Colonoscopy Findings in FIT$^+$ and mt-sDNA$^+$ Patients versus in Colonoscopy-only Patients: New Hampshire Colonoscopy Registry Data

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ABSTRACT

Few studies compare fecal immunochemical test (FIT) and multi-target stool DNA (mt-sDNA) outcomes in practice. We compared colonoscopy yield following FIT$^+$ or mt-sDNA$^+$ tests to colonoscopies without preceding stool tests in the comprehensive population-based New Hampshire Colonoscopy Registry (NHCR). Outcomes were any neoplasia and an ordered outcome: adenocarcinoma, advanced neoplasia (adenoma/serrated polyp ≥ 1 cm/villous/high-grade dysplasia), nonadvanced neoplasia, or normal. Our total sample included 306 mt-sDNA$^+$ (average age ± SD 67.0 ± 7.9), 276 FIT$^+$ (66.6 ± 8.7), and 50,990 colonoscopy-only patients (61.8 ± 8.1). Among average-risk patients (N = 240 mt-sDNA$^+$, N = 194 FIT$^+$, N = 26,221 colonoscopy only), mt-sDNA$^+$ patients had a higher risk for any neoplasia (67.1%) compared with FIT$^+$ (54.6%, $P = 0.00098$) or colonoscopy (40.8%, $P < 0.0001$). Severity of findings and histology subtypes differed across the three groups ($P < 0.0001$ for both), with a higher yield of advanced findings in mt-sDNA$^+$ patients. In particular, clinically relevant serrated polyps (hyperplastic polyps ≥10 mm/traditional serrated adenomas/sessile serrated polyps) were detected at a higher frequency in mt-sDNA$^+$ patients as compared with FIT$^+$ or colonoscopy-only patients. Even after adjustment, patients with positive mt-sDNA [OR = 2.82; 95% confidence interval (CI), 2.00–4.02] or FIT$^+$ tests (OR = 1.67; 95% CI, 1.19–2.36) were more likely to have histologically more advanced findings than colonoscopy alone. At follow-up colonoscopy, mt-sDNA$^+$ tests were more likely to predict neoplasia than FIT$^+$, largely due to increased detection of serrated polyps.

Prevention Relevance: Colorectal cancer screening options include colonoscopy and stool-based tests, including the fecal immunochemical test (FIT) and the multi-target stool DNA (mt-sDNA) test which, if positive, must be followed by a colonoscopy. Assessing "real-world" outcomes of colonoscopies following positive stool tests can inform their clinical use.

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Introduction

Colorectal cancer, the second most common cause of cancer-related death in the United States (1), develops from precancerous adenomatous or serrated polyps, and can be prevented through screening and surveillance. Screening options include colonoscopy and noninvasive stool-based tests, such as the fecal immunochemical test (FIT) and the multi-target stool DNA (mt-sDNA) test, which are both recommended for average-risk screening by the United States Multi-Society Task Force on Colorectal Cancer (USMSTF) and United States Preventive Services Task Force (USPSTF) (2, 3) and if positive, must be followed by a colonoscopy. Assessing "real-world" outcomes of colonoscopies following positive stool testing can inform their clinical use.

Colorectal polyps and cancer bleed intermittently, and this blood can be detected by stool-based tests. FIT uses an antibody specific to the globin moiety of human hemoglobin (HgB), and its sensitivity depends on bleeding from colorectal lesions. Larger size and protruding shape are predictors of bleeding and of higher stool HgB concentration (4). While elevated stool HgB levels have been found in individuals with colorectal cancer (199 ug of HgB/gram of feces), patients with serrated polyps, which are commonly flat, have lower HgB levels (46 ug/g), similar to adults with normal colons (66 ug/g) or nonadvanced adenomas (50 ug/g; ref. 4). It is not surprising, then, that FIT has been found to be less effective at identifying patients with serrated polyps than those with conventional advanced adenomas or colorectal cancer (5).

In addition to measuring stool HgB through inclusion of a FIT, mt-sDNA tests assess stool for DNA markers shed by

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Cancer Prev Res 2022;15:455–64

doi: 10.1158/1940-6207.CAPR-21-0581

colorectal neoplasia, including methylated *BMP3* and *NDRG4* and mutant *KRAS*. Some studies have shown mt-sDNA to have superior performance characteristics to fecal occult blood testing (6). Mt-sDNA has been shown to have increased sensitivity for precancerous lesions as compared with FIT both in a large trial (7) and in a study of adults presenting for screening (8). Because it includes methylated markers associated with serrated lesions, such as BMP3, mt-sDNA has higher sensitivity for these lesions (7, 8) than FIT.

Few studies have compared the use and outcomes of FIT and mt-sDNA in community clinical practice. The New Hampshire Colonoscopy Registry (NHCR) is a statewide population-based registry that has collected comprehensive data on over 200,000 colonoscopies. This study aims to compare the yield of specific colonoscopy findings in patients with preceding positive FIT and mt-sDNA tests, and in patients presenting for colonoscopy without a preceding positive stool test.

Materials and Methods

Population

Our analysis included patient data, colonoscopy outcomes including location, size, and pathology, and colonoscopy quality measures. Patients complete an NHCR Patient Questionnaire prior to colonoscopy, including detailed demographic, health behavior, and personal and family history data. Endoscopists and/or endoscopy nurses complete the NHCR Colonoscopy Procedure Form during or immediately after colonoscopy, including detailed exam indications, completion status, withdrawal time, bowel preparation quality, recommended follow-up, and the location, size, and treatment method for all findings.

Trained NHCR abstractors match polyp-level pathology data to information from the Colonoscopy Procedure Form (9).

