BMJ Open

Priority stratification for precedence colonoscopy based on two-sample faecal immunochemical test (FIT) screening

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2020-046055
Article Type:	Original research
Date Submitted by the Author:	22-Oct-2020
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Keywords:	Gastrointestinal tumours < GASTROENTEROLOGY, Endoscopy < GASTROENTEROLOGY, Gastroenterology < INTERNAL MEDICINE, PREVENTIVE MEDICINE, Protocols & guidelines < HEALTH SERVICES ADMINISTRATION & MANAGEMENT

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ORIGINAL ARTICLE

TITLE

Priority stratification for precedence colonoscopy based on two-sample faecal immunochemical test (FIT) screening

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Competing interests: None.

Funding: The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Ethics approval: [review board] Certificated Review Board, Hattori Clinic (certification no. CRB3180027), [approval date] September 6th, 2019, [approval no.] S1909-U06, [registration site] UMIN-CTR system (https://rctportal.niph.go.jp/en/detail?trial_id=UMIN000018541), [registration no.] UMIN000018541.

Informed consent statement: Written informed consents were obtained from the participants.

Patient and public involvement: Patients and/or the public were involved in the design, or conduct, or report, or dissemination plans of this research. Information on the publication of this study will be provided to the patients on the website of our clinic (https://www.ichou.com).

Provenance and peer review: Not commissioned; externally peer reviewed.

Data availability statement: Data are available upon reasonable request. Additional data can be obtained by emailing OT at t@ichou.com. All requests for data sharing should be discussed with OT at the first instance.

STROBE statement: The authors have read the STROBE Statement checklist of items, and the manuscript was prepared and revised according to the STROBE Statement checklist of items.

Running title: Triage by 2-sample FIT

Manuscript word count: 2466 words

Keywords: hemoccult; colorectal cancer; colorectal cancer screening; colonoscopy; cancer prevention

ABSTRACT

Objective Little has been reported on the yield and characteristics of colorectal neoplasia detected by the 2-sample faecal immunochemical test (FIT), particularly the difference between subjects with 2-positive results on the 2-sample FIT and those with 1-positive results. We aimed to assess risk stratification amongst patients with positive 2-sample FIT to prioritise colonoscopy.

Design Consecutive patients who underwent colonoscopy at our institute were enrolled. The indications for colonoscopy included 2-positive results on the 2-sample FIT (FIT (2+)), the other patterns of positive FIT results (FIT (+)), and other reasons (non-FIT group, including presence of symptoms, screening, or surveillance). The detection rates of colorectal cancers, including in situ cancers (all cancers) and invasive cancers, based on the indication for colonoscopy, were investigated.

Results Of the 9147 patients, 264 underwent colonoscopy following FIT (2+), 1441 following FIT (+), and 7442 for reasons other than positive FIT. Detection rates of all (and invasive) cancers in the FIT (2+), FIT (+), and non-FIT groups were 12.1% (8.3%), 1.9% (0.5%), and 0.4% (0.2%), respectively. The cancer detection rates were much higher in the FIT (2+) group than in the FIT (+) group, which in turn had higher rates than the non-FIT group. Moreover, the FIT (2+) group showed more advanced T stages on TNM classification (Tis/T1/T2/T3/T4: 10/7/4/10/1) than the FIT (+) group (20/3/2/1/1, $P<.001$).

Conclusions 2-positive results for 2-sample FIT showed a much higher yield for more advanced

colorectal cancers than the 1-positive result. High priority for diagnostic colonoscopy should be assigned to patients with 2-positive-FIT results.

(252 words)

Strengths and limitations of this study

- This study shows real-world data on 2-sample FIT in Japan, where 2-sample FIT-based colorectal screening has been conducted for many years throughout the country.
- This study shows that 2-sample FIT can be useful in triage of patients for diagnostic colonoscopy depending on the number of positive results.
- This study also suggests that 2 positive results from 2-sample FITs may be useful for colorectal cancer screening in younger patients below 50 years of age.
- The cross-sectional design at a single endoscopy clinic was a limitation.
- We could not assess the faecal haemoglobin concentration and the patients' symptoms in detail.

INTRODUCTION

Colorectal cancer is one of the leading cancers worldwide, with 1.8 million new cases and 860,000 deaths annually, and has a significant impact on public health.[1] Screening for colorectal cancer has shown significant effects on reducing the morbidity and mortality, and is also economical.[2] There are several options for colorectal cancer screening, such as primary colonoscopy, sigmoidoscopy, and stool-based tests.[2] Amongst stool-based tests, the faecal immunochemical test (FIT) is now widely used instead of the guaiac faecal occult blood test, because of its higher accuracy and ease of handling.[3,4] Although its accuracy is limited compared to that of primary colonoscopy, FIT is noninvasive and can conserve the resources required for colonoscopy and reduce human contact. Hence, FIT might facilitate the safety and prioritisation of patients during the COVID-19 pandemic.[5]

In Japan, the population-based annual 2-sample FIT has been used for colorectal cancer screening for three decades since 1992.[6] For implementation and effectiveness, the number of FIT samples required, the interval between two FITs, and the FIT brands have been estimated.[4] The 2-sample method has been reported to have the best sensitivity and specificity for colorectal cancer.[3,7] Some investigators also reported that the sensitivity for advanced neoplasia was higher by using the 2-sample method than by the 1-sample method.[8,9]

At least 1-positive result is defined as a positive result in the 2-sample FIT method.[3,7-9] Few studies have investigated the yield and characteristics of neoplasia detected by 2-sample FIT.[8-10] In particular, little is known about the differences between the subjects with 2-positive results in the 2-

sample FIT and those with 1-positive result.[11,12] In this study, we investigated the detection rates and features of invasive and in situ colorectal cancers detected by colonoscopy at our institution based on the indication for colonoscopy, focussing on the positivity patterns in the 2-sample FIT.

METHODS

Study design

This cross-sectional study included consecutive patients who underwent colonoscopy at the Toyoshima Endoscopy Clinic from April 2017 to August 2019. The indications for colonoscopy included a positive FIT result, evaluation of symptoms, screening, surveillance, and treatment. Samples for FIT measurements were collected from two consecutive bowel movements. We divided the patients who were FIT positive into two categories: FIT (2+) and FIT (+). We defined 2-positive results for 2 samples as FIT (2+) and the other positive FIT results (i.e., 1-positive result for 1 sample, 1-positive result for 2 samples, or unknown number-positive results for 2 samples) as FIT (+). The results of FIT were based on the test conducted at our clinic or at the referral medical institutions. The symptoms included abnormal bowel habits, hematochezia, and abdominal pain. The surveillance included patients with a medical history of colorectal cancer, colorectal polyps, or inflammatory bowel diseases. Treatment involved polypectomy and haemostasis. We excluded colonoscopies performed for treatment from this study. All indications other than positive FIT were divided into two categories: symptoms and screening + surveillance (asymptomatic).

Ethics

This study was approved by the Certificated Review Board, Hattori Clinic on September 6th, 2019 (approval no. S1909-U06, registration no. UMIN000018541). Written informed consent was obtained from the patients. All clinical investigations were conducted according to the ethical guidelines of the Declaration of Helsinki.

Colonoscopy

Colonoscopies were performed by certified gastroenterologists. Olympus Elite 290 endoscope series (Olympus, Tokyo, Japan) was used.[13,14] The clinical data were recorded on an electronic endoscopy reporting system, T-File System (STS Medic, Tokyo, Japan). The data included the patients' baseline characteristics (age, sex, and indication for colonoscopy) and tumour characteristics (location and size).

All colonoscopists were instructed to observe the entire colorectum, with a withdrawal time of 6 minutes or longer.[15,16] Polyps 15 mm in size or smaller were resected at the time of the examination, and if the polyp was larger than 15 mm, or if invasive cancer was suspected, the patient was referred to the hospital.[17]

Colorectal cancer

Colorectal cancer was treated by endoscopic resection, surgery, chemotherapy, and/or best

supportive care. The patients received treatment at our clinic or at the hospital they were referred to. Colorectal cancer was diagnosed by histopathology. The location of cancer was determined by colonoscopy, surgery, or CT. The location from the caecum to the transverse colon was defined as proximal colon. The size of the cancer was measured by colonoscopy, pathology, or CT. The extent of tumour invasion was determined by pathology in combination with colonoscopy and CT findings. Tumours were classified according to the T stage of the UICC TNM classification.[18] We included noninvasive carcinoma (carcinoma in situ) as a cancer.[19] The histological subtype of the cancer was determined by histopathological evaluation of the resected or biopsy specimens. Four histological subtypes of adenocarcinomas (i.e., well-differentiated, moderately-differentiated, poorly-differentiated, and mucinous adenocarcinoma) were classified into two categories: well- + moderately-differentiated and poorly-differentiated + mucinous adenocarcinoma based on the prognosis of the subtypes.[20]

Outcomes

The main outcomes were detection rates of all colorectal cancers (including carcinomas in situ) and those of invasive colorectal cancers, based on the indication for colonoscopy. The secondary outcomes were the features of the cancers, such as location, size, T stage, and histological subtype. We also divided the patients into 2 groups according to age: <50 years and ≥50 years, and analysed the detection rates and features of the cancers.

Statistical analysis

The cancer detection rates were compared using the chi-squared test or Fisher's exact test. The characteristics of the cancer were compared using the Mann-Whitney U test, chi-squared test, or Fisher's exact test. The association between the T stages of colorectal cancers and the number of positive results of FIT was analysed using Spearman's rank correlation test. A two-sided *P* value <.05 was considered statistically significant. Statistical analyses were performed using IBM SPSS version 21.0 (IBM SPSS, Armonk, NY).

Patient and public involvement

Patients and/or the public were involved in the design, or conduct, or report, or dissemination plans of this research. Information on the publication of this study will be provided to the patients on the website of our clinic (https://www.ichou.com).

RESULTS

Characteristics of the study patients

During the study period, 9321 patients underwent colonoscopy. Of these, 174 patients were excluded because they underwent colonoscopy for treatment. Finally, 9147 patients (age, mean ± SD: 53.6 ± 12.5 years; male sex: 49.6%) were eligible for this study. In all, 1705 colonoscopies were

performed following positive FIT results. Of the positive FIT results, 264 were FIT (2+) and 1441 were FIT (+). The remaining 7442 colonoscopies performed for indications other than positive FIT included 1826 for evaluation of the symptoms, and 5616 for screening (2394) + surveillance (3222), all these were performed without FIT investigation (**Table 1)**.

Cancer detection rates based on the indication for colonoscopy

The detection rates of colorectal cancer based on the indication for colonoscopy are shown in **Table 2**. All colorectal cancers (including carcinoma in situ) and invasive cancers were detected in 1.0% (87/9147 cases) and 0.4% (41 cases) of patients included in the study. The detection rates of all cancers and invasive cancers in FIT-positive patients were 3.5% (59/1705 cases) and 1.7% (29 cases), which were significantly higher than those detected patients who did not undergo FIT (0.4% for all cancers, 28/7442, $P<.001$; 0.2% for invasive cancers, 12 cases, $P<.001$).

Amongst FIT-positive patients, the rate of detection of all cancers in the FIT (2+) group was very high at 12.1% (32/264 patients) and that in the FIT (+) group was 1.9% (27/1441 patients). Invasive cancers accounted for 8.3% (22 cases) in the FIT (2+) group and 0.5% (7 cases) in the FIT (+) group. FIT (2+) had significantly higher detection rates than FIT (+) ($P<.001$ and $P<.001$).

Amongst patients who did not undergo FIT, the cancer detection rates in symptomatic patients were significantly higher than in asymptomatic patients, in whom colonoscopy was performed for screening and surveillance (0.8% and 0.2% for all cancers, $P<.001$, 0.4% and 0.1% for invasive

cancers, *P*=.01 respectively).

Additionally, the rate of cancer detection was significantly higher in patients with FIT (+) than in those with symptoms (1.9% and 0.8%, *P*<.001).

Cancer detection rates based on age and FIT positivity groups

Table 3 shows the rates of detection for colorectal cancer based on age group and FIT positivity patterns.

In patients aged ≥50 years, the FIT (2+) group showed the highest rate of cancer detection (12.9% for all cancers and 8.6% for invasive cancers). For the FIT (+) group, the respective rates were 3.5% and 1.0%, which were significantly lower than those in the FIT (2+) group (*P*<.001 and *P*<.001), suggesting that early stage cancers are more predominant.

In the <50 years age group as well, the rate of cancer detection was higher in the FIT (2+) group (11.3% for all cancers and 8.1% for invasive cancers). They were comparable to those in the ≥50 age group. However, the rates of detection in FIT (+) group were low (0.4% and 0.0%); moreover, they were lower than those in the same FIT (+) group at age ≥50 years (*P*<.001 and *P*<.001).

The features of the colorectal cancers

Table 4 shows the features of colorectal cancers based on the indication for colonoscopy. The colorectal cancers in patients with FIT (2+) were larger than those in the FIT (+) patients (31.2 mm

and 21.0 mm, *P*=.006). The T stage of FIT (2+) colorectal cancer was more advanced than that of the FIT (+) cancers (*P*<.001). Although cancers were generally likely to be located in the distal colon or rectum, the cancers detected during screening and surveillance colonoscopies were predominantly in the proximal colon (proximal colon/distal colon and rectum: 7/6 for screening and surveillance vs. 17/57 for the others, *P*=.04).

