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What do 'false-positive' stool tests really mean? Data from the New Hampshire colonoscopy registry

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ABSTRACT

We utilized the population-based New Hampshire Colonoscopy Registry to calculate false discovery rates (FDR) and positive predictive values (PPVs) using three 'positive' colonoscopy definitions. Understanding the frequency of meaningful 'true positive' mt-sDNA and Fecal Immunochemical Test (FIT) results can optimize the use of these colorectal cancer (CRC) screening tests. We calculated FDR (positive stool test followed by negative colonoscopy divided by all positive stool tests) and PPV for mt-sDNA and FIT cohorts using the following definitions:

1) DeeP-C Study (CRC, adenomas/serrated polyps ≥ 1 cm, villous/High Grade Dysplasia);

2) < 10 year US Multi-Society Task Force (USMSTF) follow-up: DeeP-C findings & ≥1 sessile serrated polyps (SSPs) < 1 cm (with/without dysplasia) or ≥ 1 tubular adenomas < 1 cm.

3) Clinically Significant: DeeP-C + USMSTF + clinically significant serrated polyps: traditional serrated adenomas, SSPs, hyperplastic polyps (HPs) > 1 cm, and 5–9 mm proximal HPs.

The sample included 549 mt-sDNA + and 410 FIT + and patients (mean age 66.4, 43.0% male). Using the most limited definition of positive colonoscopy, DeeP-C, FDR was 71.9% for mt-sDNA + and 81.7% for FIT +. Using the USMSTF definition, FDR decreased substantially: mt-sDNA+:33.2% and FIT+:47.6%. Adding all CSSPs resulted in the lowest FDR: mt-sDNA+:32.2% and FIT+:47.1%. Decreasing FDRs corresponded to increasing PPVs: mt-sDNA+:28.1% and FIT+:18.3% (DeeP-C definition) and mt-sDNA+:67.8% and FIT+:52.9% (DeeP-C + USMSTF + CSSP) (Table 1).

FDRs decreased substantially when the definition of positive exams included all significant precancerous findings. These data present a comprehensive understanding of false positive outcomes at colonoscopies following positive stool tests, which to our knowledge is the first such analysis.

1. Introduction

Stool tests including the multi-target stool DNA (mt-sDNA) and fecal immunochemical tests (FIT) are home-based, accessible options that expand opportunities for colorectal cancer (CRC) screening among average risk individuals. (Anderson et al., 2022) To complete the screening process, positive stool tests require follow-up colonoscopy to determine if a polyp caused the positive result. Preceding positive stool tests enrich the proportion of colonoscopies at which colorectal lesions will be found and removed. (Anderson et al., 2022) Detection and polypectomy of polyps prevent CRC, (Winawer et al., 1993) and early detection allows CRC to be found at a more treatable stage, resulting in

Abbreviations: DeeP-C, Detection of colorectal advanced adenomatous Polyps and Cancer; AA, Advanced Adenoma; mt-sDNA, multi-target stool DNA; FDR, False discovery rate; PPV, positive predictive value; BMI, Body Mass Index; CRC, colorectal cancer; FIT, fecal immunochemical test; NHCR, New Hampshire Colonoscopy Registry; NSAID, nonsteroidal anti-inflammatory drug; USMSTF, United States Multi-Society Task Force on Colorectal Cancer; USPSTF, United States Preventive Services Task Force.

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improved survival and decreased treatment costs.

An issue in CRC screening arises when a stool test is positive, but the subsequent colonoscopy is negative; i.e., no lesions are found to explain the positive stool test. These tests are called 'false positives', since the test did not indicate the presence of a significant colonic lesion. Clinicians often question the appropriate response to this situation. While some 'negative' colonoscopies are truly 'normal' with no polyps or CRC, in this specific context – that is, colonoscopy after a positive stool test – definitions of 'negative' colonoscopies have also included colonoscopies with various polyp findings. This stems from a lack of consistent classification of which polyps found at colonoscopy following a positive stool test should be considered positive findings.