As approved by our Institutional Review Board (IRB), through 2018 all patients provided separate written informed consent; since 2018, to indicate consent patients complete and return the Patient Information Form, which, for this minimal risk study was determined by the IRB to be "acceptable to indicate consent and authorization to participate." All data collection and study procedures were approved by the IRB of the NHCR (the Committee for the Protection of Human Subjects at Dartmouth College, Hanover, NH; CPHS#00015834) in accordance with the Belmont Report and the U.S. Common Rule.

Study cohorts

Under the IRB-approved protocol, Exact Sciences Laboratories provided the NHCR with identifiers of all patients with mt-sDNA$^+$ tests in the NHCR catchment area (New Hampshire, Vermont, Maine, and Massachusetts). Our cohort included 306 individuals with an mt-sDNA$^+$ result as part of usual clinical care and a subsequent colonoscopy. A second cohort of 276 individuals had FIT$^+$ tests and a subsequent colonoscopy. A third cohort of 50,990 individuals had screening or surveillance colonoscopy with no indication of a prior positive stool test. To avoid any potential bias due to changes in polyp detection rates over time, colonoscopy data for all three cohorts used the same time period (2015 to 2019). We also conducted a subanalysis of average-risk patients (no personal history of neoplasia or colorectal cancer and no first degree relatives with colorectal cancer), which included 240 mt-sDNA$^+$, 194 FIT$^+$, and 26,221 colonoscopy-only patients.

All mt-sDNA, FIT tests, and colonoscopies were conducted in the course of routine clinical practice. Patients with mt-sDNA$^+$ and FIT$^+$ results were referred by their primary care providers to endoscopists throughout New Hampshire for their colonoscopies. Exclusion criteria for colonoscopies in all three groups were the same. We excluded exams performed for symptomatic diagnostic indications, as well as patients with Inflammatory Bowel Disease or a genetic syndrome such as Lynch Syndrome. Colonoscopies within 12 months of one another were merged and treated as a single colonoscopy if the initial exam was incomplete or had poor bowel preparation, or if the subsequent exam was a resection or was indicated for polypectomy of a known polyp. After this merge, patients with no complete exam with adequate bowel preparation were excluded. Although the current recommendation is to start colorectal cancer screening at age 45 (10), we used 50 years as the age to begin colorectal cancer screening in order to reflect the recommendation that was in place during the time period of our analysis.

Outcomes

Study outcomes included colonoscopy findings, categorized by most advanced lesion detected: adenocarcinoma/ colorectal cancer, advanced precancerous neoplasia (tubular adenoma or serrated polyp ≥1 cm, or any size lesion with ≥25% villous elements and/or high-grade dysplasia), nonadvanced neoplasia (tubular adenoma or serrated polyp <1 cm), or normal exam [including exams with only rectosigmoid hyperplastic polyps (HP) <5 mm; refs. 7, 11]. Our analysis included all colorectal cancer and other neoplasia detected during colonoscopy or from clinical resections, incorporating data available through linkage with the New Hampshire State Cancer Registry. In a separate analysis, we stratified exam findings into conventional advanced neoplasia versus clinically relevant serrated polyps [including all traditional serrated adenomas, all sessile serrated polyps (SSP), and HPs ≥10 mm].

We also compared colonoscopy quality measures between cohorts, to determine if endoscopists change their clinical practice in response to mt-sDNA$^+$ or FIT$^+$ tests. These included endoscopist and exam factors associated with higher polyp detection rates, such as withdrawal or mucosal inspection time (12) and bowel preparation quality. We also compared the percentage of average-risk individuals with normal colonoscopy who were instructed to return for screening colonoscopy in 10 years, as recommended by colorectal cancer screening guidelines (2, 13, 14).

Covariates

Patient variables were derived from the NHCR Patient Questionnaire and included demographic factors (age, sex,

Table 1. Patient characteristics and risk factors.

Patient characteristics	After positive mt-sDNA test ($N = 306$)		After positive FIT test ($N = 276$)		Colonoscopy only ($N = 50,990$)	
	Mean	SD	Mean	SD	Mean	SD
Age (years), mean ± SD	67.0	7.9	66.6	8.7	61.8	8.1
BMI, mean ± SD	29.3	7.8	28.6	7.5	28.8	7.2
	N	%	*N*	%	*N*	%
Sex: Male	117	38.2	139	50.4	25,034	49.1
Female	189	61.8	137	49.6	25,956	50.9
Race: Caucasian	257	96.3	239	98.8	42,333	97.0
Other	10	3.7	3	1.2	1,314	3.0
Smoking status: Never	122	45.4	116	47.3	24,072	54.5
Former	119	44.2	97	39.6	16,759	37.9
Current	28	10.4	32	13.1	3,346	7.6
Overall health						
Excellent or good	235	87.0	215	86.7	41,718	94.2
Fair	34	12.6	29	11.7	2,349	5.3
Poor	1	0.4	4	1.6	199	0.4
Aspirin /NSAIDS ≥once/wk	109	44.3	117	49.8	18,859	44.2
Anticoagulant usage	20	7.5	14	5.3	759	1.6
Prior colonoscopy[a]	175	57.8	190	68.8	37,222	73.0
Increased risk[b]	66	21.6	82	29.7	24,769	48.6
History of prior neoplasia including CRC and/or surveillance indication	37	12.2	46	16.7	19,051	37.4
Family history (first degree)	41	13.6	48	17.4	11,124	21.9

Abbreviations: CRC, colorectal cancer; wk, week.

[a]History of colonoscopy as per patient self-report, surveillance indication, and prior exams in the NHCR database.