The cancer detection rates stratified by T stage based on age (≥50 and <50 years) and FIT positivity patterns (FIT (2+) and FIT (+)) are shown in **Figure 1**. FIT (2+) patients had predominantly more advanced cancers than the FIT (+) patients (Tis/T1/T2/T3/T4 for FIT [2+] vs. FIT [+]: 6/2/3/7/0 vs. 17/3/2/1/1 in ≥50 years: *P*=.008; 4/5/1/3/1 vs. 3/0/0/0/0 in <50 years: *P*=.04).

DISCUSSION

This study found that cases with 2-positive FIT results in 2 samples had significantly high rates of more advanced-stage colorectal cancers amongst all cases with positive FIT results. Although FIT has been an important screening tool for colorectal cancer and can help in selecting candidates for diagnostic colonoscopy, patients with 2-positive results were shown to be at the highest risk for life-threatening cancer. In the face of the COVID-19 pandemic, when resources for colonoscopy are limited, FIT can stratify the patients' risk. The study outcomes indicate that those with FIT (2+) should be given the highest priority for colonoscopy.

Although the sensitivity of FIT is superior to that of the guaiac test,[3,4] the sensitivity is lower for early stage cancer or high-grade dysplasia. Morikawa *et al*.[21] compared the results of 1-sample FIT and total colonoscopy in asymptomatic Japanese patients and reported that the sensitivity for invasive cancer was 78.3% (18/23) for Dukes' stages C or D, 70.0% (7/10) for Dukes' stage B, and 52.8% (19/36) for Dukes' stage A, and that for high-grade dysplasia was 32.7% (39/119). A similar study from Taiwan reported that the 1-sample FIT showed a sensitivity of 100% (5/5) for cancers in T2-4 stages and 66.7% (12/18) for those of Tis or T1.[22] The 2-sample method was adopted to improve the sensitivity of FIT.[3,7-9]

A simulation analysis based on the results of colonoscopic screening in the Japanese subjects predicted markedly higher positive predictive values (PPVs) for invasive cancers in patients with 2 positive FIT results.[23] The PPVs were estimated to be 1.7% and 26% for male subjects in their 50s with 1 and 2 positive FITs, respectively. PPVs could increase because of lower rates of false positivity in cases with 2 positive FITs. The effect of improving the sensitivity could be higher for early stage lesions than for advanced cancers, for which the sensitivity is already high in single-sample FIT. One positive result in two samples was predicted to detect predominantly earlier-stage lesions. Few investigators have reported the actual findings of colonoscopy comparing one and two positive FITs in 2-sample FIT screening. Our result is compatible with a previous Canadian study, which reported PPVs for colorectal cancer to be 1% and 8%, in patients with 1 and 2 positive FITs, respectively.[11] A recent study from the Netherlands suggested that 2-positive FITs from two samples

of the same bowel movement also have high cancer detection rates.[12] The present study showed more advanced stages of colorectal cancers were predominant in patients with 2-positive FITs.

The FIT (+) group showed higher rates of cancer detection than those in whom the colonoscopy was performed for evaluation of symptoms, screening, or surveillance. This might be partly because the patients' symptoms at our clinic were generally mild. Although further evaluations are necessary, FIT might be helpful in making decisions about performing colonoscopy in symptomatic patients[24] or at the time of surveillance for patients after polypectomy.[25]

Even in patients <50 years of age, those with FIT (2+) showed negligible rates of colorectal cancers, and in those with FIT (+), the rates were very low. Our results suggest that patients under 50 years of age with 2 positive FITs might need to receive a higher priority for colonoscopy than those over 50 years with 1 positive FIT. There is some discussion as to whether colorectal cancer screening should be started for subjects under 50 years of age, in whom the incidence of colorectal cancer is quite low but is increasing.[26] If they were screened by 2 positive results from 2 sample FITs, the cost-benefit balance might be acceptable.

Limitations and Strengths

This study has several limitations. First, it was conducted at a single endoscopy unit; hence, the results cannot be generalised. However, the indications and quality of colonoscopy as well as the criteria for diagnosis were well controlled. Two-sample FIT-based colorectal screening has been

conducted for many years throughout Japan. Our results could well represent the regular practice of colorectal screening in Japan. Second, since our institute is specialised in endoscopies, many patients were referred from other medical institutions for colonoscopy. The category of FIT (+) included various categories of positivity for FIT: 1-positive result in 2 samples, 1-positive result in 1 sample, unknown number-positive results in 2 samples, and so on. The brand names of FIT kits or cutoff values for positivity were also unknown in many cases. However, a similar trend was seen when patients with 1-positive result in 2 samples from the FIT (+) group were separately analysed (**Supplemental Table 1**). Third, we did not assess the patients' symptoms in detail, as cancer detection rates were low in symptomatic patients without FIT evaluation. However, the symptoms in our patients were generally mild. In populations with more serious symptoms, they could also be useful to urge early colonoscopy.[27]

In conclusion, 2-positive results for 2 samples of FIT showed a much higher yield of advanced colorectal cancers than the 1-positive result, which also showed a higher yield than colonoscopy performed in patients with symptoms or with an associated history. The highest priority for diagnostic colonoscopy should be assigned to patients with 2-positive-FIT results.

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TABLES

Table 1. Study patients.

	Total	Positive FIT			Other than positive FIT		
		Total	FIT (2+)	FIT (+)	Total	Symptom	Screening + surveillance
No.	9147	1705	264	1441	7442	1826	5616
Age, mean (SD), years	53.6 (12.5)	50.7 (12.3)	52.7 (13.7)	50.3 (12.0)	54.2 (12.4)	49.2 (13.4)	55.8 (11.6)
Male, n (%)	4534 (49.6)	797 (46.7)	144 (54.5)	653 (45.3)	3737 (50.2)	722 (39.5)	3015 (53.7)

Abbreviation: FIT, faecal immunochemical test. FIT (2+) indicates 2-positive FIT results. FIT (+) indicates positive FIT results other than FIT (2+).

Table 2. Detection rates of colorectal cancer based on the indication for colonoscopy.

	Total	Positive FIT			Other than positive FIT		
		Total	FIT (2+)	FIT (+)	Total	Symptom	Screening + surveillance
No.	9147	1705	264	1441	7442	1826	5616
All cancers (including in situ), n	87	59	32	27	28	15	13
Detection rate	1.0%	3.5%[a]	12.1%[b]	1.9%[c]	0.4%	0.8%[d]	0.2%
Invasive cancers, n	41	29	22	7	12	7	5
Detection rate	0.4%	1.7%[a]	8.3%[b]	0.5%	0.2%	0.4%[e]	0.1%

Abbreviation: FIT, faecal immunochemical test. FIT (2+) indicates 2-positive FIT results. FIT (+) indicates positive FIT results other than FIT (2+).

[a] $P<.001$, Positive FIT vs. Other than positive FIT.

[b] $P<.001$, FIT (2+) vs. FIT (+).

[c] $P=.008$, FIT (+) vs. Symptom.

[d] $P<.001$, Symptom vs. Screening + surveillance.

[e] $P=.01$, Symptom vs. Screening + surveillance.

Table 3. Colorectal cancer detection rates based on age and FIT positivity groups.

	Age ≥50 years			Age <50 years		
	Total	FIT (2+)	FIT (+)	Total	FIT (2+)	FIT (+)
No.	829	140	689	876	124	752
All cancers (including in situ), n	42	18	24	17	14	3
Detection rate	5.1%[a]	12.9%[b]	3.5%[a]	1.9%	11.3%[b]	0.4%
Invasive cancers, n	19	12	7	10	10	0
Detection rate	2.3%[a]	8.6%[b]	1.0%[c]	1.1%	8.1%[b]	0.0%

Abbreviation: FIT, faecal immunochemical test. FIT (2+) indicates 2-positive FIT results. FIT (+) indicates positive FIT results other than FIT (2+).

[a] $P<.001$, Age ≥50 years vs. Age <50 years.

[b] $P<.001$, FIT (2+) vs. FIT (+).

[c] $P=.006$, Age ≥50 years vs. Age <50 years.

Table 4. Features of colorectal cancers based on indication for colonoscopy.

	Total	Positive FIT		Other than positive FIT	
		FIT (2+)	FIT (+)	Symptom	Screening + Surveillance
No.	87	32	27	15	13
Location (Proximal/Distal), n	24/63	10/22[a]	6/21[a]	1/14[a]	7/6[a]
Size (SD), mm	26.5 (21.8)	31.2 (22.7)[b]	21.0 (22.0)[b]	30.0 (20.4)	22.0 (19.8)
T stage (Tis/T1/T2/T3/T4), n	46/14/8/16/3	10/7/4/10/1[c]	20/3/2/1/1[c]	8/1/1/4/1	8/3/1/1/0
Histological subtype (Well+Mod/Por+Muc), n	82/4	30/2	27/0	13/1	12/1

Abbreviation: FIT, faecal immunochemical test. FIT (2+) indicates 2-positive FIT results. FIT (+) indicates positive FIT results other than FIT (2+). "Proximal" indicates from the caecum to the transverse colon and "Distal" indicates from the descending colon to the rectum. SD: standard deviation. "T stage" of the tumour was based on the UICC TNM Classification. "Well+Mod" indicates well- and moderately-differentiated adenocarcinoma. "Por+Muc" indicates poorly-differentiated and mucinous adenocarcinoma. One squamous cell carcinoma was excluded from this analysis.

[a] P=.04, Amongst indication groups.

[b] P=.006, FIT (2+) vs. FIT (+).

[c] P<.001, FIT (2+) vs. FIT (+).

FIGURE LEGEND

Figure 1. Cancer detection rates stratified by T stages based on age and FIT positivity groups.

T stage was classified according to the UICC TNM Classification.

FIT (2+) had a higher percentage of invasive cancers than in FIT (+) in both age groups.

Abbreviation: FIT, faecal immunochemical test. FIT (2+) indicates 2-positive FIT results. FIT (+) indicates positive FIT results other than FIT (2+).

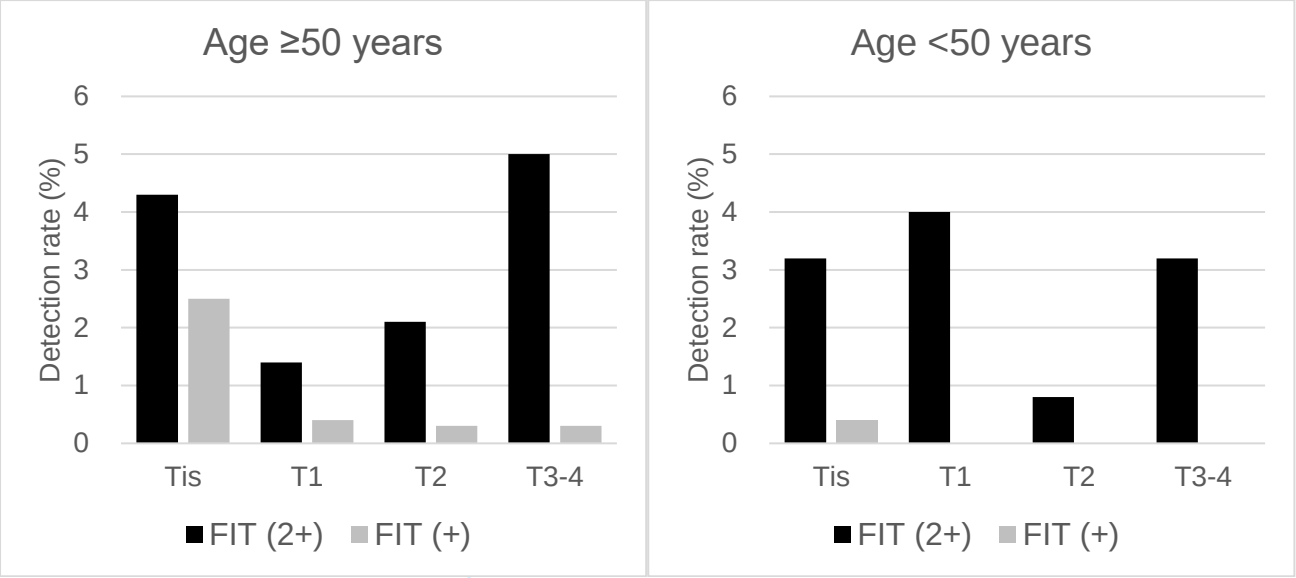

Figure 1.

Supplemental Table 1. Details of the FIT (+) groups.