Different classifications of 'positive colonoscopy' outcomes exist: e.g., the definition used for the primary analysis in the first major mt-sDNA paper (which excluded small (<10 mm) serrated and adenomatous polyps as positive findings) (Imperiale et al., 2014) and more recent definitions which include potentially pre-cancerous serrated polyps that have emerged as clinically relevant over the subsequent decade. (Rex et al., 2012; Pai et al., 2019) Two other factors that contribute significantly to increased risk are the presence of either *multiple* small polyps or *synchronous* adenomatous and serrated polyps. Both scenarios confer increased risk and should be considered positive outcomes.

Our understanding of the CRC risk associated with different types of polyps has been informed by recent research, especially for serrated polyps. (Anderson et al., 2018) The initial study comparing mt-sDNA and FIT considered as positive only those tests in which advanced neoplasia and/or CRC were found at follow-up colonoscopy. (Imperiale et al., 2014) This classification excludes small adenomas or sessile serrated polyps (SSPs), which both indicate heightened risk for future advanced neoplasia, as highlighted in current surveillance guidelines. (Gupta et al., 2020) Which definition of a true positive colonoscopy is most clinically useful? To answer that question, we analyzed colonoscopy outcomes following positive stool tests using various definitions of a true positive colonoscopy.

Understanding different measures involving 'false positives' and how they are calculated is also informative. One clinically important assessment considers the question: of all positive stool tests, what proportion are *false* positives, reflecting the probability a patient with a positive stool test does not actually have a significant colorectal finding at follow-up colonoscopy? This is distinct from the *false positive rate* which is the probability that a patient without the condition (significant colorectal finding) will test positive for it (have a positive stool test). The calculation for the probability that a patient with a positive stool test does not actually have a significant colorectal finding at follow-up colonoscopy is called the ***false discovery rate***, (Benjamini and Hochberg, 1995; Clarke et al., 2006; Malagón et al., 2020; Soric, 1989) which is equal to the number of false positive stool tests (those followed by negative colonoscopy) divided by the total number of positive stool tests:

False discovery rate = # of false positives.
(# of false positives + # of true positives).

False discovery rate is distinct from the ***false positive rate,*** which is calculated as the number of false positive stool tests (those followed by a negative colonoscopy) divided by the # of true negatives plus the # of false positives, i.e., the percent of patients without disease:

False positive rate = # of false positives.
(# of true negatives + # of false positives).

The false discovery rate is 1 minus the ***positive predictive value*** (PPV). PPV answers the question: of all the positive stool tests, what proportion are *true* positives?

PPV is equal to the number of *true positive* stool tests (those with findings on colonoscopy) divided by the total number of positive stool tests:

Positive predictive value = # of true positives.
(# of false positives + # of true positives).

Accurate understanding of the frequency of meaningful 'true positive' mt-sDNA and FIT results is essential to optimizing the use of these important and common tests. 'True positives' are complementary to *false* positive outcomes, by which we mean a positive stool test followed by a colonoscopy where no polyp considered significant (according to the specific categorization being assessed) is detected. We utilized the statewide, population-based New Hampshire Colonoscopy Registry (NHCR) to investigate colonoscopy outcomes using three distinct but commonly used definitions of a 'positive' colonoscopy, and present the corresponding false discovery rate and PPVs.

2. Methods

2.1 Population

The NHCR has collected data from patients, providers, and pathology laboratories across New Hampshire since 2004, under IRB review by the Committee for the Protection of Human Subjects at Dartmouth College (CPHS#15834). (Anderson et al., 2022).

2.2 Study sample population

Under an IRB-approved protocol, Exact Sciences Laboratories, LLC (Madison, Wisconsin) provided the NHCR with identifiers of all patients in the NHCR catchment area (New Hampshire, Vermont, Maine and Massachusetts) with positive mt-sDNA tests.

2.3 Exposure cohorts

Our study population consisted of two cohorts: 1) 549 individuals with a mt-sDNA + result and 2) 410 individuals with a FIT + result during the same time interval. All patients had colonoscopy after their positive stool tests, and were referred by their primary care providers to endoscopists throughout New Hampshire who performed their colonoscopies in the course of routine clinical practice (2015-21). While all patients were asymptomatic, in this real-world study, some had stool tests for off-label indications, such as personal or family history of CRC (Table 1).