[b]Increased risk includes patients with prior neoplasia (including colorectal cancer) and / or a family history of colorectal cancer in a first-degree relative.

race) health behaviors [smoking status, body mass index (BMI), overall health status], aspirin/nonsteroidal anti-inflammatory drug (NSAID) or anticoagulant use, and history of prior colonoscopy.

Statistical and analytic approach

Our examination of real-world mt-sDNA and FIT use assessed colonoscopy outcomes in all patients, including those at increased risk for colorectal cancer and average-risk individuals. For univariate analyses, we used Monte Carlo estimates of Fisher exact tests with 50,000 replications or χ^2 tests for comparisons involving proportions or discrete variables. We used logistic and ordered logistic regression to account for the influence of covariates when examining the relationship between study cohorts and neoplastic findings.

Data availability

Data were generated by the authors but are not publicly available because confidentiality of endoscopists and patients might be compromised but the non protected health information data used in the analyses are available upon reasonable request.

Results

After exclusions, 51,572 patients with colonoscopy remained: 306 after a mt-sDNA$^+$ (average age ± SD 67.0 ± 7.9), 276 after a FIT$^+$ (66.6 ± 8.7) and 50,990 with colonoscopy with no prior stool test (61.8 ± 8.1; **Table 1**). More mt-sDNA$^+$ patients were female (61.8%), while the FIT$^+$ and colonoscopy-only groups were evenly split by sex (49.6% and 50.9% female, respectively). Both the FIT$^+$ and mt-sDNA$^+$ cohorts were older than the colonoscopy-only cohort, with more former or current smokers, more patients taking anticoagulants, and fewer reporting good/excellent health. More FIT$^+$ and colonoscopy-only patients had a known prior colonoscopy than in the mt-sDNA$^+$ cohort (68.8% FIT$^+$, 73.0% colonoscopy, 57.8% mt-sDNA). Nearly half (48.6%) of the colonoscopy-only cohort were at increased risk for colorectal cancer, compared with 21.6% of the mt-sDNA$^+$ and 29.7% of the FIT$^+$ cohorts. There were 66 increased risk patients in the mt-sDNA group, 82 in the FIT$^+$ group, and 24,769 in the colonoscopy-only group. A higher percentage of FIT$^+$ patients (29.7%, 82/276) than mt-sDNA$^+$ (21.5%, 66/306) were at increased risk. Endoscopist performance quality as measured by Adenoma Detection Rate (ADR) was analyzed and did not differ between the three groups.

In the average-risk group, mt-sDNA$^+$ patients had a substantially higher risk for any neoplasia [67.1.0%; 95% confidence interval (CI), 0.61–0.73] compared with FIT$^+$ (54.6%; 95% CI, 0.48–0.62; $P = 0.0098$) or colonoscopy-only patients (40.8%; 95% CI, 0.40–0.41; $P < 0.0001$), but the comparison of most advanced finding between cohorts was not significant (**Table 2**). The mt-sDNA$^+$ group had a higher frequency of both advanced noncancerous neoplasia (mt-sDNA$^+$ 25.0%, FIT$^+$ 22.7%, colonoscopy-only 7.7%) and nonadvanced neoplasia (40.4%, 30.9%, and 32.8%, respectively), while colorectal

Table 2. Most advanced finding on colonoscopy.

	Colonoscopy after positive mt-sDNA test		Colonoscopy after positive FIT test		Colonoscopy only		Overall *P*	*P* value (mt-sDNA vs. FIT)	*P* value (mt-sDNA vs. colo only)	*P* value (FIT vs. colo only)
Average-risk patients	**(*N* = 240)**		**(*N* = 194)**		**(*N* = 26,221)**					
	N	**%**	***N***	**%**	***N***	**%**				
Any neoplasia*	161	67.1	106	54.6	10,694	40.8	<0.0001	0.0098	<0.0001	0.0001
Most advanced finding on colonoscopy							<0.0001	0.0529	<0.0001	<0.0001
Adenocarcinoma/colorectal cancer**	4	1.7	2	1.0	79	0.3				
Advanced noncancerous neoplasia[a]	60	25.0	44	22.7	2,021	7.7				
Nonadvanced neoplasia[b]	97	40.4	60	30.9	8,594	32.8				
Normal exam[c]	79	32.9	88	45.4	15,506	59.2				
Unknown outcome	0		0		21					
All patients	**(*N* = 306)**		**(*N* = 276)**		**(*N* = 50,990)**					
	N	**%**	***N***	**%**	***N***	**%**				
Any neoplasia*	208	68.0	149	54.0	23,718	46.6	<0.0001	0.0006	<0.0001	0.0154
Most advanced finding on colonoscopy							<0.0001	0.0054	<0.0001	<0.0001
Adenocarcinoma/colorectal cancer**	4	1.3	4	1.4	137	0.3				
Advanced noncancerous neoplasia[a]	83	27.1	55	19.9	4,273	8.4				
Nonadvanced neoplasia[b]	121	39.5	90	32.6	19,308	37.9				
Normal exam[c]	98	32.0	127	46.0	27,224	53.4				
Unknown outcome	0		0		48					
Increased risk patients	**(*N* = 66)**		**(*N* = 82)**		**(*N* = 24,769)**					
	N	**%**	***N***	**%**	***N***	**%**				
Any neoplasia*	47	71.2	43	52.4	13,024	52.6	0.0094	0.0274	0.0028	1.0000
Most advanced finding on colonoscopy							<0.0001	0.0052	<0.0001	0.0143
Adenocarcinoma/colorectal cancer**	0	0.0	2	2.4	58	0.2				
Advanced noncancerous neoplasia[a]	23	34.8	11	13.4	2,252	9.1				
Nonadvanced neoplasia[b]	24	36.4	30	36.6	10,714	43.3				
Normal exam[c]	19	28.8	39	47.6	11,718	47.4				
Unknown outcome	0		0		27					
Average-risk patients, first colonoscopy only	**(*N* = 120)**		**(*N* = 80)**		**(*N* = 12,368)**					
	N	**%**	***N***	**%**	***N***	**%**				
Any neoplasia*	86	71.7	50	62.5	5,192	42.0	<0.0001	0.2158	<0.0001	0.0003
Most advanced finding on colonoscopy							<0.0001	0.2233	<0.0001	<0.0001
Adenocarcinoma/colorectal cancer**	4	3.3	1	1.3	50	0.4				
Advanced noncancerous neoplasia[a]	33	27.5	26	32.5	1,131	9.1				
Nonadvanced neoplasia[b]	49	40.8	23	28.8	4,011	32.4				
Normal exam[c]	34	28.3	30	37.5	7,169	58.0				
Unknown outcome	0		0		7					

