COLORECTAL CANCER

Evaluation of a card collection-based faecal immunochemical test in screening for colorectal cancer using a two-tier reflex approach

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Background: The guaiac faecal occult blood test (gFOBT) has been proved as a screening investigation for colorectal cancer, but has disadvantages. Newer faecal immunochemical tests (FITs) have many advantages, but yield higher positivity rates and are expensive. A two-tier reflex follow-up of gFOBT-positive individuals with a FIT before colonoscopy has been advocated as an efficient and effective approach.
Methods: A new simple and stable card collection FIT was evaluated.
Results: 1124 individuals who were gFOBT positive were asked to provide samples. 558 individuals participated, 320 refused and 246 did not return samples. No evidence of sampling bias was found. 302 individuals tested FIT negative and 256 tested positive. In the 302 FIT-negative individuals, 2 (0.7%) had cancer and 12 (4.0%) had large or multiple (high-risk) adenomatous polyps. In contrast, of 254 positive individuals, 47 (18.5%) had cancer and 54 (21.3%) had high-risk polyps. 93 (30.8%) of the FIT-negative individuals had a normal colonoscopy, but only 34 (13.4%) of the FIT-positive individuals had no pathology. Sensitivity, specificity, and positive and negative likelihood ratios (and 95% CIs) for cancer were 95.9% (84.8 to 99.3), 59.2% (54.7 to 63.5), 2.35 (2.08 to 2.65) and 0.07 (0.02 to 0.27), and for cancer and high-risk polyps were 87.8% (80.1 to 92.9), 65.3% (60.6 to 69.7), 2.53 (2.19 to 2.93) and 0.19 (0.11 to 0.31), respectively.
Conclusions: A two-tier reflex screening algorithm, in which gFOBT-positive participants are tested with a FIT, is effective in identifying individuals at high risk of significant colorectal neoplasia. This strategy is transferable across different FIT formats. This approach has been adopted for the Scottish Bowel Screening Programme.

Guaiac-based faecal occult blood tests (gFOBTs) are effective in population colorectal cancer screening.[1–4] gFOBTs are inexpensive and easy to use and transport by mail. However, they have many disadvantages including low sensitivity and low specificity,[5] and require to be developed and evaluated by well-trained personnel.[6] For these reasons, there is considerable current interest in faecal immunochemical tests (FITs),[7] and an ever-increasing body of work suggests that FITs could be used in population screening programmes.[8] These tests are specific for human haemoglobin and hence do not require any drug or dietary restriction and are easy to use.[5]

However, FITs are expensive, and qualitative FITs have lower analytical detection limits than gFOBTs, which leads to positivity rates that would place an intolerable burden on the scarce colonoscopy services available in many countries if used as the preferred screening test. For this reason, we have explored previously the use of a reflex two-tier strategy in which individuals who are gFOBT positive are asked to perform a FIT before colonoscopy, and we proposed that a population-based screening programme for colorectal cancer could be based on a combination of initial gFOBTs for all, followed by FITs for individuals with positive gFOBT.[9] We have demonstrated that use of this strategy would decrease the need for colonoscopy by about 30%.

However, the FIT that was used required the collection of faeces into tubes containing buffer solution. These tubes would be difficult and expensive to post to and from participants in a screening programme. Moreover, the material in the tubes must be analysed within 7–8 days of faecal collection, which would provide logistic difficulties.

Recently, a FIT has become available with an innovative sampling method that uses a single simple card for collection of two specimens of faeces, is stable for 30 days after the first collection and requires only one specimen preparation tube and one test cassette system for analysis. Being card based, this is familiar to participants and is also easy and inexpensive to post to and from them. We evaluated this system in phase 1 of the Scottish Bowel Screening Programme to determine the clinical characteristics of this particular FIT in our planned reflex two-tier gFOBT/FIT approach to colorectal cancer screening[9] and to assess, whether our previous results were transferable over different FIT formats.