2.4 Exclusion criteria

We excluded patients with Inflammatory Bowel Disease,genetic syndromes (e.g. Lynch), and those under 50 years old, who were below the recommended screening age during our study period. Colonoscopy outcomes were merged and treated as a single exam if 2 colonoscopies were performed within 12 months and the initial exam was incomplete, had poor bowel preparation, or the subsequent exam was indicated for polypectomy or completion of polypectomy. After merging, patients with no complete exam with adequate bowel preparation were excluded, as were patients with incomplete outcome data.

2.5 Outcomes

Our primary outcome was the false discovery rate, defined as the number of false positives (positive stool test followed by negative colonoscopy) divided by the total number of positive stool tests (both true and false positives). We calculated false discovery rates and the corresponding PPVs for both cohorts, using 3 increasingly comprehensive definitions of positive colonoscopy (Fig. 1):

1) Detection of colorectal advanced adenomatous Polyps and Cancer: DeeP-C Study (Imperiale et al., 2014).

Exams with any CRC, adenomas ≥ 1 cm or with villous/tubulovillous histology or high grade dysplasia or any serrated polyp ≥ 1 cm were considered positive. The primary DeepC study (Imperiale et al., 2014) outcome was the ability of the DNA test to detect CRC (i.e., adenocarcinoma), using the American Joint Committee on Cancer (AJCC) staging system. The secondary outcome was the detection of advanced

	DeeP-C*	USMSTF <10 years**	Clinically Significant***
Colorectal cancer	POSITIVE	POSITIVE	POSITIVE
Advanced adenomas (>=1 cm, with villous/tubulovillous histology or high grade dysplasia) or serrated polyps >=1cm	POSITIVE	POSITIVE	POSITIVE
traditional serrated adenomas <1 cm	NEGATIVE	POSITIVE	POSITIVE
sessile serrated polyps < 1 cm	NEGATIVE	POSITIVE	POSITIVE
tubular adenomas < 1 cm	NEGATIVE	POSITIVE	POSITIVE
5-9 mm hyperplastic polyps in the proximal colon	NEGATIVE	NEGATIVE	POSITIVE

* DeeP-C: Detection of colorectal advanced adenomatous Polyps and Cancer[3]
** USMSTF: United States Multi-Society Task Force on Colorectal Cancer Screening[8]
*** Clinically Significant[14]

Fig. 1. False discovery rates and the corresponding PPVs were calculated for our mt-sDNA + and FIT + cohorts, using 3 increasingly comprehensive definitions of positive colonoscopy.

precancerous lesions, including advanced adenomas (high-grade dysplasia or with ≥ 25% villous histologic features or ≥ 1 cm in the greatest dimension) and SSPs measuring ≥ 1 cm.

2) USMSTF < 10 years: Exams with lesions requiring < 10 year follow up per USMSTF (Gupta et al., 2020) guidelines were considered positive. This group includes the DeeP-C findings in addition to 1 or more SSPs < 1 cm (with/without dysplasia) or 1 or more tubular adenomas < 1 cm.

3) Clinically Significant: Positive colonoscopies included findings from the categories above plus clinically significant serrated polyps (CSSPs): all traditional serrated adenomas, all SSPs, all hyperplastic polyps (HPs) > 1 cm, and 5–9 mm HP located in the proximal colon. The major difference between CSSPs and the USMSTF is the inclusion of proximal 5–9 mm HPs which have been shown to increase risk for future neoplasia and may represent misdiagnosed SSPs. (Anderson et al., 2018; Anderson et al., 2020).

2.6 Analytic approach

We explored how definitions of positive colonoscopy designed for different purposes – DeeP-C for assessing and documenting the clinical viability of a new screening test, USMSTF as the basis for population-based surveillance colonoscopy recommendations, and "Clinically Significant" as an all-inclusive definition which incorporates all polyps of any size or histology that are recognized as conferring an increase in risk (Rex et al., 2012; Rosty et al., 2019) – can lead to substantially different false discovery rates when applied to the same set of patients and exams. Our goal is to provide insight into a more updated and clinically useful definition, given current understanding of CRC risk stratification and guideline-endorsed surveillance recommendations.