Abbreviation: colo, colonoscopy.

*P value for any neoplasia vs. normal exam.

**P value for ordered outcome.

[a]Advanced noncancerous neoplasia: adenoma or serrated polyp ≥1 cm, or with ≥25% villous elements and/or high-grade dysplasia of any size.

[b]Nonadvanced neoplasia: tubular adenoma or serrated polyps <1 cm (other than rectosigmoid hyperplastic polyps <5 mm).

[c]Normal exam: includes colonoscopies with only rectosigmoid hyperplastic polyps <5 mm.

cancer rates were higher in both mt-sDNA$^+$ (1.7%) and FIT$^+$ (1.0%) groups compared with colonoscopy-only (0.3%; **Table 2**). Given the multiple comparisons in **Table 2**, the appropriate adjusted threshold for significance corresponding to 0.05 for a single test is 0.0016 as per Bonferroni correction.

We found that mt-sDNA$^+$ patients in the total (all patients) sample had a substantially higher risk for any neoplasia (68.0%; 95% CI, 0.63–0.73) compared with FIT$^+$ (54.0%; 95% CI, 0.48–0.60; $P = 0.0006$) or colonoscopy-only patients (46.6%; 95% CI, 0.46–0.47; $P < 0.0001$; **Table 2**). FIT$^+$ patients also had a statistically significant higher rate of neoplastic findings than colonoscopy-only patients (54.0% vs. 46.6%). Similarly, patients with mt-sDNA$^+$ had a higher risk of histologically more advanced findings than the FIT$^+$ ($P = 0.0054$) and colonoscopy-only cohorts ($P < 0.0001$). The mt-sDNA$^+$ group had a higher frequency of both advanced noncancerous neoplasia (mt-sDNA$^+$ 27.1%, FIT$^+$ 19.9%, colonoscopy-only 8.4%) and nonadvanced neoplasia (39.5%, 32.6%, and 37.9%, respectively), while colorectal cancer rates were higher in both mt-sDNA$^+$ (1.3%) and FIT$^+$ (1.4%) groups compared with colonoscopy-only (0.3%; **Table 2**). The outcomes in the increased risk group were similar to those in the total (all patients) sample (**Table 2**). Finally, a subanalysis restricted to average-risk patients with no prior colonoscopy (**Table 2**) also

Table 3. Advanced adenomas and CRSPs detected on colonoscopy.

All patients	Colonoscopy after positive mt-sDNA test ($N = 306$)		Colonoscopy after positive FIT test ($n = 276$)		Colonoscopy only ($N = 50,990$)		Overall *P* value	*P* value (mt-sDNA vs. FIT)	*P* value (mt-sDNA vs. colo only)	P value (FIT vs. colo only)
	N	%	*N*	%	*N*	%	<0.0001	0.0053	<0.0001	<0.0001
Advanced adenoma and CRSP	9	2.9	7	2.5	370	0.7				
Advanced adenoma with no CRSP	45	14.7	41	14.9	2,313	4.5				
CRSP with no advanced adenoma	57	18.6	24	8.7	3,856	7.6				
No advanced adenoma or CRSP	195	63.7	204	73.9	44,403	87.2				
Unknown outcome	0	—	0	—	48	—				
Average-risk patients	($N = 240$)		($n = 194$)		($N = 26,221$)					
	N	%	*N*	%	*N*	%	<0.0001	0.0638	<0.0001	<0.0001
Advanced adenoma and CRSP	8	3.3	5	2.6	183	0.7				
Advanced adenoma with no CRSP	33	13.8	31	16.0	1,092	4.2				
CRSP with no advanced adenoma	43	17.9	18	9.3	1,878	7.2				
No advanced adenoma or CRSP	156	65.0	140	72.2	23,047	88.0				
Unknown outcome	0	—	0	—	21	—				

Note: CRSP includes all traditional serrated adenomas, all SSPs, and HPs ≥ 10 mm.
Abbreviation: colo, colonoscopy.

found a higher rate of any neoplasia in patients with mt-sDNA$^+$ (71.7%) than in those with FIT$^+$ (62.5%) or colonoscopy only (42%), but in this sample the difference between mt-sDNA$^+$ and FIT$^+$ patients was not statistically significant.

To explore detection rates of serrated polyps versus conventional adenomas, we separated findings by histology into four groups: exams with advanced adenomas (AA) only, those with clinically relevant serrated polyps (CRSP) with no AAs, those with both AAs and CRSPs, and those with neither (**Table 3**). Findings were significantly different among the three cohorts ($P < 0.0001$ for all comparisons except 0.0053 for mt-sDNA$^+$ versus FIT$^+$); while mt-sDNA$^+$ and FIT$^+$ patients were both more likely than colonoscopy-only patients to have AAs only (mt-sDNA$^+$ 14.7%, FIT$^+$ 14.9%, colonoscopy-only 4.5%), patients with mt-sDNA$^+$ tests were more likely than both FIT$^+$ or colonoscopy-only patients to have CRSPs with no AAs (18.6%, 8.7%, and 7.6%, respectively). Frequencies were similar in our average-risk subanalysis, but the comparison between the mt-sDNA$^+$ and FIT$^+$ cohorts was not significant (**Table 3**). In **Table 3**, there are eight tests, so the appropriate threshold for significance corresponding to 0.05 for a single test is 0.00625.