PARTICIPANTS AND METHODS

Study population

All participants in the first 13.5 months of phase 1 of the Scottish Bowel Screening Programme who had a positive gFOBT were offered colonoscopy: the population here was not the same as in our previous work.[9] Exactly as per the pilot study in the UK,[4] the population invited for screening comprised individuals aged 50–69 years. A conventional gFOBT kit (hema-screen, Immunostics, Ocean, New Jersey, USA) was used. The screening algorithm was similar to that mandated for the UK pilot study[4]: participants collected two samples of faeces without any dietary restriction from each of three consecutive bowel movements directly on to filter paper containing guaiac

Abbreviations: FIT, faecal immunochemical test; gFOBT, guaiac faecal occult blood test; Hb, haemoglobin

Table 1 Number of participants by centre, gender and faecal occult blood test positivity

	Potential participants	Returned FIT (%)	Refused or regarded as inappropriate (%)	Did not respond (%)
Overall	1124	558 (49.6)	320 (28.5)	246 (21.9)
By centre				
Centre A	323	171 (52.9)	122 (37.8)	30 (9.3)
Centre B	84	44 (52.4)	31 (36.9)	9 (10.7)
Centre C	328	179 (54.6)	45 (13.7)	104 (31.7)
Centre D	389	164 (42.2)	122 (31.4)	103 (26.4)
By gender				
Male	673	333 (49.5)	194 (28.8)	196 (21.7)
Female	451	225 (49.9)	126 (27.9)	100 (22.2)
By gFOBT positivity				
Strongly positive	181	87 (48.1)	60 (33.1)	34 (18.8)
Weakly positive	943	471 (49.9)	260 (27.6)	212 (22.5)

FIT, faecal immunochemical test; gFOBT, guaiac faecal occult blood test.
The percentages are across rows.

gum through oval shapes in the gFOBT kit card. Participants who had five or six positive ovals on the gFOBT were classified as strongly positive, and were offered colonoscopy without further testing. Participants with one to four positive ovals were classified as weakly positive and asked to perform a second gFOBT (again without dietary restriction) and, if any oval was positive, colonoscopy was offered.

Study design

Participants with a positive gFOBT were offered an appointment to discuss colonoscopy with a specialist nurse at one of four centres. At the end of the discussion, nurses asked attendees to take part in this study. Attendees were told that the results of the FIT would not be used to influence the decision to proceed with colonoscopy and that the result would be unavailable to them so that their decision to proceed with further investigation would not be influenced. The study was approved by the National Health Service Tayside Medical Ethics Committee, and all participants gave written informed consent.

gFOBT and FIT analyses

All gFOBTs (hema-screen, Immunostics, supplied by Alpha Laboratories, Eastleigh, Hampshire, UK) were analysed in the Scottish Bowel Screening Centre Laboratory, which is accredited by Clinical Pathology Accreditation (CPA, UK) to ISO 15189-based standards and only performs analyses of faecal samples. In general, four bowel screener staff performed the analyses, and they had all received detailed training as required by CPA and by the Bowel Screening Programme standards of National Health Service Quality Improvement Scotland.[10] The FIT analyses were also performed in the Centre Laboratory, generally by a single senior bowel screener. The faeces collected on the card (hema-screen DEVEL-A-TAB) were delivered via the tab into a specimen preparation tube containing phosphate-buffered saline (hema-screen SPECIFIC). After mixing on a horizontal rotary mixer for 30 min, a portion of the faeces in buffer was dropped into the specimen well of a test cassette. At 5 min, positive results are detected for haemoglobin (Hb) at a concentration of 50 ng Hb/ml buffer. This is equivalent to 50 μg Hb/g faeces. Faeces in buffer flows up through the test cassette and labelled–antibody-dye conjugate binds to the globin of any haemoglobin present in the specimen, forming an antibody–antigen complex. This complex binds to anti-globin antibody in the positive test reaction zone and produces a coloured line. In the absence of Hb, there is no line in the test reaction zone. A coloured line in the control reaction zone demonstrates that the reagents and devices are functioning correctly.