We compare relevant patient and exam characteristics of the mt-sDNA + and FIT + cohorts in Table 1. In Table 2 we present the false discovery (negative colonoscopy) rates within our mt-sDNA + and FIT + cohorts for each definition of positive colonoscopy. Table 3 stratifies exams by the most advanced finding, highlighting the DeeP-C break point between positive versus negative exams. Tables 4 and 5 provide further detail on findings in exams identified by DeeP-C as negative colonoscopies, reporting on multiple small adenomas and multiple SSPs, and the presence of both CSSPs and TAs in the same exam (Table 4). Multiple serrated polyps (Egoavil et al., 2017), multiple adenomas (Sullivan et al., 2022) and synchronous adenomatous and serrated polyps (Anderson et al., 2018; IJspeert et al., 2017) have been associated with an increased risk of advanced neoplasia and CRC.

3. Results

The sample included 549 mt-sDNA + and 410 FIT + patients. More women than men were present in the mt-sDNA + cohort (61% female) while the FIT + cohort was more evenly split (52% female). Aspirin use was more common among FIT + patients than mt-sDNA + patients, as was prior colonoscopy (62% FIT + vs 52% mt-sDNA +).

Using the most limited definition of positive colonoscopy, the DeeP-C classification (Imperiale et al., 2014), the false discovery rate in our sample was 71.9% for mt-sDNA + and 81.7% for FIT+ (Table 2). Using the USMSTF definition, where any exam with a < 10 year follow up recommendation is a positive colonoscopy, the false discovery rate decreased to 33.2% for mt-sDNA + and 47.6% for FIT +. Finally, adding all CSSPs, including 5–9 mm proximal HPs to the USMSTF < 10 year definition resulted in the lowest false discovery rates, 32.2% for mt-sDNA + and 47.1% for FIT + results. These decreasing false discovery rates correspond to increasing positive predictive values (PPVs), ranging from 28.1% for mt-sDNA + and 18.3% for FIT+ (DeeP-C) to 67.8% for mt-sDNA + and 52.9% for FIT+ (DeeP-C + USMSTF + CSSP).

Table 3 shows the colonoscopy outcomes classified by most advanced polyp. Out of exams classified by DeeP-C as negative colonoscopies, 31.4% of mt-sDNA + colonoscopies (124/395) and 49.6% of FIT + colonoscopies (166/335) were true 'normal' exams with no findings.

Table 4 reports detailed findings for exams considered false positives by DeeP-C, highlighting the frequency of exams with multiple polyps. Nearly a fifth (18.2%) of mt-sDNA + cohort exams classified as false positives by DeeP-C had >=3 small (<10 mm) non-advanced polyps; 9.1% had three to four small tubular adenomas, while another 4.1% had five to ten small tubular adenomas, a finding for which the USMSTF recommends a 3-year follow-up interval. (Gupta et al., 2020) Similarly, 6.6% of FIT + cohort exams classified as false positives by DeeP-C had

Table 1
Characteristics of adult patients in the New Hampshire Colonoscopy Registry having colonoscopy after a positive stool test (2015–2020).

	Mt-sDNA+ N = 549		FIT+ N = 410		P
	N/ Mean	%/SD	N/ Mean	%/SD	
Age (continuous)	66.5	7.8	66.3	8.9	0.657
Age categories					
<65 years	218	39.7	182	44.4	0.164
>=65 years	331	60.3	228	55.6	
Patient sex					
Male	214	39.0	198	48.3	0.005
Female	335	61.0	212	51.7	
Patient risk					
Average risk	433	78.9	303	73.9	0.076
Increased risk	116	21.1	107	26.1	
Race					
Caucasian	435	96.9	338	97.7	0.523
Other	14	3.1	8	2.3	
BMI (continuous)	29.3	7.3	28.8	7.6	0.182
BMI categories					
Underweight	94	22.9	89	27.7	0.509
Normal	148	36.0	107	33.3	
Overweight	97	23.6	70	21.8	
Obese	72	17.5	55	17.1	
Smoking status					
Never smoker	214	46.3	160	45.6	0.958
Former smoker	194	42.0	148	42.2	
Current smoker	54	11.7	43	12.3	
Self-reported health					
Good to excellent	410	89.7	309	87.3	0.196
Fair	44	9.6	38	10.7	
Poor	3	0.7	7	2.0	
Aspirin use					
Once or more per week	124	29.5	126	38.5	0.010
NSAIDs use					
Once or more per week	49	11.0	35	10.2	0.728
Blood thinner use					
Yes	37	8.3	27	7.6	0.793
Patient endoscopy history					
Prior exam	286	52.1	253	61.7	0.003
Patient history of neoplastic findings					
Prior neoplastic findings	69	12.6	60	14.6	0.390
First-degree family history of colorectal cancer					
First-degree family history of CRC	56	12.8	60	18.1	0.053