We used logistic and ordered logistic regression to account for the influence of age, sex, aspirin/NSAID use, anticoagulant use, smoking, BMI, risk-status, and history of prior colonoscopy when examining the relationship between cohorts and likelihood of neoplastic findings in average-risk patients. Exams following mt-sDNA$^+$ tests were more likely to find neoplastic findings (**Table 4**; OR = 2.82; 95% CI, 2.00–4.02); and over three times more likely to uncover histologically more advanced findings relative to those in the colonoscopy-only group (**Table 4**; OR = 3.25; 95% CI, 2.40–4.41). FIT$^+$ patients were also likely to have histologically more advanced findings relative to the colonoscopy-only group, but to a lesser degree than the mt-sDNA$^+$ cohort (**Table 4**; OR = 1.67; 95% CI, 1.19–2.36). Regression analyses for the total sample had similar results (Supplementary Table S1).

In our comparison of colonoscopy quality metrics (**Table 5**), we found that fair bowel preparation quality was slightly more common after positive stool tests. We also examined the impact of positive stool tests on endoscopist behavior by comparing withdrawal time and endoscopist-recommended follow-up intervals in exams with no findings. There were no significant differences in either of these factors.

Discussion

Average-risk colorectal cancer screening options include stool tests such as FIT or mt-sDNA, followed by colonoscopy when these tests are positive, or colonoscopy only. Each screening pathway may yield a different frequency of specific polyp and colorectal cancer findings. Importantly, the addition of molecular markers in mt-sDNA may increase detection of precancerous serrated lesions compared with FIT (7). The use of FIT and mt-sDNA as initial tests can enrich the frequency of advanced findings at colonoscopy, as demonstrated by our finding of higher colonoscopy yield following either positive stool test. This is consistent with previous studies (15–17), and intuitively reasonable, since both stool tests are designed to detect neoplasia.

We found that patients with preceding mt-sDNA$^+$ tests had a significantly higher prevalence of any neoplasia than patients with preceding FIT$^+$ tests or with colonoscopy only. Furthermore, the prevalence of more advanced pathology was substantially higher in the mt-sDNA$^+$ group than in the FIT$^+$ group (27.1% mt-sDNA$^+$, 19.9% FIT$^+$, and 8.4% colonoscopy

Table 4. Logistic regression, average-risk patients ($N = 20{,}281$[a]), colonoscopy only as reference.

Parameter	Binary regression[b] OR (95% CI)	Ordered regression (most advanced finding[c]) OR (95% CI)
Colonoscopy after positive mt-sDNA	2.82 (2.00–4.02)	3.25 (2.40–4.41)
Colonoscopy after positive FIT	1.67 (1.19–2.36)	2.06 (1.49–2.85)
Age (per year over age 50)	1.02 (1.02–1.03)	1.02 (1.02–1.03)
Female	0.59 (0.56–0.63)	0.60 (0.57–0.64)
Aspirin and/or NSAID use ≥ once/week	0.84 (0.79–0.89)	0.83 (0.79–0.88)
Anticoagulant use	1.42 (1.07–1.89)	1.32 (1.01–1.71)
Smoking status: Former smoker	1.26 (1.18–1.34)	1.26 (1.18–1.33)
Current smoker	2.33 (2.08–2.61)	2.44 (2.19–2.71)
BMI	1.02 (1.02–1.03)	1.02 (1.02–1.02)
History of prior colonoscopy	0.81 (0.75–0.87)	0.76 (0.71–0.82)

[a]Sample sizes are accurate and reflect the number of observations with complete data for all of the variables in the regression (both the outcome and explanatory variables).
[b]Binary: any neoplasia versus no significant findings.
[c]Ordered by most advanced finding: no significant findings, nonadvanced neoplasia, advanced neoplasia.

only). A recent study found similar results (8), reporting that mt-sDNA was both more sensitive at identifying patients with advanced precancerous lesions and was associated with a higher risk of advanced pathology than FIT^{+}.

We also observed fewer normal exams following a positive stool test in the mt-sDNA group versus the FIT group (32% versus 46%). Given the concern that endoscopists may have about normal colonoscopies following positive stool tests, this lower rate might suggest an additional benefit for endoscopists and patients. The differences in colonoscopy findings between mt-sDNA and FIT do not appear to be related to differences in endoscopist performance; for example, there was no significant variation in withdrawal times between the two stool test cohorts, and did not differ between the three groups. While

Table 5. Colonoscopy quality metrics following a positive mt-sDNA test, following a positive FIT test, or colonoscopy alone (average-risk patients[a]).