Comparison of FIT analytical detection limit

To compare the analytical detection limit of the FIT under study with that of the method used in our previous study,[9] 200 consecutive faecal specimens were analysed using Instant-View FIT (Alfa Scientific Designs, Poway, California, USA) test cassettes using the samples in the SPECIFIC specimen collection tubes.

Colonoscopy and pathology

Colonoscopy was performed in each of the four centres. Data on colonoscopy were recorded on a specific form with information on the quality of the investigation (quality of preparation and completeness of colonoscopy) and on the results including number, size and localisation of colorectal cancers and adenomas, and whether biopsy was performed. Full pathological data were available on all excised biopsy specimens, including polyp type, presence or absence of malignancy and, in all adenomas, the severity of dysplasia.

Analysis of results

Exactly as described previously,[9] sampling bias was analysed between those who participated, refused or did not participate by gender and by degree of gFOBT positivity. Colonoscopy results in FIT-positive and FIT-negative groups were compared using Fisher's exact test. Sensitivity, specificity, and positive and negative likelihood ratios (all with 95% CIs) of FIT were calculated for identification of cancer, with or without high-risk[11] adenomatous polyps primarily to facilitate comparison of the results with data on the previously studied FIT.[9]

RESULTS

Table 1 shows the number of participants by centre, gender and gFOBT positivity. Of the 1124 invited, 558 (49.6%) individuals participated, 320 (28.5%) refused or were judged inappropriate for colonoscopy by the specialist nurses, and 246 (21.9%) did not return samples. No evidence of sampling bias according to gender was found, since the percentages of men and women who returned the FIT, refused to take part or were judged inappropriate by the specialist nurses, and took the FIT but did not return it were similar. This lack of sampling bias also held for degree of gFOBT positivity.

Of the 200 samples analysed using the FIT, 112 were negative and 88 were positive. Identical results were obtained with the

Table 2 Colonoscopy results

	FIT-positive (%)	FIT-negative (%)	p Value
Participants	254	302	
Cancer	47 (18.5)	2 (0.7)	<0.0001
Adenoma	106 (41.7)	60 (19.9)	<0.0001
High-risk adenomas	54 (21.3)	12 (4.0)	<0.0001
Hyper-/metaplastic polyps	32 (12.6)	26 (8.6)	>0.05
Other pathology	96 (37.8)	121 (39.2)	>0.05
Incomplete colonoscopy	22 (8.8)	16 (5.3)	>0.05
Did not attend	6 (2.4)	5 (1.7)	>0.05
Normal colonoscopy	34 (13.4)	93 (30.8)	<0.0001

FIT, faecal immunochemical test.
The percentages are down columns: other pathology encompasses inflammatory bowel disease, diverticular disease, angiodysplasia and non-significant lesions.

previously studied FIT. In consequence, κ, the preferred statistic because it accounts for chance, was 1.00.

Table 2 shows data for the 556 participants who had information on colonoscopy. The numbers of cancers, adenomas and high-risk adenomas were statistically significantly higher in the FIT-positive participants than in the FIT-negative participants, and the number of normal colonoscopies was significantly lower. Other pathology, comprising inflammatory bowel disease, diverticular disease and angiodysplasia, was not different in the groups, nor were the number of incomplete colonoscopies and the number of participants who did not attend.

Table 3 shows the sensitivity, specificity and positive and negative likelihood ratios for cancer alone and cancer and high-risk adenomatous polyps in the gFOBT-positive participants.