	FIT (2+)	FIT (+/-)	FIT (+/?)
No.	264	1018	423
Age, mean (SD), years	52.7 (13.7)	50.3 (11.9)	50.3 (12.3)
Male, n (%)	144 (54.5)	469 (46.1)	184 (43.5)
All cancers (including in situ), n	32	19	8
Detection rate	12.1%	1.9%	1.9%
Invasive cancers, n	22	3	4
Detection rate	8.3%	0.3%	0.9%
Age ≥50 years			
No.	140	490	199
All cancers (including in situ), n	18	17	7
Detection rate	12.9%	3.5%	3.5%
Invasive cancers, n	12	3	4
Detection rate	8.6%	0.6%	2.0%
Age <50 years			
No.	124	528	224
All cancers (including in situ), n	14	2	1
Detection rate	11.3%	0.4%	0.4%
Invasive cancers, n	10	0	0
Detection rate	8.1%	0.0%	0.0%
Features of cancers			
Location (Proximal/Distal), n	10/22	5/14	1/7
Size (SD), mm	31.2 (22.7)	17.4 (9.7)	29.6 (37.9)
T stage (Tis/T1/T2/T3/T4), n	10/7/4/10/1	16/1/2/0/0	4/2/0/1/1
Histological subtype (Well+Mod/Por+Muc), n	30/2	19/0	8/0

Abbreviation: FIT, fecal immunochemical test. FIT (2+) indicates 2-positive results for the 2-sample FIT. FIT (+/-) indicates a 1-positive result for the 2-sample FIT. FIT (+/?) includes a 1-positive result for the 1-sample FIT and unknown number-positive results for the 2-sample FIT. SD: standard deviation. “Proximal” indicates from the cecum to the transverse colon and “Distal” indicates from the descending colon to the rectum. “T stage” of the tumor was based on the UICC TNM Classification. “Well+Mod” indicates well- and moderately-differentiated adenocarcinoma. “Por+Muc” indicates poorly-differentiated and mucinous adenocarcinoma.

BMJ Open

Priority stratification for colonoscopy based on 2-sample faecal immunochemical test screening: results from a cross-sectional study at an endoscopy clinic in Japan

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2020-046055.R1
Article Type:	Original research
Date Submitted by the Author:	30-Mar-2021
Complete List of Authors:	Toyoshima, Osamu; Toyoshima Endoscopy Clinic, Gastroenterology; The University of Tokyo, Department of Gastroenterology Yamaji, Yutaka; Tokyo Pharmaceutical Industry Health Insurance Society, Health Development Center Nishizawa, Toshihiro; Toyoshima Endoscopy Clinic, Gastroenterology; International Medical and Welfare College, Department of Gastroenterology and Hepatology Yoshida, Shuntaro; Toyoshima Endoscopy Clinic, Gastroenterology Yamada, Tomoharu; The University of Tokyo, Department of Gastroenterology; Toyoshima Endoscopy Clinic, Gastroenterology Kurokawa, Ken; The University of Tokyo, Gastroenterology; Toyoshima Endoscopy Clinic, Gastroenterology Obata, Miho; The University of Tokyo, Department of Gastroenterology; Toyoshima Endoscopy Clinic, Gastroenterology Kondo, Ryo; The University of Tokyo, Department of Gastroenterology; Toyoshima Endoscopy Clinic, Gastroenterology Toba, Masahito; Toba Clinic, Gastroenterology Koike, Kazuhiko; The University of Tokyo, Department of Gastroenterology
Primary Subject Heading:	Gastroenterology and hepatology
Secondary Subject Heading:	Health informatics, Health policy, General practice / Family practice
Keywords:	Gastrointestinal tumours < GASTROENTEROLOGY, Endoscopy < GASTROENTEROLOGY, Gastroenterology < INTERNAL MEDICINE, PREVENTIVE MEDICINE, Protocols & guidelines < HEALTH SERVICES ADMINISTRATION & MANAGEMENT

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ORIGINAL ARTICLE

TITLE

Priority stratification for colonoscopy based on 2-sample faecal immunochemical test screening: results from a cross-sectional study at an endoscopy clinic in Japan

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Competing interests: None.

Funding: The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Ethics approval: [review board] Certificated Review Board, Hattori Clinic (certification no. CRB3180027), [approval date] September 6th, 2019, [approval no.] S1909-U06, [registration site] UMIN-CTR system (https://rctportal.niph.go.jp/en/detail?trial_id=UMIN000018541), [registration

no.] UMIN000018541.

Informed consent statement: Written informed consents were obtained from the participants.

Patient and public involvement: Patients and/or the public were not involved in the design, conduct, report, or dissemination of this research.

Provenance and peer review: Not commissioned; externally peer reviewed.

Data availability statement: Data are available upon reasonable request. Additional data can be obtained by emailing OT at t@ichou.com. All requests for data sharing should be discussed with OT at the first instance.

STROBE statement: The authors have read the STROBE Statement checklist of items, and the manuscript was prepared and revised according to the STROBE Statement checklist of items.

Running title: Triage by 2-sample FIT

Manuscript word count: 3139 words

Keywords: hemoccult; colorectal cancer; colorectal cancer screening; colonoscopy; cancer prevention

ABSTRACT

Objectives Little has been reported on the yield and characteristics of colorectal neoplasia detected by the 2-sample faecal immunochemical test (FIT), particularly the difference between subjects with 2-positive results on the 2-sample FIT and those with 1-positive results. We aimed to assess risk stratification amongst patients with positive 2-sample FIT to prioritise colonoscopy.

Design A retrospective cross-sectional study

Setting A single-centre, representative endoscopy clinic in Japan

Participants Consecutive patients who underwent colonoscopy were enrolled. Indications for colonoscopy included 2-positive results on the 2-sample FIT (FIT (+/+)), 1-positive results on the 2-sample FIT (FIT (+/-)), and other reasons (non-FIT group, including presence of symptoms, screening, or surveillance).

Primary and secondary outcome measures Primary outcomes were detection rates of colorectal cancers, including in situ (all cancers) and invasive cancers, based on the indications for colonoscopy. Secondary outcomes were cancer features, such as location, size, T stage, and histological subtype.

Results Of the 9147 patients, 264 underwent colonoscopy following FIT (+/+), 1018 following FIT (+/-), and 7442 for reasons other than positive FIT. Detection rates of all (and invasive) cancers in the FIT (+/+), FIT (+/-), and non-FIT groups were 12.1% (8.3%), 1.9% (0.3%), and 0.4% (0.2%), respectively. The cancer detection rates were much higher in the FIT (+/+) group than in the FIT

(+/-) group, which in turn had higher rates than the non-FIT group. Moreover, the FIT (+/+) group showed more advanced T stages on TNM classification (Tis/T1/T2/T3/T4: 10/7/4/10/1) than the FIT (+/-) group (16/1/2/0/0, *P*<.001).

Conclusions Two-positive results for 2-sample FIT showed a much higher yield for more advanced colorectal cancers than the 1-positive result. High priority for diagnostic colonoscopy should be assigned to patients with 2-positive-FIT results.

(278 words)

Strengths and limitations of this study

- This study shows real-world data on 2-sample FIT in Japan, where 2-sample FIT-based colorectal screening has been conducted for many years throughout the country.
- This study investigated detection rates and features of colorectal cancers in patients with 1- and 2-positive results for the 2-sample FIT.
- This study also evaluated colorectal cancers in patients aged <50 years with 2-positive results for the 2-sample FIT.
- The retrospective cross-sectional design at a single endoscopy clinic was a limitation.
- We could not assess the FIT kit brand, faecal haemoglobin concentration, or patients' symptoms in detail.

INTRODUCTION

Colorectal cancer is one of the leading cancers worldwide, with 1.8 million new cases and 860,000 deaths annually, and has a significant impact on public health.[1] Screening for colorectal cancer has shown significant effects on reducing the morbidity and mortality, and is also economical.[2] There are several options for colorectal cancer screening, such as primary colonoscopy, sigmoidoscopy, and stool-based tests.[2] Amongst stool-based tests, the faecal immunochemical test (FIT) is now widely used instead of the guaiac faecal occult blood test, because of its higher accuracy and ease of handling.[3,4] Although its accuracy is limited compared to that of primary colonoscopy, FIT is noninvasive and can conserve the resources required for colonoscopy and reduce human contact. Hence, FIT might facilitate the safety and prioritisation of patients during the COVID-19 pandemic.[5]

In Japan, the population-based annual 2-sample FIT has been used for colorectal cancer screening for three decades since 1992.[6] For implementation and effectiveness, the number of FIT samples required, the interval between two FITs, and the FIT brands have been estimated.[4] The 2-sample method has been reported to have the best sensitivity and specificity for colorectal cancer.[3,7] Some investigators also reported that the sensitivity for advanced neoplasia was higher by using the 2-sample method than by the 1-sample method.[8,9]

At least 1-positive result is defined as a positive result in the 2-sample FIT method.[3,7-9] Few studies have investigated the yield and characteristics of neoplasia detected by 2-sample FIT.[8-10] In particular, little is known about the differences between the subjects with 2-positive results in the 2-

sample FIT and those with 1-positive result.[11,12] In this study, we investigated the detection rates and features of invasive and in situ colorectal cancers detected by colonoscopy at our institution based on the indication for colonoscopy, focussing on the positivity patterns in the 2-sample FIT.

METHODS

Study design

This cross-sectional study included consecutive patients who underwent colonoscopy at the Toyoshima Endoscopy Clinic from April 2017 to August 2019. The indications for colonoscopy included a positive FIT result, evaluation of symptoms, screening, surveillance, and treatment. Samples for FIT measurements were collected from two consecutive bowel movements. FITs were conducted at our clinic or at referral medical institutions. The FIT kits included both qualitative and quantitative types. The FIT kit brand and cutoff values for positivity were chosen by the institutes conducting the FIT. At our institute, FIT was performed using OC-Auto Sampling Bottle 3 (Eiken Chemical Co., Ltd., Tokyo, Japan) with the threshold of 32 μg haemoglobin/g faeces. We divided the patients who were FIT positive into two categories: FIT (+/+) and FIT (+/-). We defined 2-positive results for 2 samples as FIT (+/+) and 1-positive result for 2 samples as FIT (+/-). Patients with a 1-positive result for the 1-sample FIT and positive FIT results with unknown number of positivity were excluded from this study; these findings are summarised in **Supplementary Table 1**. The symptoms included abnormal bowel habits, haematochezia, and abdominal pain. The surveillance included

patients with a medical history of colorectal cancer, colorectal polyps, or inflammatory bowel diseases. Treatment involved polypectomy and haemostasis. We excluded colonoscopies performed for treatment from this study. All indications other than positive FIT were divided into two categories: symptoms and screening + surveillance (asymptomatic).

Ethics

This study was conducted in accordance with ethical guidelines for medical studies in Japan. Written informed consent was obtained from patients at the time of colonoscopy to use their data for research purposes. The study design was described in a protocol prepared by Toyoshima Endoscopy Clinic and was approved by the Certificated Review Board, Hattori Clinic on 6 September 2019 (approval no. S1909-U06, registration no. UMIN000018541). We published this study's protocol on our institute's website (http://www.ichou.com), so that patients can opt out of the study. All clinical investigations were conducted according to the ethical guidelines of the Declaration of Helsinki.

Colonoscopy

Colonoscopies were performed by certified gastroenterologists. Olympus Elite 290 endoscope series (Olympus, Tokyo, Japan) was used.[13,14] The clinical data were recorded on an electronic endoscopy reporting system, T-File System (STS Medic, Tokyo, Japan). The data included the patients' baseline characteristics (age, sex, and indication for colonoscopy) and tumour

characteristics (location and size).

All colonoscopists were instructed to observe the entire colorectum, with a withdrawal time of 6 minutes or longer.[15,16] Polyps 15 mm in size or smaller were resected at the time of the examination, and if the polyp was larger than 15 mm, or if invasive cancer was suspected, the patient was referred to the hospital.[17]

Colorectal cancer

Colorectal cancer was treated by endoscopic resection, surgery, chemotherapy, and/or best supportive care. The patients received treatment at our clinic or at the hospital they were referred to. Colorectal cancer was diagnosed by histopathology. The location of cancer was determined by colonoscopy, surgery, or CT. The location from the caecum to the transverse colon was defined as proximal colon. The size of the cancer was measured by colonoscopy, pathology, or CT. The extent of tumour invasion was determined by pathology in combination with colonoscopy and CT findings. Tumours were classified according to the T stage of the UICC TNM classification.[18] We included noninvasive carcinoma (carcinoma in situ) as a cancer.[19] The histological subtype of the cancer was determined by histopathological evaluation of the resected or biopsy specimens. Four histological subtypes of adenocarcinomas (i.e., well-differentiated, moderately-differentiated, poorly-differentiated, and mucinous adenocarcinoma) were classified into two categories: well- + moderately-differentiated and poorly-differentiated + mucinous adenocarcinoma based on the

prognosis of the subtypes.[20]

Outcomes

The main outcomes were detection rates of all colorectal cancers (including carcinomas in situ) and those of invasive colorectal cancers, based on the indication for colonoscopy. The secondary outcomes were the features of the cancers, such as location, size, T stage, and histological subtype. We also divided the patients into 2 groups according to age: <50 years and ≥50 years, and analysed the detection rates and features of the cancers.

Statistical analysis

The detection rates were compared using the chi-squared test or Fisher's exact test. The characteristics of the cancer were compared using the Mann-Whitney U test, chi-squared test, or Fisher's exact test. The association between the T stages of colorectal cancers and the number of positive results of FIT was analysed using Spearman's rank correlation test. A two-sided *P* value <.05 was considered statistically significant. Statistical analyses were performed using IBM SPSS version 21.0 (IBM SPSS, Armonk, NY).

Patient and public involvement

Patients and/or the public were not involved in the design, or conduct, or report, or dissemination

plans of this research.