Colonoscopy preparation quality

	Colonoscopy after positive mt-sDNA test		Colonoscopy after positive FIT test		Colonoscopy only		Overall *P*	*P* value (mt-sDNA vs. FIT)	*P* value (mt-sDNA vs. colo only)	*P* value (FIT vs. colo only)
Total number of patients	**($N = 240$)**		**($N = 194$)**		**($N = 26{,}221$)**					
Colonoscopy preparation quality	*N*	%	*N*	%	*N*	%				
Excellent or good	191	91.4	170	91.4	22,347	94.5	0.0280	1.0000	0.0648	0.0736
Fair	18	8.6	16	8.6	1,296	5.5				
Missing	31		8		2,578					

Withdrawal time and recommended rescreening interval among patients with normal exams with no findings

	Colonoscopy after positive mt-sDNA test		Colonoscopy after positive FIT test		Colonoscopy only		Overall *P*	*P* value (mt-sDNA vs. FIT)	*P* value (mt-sDNA vs. colo only)	*P* value (FIT vs. colo only)
Total number of patients	**($N = 55$)**		**($N = 68$)**		**($N = 12{,}813$)**					
Withdrawal time[b]	*N*	%	*N*	%	*N*	%				
Less than 9 minutes	20	48.8	27	51.9	4,705	47.5	0.8148	0.8358	0.8771	0.5787
Greater than or equal to 9 minutes	21	51.2	25	48.1	5,191	52.5				
Missing	14		16		2,917					
Recommended colonoscopy rescreening interval										
	N	**%**	*N*	**%**	*N*	**%**	0.0129	0.5673	0.2419	0.0104
Less than 10 years	5	14.7	10	21.3	910	9.3				
Greater than or equal to 10 years	29	85.3	37	78.7	8,871	90.7				
Missing	21		21		3,032					

Abbreviation: colo, colonoscopy.
[a]Average-risk definition: No prior personal history of polyps or colorectal cancer and no first degree family history of colorectal cancer.
[b]Withdrawal time data is censored at <2 and >10 minutes.

concern about normal colonoscopy following positive stool testing is prevalent, data from a retrospective study of 1,216 subjects with median follow-up time of over 5 years demonstrated that incident aerodigestive cancers were uncommon among mt-sDNA$^+$ subjects with negative colonoscopies (18), suggesting that patients with no polyps detected during a high-quality colonoscopy may not need to undergo further testing.

Since colorectal cancer arises from serrated lesions as well as adenomas, we examined the specific histology found following FIT$^+$ or mt-sDNA$^+$ testing. Serrated polyps include HPs, SSPs, also known as sessile serrated lesions, and traditional serrated adenomas (TSA; refs. 19, 20). While SSPs and TSAs can develop into cancer, in the past HPs were believed to have no malignant potential. However, due to challenges in pathologically differentiating SSPs from HPs, many experts consider HPs more than 1 cm as SSP-equivalent polyps (21), so we included them with SSPs and TSAs in our CRSP category.

Patients with mt-sDNA$^+$ tests were more likely to have CRSPs with no synchronous AAs than those with FIT$^+$ tests or those with colonoscopy only (18.6%, 8.7%, and 7.6%, respectively). This is consistent with current knowledge regarding detection of serrated lesions by stool based tests. Because serrated lesions are less likely to protrude and to bleed, they are less likely to be detected by FIT as compared with mt-sDNA, which detects both HgB and methylation markers indicative of serrated polyps (4, 5, 22). A large Italian study examining polyp detection during multiple rounds of FIT testing found SSP prevalence among FIT$^+$ patients to be lower than expected (23), while prevalence for adenomas (45%) and AAs (29%) was much higher than in a primary screening colonoscopy setting. After the second round of FIT testing, detection of AAs decreased, suggesting that they had been detected by the first round of FIT and resected by the subsequent colonoscopy. However, SSP prevalence did not change in the second round, suggesting that SSPs had *not* been detected by the initial round of FIT testing. Similarly, a Dutch study compared colonoscopy findings after FIT$^+$ and mt-sDNA$^+$, and found that the sensitivity for advanced serrated polyps (>1 cm or with dysplasia), was higher for mt-sDNA than for FIT (8). All screening tests have advantages and disadvantages; an important advantage for mt-sDNA is its detection of serrated lesions, which may be responsible for up to 30% of colorectal cancer (24).

Our aim was to assess outcomes among three cohorts in community practice. As we and others have noted, while FIT and mt-sDNA are intended for people who are at average-risk for colorectal cancer, in practice some patients at increased risk undergo stool testing. Stool testing for off-label indications occurs for both FIT and mt-sDNA. In our study, a higher percentage of FIT$^+$ patients (29.7%, 82/276) than mt-sDNA$^+$ (21.5%, 66/306) were at increased risk, a finding that has been found in previous research (25). Therefore, we investigated outcomes for average-risk patients as well as those at increased risk, finding similar frequencies in both the full sample and average-risk subanalysis (**Table 2**). In our study, a higher percentage of FIT patients were at increased risk than mt-sDNA patients (31% versus 22%; $P = 0.02$), implying that additional education as to approved indications may be helpful for both tests.

Patients with stool tests were more likely to be older, to be current or former smokers, to take anticoagulants, and to report fair (vs. good or excellent) health; they were also less likely to be at increased risk for colorectal cancer than patients undergoing colonoscopy alone. Although the mt-sDNA and FIT cohorts for this analysis only include patients with positive mt-sDNA or FIT tests, these data suggest that primary care providers may be more likely to use stool tests to screen patients with comorbidities or those at a higher potential risk of experiencing complications from colonoscopy. Another difference was a higher proportion of women in the mt-sDNA$^+$ group than in the FIT$^+$ group. Despite having a higher proportion of women, the mt-sDNA$^+$ group had a greater prevalence of neoplasia.

Our comparison of outcomes in average-risk patients using these three commonly used colorectal cancer testing modalities provides important insights regarding their use in general practice. Because differences in our cohort populations could lead to variations in neoplasia prevalence, we accounted for all known risk factors for both adenomatous and serrated polyps (26, 27) in our binary and ordered logistic models, and observed no change in our results. Specifically, those with colonoscopy after mt-sDNA$^+$ tests were over three times more likely to have more advanced findings relative to those in the colonoscopy-only group (OR = 3.34; 95% CI, 2.54 – 4.38).