mt-sDNA: multi-target stool DNA test, FIT: Fecal Immunochemical Test; BMI: Body Mass Index; NSAIDS: Nonsteroidal anti-inflammatory drugs; Missing data (#, %): Race (164, 17.1%), BMI (227, 23.7%), smoking status (146, 15.2%), Health status (148, 15.4%), Aspirin use (211 (22.0%), NSAIDs (172, 17.9%), blood thinner (158, 16.5%), history of neoplastic findings (1, 0.1%), First-degree family history of CRC (190, 19.8%).

Table 2
False discovery rates and positive predictive value (PPV) among adult patients in the New Hampshire Colonoscopy Registry having colonoscopy after a positive stool test (2015–2020), according to different definitions of positive colonoscopy.

False discovery rate ("negative" colonoscopy)				
	Mt-sDNA N = 549		FIT N = 410	
	#	% (95% CI)	#	% (95% CI)
Deep-C*	395	71.9 (68.2—75.7)	335	81.7 (78.0—85.4)
DeeP C* + USMSTF** <10 yrs	182	33.2 (29.2—37.1)	195	47.6 (42.7—52.4)
DeeP C*+ USMSTF** <10 yrs + CSSP	177	32.2 (28.3—36.2)	193	47.1 (42.2—51.9)
Positive Predictive Value (PPV)				
	#	%	#	%
Deep-C*	154	28.1 (24.3—31.8)	75	18.3 (14.6—22.0)
DeeP C* + USMSTF** <10 yrs	367	66.8 (62.9—70.8)	215	52.4 (47.6—57.3)
DeeP C*+ USMSTF** <10 yrs + CSSP	372	67.8 (63.8—71.7)	217	52.9 (48.1—57.8)

mt-sDNA: multi-target stool DNA test, FIT: Fecal Immunochemical Test;
* DeeP-C: Detection of colorectal advanced adenomatous Polyps and Cancer[3].
** USMSTF: United States Multi-Society Task Force on Colorectal Cancer Screening[7].

three to four small tubular adenomas and another 3.3% had five to ten small tubular adenomas.

Almost 10% of all mt-sDNA + cohort exams considered false positives by DeeP-C were CSSPs synchronous with tubular adenomas (Table 4).

4. Discussion

Stool tests greatly expand the accessibility of average-risk screening for CRC. Since any positive stool test requires follow up colonoscopy to complete screening, and a proportion of those colonoscopies will yield polyps or CRC, stool testing enriches the frequency of significant polyp findings at colonoscopy. (Anderson et al., 2022) However, there are various definitions of "true positive" polyp findings. The original definition for positive findings following mt-sDNA + testing was derived to be consistent with FDA-labelling requirements at that time. (Imperiale et al., 2014) While the lesions identified as positive at the time remain clinically important, this initial definition did not include other polyps more recently understood to be CRC precursors within the serrated and adenomatous pathways.

From a clinical perspective, it is reasonable to consider any polyps that confer an increased risk for CRC as significant and therefore as positive colonoscopy findings. These include not only advanced neoplasms and CRC, but also other polyps for which the USMSTF recommends a surveillance interval under 10 years. (Gupta et al., 2020) Similarly, polyps identified as potentially precancerous by the WHO [195], expert consensus, (Rex et al., 2012), and other literature investigating serrated polyps could also be considered significant, and therefore positive findings. (Rex et al., 2012; Pai et al., 2019; Pai et al., 2019) Recalibrating 'positive' outcomes to include polyps considered significant based on current evidence provides a clearer understanding of stool test outcomes. This reassessment also aligns the definition of positive results following a positive stool test with the findings considered positive at colonoscopy without a preceding stool test, and can therefore better inform clinical practice.