RESULTS

Characteristics of the study patients

During the study period, 9321 patients underwent colonoscopy. Of them, we excluded 174 patients for undergoing colonoscopy for treatment, 136 patients for a 1-positive result for the 1-sample FIT, and 287 patients for positive FIT results with unknown number of positivity. Finally, 8724 patients (age, mean ± SD: 53.7 ± 12.5 years; male sex: 49.9%) were eligible for this study. In all, 1282 colonoscopies were performed following positive FIT results. Of the positive FIT results, 264 were FIT (+/+) and 1018 were FIT (+/-). The remaining 7442 colonoscopies performed for indications other than positive FIT included 1826 for evaluation of the symptoms, and 5616 for screening (2394) + surveillance (3222), all these were performed without FIT investigation (**Table 1)**.

Cancer detection rates based on the indication for colonoscopy

The detection rates of colorectal cancer based on the indication for colonoscopy are shown in **Table 2**. All colorectal cancers (including carcinoma in situ) and invasive cancers were detected in 0.9% (79/8724 cases) and 0.4% (37 cases) of patients included in the study. The detection rates of all cancers and invasive cancers in FIT-positive patients were 4.0% (51/1282 cases) and 2.0% (25 cases), which were significantly higher than those detected patients who did not undergo FIT (0.4%

for all cancers, 28/7442, *P*<.001; 0.2% for invasive cancers, 12 cases, *P*<.001).

Amongst FIT-positive patients, the detection rate of all cancers in the FIT (+/+) group was very high at 12.1% (32/264 patients) and that in the FIT (+/-) group was 1.9% (19/1018 patients). Invasive cancers accounted for 8.3% (22 cases) in the FIT (+/+) group and 0.3% (3 cases) in the FIT (+/-) group. FIT (+/+) had significantly higher detection rates than FIT (+/-) (*P*<.001 and *P*<.001, respectively).

Amongst patients who did not undergo FIT, the cancer detection rates in symptomatic patients were significantly higher than in asymptomatic patients, in whom colonoscopy was performed for screening and surveillance (0.8% and 0.2% for all cancers, *P*<.001; 0.4% and 0.1% for invasive cancers, *P*=.01).

Additionally, the rate of cancer detection was significantly higher in patients with FIT (+/-) than in those with symptoms (1.9% and 0.8%, *P*=.02, respectively).

The detection rate of benign adenomas was significantly higher in the FIT (+/+) group than in the FIT (+/-) group (61.4% vs. 47.7%, *P*<.001). The difference in the detection rates of adenomas between the FIT (+/+) group and the FIT (+/-) group was less remarkable than those of cancers.

Cancer detection rates based on age and FIT positivity groups

Table 3 shows the rates of detection for colorectal cancer based on age group and FIT positivity patterns.

In patients aged ≥50 years, the FIT (+/+) group showed the highest rate of cancer detection (12.9% for all cancers and 8.6% for invasive cancers). For the FIT (+/-) group, the respective rates were 3.5% and 0.6%, which were significantly lower than those in the FIT (+/+) group (P<.001 and P<.001), suggesting that early stage cancers are more predominant.

In the <50 years age group as well, the rate of cancer detection was higher in the FIT (+/+) group (11.3% for all cancers and 8.1% for invasive cancers). They were comparable to those in the ≥50 age group. However, the detection rate in the FIT (+/-) group was low (0.4% for all cancers and 0.0% for invasive cancers, respectively); moreover, the detection rate for all cancers was lower than that in the same FIT (+/-) group at age ≥50 years (P<.001).

The features of the colorectal cancers

Table 4 shows the features of colorectal cancers based on the indication for colonoscopy. The colorectal cancers in patients with FIT (+/+) were larger than those in the FIT (+/-) patients (31.2 mm and 17.4 mm, P=.004). The T stage of FIT (+/+) colorectal cancer was more advanced than that of the FIT (+/-) cancers (P<.001). Although cancers were generally likely to be located in the distal colon or rectum, the cancers detected during screening and surveillance colonoscopies were predominantly in the proximal colon (proximal colon/distal colon and rectum: 7/6 for screening and surveillance vs. 16/50 for the others, P=.046).

The cancer detection rates stratified by T stage based on age (≥50 and <50 years) and FIT

positivity patterns (FIT [+/+] and FIT [+/-]) are shown in **Figure 1**. FIT (+/+) patients had predominantly more advanced cancers than the FIT (+/-) patients (Tis/T1/T2/T3/T4 for FIT [+/+] vs. FIT [+/-]: 6/2/3/7/0 vs. 14/1/2/0/0 in ≥50 years: $P<.001$; 4/5/1/3/1 vs. 2/0/0/0/0 in <50 years: $P=.10$).

Patients with positive FIT overlapping symptoms or history of colorectal lesions

Because FIT was conducted annually as part of colorectal cancer screening system, independent of symptoms or history of colorectal lesions, the FIT groups included patients with accompanying symptoms or history of polypectomy. In the positive FIT groups, 31 patients were symptomatic and 19 had a history of colorectal lesions. In situ cancers were found in three patients with 2-positive FIT results and haematochezia. No cancer was detected in patients with positive FIT results and history of colorectal lesions.

DISCUSSION

This study found that cases with 2-positive FIT results in 2 samples had significantly high rates of more advanced-stage colorectal cancers amongst all cases with positive FIT results. Although FIT has been an important screening tool for colorectal cancer and can help in selecting candidates for diagnostic colonoscopy, patients with 2-positive results were shown to be at the highest risk for life-threatening cancer. In the face of the COVID-19 pandemic, when resources for colonoscopy are

limited, FIT can stratify the patients' risk. The study outcomes indicate that those with FIT (2+) should be given the highest priority for colonoscopy.

Although the sensitivity of FIT is superior to that of the guaiac test,[3,4,21,22] it decreases considerably for early-stage cancer or high-grade dysplasia compared with direct colonoscopy. Morikawa *et al*.[23] compared the results of 1-sample FIT and total colonoscopy in asymptomatic Japanese patients and reported that the sensitivity for invasive cancer was 78.3% (18/23) for Dukes' stages C or D, 70.0% (7/10) for Dukes' stage B, and 52.8% (19/36) for Dukes' stage A, and that for high-grade dysplasia was 32.7% (39/119). A similar study from Taiwan reported that the 1-sample FIT showed a sensitivity of 100% (5/5) for cancers in T2-4 stages and 66.7% (12/18) for those of Tis or T1.[24] The 2-sample method was adopted to improve the sensitivity of FIT.[3,7-9]

A simulation analysis based on the results of colonoscopic screening in the Japanese subjects predicted markedly higher positive predictive values (PPVs) for invasive cancers in patients with 2 positive-FIT results.[25] The PPVs were estimated to be 1.7% and 26% for male subjects in their 50s with 1 and 2 positive FITs, respectively. PPVs could increase because of lower rates of false positivity in cases with 2 positive FITs. The effect of improving the sensitivity could be higher for early stage lesions than for advanced cancers, for which the sensitivity is already high in single-sample FIT. One positive result in two samples was predicted to detect predominantly earlier-stage lesions. Few investigators have reported the actual findings of colonoscopy comparing one and two positive FITs in 2-sample FIT screening. Our result is compatible with a previous Canadian study,

which reported PPVs for colorectal cancer to be 1% and 8%, in patients with 1 and 2 positive FITs, respectively.[11] A recent study from the Netherlands suggested that 2-positive FITs from two samples of the same bowel movement also have high cancer detection rates.[12] The present study showed more advanced stages of colorectal cancers were predominant in patients with 2-positive FITs.

The FIT (+/-) group showed higher rates of cancer detection than those in whom the colonoscopy was performed for evaluation of symptoms, screening, or surveillance. This might be partly because the patients' symptoms at our clinic were generally mild. Although further evaluations are necessary, FIT might be helpful in making decisions about performing colonoscopy in symptomatic patients[26] or at the time of surveillance for patients after polypectomy.[27]

Even in patients <50 years of age, those with FIT (+/+) showed negligible rates of colorectal cancers, and in those with FIT (+/-), the rates were very low. Our results suggest that patients under 50 years of age with 2 positive FITs might need to receive a higher priority for colonoscopy than those over 50 years with 1 positive FIT. There is some discussion as to whether colorectal cancer screening should be started for subjects under 50 years of age, in whom the incidence of colorectal cancer is quite low but is increasing.[28] If they were screened by 2-positive results from 2-sample FITs, the cost-benefit balance might be acceptable.

The present study cannot answer whether the 2-sample FIT is superior to the 1-sample quantitative FIT as a tool for organised colorectal cancer screening program. The 1-sample FIT is simpler and less expensive at the primary screening step. Careful and wide-range evaluations are necessary to

select the best method, which should depend on the various conditions of the population. An advantage of the 2-sample FIT is based on the considerable discordance in FIT results between samples collected even from the same person. The result can sometimes change from 1 ng/mL to 1000 ng/mL (cutoff: 100 ng/mL = 20 μg Hb/g faeces) by the next day. The 2-sample FIT may have advantages over the 1-sample FIT, even after adjusting the threshold, under some circumstances. On the other hand, for risk stratification, the appropriate secondary cutoff values for the 1-sample quantitative FIT need to be decided for each FIT kit. The 2-sample FIT, using the established threshold for each FIT kit, has two possible results: 2-positive or 1-positive result.

We propose that patients with 2-positive results should be prioritised for colonoscopy, especially when resources are limited. In addition, given the COVID-19 pandemic, patients are likely to hesitate to undergo colonoscopy. In such cases, they should be strongly encouraged to receive colonoscopy with high priority. It may be useful to stratify patients with symptoms in a primary care setting. In the setting of 1-sample FIT screening, our results suggest that secondary FIT administered to patients with a positive primary FIT result can help identify patients at higher risk for whom colonoscopy should not be delayed.

Limitations and Strengths

This study has several limitations. First, it was conducted at a single endoscopy unit; hence, the results cannot be generalised. However, the indications and quality of colonoscopy as well as the

criteria for diagnosis were well controlled. Two-sample FIT-based colorectal screening has been conducted for many years throughout Japan. Our results could well represent the regular practice of colorectal screening in Japan. Second, the FIT kit brands and cutoff values for positivity were various and unknown in many cases that were referred from other medical institutions for colonoscopy. The guidelines for colorectal cancer screening in Japan only recommend the 2-sample FIT as standard, with no specific kits or cutoff values. As differences in FIT kit features and thresholds have been known to affect screening performance,[29] these variations are certainly a limitation of our study. However, a notable difference in the results between 2-positive and 1-positive FIT groups shown in our study suggests a common trend irrespective of kits brand. Third, we did not assess the patients' symptoms in detail, as cancer detection rates were low in symptomatic patients without FIT evaluation. However, the symptoms in our patients were generally mild. In populations with more serious symptoms, they could also be useful to urge early colonoscopy.[30] Fourth, positive predictive values are highly associated with the expected prevalence of lesions in the study population. Our results are susceptible to bias due to heterogeneity among our patients, which is a limitation of our study design. However, based on our results, detection rates of more advanced tumours were excellent in patients with 2-positive results, whereas they were generally quite low in the other positive groups. Further, this trend was observed irrespective of age groups. Although the results could change according to the study population, we assume that higher risk for advanced-stage lesions in 2-positive FIT results is generally true for various populations.

In conclusion, 2-positive results for 2 samples of FIT showed a much higher yield of advanced colorectal cancers than the 1-positive result, which also showed a higher yield than colonoscopy performed in patients with symptoms or with an associated history. The highest priority for diagnostic colonoscopy should be assigned to patients with 2-positive-FIT results.

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TABLES

Table 1. Study patients.

	Total	Positive FIT			Other than positive FIT		
		Total	FIT (+/+)	FIT (+/-)	Total	Symptom	Screening + surveillance
No.	8724	1282	264	1018	7442	1826	5616
Age, mean (SD), years	53.7 (12.5)	50.8 (12.4)	52.7 (13.7)	50.3 (12.0)	54.2 (12.4)	49.2 (13.4)	55.8 (11.6)
Male, n (%)	4350 (49.9)	613 (47.8)	144 (54.5)	653 (45.3)	3737 (50.2)	722 (39.5)	3015 (53.7)

Abbreviation: FIT, faecal immunochemical test. FIT (+/+) indicates 2-positive results for the 2-sample FIT. FIT (+/-) indicates a 1-positive result for the 2-sample FIT.

Table 2. Detection rates of colorectal cancer based on the indication for colonoscopy.

	Total	Positive FIT			Other than positive FIT		
		Total	FIT (+/+)	FIT (+/-)	Total	Symptom	Screening + surveillance
No.	8724	1282	264	1018	7442	1826	5616
All cancers (including in situ), n	79	51	32	19	28	15	13
Detection rate	0.9%	4.0%[a]	12.1%[b]	1.9%[c]	0.4%	0.8%[d]	0.2%
Invasive cancers, n	37	25	22	3	12	7	5
Detection rate	0.4%	2.0%[a]	8.3%[b]	0.3%	0.2%	0.4%[e]	0.1%

Abbreviation: FIT, faecal immunochemical test. FIT (+/+) indicates 2-positive results for the 2-sample FIT. FIT (+/-) indicates a 1-positive result for the 2-sample FIT.

[a] $P<.001$, Positive FIT vs. Other than positive FIT.

[b] $P<.001$, FIT (+/+) vs. FIT (+/-).

[c] $P=.02$, FIT (+/-) vs. Symptom.

[d] $P<.001$, Symptom vs. Screening + surveillance.

