findings and according to age group, with the latter results showing an expected decrement in specificity with increasing age. Another strength of the study is the central, blinded adjudication of all the colorectal cancers for histologic features and disease stage, which ensured diagnostic accuracy against which to assess performance of both the next-generation multitarget stool DNA test and the commercial FIT.

A limitation of this study is the relatively high proportion of persons who provided informed consent and were enrolled but whose samples could not be evaluated according to the protocol. A contributing factor may have been conduct of the study during the coronavirus disease 2019 pandemic, which probably affected enrollment and access to colonoscopy. Multiple imputation analyses that accounted for all the participants showed results consistent with those from the population of participants with evaluable samples. Another limitation is that we did not directly compare the performance of the next-generation multitarget stool DNA test with the current version of the multitarget stool DNA test. Thus, the results from this study cannot be reliably compared with published findings for the multitarget stool DNA test that is currently available for screening purposes, and valid comparisons would require the assessment of both tests in the same persons and specimens concurrently in the context of screening.

In this study, we found that the next-generation multitarget stool DNA test showed 93.9% sensitivity for colorectal cancer, 43.4% sensitivity for advanced precancerous lesions, and 90.6% specificity for advanced neoplasia. This new version of the test was more sensitive than a commercial FIT for all screening-relevant lesions, but the FIT had higher specificity.

Supported by Exact Sciences.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

A data sharing statement provided by the authors is available with the full text of this article at NEJM.org.

We thank David Ahlquist, M.D., and Graham Lidgard, Ph.D., whose pioneering work was instrumental in the creation of multitarget stool DNA testing; William J. Kelley, Ph.D., and Charlotte Majerczyk, Ph.D., of Lumanity Communications, for medical writing and editorial assistance with an earlier version of the manuscript; Varun Sama, M.S., and Nishitha Therala, M.S., of Exact Sciences, for statistical programming assistance and verification of results; and Feyza Sancar, Ph.D., and Rukaiyya Khan, Ph.D., of Exact Sciences, for editorial review and assistance with an earlier version of the manuscript.

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