DISCUSSION

Overall, 558 (49.6%) invitees returned satisfactory samples for analysis. It was surprising that the response was not higher than in our previous study (53.9%) since the card collection technique is simple and since all had already completed at least one gFOBT and the collection format was familiar. Moreover, it has been shown that, by simplifying sampling (and removing the need for restrictions of diet and drugs), FIT increases participation in screening.[12] As in our previous study,[9] it was disappointing that 320 (28.5%) potential participants who attended an interview with a specialist nurse either refused to take part or were regarded as ineligible for the study. This low uptake might have arisen because they were reluctant to do yet another collection of faeces since most had performed two gFOBTs already. In addition, the results could not influence the decision regarding colonoscopy. Moreover, the request to participate came at the end of an interview. Further, 246 (21.9%) took information and materials home, but did not participate. However, it is highly likely that our two-tier approach would be successful with regard to participation in our national programme since we have found that most who are weakly positive on initial gFOBT, and are then asked to undertake a second, do comply.[13]

We again found that the four sites of interview location differed slightly in the number of participants. In contrast to our previous study,[9] the percentage of men and women who participated was the same. Although it could be argued that a higher uptake in this study could have affected the results, no evidence for sampling bias was found between those who participated and those who did not with regard to both gender and gFOBT positivity.

We showed that the analytical detection limit of the FIT cassettes used in this study and in our previous study[9] were identical and, in consequence, differences in findings would be due to either the characteristics of the participants or the sample collection technique, particularly the amount of faeces, since the participants were from the same geographical area.

Colonoscopy revealed that this FIT was highly sensitive for colorectal cancer in this group of gFOBT-positive individuals since, of the 49 cancers found, 47 were FIT-positive and only 2 negative. This was similar to our earlier results,[9] where 38 were FIT-positive and 2 negative. The two cancers were from the weakly positive gFOBT group and both were polyp cancers, one of 2 mm diameter and the other 18 mm. Of the 12 FIT-negative individuals with high-risk adenoma, again all were gFOBT weakly positive and 7 had a single adenoma ranging from 11 to 17 mm, 3 had 2 adenomas, all <15 mm, and 2 had 3 adenomas, all <12 mm. In contrast, in the FIT-positive group, 7 were polyp cancers and 13, 10 and 17 were Dukes' stages A, B and C, respectively.

For cancer and high-risk adenoma, the findings were again similar to our results with the previously studied FIT.[9] However, for detection of all polyps, 139 (54.6%) were in FIT-positive participants and 116 (45.4%) in the FIT-negative participants, in contrast to the 78.8% and 21.2%. This is highly likely to be due to a smaller amount of faeces being collected on the card compared with into the tubes when used as collection devices. Thus, about half of those individuals with low-risk adenomas would be missed by this approach, but since 96% of cancers and 88% of cancers and high-risk adenomas were detected, this represents an efficient and effective disease detection strategy while simultaneously reducing the need for screening colonoscopy. Over 80% of those with high-risk polyps are detected with the FIT, and there now seems to be a growing view that there is little harm in not detecting, removing and following up those with single small polyps. Even large polyps (>1 cm) become cancers at the low rate of ca 1% per year[14] and the development of invasive cancer from a small (<10 mm) adenoma is extremely unlikely in <5 years.[15]

Our strategy would reduce the colonoscopy demands. The actual reduction would depend on the screening round. In the second Scottish screening round, the reduction in colonoscopy demand was shown to be ca 30%.[9] In this study, which involved the third screening round of essentially the same population, the gFOBT positivity rate had fallen from 1.9% to 1.0% and the proposed strategy of gFOBT followed by FIT would save ca 50% of colonoscopies. Although FITs are more expensive than gFOBTs, often by a factor of 10, and cost about £5 (US$9.85, €7.34), colonoscopy in Scotland is documented to cost £350

Table 3 Sensitivity, specificity, and positive and negative likelihood ratios for cancer alone and cancer and high-risk adenomatous polyps

	Sensitivity, % (95% CI)	Specificity, % (95% CI)	Positive likelihood ratio (95% CI)	Negative likelihood ratio (95% CI)
Cancer	95.9 (84.8 to 99.3)	59.2 (54.7 to 63.5)	2.35 (2.08 to 2.65)	0.07 (0.02 to 0.27)
Cancer and high-risk adenomatous polyps	87.8 (80.1 to 92.9)	65.3 (60.6 to 69.7)	2.53 (2.19 to 2.93)	0.19 (0.11 to 0.31)

High-risk adenomatous polyps are either >10 mm diameter or >3 polyps or both in an individual participant.