We acknowledge some limitations in our study. In this real-world study, there were differences in patient characteristics between the three groups, which we accounted for by adjusting for risk factors in a logistic regression (**Table 4**). In addition, there may be potential confounders for which we did not control in our analysis. Another potential limitation is that the cohort is predominantly white, which limits generalizability. However, considerable ethnic, urban/rural, and socioeconomic diversity is present in the population captured within the NHCR (28). Further research will clarify the findings in more racially diverse populations. Fewer patients are included in the stool test groups than in the colonoscopy only group, which reflects tests done during the timeframe of the analysis. We aimed to compare neoplasia yield using three different approaches to colorectal cancer screening; however, it should be noted that both stool tests and colonoscopy must be used in a program of serial testing to achieve effective colorectal cancer prevention and early detection.

In summary, we compared colonoscopy findings following positive stool based tests and from exams with colonoscopy as the initial test. More polyps and colorectal cancer were found in those with a preceding positive stool test compared with colonoscopy only, and patients with a mt-sDNA$^+$ test had a higher frequency of advanced precancerous lesions than those with FIT$^+$ tests. Perhaps most notably, there was a higher prevalence of clinically relevant serrated polyps in mt-sDNA$^+$ patients. These data support the recognized "enrichment" of

findings at colonoscopies preceded by a positive stool test, and also clarify specific differences in outcomes at colonoscopy preceded by FIT^+ versus mt-$sDNA^+$ tests, respectively. Our data also help to provide real world outcomes for colonoscopies performed in patients with positive mt-sDNA and FIT tests.

Authors' Disclosures

C.M. Robinson reports grants from Exact Sciences during the conduct of the study. W. Hisey reports grants from Exact Sciences during the conduct of the study. P.J. Limburg reports other support from Exact Sciences during the conduct of the study; other support from Exact Sciences outside the submitted work; in addition, P.J. Limburg has a patent for Patent Number: 5,891,651 issued. L.F. Butterly reports grants from Exact Sciences during the conduct of the study. Dr. Limburg serves as Chief Medical Officer for exact Sciences through a contracted services agreement with Mayo Clinic. Dr. Limburg and Mayo Clinic have contractual rights to receive royalties through this agreement. No disclosures were reported by the other authors.

Authors' Contributions

J.C. Anderson: Conceptualization, formal analysis, investigation, methodology, writing–original draft, writing–review and editing. **C.M. Robinson:** Conceptualization, resources, data curation, formal analysis, supervision, funding acquisition, investigation, methodology, writing–original draft, project administration, writing–review and editing. **W. Hisey:** Data curation, formal analysis, validation, investigation, methodology, writing–original draft, writing–review and editing. **P.J. Limburg:** Conceptualization, methodology, writing–review and editing. **L.F. Butterly:** Conceptualization, resources, data curation, software, formal analysis, supervision, funding acquisition, validation, investigation, visualization, methodology, writing–original draft, project administration, writing–review and editing.

Acknowledgments

We would like to thank Taylor Larson, MPH, Emily Weiser, MPH, and Bonny Kneedler, all of Exact Sciences, for assisting with the linked data, project timeline, and feedback. We would also like to thank Jill Mullaly and Julie Blain of the NHCR and the endoscopists, endoscopy practices and pathology laboratories participating in the research of the NHCR. Exact Sciences provided funding support for this analysis to the NHCR (to L. Butterly, W. Hisey, C.M. Robinson). The funding agreement ensured that the authors had independence in designing the study, conducting the analyses, and writing and publishing the results. The contents of this work do not represent the views of the Department of Veterans Affairs or the United States Government. This project included data from the NH State Cancer Registry, which was supported in part by the Centers for Disease Control and Prevention's National Program of Cancer Registries, cooperative agreement 5U58DP003930 awarded to the New Hampshire Department of Health and Human Services, Division of Public Health Services, Bureau of Public Health Statistics and Informatics, Office of Health Statistics and Data Management. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the Centers for Disease Control and Prevention or New Hampshire Department of Health and Human Services.

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Note

Supplementary data for this article are available at Cancer Prevention Research Online (http://cancerprevres.aacrjournals.org/).

Received December 13, 2021; revised February 1, 2022; accepted March 30, 2022; published first April 4, 2022.