[e] $P=.01$, Symptom vs. Screening + surveillance.

Table 3. Colorectal cancer detection rates based on age and FIT positivity groups.

	Age ≥50 years			Age <50 years		
	Total	FIT (+/+)	FIT (+/-)	Total	FIT (+/+)	FIT (+/-)
No.	630	140	490	652	124	528
All cancers (including in situ), n	35	18	17	16	14	2
Detection rate	5.6%[a]	12.9%[b]	3.5%[c]	2.5%	11.3%[b]	0.4%
Invasive cancers, n	15	12	3	10	10	0
Detection rate	2.4%	8.6%[b]	0.6%	1.5%	8.1%[b]	0%

Abbreviation: FIT, faecal immunochemical test. FIT (+/+) indicates 2-positive results for the 2-sample FIT. FIT (+/-) indicates a 1-positive result for the 2-sample FIT.

[a] *P*=.006, Age ≥50 years vs. Age <50 years.

[b] *P*<.001, FIT (+/+) vs. FIT (+/-).

[c] *P*<.001, Age ≥50 years vs. Age <50 years.

Table 4. Features of colorectal cancers based on indication for colonoscopy.

	Total	Positive FIT		Other than positive FIT	
		FIT (+/+)	FIT (+/-)	Symptom	Screening + Surveillance
No.	79	32	19	15	13
Location (Proximal/Distal), n	23/56	10/22	5/14	1/14	7/6[a]
Size (SD), mm	26.1 (19.9)	31.2 (22.7)[b]	17.4 (9.7)	30.0 (20.4)	22.0 (19.8)
T stage (Tis/T1/T2/T3/T4), n	42/12/8/15/2	10/7/4/10/1[c]	16/1/2/0/0	8/1/1/4/1	8/3/1/1/0
Histological subtype (Well+Mod/Por+Muc), n	74/4	30/2	19/0	13/1	12/1

Abbreviation: FIT, faecal immunochemical test. FIT (+/+) indicates 2-positive results for the 2-sample FIT. FIT (+/-) indicates a 1-positive result for the 2-sample FIT. "Proximal" indicates from the caecum to the transverse colon and "Distal" indicates from the descending colon to the rectum. SD: standard deviation. "T stage" of the tumour was based on the UICC TNM Classification. "Well+Mod" indicates well- and moderately-differentiated adenocarcinoma. "Por+Muc" indicates poorly-differentiated and mucinous adenocarcinoma. One squamous cell carcinoma was excluded from this analysis.

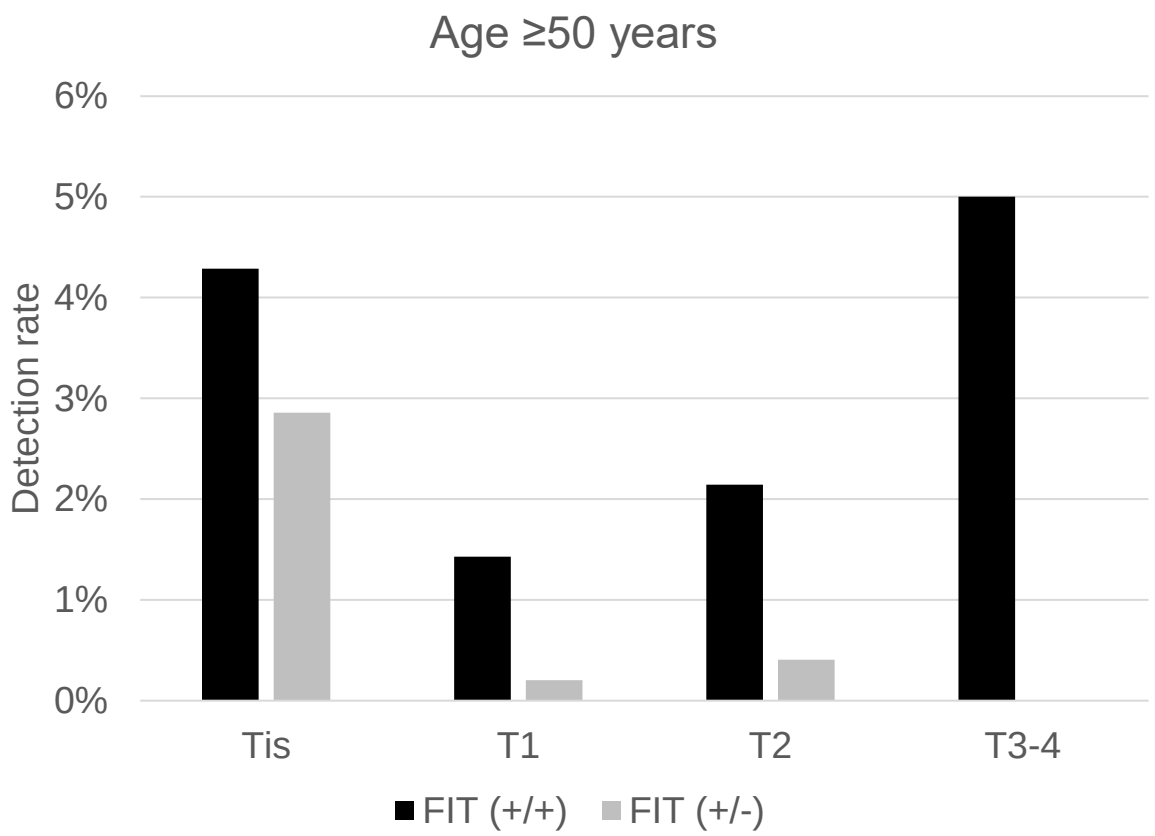
[a] P=.046, Screening + Surveillance vs. the others.

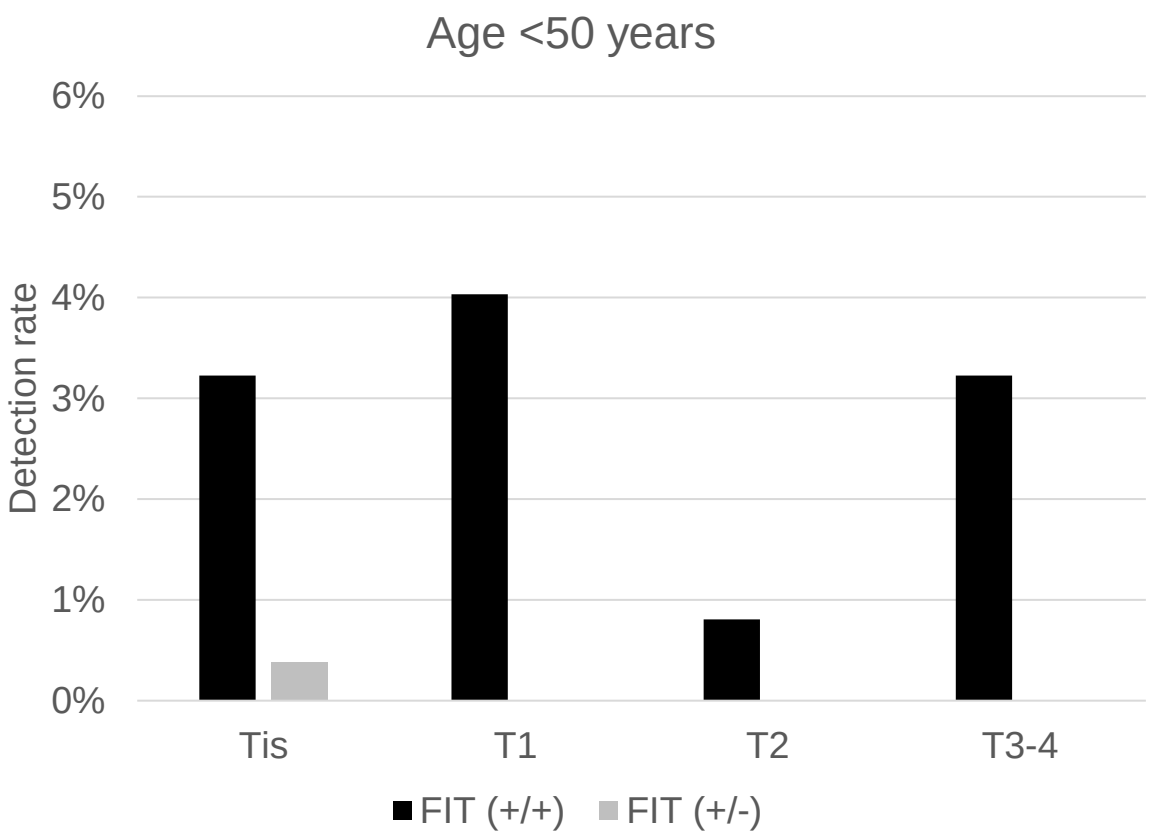
[b] P=.004, FIT (+/+) vs. FIT (+/-).

[c] P<.001, FIT (+/+) vs. FIT (+/-).

FIGURE LEGEND

Figure 1. Cancer detection rates stratified by T stages based on age and FIT positivity groups.
T stage was classified according to the UICC TNM Classification.
FIT (+/+) had a higher percentage of invasive cancers than in FIT (+/-).
Abbreviation: FIT, faecal immunochemical test. FIT (+/+) indicates 2-positive results for the 2-sample FIT. FIT (+/-) indicates a 1-positive result for the 2-sample FIT.


Age ≥50 years
6%
5%
4%
3%
2%
1%
0%
Detection rate
Tis
T1
T2
T3-4
FIT (+/+)
FIT (+/-)


Age <50 years
6%
5%
4%
3%
2%
1%
0%
Detection rate
Tis
T1
T2
T3-4
FIT (+/+)
FIT (+/-)

Supplementary Table 1. Details of the positive FIT groups

	FIT (+/+)	FIT (+/-)	FIT (+)	FIT (+/?)
No.	264	1018	136	287
Age, mean (SD), years	52.7 (13.7)	50.3 (11.9)	48.3 (12.1)	51.2 (12.2)
Male, n (%)	144 (54.5)	469 (46.1)	65 (47.8)	119 (41.5)
All cancers (including in situ), n	32	19	2	6
Detection rate	12.1%	1.9%[a]	1.5%[a]	2.1%[a]
Invasive cancers, n	22	3	0	4
Detection rate	8.3%	0.3%[a]	0.0%[a]	1.4%[a]
Age ≥50 years				
No.	140	490	54	145
All cancers (including in situ), n	18	17	2	5
Detection rate	12.9%	3.5%[a]	3.7%	3.4%[b]
Invasive cancers, n	12	3	0	4
Detection rate	8.6%	0.6%[a]	0.0%[c]	2.8%[c]
Age <50 years				
No.	124	528	82	142
All cancers (including in situ), n	14	2	0	1
Detection rate	11.3%	0.4%[a]	0.0%[b]	0.7%[a]
Invasive cancers, n	10	0	0	0
Detection rate	8.1%	0.0%[a]	0.0%[c]	0.0%[b]
Features of cancers				
Location (Proximal/Distal), n	10/22	5/14	1/1	0/6
Size (SD), mm	31.2 (22.7)	17.4[b] (9.7)	9.0 (8.5)	36.5 (42.0)
T stage (Tis/T1/T2/T3/T4), n	10/7/4/10/1	16/1/2/0/0	2/0/0/0/0	2/2/0/1/1
Histological subtype (Well+Mod/Por+Muc), n	30/2	19/0	2/0	6/0

Abbreviation: FIT, fecal immunochemical test. FIT (+/+) indicates 2-positive results for the 2-sample FIT. FIT (+/-) indicates a 1-positive result for the 2-sample FIT. FIT (+) indicates a 1-positive result for the 1-sample FIT. FIT (+/?) indicates positive FIT results with unknown number of positivity. SD: standard deviation. “Proximal” indicates from the cecum to the transverse colon and “Distal” indicates from the descending colon to the rectum. “T stage” of the tumor was based on the UICC TNM Classification. “Well+Mod” indicates well- and moderately-differentiated adenocarcinoma. “Por+Muc” indicates poorly-differentiated and mucinous adenocarcinoma.

[a] $P<.001$, vs. FIT (+/+).

[b] $P<.01$, vs. FIT (+/+).

[c] $P<.05$, vs. FIT (+/+).