A common concern among patients and physicians who order stool tests is the yield or findings on colonoscopy. While specificity and false positive rate provide important information about the test, the false

Table 3
Most advanced colonoscopy findings in adult patients in the New Hampshire Colonoscopy Registry having colonoscopy after a positive stool test (2015–2020), with DeeP-C classification.

Most advanced finding on exam	Mt-sDNA (N = 549)		FIT (N = 410)		DeeP-C* classification
	N	% (95% CI)	N	% (95% CI)	
Colorectal cancer	9	1.6 (0.58—2.70)	8	2.0 (0.61—3.29)	Positive colonoscopy
Adenoma with high-grade dysplasia	17	3.1 (1.65—4.55)	8	2.0 (0.61—3.29)	
Villous or tubulovillous adenoma	36	6.6 (4.49—8.63)	22	5.4 (3.18—7.55)	
Conventional adenoma or serrated lesion>=10 mm	92	16.8 (13.63—19.88)	37	9.0 (6.25—11.80)	
1–2 non-advanced adenomas or serrated polyps 5—10 mm	101	18.4 (15.16—21.64)	61	14.9 (11.43—18.32)	Negative colonoscopy
3 or more non-advanced adenomas or serrated polyps < 10 mm	39	7.1 (4.95—9.25)	27	6.6 (4.18—8.99)	
1–2 non-advanced adenomas or serrated polyps < 5 mm	73	13.3 (10.46—16.14)	52	12.7 (9.46—15.90)	
Only hyperplastic polyp(s) < 10 mm	58	10.6 (7.99—13.14)	29	7.1 (4.59—9.55)	
Negative (normal) exam	124	22.6 (19.09—26.08)	166	40.5 (35.74—45.24)	

mt-sDNA: multi-target stool DNA test, FIT: Fecal Immunochemical Test;
* DeeP-C: Detection of colorectal advanced adenomatous Polyps and Cancer[3].

Table 4
Multiplicity and synchronicity of adenomatous and serrated polyps in adult patients in the New Hampshire Colonoscopy Registry having colonoscopy after a positive stool test (2015–2020), in colonoscopies classified as negative according to DeeP-C* criteria.

Exam finding	Mt-sDNA (N = 549)		FIT (N = 410)	
	N	% (95% CI)	N	% (95% CI)
Count of tubular adenomas (TAs)				
Five to ten TAs < 10 mm	16	4.1 (2.40–5.70)	11	3.3 (1.56—5.01)
Three or four TAs < 10 mm	36	9.1 (6.71–11.52)	22	6.6 (4.17—8.96)
One or two TAs < 10 mm	134	33.9 (29.96–37.88)	89	26.6 (22.29—30.84)
Count of sessile serrated polyps				
Five to ten SSPs < 10 mm	4	1.0 (0.18–1.85)	1	0.3 (-0.23—0.83)
Three or four SSPs < 10 mm	15	3.8 (2.20–5.40)	0	0.0 (0.00 – 0.00)
One or two SSPs < 10 mm	39	9.9 (7.38–12.37)	31	9.3 (6.45—12.06)
Synchronicity of TAs and Clinically Significant Serrated Polyps (CSSPs)				
Both CSSP and TA	35	8.9 (6.48—11.24)	17	5.1 (2.95—7.20)
CSSP without TA	31	7.8 (5.60—10.10)	19	5.7 (3.43—7.91)
no CSSP	329	83.3 (80.17—86.41)	299	89.3 (86.26—92.25)

mt-sDNA: multi-target stool DNA test, FIT: Fecal Immunochemical Test;
* DeeP-C: Detection of colorectal advanced adenomatous Polyps and Cancer[3].

discovery rate (FDR) and 1-FDR or positive predictive value (PPV) provide data that may be more useful in clinical practice, where only patients with positive tests have a subsequent colonoscopy. It is important to know the probability of a clinically meaningful outcome on a colonoscopy following a positive stool test. False discovery rate and false positive rate are two ratios that are transposed conditional probabilities and are superficially easy to confuse, especially in the context of population-based studies. Previous studies have calculated the false discovery rate or rate of type I error but did not label it as FDR in the paper. (Imperiale et al., 2014) Others use the term "false positive rate" to refer to the rate of type I errors or 1-PPV - what we have more precisely called the false discovery rate. (Clarke et al., 2006; Malagón et al., 2020).