References

1. Siegel RL, Miller KD, Goding Sauer A, Fedewa SA, Butterly LF, Anderson JC, et al. Colorectal cancer statistics, 2020. CA Cancer J Clin 2020;70:145–64.
2. Force USPST, Bibbins-Domingo K, Grossman DC, Curry SJ, Davidson KW, Epling JW Jr, et al. Screening for colorectal cancer: US Preventive Services Task Force recommendation statement. JAMA 2016;315:2564–75.
3. Rex DK, Boland CR, Dominitz JA, Giardiello FM, Johnson DA, Kaltenbach T, et al. Colorectal cancer screening: Recommendations for physicians and patients from the U.S. Multi-Society Task Force on Colorectal Cancer. Gastrointest Endosc 2017;86:18–33.
4. van Doorn SC, Stegeman I, Stroobants AK, Mundt MW, de Wijkerslooth TR, Fockens P, et al. Fecal immunochemical testing results and characteristics of colonic lesions. Endoscopy 2015;47:1011–7.
5. Chang LC, Shun CT, Hsu WF, Tu CH, Tsai PY, Lin BR, et al. Fecal immunochemical test detects sessile serrated adenomas and polyps with a low level of sensitivity. Clin Gastroenterol Hepatol 2016;15:872–9.
6. Yang C, Wu W, Yang Y, Yang X, Sun J, Zhang W, et al. Multitarget stool DNA test compared with fecal occult blood test for colorectal cancer screening. Oncol Lett 2020;20:1193–200.
7. Imperiale TF, Ransohoff DF, Itzkowitz SH, Levin TR, Lavin P, Lidgard GP, et al. Multitarget stool DNA testing for colorectal-cancer screening. N Engl J Med 2014;370:1287–97.
8. Bosch LJW, Melotte V, Mongera S, Daenen KLJ, Coupe VMH, van Turenhout ST, et al. Multitarget stool DNA test performance in an average-risk colorectal cancer screening population. Am J Gastroenterol 2019;114:1909–18.
9. Greene MA, Butterly LF, Goodrich M, Onega T, Baron JA, Lieberman DA, et al. Matching colonoscopy and pathology data in population-based registries: development of a novel algorithm and the initial experience of the New Hampshire Colonoscopy Registry. Gastrointest Endosc 2011;74:334–40.
10. Patel SG, May FP, Anderson JC, Burke CA, Dominitz JA, Gross SA, et al. Updates on age to start and stop colorectal cancer screening: recommendations from the U.S. Multi-Society Task Force on Colorectal Cancer. Gastrointest Endosc 2022;95:1–15.
11. Gupta S, Lieberman D, Anderson JC, Burke CA, Dominitz JA, Kaltenbach T, et al. Recommendations for follow-up after colonoscopy and polypectomy: a consensus update by the US Multi-Society Task Force on colorectal cancer. Gastroenterology 2020;158:1131–53.
12. Butterly L, Robinson CM, Anderson JC, Weiss JE, Goodrich M, Onega TL, et al. Serrated and adenomatous polyp detection increases with longer withdrawal time: results from the New Hampshire Colonoscopy Registry. Am J Gastroenterol 2014;109:417–26.
13. Wolf AMD, Fontham ETH, Church TR, Flowers CR, Guerra CE, LaMonte SJ, et al. Colorectal cancer screening for average-risk adults: 2018 guideline update from the American Cancer Society. CA Cancer J Clin 2018;68:250–81.
14. Rex DK, Boland CR, Dominitz JA, Giardiello FM, Johnson DA, Kaltenbach T, et al. Colorectal cancer screening: recommendations

for physicians and patients from the U.S. Multi-Society Task Force on colorectal cancer. Am J Gastroenterol 2017;112:1016–30.
15. Cubiella J, Castells A, Andreu M, Bujanda L, Carballo F, Jover R, et al. Correlation between adenoma detection rate in colonoscopy- and fecal immunochemical testing-based colorectal cancer screening programs. United European Gastroenterol J 2017;5:255–60.
16. Hassan C, Repici A, Rex DK. FITting ADR to colonoscopy indication. United European Gastroenterol J 2017;5:149–52.
17. Kligman E, Li W, Eckert GJ, Kahi C. Adenoma detection rate in asymptomatic patients with positive fecal immunochemical tests. Dig Dis Sci 2018;63:1167–72.
18. Berger BM, Kisiel JB, Imperiale TF, Geenen DJ, Heigh RI, Mahoney DW, et al. Low incidence of aerodigestive cancers in patients with negative results from colonoscopies, regardless of findings from multitarget stool DNA tests. Clin Gastroenterol Hepatol 2019;18:864–71.
19. Anderson JC, Srivastava A. Colorectal cancer screening for the serrated pathway. Gastrointest Endosc Clin N Am 2020;30:457–78.
20. Bell PD, Anderson JC, Srivastava A. The frontiers of serrated polyps. Am J Surg Pathol 2022;46:e64–70.
21. Anderson JC, Lisovsky M, Greene MA, Hagen C, Srivastava A. Factors associated with classification of hyperplastic polyps as sessile serrated adenomas/polyps on morphologic review. J Clin Gastroenterol 2018; 52:524–9.
22. Heigh RI, Yab TC, Taylor WR, Hussain FT, Smyrk TC, Mahoney DW, et al. Detection of colorectal serrated polyps by stool DNA testing: comparison with fecal immunochemical testing for occult blood (FIT). PLoS One 2014;9:e85659.
23. Zorzi M, Senore C, Da Re F, Barca A, Bonelli LA, Cannizzaro R, et al. Detection rate and predictive factors of sessile serrated polyps in an organised colorectal cancer screening programme with immunochemical faecal occult blood test: the EQuIPE study (Evaluating Quality Indicators of the Performance of Endoscopy). Gut 2017;66: 1233–40.
24. Rex DK, Ahnen DJ, Baron JA, Batts KP, Burke CA, Burt RW, et al. Serrated lesions of the colorectum: review and recommendations from an expert panel. Am J Gastroenterol 2012;107: 1315–29.
25. Powell AA, Saini SD, Breitenstein MK, Noorbaloochi S, Cutting A, Fisher DA, et al. Rates and correlates of potentially inappropriate colorectal cancer screening in the Veterans Health Administration. J Gen Intern Med 2015;30:732–41.
26. Anderson JC, Alpern ZA. Smoking and the increased risk for serrated polyps: Implications for screening and surveillance. J Clin Gastroenterol 2019;53:319–21.
27. Anderson JC, Calderwood AH, Christensen BC, Robinson CM, Amos CI, Butterly L. Smoking and other risk factors in individuals with synchronous conventional high-risk adenomas and clinically significant serrated polyps. Am J Gastroenterol 2018; 113:1828–35.
28. Rice K, Gressard L, DeGroff A, Gersten J, Robie J, Leadbetter S, et al. Increasing colonoscopy screening in disparate populations: Results from an evaluation of patient navigation in the New Hampshire Colorectal Cancer Screening Program. Cancer. 2017;123:3356–66.