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cross-sectional studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	P1 line3-5, P4 line6-7
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	P4 line6-P5 line6
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	P6 line2-16
Objectives	3	State specific objectives, including any prespecified hypotheses	P6 line17-P7 line3
Methods			
Study design	4	Present key elements of study design early in the paper	P7 line6-P8 line4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	P7 line7-8
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	P7 line7-P8 line4
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	P10 line2-7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	P7 line10-P8 line4, P8 line15-P9 line19
Bias	9	Describe any efforts to address potential sources of bias	P7 line16-18, P10 line6-7
Study size	10	Explain how the study size was arrived at	P7 line7-9
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	P9 line10-19
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	P10 line10-15
		(*b*) Describe any methods used to examine subgroups and interactions	P10 line6-7, 10-15
		(*c*) Explain how missing data were addressed	N.A.
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	N.A.
		(*e*) Describe any sensitivity analyses	N.A.
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	P11 line3-11
		(b) Give reasons for non-participation at each stage	P11 line4-6
		(c) Consider use of a flow diagram	N.A.
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	P11 line6-11
		(b) Indicate number of participants with missing data for each variable of interest	N.A.
Outcome data	15*	Report numbers of outcome events or summary measures	P11 line14-19
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	P12 line1-9
		(*b*) Report category boundaries when continuous variables were categorized	N.A.
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N.A.
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	P12 line16-P13 line8, P14 line4-10
Discussion			
Key results	18	Summarise key results with reference to study objectives	P14 line13-19
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	P17 line16-P18 line18
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	P19 line1-4
Generalisability	21	Discuss the generalisability (external validity) of the study results	P17 line16-P18 line18
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	P2 line14-15

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Priority stratification for colonoscopy based on 2-sample faecal immunochemical test screening: results from a cross-sectional study at an endoscopy clinic in Japan

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2020-046055.R2
Article Type:	Original research
Date Submitted by the Author:	20-Apr-2021
Complete List of Authors:	Toyoshima, Osamu; Toyoshima Endoscopy Clinic, Gastroenterology; The University of Tokyo, Department of Gastroenterology Yamaji, Yutaka; Tokyo Pharmaceutical Industry Health Insurance Society, Health Development Center Nishizawa, Toshihiro; Toyoshima Endoscopy Clinic, Gastroenterology; International Medical and Welfare College, Department of Gastroenterology and Hepatology Yoshida, Shuntaro; Toyoshima Endoscopy Clinic, Gastroenterology Yamada, Tomoharu; The University of Tokyo, Department of Gastroenterology; Toyoshima Endoscopy Clinic, Gastroenterology Kurokawa, Ken; The University of Tokyo, Gastroenterology; Toyoshima Endoscopy Clinic, Gastroenterology Obata, Miho; The University of Tokyo, Department of Gastroenterology; Toyoshima Endoscopy Clinic, Gastroenterology Kondo, Ryo; The University of Tokyo, Department of Gastroenterology; Toyoshima Endoscopy Clinic, Gastroenterology Toba, Masahito; Toba Clinic, Gastroenterology Koike, Kazuhiko; The University of Tokyo, Department of Gastroenterology
Primary Subject Heading:	Gastroenterology and hepatology
Secondary Subject Heading:	Health informatics, Health policy, General practice / Family practice
Keywords:	Gastrointestinal tumours < GASTROENTEROLOGY, Endoscopy < GASTROENTEROLOGY, Gastroenterology < INTERNAL MEDICINE, PREVENTIVE MEDICINE, Protocols & guidelines < HEALTH SERVICES ADMINISTRATION & MANAGEMENT

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ORIGINAL ARTICLE

TITLE

Priority stratification for colonoscopy based on 2-sample faecal immunochemical test screening: results from a cross-sectional study at an endoscopy clinic in Japan

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Competing interests: None.

Funding: The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Ethics approval: [review board] Certificated Review Board, Hattori Clinic (certification no. CRB3180027), [approval date] September 6th, 2019, [approval no.] S1909-U06, [registration site] UMIN-CTR system (https://rctportal.niph.go.jp/en/detail?trial_id=UMIN000018541), [registration

no.] UMIN000018541.

Informed consent statement: Written informed consents were obtained from the participants.

Patient and public involvement: Patients and/or the public were not involved in the design, conduct, report, or dissemination of this research.

Provenance and peer review: Not commissioned; externally peer reviewed.

Data availability statement: Data are available upon reasonable request. Additional data can be obtained by emailing OT at t@ichou.com. All requests for data sharing should be discussed with OT at the first instance.

STROBE statement: The authors have read the STROBE Statement checklist of items, and the manuscript was prepared and revised according to the STROBE Statement checklist of items.

Running title: Triage by 2-sample FIT

Manuscript word count: 3165 words

Keywords: hemoccult; colorectal cancer; colorectal cancer screening; colonoscopy; cancer prevention

ABSTRACT

Objectives Little has been reported on the yield and characteristics of colorectal neoplasia detected by the 2-sample faecal immunochemical test (FIT), particularly the difference between subjects with 2-positive results on the 2-sample FIT and those with 1-positive results. We aimed to assess risk stratification amongst patients with positive 2-sample FIT to prioritise colonoscopy.

Design A retrospective cross-sectional study

Setting A single-centre, representative endoscopy clinic in Japan

Participants Consecutive patients who underwent colonoscopy were enrolled. Indications for colonoscopy included 2-positive results on the 2-sample FIT (FIT (+/+)), 1-positive results on the 2-sample FIT (FIT (+/-)), and other reasons (non-FIT group, including presence of symptoms, screening, or surveillance).

Primary and secondary outcome measures Primary outcomes were detection rates of colorectal cancers, including in situ (all cancers) and invasive cancers, based on the indications for colonoscopy. Secondary outcomes were cancer features, such as location, size, T stage, and histological subtype.

Results Of the 9147 patients, 264 underwent colonoscopy following FIT (+/+), 1018 following FIT (+/-), and 7442 for reasons other than positive FIT. Detection rates of all (and invasive) cancers in the FIT (+/+), FIT (+/-), and non-FIT groups were 12.1% (8.3%), 1.9% (0.3%), and 0.4% (0.2%), respectively. The cancer detection rates were much higher in the FIT (+/+) group than in the FIT

(+/-) group, which in turn had higher rates than the non-FIT group. Moreover, the FIT (+/+) group showed more advanced T stages on TNM classification (Tis/T1/T2/T3/T4: 10/7/4/10/1) than the FIT (+/-) group (16/1/2/0/0, *P*<.001).

Conclusions Two-positive results for 2-sample FIT showed a much higher yield for more advanced colorectal cancers than the 1-positive result. High priority for diagnostic colonoscopy should be assigned to patients with 2-positive-FIT results.

(278 words)

Strengths and limitations of this study

- This study shows real-world data on 2-sample FIT in Japan, where 2-sample FIT-based colorectal screening has been conducted for many years throughout the country.
- This study investigated detection rates and features of colorectal cancers in patients with 1- and 2-positive results for the 2-sample FIT.
- This study also evaluated colorectal cancers in patients aged <50 years with 2-positive results for the 2-sample FIT.
- The retrospective cross-sectional design at a single endoscopy clinic was a limitation.
- We could not assess the FIT kit brand, faecal haemoglobin concentration, or patients' symptoms in detail.

INTRODUCTION

Colorectal cancer is one of the leading cancers worldwide, with 1.8 million new cases and 860,000 deaths annually, and has a significant impact on public health.[1] Screening for colorectal cancer has shown significant effects on reducing the morbidity and mortality, and is also economical.[2] There are several options for colorectal cancer screening, such as primary colonoscopy, sigmoidoscopy, and stool-based tests.[2] Amongst stool-based tests, the faecal immunochemical test (FIT) is now widely used instead of the guaiac faecal occult blood test, because of its higher accuracy and ease of handling.[3,4] Although its accuracy is limited compared to that of primary colonoscopy, FIT is noninvasive and can conserve the resources required for colonoscopy and reduce human contact. Hence, FIT might facilitate the safety and prioritisation of patients during the COVID-19 pandemic.[5]

In Japan, the population-based annual 2-sample FIT has been used for colorectal cancer screening for three decades since 1992.[6] For implementation and effectiveness, the number of FIT samples required, the interval between two FITs, and the FIT brands have been estimated.[4] The 2-sample method has been reported to have the best sensitivity and specificity for colorectal cancer.[3,7] Some investigators also reported that the sensitivity for advanced neoplasia was higher by using the 2-sample method than by the 1-sample method.[8,9]

At least 1-positive result is defined as a positive result in the 2-sample FIT method.[3,7-9] Few studies have investigated the yield and characteristics of neoplasia detected by 2-sample FIT.[8-10] In particular, little is known about the differences between the subjects with 2-positive results in the

2-sample FIT and those with 1-positive result.[11,12] In this study, we investigated the detection rates and features of invasive and in situ colorectal cancers detected by colonoscopy at our institution based on the indication for colonoscopy, focussing on the positivity patterns in the 2-sample FIT.

METHODS

Study design

This cross-sectional study included consecutive patients who underwent colonoscopy at the Toyoshima Endoscopy Clinic from April 2017 to August 2019. The indications for colonoscopy included a positive FIT result, evaluation of symptoms, screening, surveillance, and treatment. Samples for FIT measurements were collected from two consecutive bowel movements. FITs were conducted at our clinic or at referral medical institutions. The FIT kits included both qualitative and quantitative types. The FIT kit brand and cutoff values for positivity were chosen by the institutes conducting the FIT. At our institute, FIT was performed using OC-Auto Sampling Bottle 3 (Eiken Chemical Co., Ltd., Tokyo, Japan) with the threshold of 32 μg haemoglobin/g faeces. We divided the patients who were FIT positive into two categories: FIT (+/+) and FIT (+/-). We defined 2-positive results for 2 samples as FIT (+/+) and 1-positive result for 2 samples as FIT (+/-). Patients with a 1-positive result for the 1-sample FIT and positive FIT results with unknown number of positivity were excluded from this study; these findings are summarised in **Supplementary Table 1**. The symptoms included abnormal bowel habits, haematochezia, and abdominal pain. The surveillance

included patients with a medical history of colorectal cancer, colorectal polyps, or inflammatory bowel diseases. Treatment involved polypectomy and haemostasis. We excluded colonoscopies performed for treatment from this study. All indications other than positive FIT were divided into two categories: symptoms and screening + surveillance (asymptomatic).

Ethics

This study was conducted in accordance with ethical guidelines for medical studies in Japan. Written informed consent was obtained from patients at the time of colonoscopy to use their data for research purposes. The study design was described in a protocol prepared by Toyoshima Endoscopy Clinic and was approved by the Certificated Review Board, Hattori Clinic on 6 September 2019 (approval no. S1909-U06, registration no. UMIN000018541). We published this study's protocol on our institute's website (http://www.ichou.com), so that patients can opt out of the study. All clinical investigations were conducted according to the ethical guidelines of the Declaration of Helsinki.

Colonoscopy

Colonoscopies were performed by certified gastroenterologists. Olympus Elite 290 endoscope series (Olympus, Tokyo, Japan) was used.[13,14] The clinical data were recorded on an electronic endoscopy reporting system, T-File System (STS Medic, Tokyo, Japan). The data included the patients' baseline characteristics (age, sex, and indication for colonoscopy) and tumour

characteristics (location and size).

All colonoscopists were instructed to observe the entire colorectum, with a withdrawal time of 6 minutes or longer.[15,16] Polyps 15 mm in size or smaller were resected at the time of the examination, and if the polyp was larger than 15 mm, or if invasive cancer was suspected, the patient was referred to the hospital.[17]

Colorectal cancer

Colorectal cancer was treated by endoscopic resection, surgery, chemotherapy, and/or best supportive care. The patients received treatment at our clinic or at the hospital they were referred to. Colorectal cancer was diagnosed by histopathology. The location of cancer was determined by colonoscopy, surgery, or CT. The location from the caecum to the transverse colon was defined as proximal colon. The size of the cancer was measured by colonoscopy, pathology, or CT. The extent of tumour invasion was determined by pathology in combination with colonoscopy and CT findings. Tumours were classified according to the T stage of the UICC TNM classification.[18] We included noninvasive carcinoma (carcinoma in situ) as a cancer.[19] The histological subtype of the cancer was determined by histopathological evaluation of the resected or biopsy specimens. Four histological subtypes of adenocarcinomas (i.e., well-differentiated, moderately-differentiated, poorly-differentiated, and mucinous adenocarcinoma) were classified into two categories: well- + moderately-differentiated and poorly-differentiated + mucinous adenocarcinoma based on the

prognosis of the subtypes.[20]

Outcomes

The main outcomes were detection rates of all colorectal cancers (including carcinomas in situ) and those of invasive colorectal cancers, based on the indication for colonoscopy. The secondary outcomes were the features of the cancers, such as location, size, T stage, and histological subtype. We also divided the patients into 2 groups according to age: <50 years and ≥50 years, and analysed the detection rates and features of the cancers.

Statistical analysis

The detection rates were compared using the chi-squared test or Fisher's exact test. The characteristics of the cancer were compared using the Mann-Whitney U test, chi-squared test, or Fisher's exact test. The association between the T stages of colorectal cancers and the number of positive results of FIT was analysed using Spearman's rank correlation test. A two-sided *P* value <.05 was considered statistically significant. Statistical analyses were performed using IBM SPSS version 21.0 (IBM SPSS, Armonk, NY).

Patient and public involvement

Patients and/or the public were not involved in the design, or conduct, or report, or dissemination

plans of this research.

RESULTS

Characteristics of the study patients

During the study period, 9321 patients underwent colonoscopy. Of them, we excluded 174 patients for undergoing colonoscopy for treatment, 136 patients for a 1-positive result for the 1-sample FIT, and 287 patients for positive FIT results with unknown number of positivity. Finally, 8724 patients (age, mean ± SD: 53.7 ± 12.5 years; male sex: 49.9%) were eligible for this study. In all, 1282 colonoscopies were performed following positive FIT results. Of the positive FIT results, 264 were FIT (+/+) and 1018 were FIT (+/-). The remaining 7442 colonoscopies performed for indications other than positive FIT included 1826 for evaluation of the symptoms, and 5616 for screening (2394) + surveillance (3222), all these were performed without FIT investigation (**Table 1)**.