To assess the proportion of false positives out of all positive stool tests, we investigated false discovery rates using three categorizations of significant (or 'positive') polyps: A) the DeeP-C definition used in the primary analysis from the pivotal Imperiale mt-sDNA study,

B) DeeP-C positives plus findings that have USMSTF recommendations of < 10 year follow-up, C) the USMSTF < 10 year definition plus all CSSPs. Additionally, we investigated the frequency of multiple small polyps and of synchronous adenomatous and serrated small polyps, respectively, which could be considered highly significant findings. Understanding the frequencies of different findings and the resulting false discovery rates for stool tests can inform physicians about how best to screen and follow their patients.

As outlined in the name of the protocol defining the primary analysis for the Imperiale et al study, "***Dete***ction of colorectal *advanced adenomatous **P**olyps* and ***Cancer***", or DeeP-C, this classification of positive colonoscopy is centered around advanced adenomas and CRC. It also included serrated polyps >=1 cm, which have been associated with increased long term risk for CRC. (Holme et al., 2015) However, the primary outcome did not include adenomas < 1 cm (even if there were multiple), nor small SSPs or TSAs, both of which are now understood to be associated with a long term risk of CRC. (Erichsen et al., 2016; Li et al., 2022) Any number of these small polyps were included in a secondary outcome as an additional "non-advanced lesion" category. Notably, a number of patients with 'negative' exams according to DeeP-C had multiple SSPs (6.8% mt-sDNA + and 2.4% FIT +), which confer an increased CRC risk, (Erichsen et al., 2016; Li et al., 2022) and could also reflect serrated polyposis syndrome, also associated with an increased CRC risk. (Anderson and Srivastava, 2020) The DeeP-C 'true positive' definition also excluded proximal HPs ≥ 5 mm; which may represent misclassified SSPs (Anderson et al., 2018) and be equally as predictive of future large serrated polyps as SSPs ≥ 1 cm, which DeeP-C classified as positive findings. (Anderson et al., 2020).

When including lesions with < 10 year USMSTF-recommended follow-up, the false discovery rate decreased from 72% to 33.2% (a 53.9% decrease) in mt-sDNA + patients and from 81.7% to 47.6% (a 41.8% decrease) in FIT + patients (Table 2). The addition of CSSPs decreased the false discovery rate further (Table 2). Correspondingly, when including USMSTF polyps meriting closer follow-up, the PPV increased from 28% to 67% for mt-sDNA+, and from 18 % to 52% for FIT +. Thus, PPV is almost 70% for mt-sDNA and just over 50% for FIT, when using the more clinically relevant definition of positive colonoscopy findings. These results demonstrate the importance of classifying all potentially precancerous polyps as positive findings in order to better understand expected outcomes of positive stool tests.

Two other important polyp outcomes associated with increased CRC risk should be considered in assessments of positive colonoscopy outcomes: synchronous adenomas and serrated polyps, and multiple small

polyps (multiplicity). Patients with synchronous adenomatous and serrated polyps have been shown to be at higher risk for advanced neoplasia than those with either serrated or adenomatous polyps alone. (Anderson et al., 2018) Nearly one in ten (8.9%) mt-sDNA + patients and 5.1% of FIT + patients with negative exams as per DeeP-C criteria had both CSSPs and tubular adenomas (Table 4).

The increased detection in both the mt-sDNA + and FIT + cohorts using a broader definition of 'positive colonoscopy findings' reflects the inclusion of small non-advanced adenomas as well as serrated polyps. In particular, mt-sDNA + is associated with more serrated polyps compared to FIT + patients. However, patients with either positive test are more likely to have small adenomas. While these small adenomas may not have a similar risk for future colorectal neoplasia as advanced adenomas, published data indicate that individuals with these index lesions also have an increased risk for future advanced adenomas. (Cheng et al., 2023; Hartstein et al., 2020; Jung et al., 2020; Kim et al., 2019; Kim et al., 2020).