Cancer detection rates based on the indication for colonoscopy

The detection rates of colorectal cancer based on the indication for colonoscopy are shown in **Table 2**. All colorectal cancers (including carcinoma in situ) and invasive cancers were detected in 0.9% (79/8724 cases) and 0.4% (37 cases) of patients included in the study. The detection rates of all cancers and invasive cancers in FIT-positive patients were 4.0% (51/1282 cases) and 2.0% (25 cases), which were significantly higher than those detected patients who did not undergo FIT (0.4%

for all cancers, 28/7442, *P*<.001; 0.2% for invasive cancers, 12 cases, *P*<.001).

Amongst FIT-positive patients, the detection rate of all cancers in the FIT (+/+) group was very high at 12.1% (32/264 patients) and that in the FIT (+/-) group was 1.9% (19/1018 patients). Invasive cancers accounted for 8.3% (22 cases) in the FIT (+/+) group and 0.3% (3 cases) in the FIT (+/-) group. FIT (+/+) had significantly higher detection rates than FIT (+/-) (*P*<.001 and *P*<.001, respectively).

Amongst patients who did not undergo FIT, the cancer detection rates in symptomatic patients were significantly higher than in asymptomatic patients, in whom colonoscopy was performed for screening and surveillance (0.8% and 0.2% for all cancers, *P*<.001; 0.4% and 0.1% for invasive cancers, *P*=.01).

Additionally, the rate of cancer detection was significantly higher in patients with FIT (+/-) than in those with symptoms (1.9% and 0.8%, *P*=.02, respectively).

The detection rate of benign adenomas was significantly higher in the FIT (+/+) group than in the FIT (+/-) group (61.4% vs. 47.7%, *P*<.001). The difference in the detection rates of adenomas between the FIT (+/+) group and the FIT (+/-) group was less remarkable than those of cancers.

Cancer detection rates based on age and FIT positivity groups

Table 3 shows the rates of detection for colorectal cancer based on age group and FIT positivity patterns.

In patients aged ≥50 years, the FIT (+/+) group showed the highest rate of cancer detection (12.9% for all cancers and 8.6% for invasive cancers). For the FIT (+/-) group, the respective rates were 3.5% and 0.6%, which were significantly lower than those in the FIT (+/+) group (P<.001 and P<.001), suggesting that early stage cancers are more predominant.

In the <50 years age group as well, the rate of cancer detection was higher in the FIT (+/+) group (11.3% for all cancers and 8.1% for invasive cancers). They were comparable to those in the ≥50 age group. However, the detection rate in the FIT (+/-) group was low (0.4% for all cancers and 0.0% for invasive cancers, respectively); moreover, the detection rate for all cancers was lower than that in the same FIT (+/-) group at age ≥50 years (P<.001).

The features of the colorectal cancers

Table 4 shows the features of colorectal cancers based on the indication for colonoscopy. The colorectal cancers in patients with FIT (+/+) were larger than those in the FIT (+/-) patients (31.2 mm and 17.4 mm, P=.004). The T stage of FIT (+/+) colorectal cancer was more advanced than that of the FIT (+/-) cancers (P<.001). Although cancers were generally likely to be located in the distal colon or rectum, the cancers detected during screening and surveillance colonoscopies were predominantly in the proximal colon (proximal colon/distal colon and rectum: 7/6 for screening and surveillance vs. 16/50 for the others, P=.046).

The cancer detection rates stratified by T stage based on age (≥50 and <50 years) and FIT

positivity patterns (FIT [+/+] and FIT [+/-]) are shown in **Figure 1**. FIT (+/+) patients had predominantly more advanced cancers than the FIT (+/-) patients (Tis/T1/T2/T3/T4 for FIT [+/+] vs. FIT [+/-]: 6/2/3/7/0 vs. 14/1/2/0/0 in ≥50 years: *P*<.001; 4/5/1/3/1 vs. 2/0/0/0/0 in <50 years: *P*=.10).

Patients with positive FIT overlapping symptoms or history of colorectal lesions

Because FIT was conducted annually as part of colorectal cancer screening system, independent of symptoms or history of colorectal lesions, the FIT groups included patients with accompanying symptoms or history of polypectomy. In the positive FIT groups, 31 patients were symptomatic and 19 had a history of colorectal lesions. In situ cancers were found in three patients with 2-positive FIT results and haematochezia. No cancer was detected in patients with positive FIT results and history of colorectal lesions.

DISCUSSION

This study found that cases with 2-positive FIT results in 2 samples had significantly high rates of more advanced-stage colorectal cancers amongst all cases with positive FIT results. Although FIT has been an important screening tool for colorectal cancer and can help in selecting candidates for diagnostic colonoscopy, patients with 2-positive results were shown to be at the highest risk for life-threatening cancer. In the face of the COVID-19 pandemic, when resources for colonoscopy are

limited, FIT can stratify the patients' risk. The study outcomes indicate that those with FIT (2+) should be given the highest priority for colonoscopy.

Although the sensitivity of FIT is superior to that of the guaiac test,[3,4,21,22] it decreases considerably for early-stage cancer or high-grade dysplasia compared with direct colonoscopy. Morikawa *et al*.[23] compared the results of 1-sample FIT and total colonoscopy in asymptomatic Japanese patients and reported that the sensitivity for invasive cancer was 78.3% (18/23) for Dukes' stages C or D, 70.0% (7/10) for Dukes' stage B, and 52.8% (19/36) for Dukes' stage A, and that for high-grade dysplasia was 32.7% (39/119). A similar study from Taiwan reported that the 1-sample FIT showed a sensitivity of 100% (5/5) for cancers in T2-4 stages and 66.7% (12/18) for those of Tis or T1.[24] The 2-sample method was adopted to improve the sensitivity of FIT.[3,7-9]

A simulation analysis based on the results of colonoscopic screening in the Japanese subjects predicted markedly higher positive predictive values (PPVs) for invasive cancers in patients with 2 positive-FIT results.[25] The PPVs were estimated to be 1.7% and 26% for male subjects in their 50s with 1 and 2 positive FITs, respectively. PPVs could increase because of lower rates of false positivity in cases with 2 positive FITs. The effect of improving the sensitivity could be higher for early stage lesions than for advanced cancers, for which the sensitivity is already high in single-sample FIT. One positive result in two samples was predicted to detect predominantly earlier-stage lesions. Few investigators have reported the actual findings of colonoscopy comparing one and two positive FITs in 2-sample FIT screening. Our result is compatible with a previous

Canadian study, which reported PPVs for colorectal cancer to be 1% and 8%, in patients with 1 and 2 positive FITs, respectively.[11] A recent study from the Netherlands suggested that 2-positive FITs from two samples of the same bowel movement also have high cancer detection rates.[12] The present study showed more advanced stages of colorectal cancers were predominant in patients with 2-positive FITs.

The FIT (+/-) group showed higher rates of cancer detection than those in whom the colonoscopy was performed for evaluation of symptoms, screening, or surveillance. This might be partly because the patients' symptoms at our clinic were generally mild. Although further evaluations are necessary, FIT might be helpful in making decisions about performing colonoscopy in symptomatic patients[26] or at the time of surveillance for patients after polypectomy.[27]

Even in patients <50 years of age, those with FIT (+/+) showed negligible rates of colorectal cancers, and in those with FIT (+/-), the rates were very low. Our results suggest that patients under 50 years of age with 2 positive FITs might need to receive a higher priority for colonoscopy than those over 50 years with 1 positive FIT. There is some discussion as to whether colorectal cancer screening should be started for subjects under 50 years of age, in whom the incidence of colorectal cancer is quite low but is increasing.[28] If they were screened by 2-positive results from 2-sample FITs, the cost-benefit balance might be acceptable.

The present study cannot answer whether the 2-sample FIT is superior to the 1-sample quantitative FIT as a tool for organised colorectal cancer screening program. The 1-sample FIT is simpler and

less expensive at the primary screening step. Careful and wide-range evaluations are necessary to select the best method, which should depend on the various conditions of the population. An advantage of the 2-sample FIT is based on the considerable discordance in FIT results between samples collected even from the same person. The result can sometimes change from 1 ng/mL of the first sample to 1000 ng/mL of the second sample on the next day (cutoff: 100 ng/mL = 20 μg Hb/g faeces, in the case of the OC Sensor method, Eiken Chemical Co., Ltd., Tokyo, Japan) by the next day. The 2-sample FIT may have advantages over the 1-sample FIT, even after adjusting the threshold, under some circumstances. On the other hand, for risk stratification, the appropriate secondary cutoff values for the 1-sample quantitative FIT need to be decided for each FIT kit. The 2-sample FIT, using the established threshold for each FIT kit, has two possible results: 2-positive or 1-positive result.

We propose that patients with 2-positive results should be prioritised for colonoscopy, especially when resources are limited. In addition, given the COVID-19 pandemic, patients are likely to hesitate to undergo colonoscopy. In such cases, they should be strongly encouraged to receive colonoscopy with high priority. It may be useful to stratify patients with symptoms in a primary care setting. In the setting of 1-sample FIT screening, our results suggest that secondary FIT administered to patients with a positive primary FIT result can help identify patients at higher risk for whom colonoscopy should not be delayed.

Limitations and Strengths

This study has several limitations. First, it was conducted at a single endoscopy unit; hence, the results cannot be generalised. However, the indications and quality of colonoscopy as well as the criteria for diagnosis were well controlled. Two-sample FIT-based colorectal screening has been conducted for many years throughout Japan. Our results could well represent the regular practice of colorectal screening in Japan. Second, the FIT kit brands and cutoff values for positivity were various and unknown in many cases that were referred from other medical institutions for colonoscopy. The guidelines for colorectal cancer screening in Japan only recommend the 2-sample FIT as standard, with no specific kits or cutoff values. As differences in FIT kit features and thresholds have been known to affect screening performance,[29] these variations are certainly a limitation of our study. However, a notable difference in the results between 2-positive and 1-positive FIT groups shown in our study suggests a common trend irrespective of kits brand. Third, we did not assess the patients' symptoms in detail, as cancer detection rates were low in symptomatic patients without FIT evaluation. However, the symptoms in our patients were generally mild. In populations with more serious symptoms, they could also be useful to urge early colonoscopy.[30] Fourth, positive predictive values are highly associated with the expected prevalence of lesions in the study population. Our results are susceptible to bias due to heterogeneity among our patients, which is a limitation of our study design. However, based on our results, detection rates of more advanced tumours were excellent in patients with 2-positive results, whereas they were generally quite low in

the other positive groups. Further, this trend was observed irrespective of age groups. Although the results could change according to the study population, we assume that higher risk for advanced-stage lesions in 2-positive FIT results is generally true for various populations.

In conclusion, 2-positive results for 2 samples of FIT showed a much higher yield of advanced colorectal cancers than the 1-positive result, which also showed a higher yield than colonoscopy performed in patients with symptoms or with an associated history. The highest priority for diagnostic colonoscopy should be assigned to patients with 2-positive-FIT results.

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TABLES

Table 1. Study patients.

	Total	Positive FIT			Other than positive FIT		
		Total	FIT (+/+)	FIT (+/-)	Total	Symptom	Screening + surveillance
No.	8724	1282	264	1018	7442	1826	5616
Age, mean (SD), years	53.7 (12.5)	50.8 (12.4)	52.7 (13.7)	50.3 (11.9)	54.2 (12.4)	49.2 (13.4)	55.8 (11.6)
Male, n (%)	4350 (49.9)	613 (47.8)	144 (54.5)	469 (46.1)	3737 (50.2)	722 (39.5)	3015 (53.7)

Abbreviation: FIT, faecal immunochemical test. FIT (+/+) indicates 2-positive results for the 2-sample FIT. FIT (+/-) indicates a 1-positive result for the 2-sample FIT.

Table 2. Detection rates of colorectal cancer based on the indication for colonoscopy.

	Total	Positive FIT			Other than positive FIT		
		Total	FIT (+/+)	FIT (+/-)	Total	Symptom	Screening + surveillance
No.	8724	1282	264	1018	7442	1826	5616
All cancers (including in situ), n	79	51	32	19	28	15	13
Detection rate	0.9%	4.0%[a]	12.1%[b]	1.9%[c]	0.4%	0.8%[d]	0.2%
Invasive cancers, n	37	25	22	3	12	7	5
Detection rate	0.4%	2.0%[a]	8.3%[b]	0.3%	0.2%	0.4%[e]	0.1%

Abbreviation: FIT, faecal immunochemical test. FIT (+/+) indicates 2-positive results for the 2-sample FIT. FIT (+/-) indicates a 1-positive result for the 2-sample FIT.

[a] $P<.001$, Positive FIT vs. Other than positive FIT.

[b] $P<.001$, FIT (+/+) vs. FIT (+/-).

[c] $P=.02$, FIT (+/-) vs. Symptom.

[d] $P<.001$, Symptom vs. Screening + surveillance.

[e] $P=.01$, Symptom vs. Screening + surveillance.

Table 3. Colorectal cancer detection rates based on age and FIT positivity groups.

	Age ≥50 years			Age <50 years		
	Total	**FIT (+/+)**	**FIT (+/-)**	**Total**	**FIT (+/+)**	**FIT (+/-)**
No.	630	140	490	652	124	528
All cancers (including in situ), n	35	18	17	16	14	2
Detection rate	5.6%[a]	12.9%[b]	3.5%[c]	2.5%	11.3%[b]	0.4%
Invasive cancers, n	15	12	3	10	10	0
Detection rate	2.4%	8.6%[b]	0.6%	1.5%	8.1%[b]	0%

Abbreviation: FIT, faecal immunochemical test. FIT (+/+) indicates 2-positive results for the 2-sample FIT. FIT (+/-) indicates a 1-positive result for the 2-sample FIT.

[a] P=.006, Age ≥50 years vs. Age <50 years.

[b] P<.001, FIT (+/+) vs. FIT (+/-).

[c] P<.001, Age ≥50 years vs. Age <50 years.

Table 4. Features of colorectal cancers based on indication for colonoscopy.

	Total	Positive FIT		Other than positive FIT	
		FIT (+/+)	FIT (+/-)	Symptom	Screening + Surveillance
No.	79	32	19	15	13
Location (Proximal/Distal), n	23/56	10/22	5/14	1/14	7/6[a]
Size (SD), mm	26.1 (19.9)	31.2 (22.7)[b]	17.4 (9.7)	30.0 (20.4)	22.0 (19.8)
T stage (Tis/T1/T2/T3/T4), n	42/12/8/15/2	10/7/4/10/1[c]	16/1/2/0/0	8/1/1/4/1	8/3/1/1/0
Histological subtype (Well+Mod/Por+Muc), n	74/4	30/2	19/0	13/1	12/1

Abbreviation: FIT, faecal immunochemical test. FIT (+/+) indicates 2-positive results for the 2-sample FIT. FIT (+/-) indicates a 1-positive result for the 2-sample FIT. "Proximal" indicates from the caecum to the transverse colon and "Distal" indicates from the descending colon to the rectum. SD: standard deviation. "T stage" of the tumour was based on the UICC TNM Classification. "Well+Mod" indicates well- and moderately-differentiated adenocarcinoma. "Por+Muc" indicates poorly-differentiated and mucinous adenocarcinoma. One squamous cell carcinoma was excluded from this analysis.

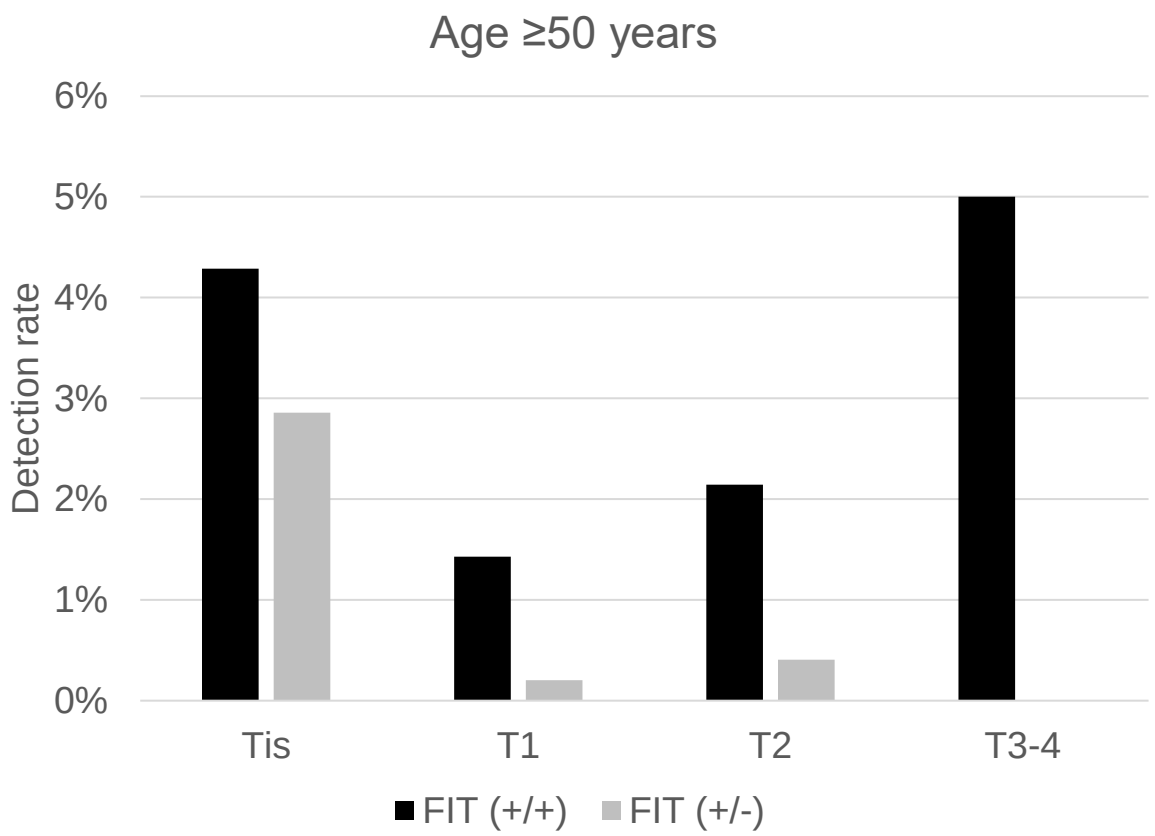
[a] P=.046, Screening + Surveillance vs. the others.

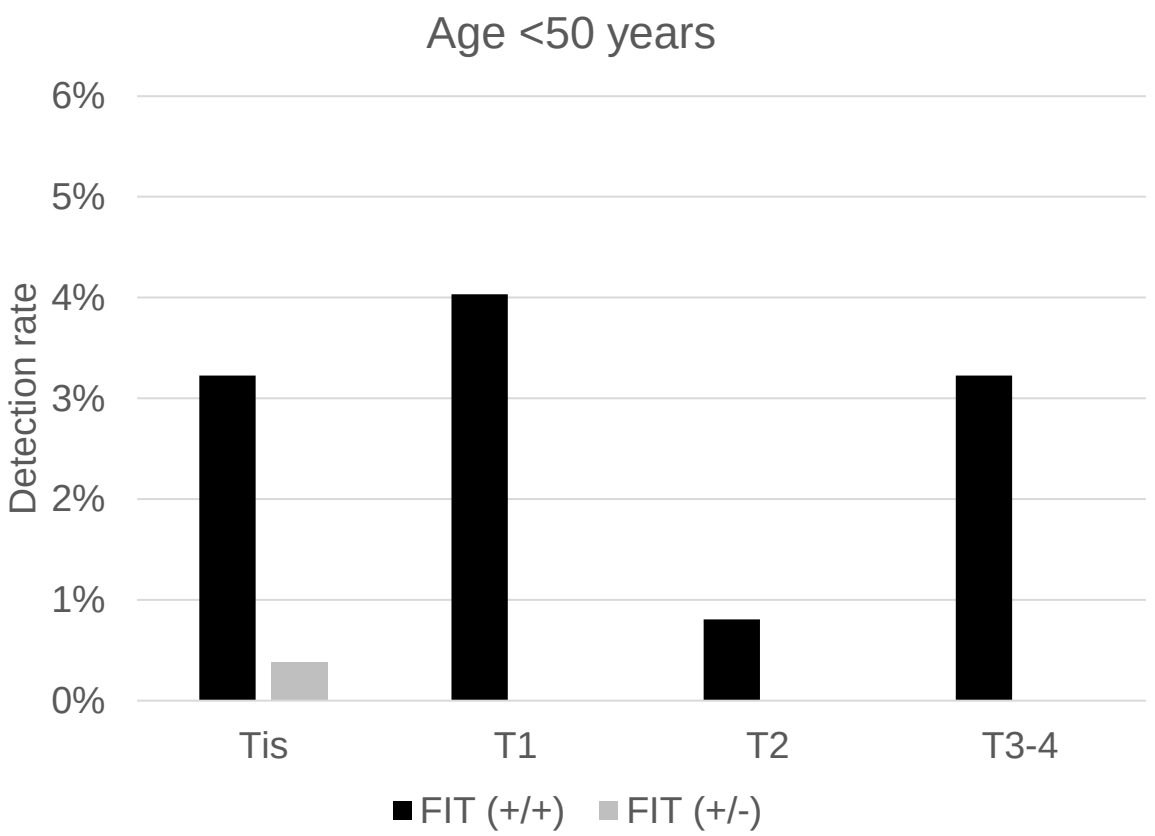
[b] P=.004, FIT (+/+) vs. FIT (+/-).

[c] P<.001, FIT (+/+) vs. FIT (+/-).

FIGURE LEGEND

Figure 1. Cancer detection rates stratified by T stages based on age and FIT positivity groups.
T stage was classified according to the UICC TNM Classification.
FIT (+/+) had a higher percentage of invasive cancers than in FIT (+/-).
Abbreviation: FIT, faecal immunochemical test. FIT (+/+) indicates 2-positive results for the 2-sample FIT. FIT (+/-) indicates a 1-positive result for the 2-sample FIT.


Age ≥50 years
6%
5%
4%
3%
2%
1%
0%
Detection rate
Tis
T1
T2
T3-4
FIT (+/+)
FIT (+/-)


Age <50 years
6%
5%
4%
3%
2%
1%
0%
Detection rate
Tis
T1
T2
T3-4
FIT (+/+)
FIT (+/-)

Supplementary Table 1. Details of the positive FIT groups

	FIT (+/+)	FIT (+/-)	FIT (+)	FIT (+/?)
No.	264	1018	136	287
Age, mean (SD), years	52.7 (13.7)	50.3 (11.9)	48.3 (12.1)	51.2 (12.2)
Male, n (%)	144 (54.5)	469 (46.1)	65 (47.8)	119 (41.5)
All cancers (including in situ), n	32	19	2	6
Detection rate	12.1%	1.9%[a]	1.5%[a]	2.1%[a]
Invasive cancers, n	22	3	0	4
Detection rate	8.3%	0.3%[a]	0.0%[a]	1.4%[a]
Age ≥50 years				
No.	140	490	54	145
All cancers (including in situ), n	18	17	2	5
Detection rate	12.9%	3.5%[a]	3.7%	3.4%[b]
Invasive cancers, n	12	3	0	4
Detection rate	8.6%	0.6%[a]	0.0%[c]	2.8%[c]
Age <50 years				
No.	124	528	82	142
All cancers (including in situ), n	14	2	0	1
Detection rate	11.3%	0.4%[a]	0.0%[b]	0.7%[a]
Invasive cancers, n	10	0	0	0
Detection rate	8.1%	0.0%[a]	0.0%[c]	0.0%[b]
Features of cancers				
Location (Proximal/Distal), n	10/22	5/14	1/1	0/6
Size (SD), mm	31.2 (22.7)	17.4[b] (9.7)	9.0 (8.5)	36.5 (42.0)
T stage (Tis/T1/T2/T3/T4), n	10/7/4/10/1	16/1/2/0/0	2/0/0/0/0	2/2/0/1/1
Histological subtype (Well+Mod/Por+Muc), n	30/2	19/0	2/0	6/0

Abbreviation: FIT, fecal immunochemical test. FIT (+/+) indicates 2-positive results for the 2-sample FIT. FIT (+/-) indicates a 1-positive result for the 2-sample FIT. FIT (+) indicates a 1-positive result for the 1-sample FIT. FIT (+/?) indicates positive FIT results with unknown number of positivity. SD: standard deviation. “Proximal” indicates from the cecum to the transverse colon and “Distal” indicates from the descending colon to the rectum. “T stage” of the tumor was based on the UICC TNM Classification. “Well+Mod” indicates well- and moderately-differentiated adenocarcinoma. “Por+Muc” indicates poorly-differentiated and mucinous adenocarcinoma.

[a] $P<.001$, vs. FIT (+/+).

[b] $P<.01$, vs. FIT (+/+).

[c] $P<.05$, vs. FIT (+/+).

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cross-sectional studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	P1 line3-5, P4 line6-7
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	P4 line6-P5 line6
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	P6 line2-16
Objectives	3	State specific objectives, including any prespecified hypotheses	P6 line17-P7 line3
Methods			
Study design	4	Present key elements of study design early in the paper	P7 line6-P8 line4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	P7 line7-8
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	P7 line7-P8 line4
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	P10 line2-7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	P7 line10-P8 line4, P8 line15-P9 line19
Bias	9	Describe any efforts to address potential sources of bias	P7 line16-18, P10 line6-7
Study size	10	Explain how the study size was arrived at	P7 line7-9
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	P9 line10-19
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	P10 line10-15
		(*b*) Describe any methods used to examine subgroups and interactions	P10 line6-7, 10-15
		(*c*) Explain how missing data were addressed	N.A.
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	N.A.
		(*e*) Describe any sensitivity analyses	N.A.
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	P11 line3-11
		(b) Give reasons for non-participation at each stage	P11 line4-6
		(c) Consider use of a flow diagram	N.A.
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	P11 line6-11
		(b) Indicate number of participants with missing data for each variable of interest	N.A.
Outcome data	15*	Report numbers of outcome events or summary measures	P11 line14-19
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	P12 line1-9
		(*b*) Report category boundaries when continuous variables were categorized	N.A.
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N.A.
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	P12 line16-P13 line8, P14 line4-10
Discussion			
Key results	18	Summarise key results with reference to study objectives	P14 line13-19
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	P17 line16-P18 line18
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	P19 line1-4
Generalisability	21	Discuss the generalisability (external validity) of the study results	P17 line16-P18 line18
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	P2 line14-15

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.