Resection of adenomas, even small, is an important part of CRC screening as evident by the inverse relationship between adenoma detection rate (proportion of screening colonoscopies per endoscopist with at least one adenoma of any size) and risk for post colonoscopy CRC. (Corley et al., 2014) Furthermore, many experts believe that another metric, APC, which measures the number of adenomas of any size per colonoscopy, may be superior in differentiating endoscopists' ability to prevent CRC. (Rex, 2020).

From a clinical standpoint, adenomas < 1 cm should sometimes be considered significant findings, particularly when more than one is present. Furthermore, it is recognized that multiple small adenomas confer greater risk than 1–2 adenomas, leading to the USMulti-Society Task Force (USMSTF) guidelines recommending shorter intervals for colonoscopy following findings of 3 or more adenomas. (Gupta et al., 2020) It is also well known that 5–10 small tubular adenomas (TAs) found on a single exam are associated with significantly increased CRC risk, for which the USMSTF recommends colonoscopy follow-up in 3 years[7]. In contrast, early assessments of false discovery included 5–10 TAs < 10 mm within the 'non-advanced' group classified as negative colonoscopy. Furthermore, several studies suggest that 5–9 mm adenomas confer an increased risk compared to diminutive (<5 mm) adenomas. (Cheng et al., 2023; Hartstein et al., 2020; Jung et al., 2020; Kim et al., 2019; Kim et al., 2020; Anderson et al., 2019) Five to ten small TAs were found in 4.1% of mt-sDNA + exams and 3.3% of FIT + exams classified as negative according to DeeP-C.

In addition, the cumulative number of small adenomas can be the only clue for the endoscopist that the patient may have a polyposis syndrome. This is especially crucial since up to 1/3 of these syndromes may be de novo and therefore an increased cumulative number of small adenomas may be the only indication of a genetic syndrome since family history will be unremarkable. (Syngal et al., 2015).

It was the intent of the current analysis to compare outcomes for primary screening colonoscopies to outcomes for colonoscopies following positive stool tests, using the current definitions for positive outcomes at primary screening colonoscopy described above. While we examined morphological colonoscopy outcomes, which is the accepted outcomes measure, molecular profiles of polyps would provide an important investigation of the risk posed by various polyps, and expanding existing evidence on that topic would significantly contribute to our understanding of test effectiveness. It should be recognized that some small adenomas might be coincidental colonoscopy findings in patients with positive stool tests, rather than the reason that the stool test was positive.

Our data have several implications for endoscopists and their patients. NHCR results, while representative of one state, are population-based and prospectively collected, and present valuable information that can be further assessed in more racially diverse populations. We report outcomes from a cohort of individuals *who had a positive stool test* followed by a colonoscopy *in the course of usual practice*. While other studies have reported on the diagnostic yield of colonoscopy after positive stool tests, to our knowledge there are no larger cohorts that report on the yield of colonoscopy after positive stool tests using different, increasingly inclusive categories of polyps that are clinically considered to be "positive findings".

Understanding of the increased risk of specific polyp types has evolved over the past decade, with new categories of polyps recognized as requiring more intensive surveillance as more evidence becomes available. Newer evidence prompts reconsideration of the outcomes of positive stool tests, including considerations of the risks conferred by synchronicity and multiplicity of polyps. A more current perspective might suggest that polyps associated with any increase in CRC risk should be considered clinically meaningful results, rather than *false* positive outcomes. Our analysis presents a more comprehensive understanding of false positive outcomes at colonoscopies following positive stool tests, and to our knowledge is the first such assessment of these outcomes. These data should serve to better inform the application, interpretation, and management of stool-based CRC screening results.

CRediT authorship contribution statement

Lynn F. Butterly: Supervision, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **William M. Hisey:** Validation, Software, Methodology, Investigation, Formal analysis, Data curation. **Christina M. Robinson:** Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization, Data curation. **Paul J. Limburg:** Methodology, Conceptualization. **Bonny L. Kneedler:** Formal analysis, Data curation. **Joseph C. Anderson:** Investigation, Supervision, Conceptualization, Methodology.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that has been used is confidential.

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