(US$689.62, €514.17)[16]: thus, significant overall cost savings for a bowel screening programme would be achieved through adoption of the approach advocated here.

Although using FIT as the preferred test in bowel screening programmes has many advantages,[8] the positivity rate and the expense mean that, at this stage at least, our two-tier reflex strategy, combining gFOBT and FIT, would seem to have advantages when colonoscopy is limited. Moreover, although there is information on the clinical performance characteristics in large average-risk populations for certain FITs,[17] comparisons in large populations are not yet available. However, since the basic immunochemistry seems to be similar for all, the real advantages for one over another may be in sampling methods and development: a simple stable card collection system would seem to have advantages.

Our current findings again show that a population with a high probability of cancer and high-risk adenomatous polyps can be identified by the use of FIT and that results from different FITs for this purpose are transferable. Our findings have informed the Scottish Bowel Screening Programme that will be rolled out to all 50–74-year olds, an extended age range compared with this study, beginning in 2007.[16] At this point in time at least, our two-tier reflex strategy, combining gFOBT and FIT, will be used. All participants will initially be sent a gFOBT. Participants who have strong positive gFOBT results will be offered colonoscopy immediately. Participants who are weakly positive will be asked to perform a FIT rather than a repeat gFOBT, since this strategy directs colonoscopy to those with significant disease, Individuals who are initially gFOBT negative or FIT-negative will be invited for screening again after 2 years.

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REFERENCES

1 **Hardcastle JD**, Chamberlain JO, Robinson MH, *et al*. Randomised controlled trial of faecal occult-blood screening for colorectal cancer. *Lancet* 1996;**348**:1472–7.
2 **Kronborg O**, Fenger C, Olsen J, *et al*. Randomised study of screening for colorectal cancer using faecal occult blood test. *Lancet* 1996;**348**:1467–71.
3 **Mandel JS**, Bond JH, Church TR, *et al*. Reducing mortality from colorectal cancer by screening for fecal occult blood. Minnesota Colon Cancer Control Study. *N Engl J Med* 1993;**328**:1365–71.
4 **Steele RJC**, Parker R, Patnick J, *et al*. A demonstration pilot trial for colorectal cancer screening in the United Kingdom: a new concept in the introduction of healthcare strategies. *J Med Screen* 2001;**8**:197–202.
5 **Young GP**, St John DJ, Winawer SJ, *et al* for WHO and OMED (World Organization for Digestive Endoscopy). Choice of fecal occult blood tests for colorectal cancer screening: recommendations based on performance characteristics in population studies: a WHO and OMED report. *Am J Gastroenterol* 2002;**97**:2499–507.
6 **Selinger RR**, Norman S, Domnitz JA. Failure of health care professionals to interpret fecal occult blood tests accurately. *Am J Med* 2003;**114**:64–7.
7 **Allison JE**. Colon cancer screening guidelines 2005: the fecal occult blood test option has become a better FIT. *Gastroenterology* 2005;**129**:745–48.
8 **Allison JE**, Lawson M. Screening tests for colorectal cancer: a menu of options remains relevant. *Curr Oncol Rep* 2006;**8**:492–8.
9 **Fraser CG**, Matthew CM, Mowat NAG, *et al*. Immunochemical testing of individuals positive for guaiac faecal occult blood test in a screening programme for colorectal cancer: an observational study. *Lancet Oncol* 2006;**7**:127–31.
10 **NHS Quality Improvement Scotland**. Clinical standards: Bowel Screening Programme 2007.
11 **Atkin WS**, Saunders BP. Surveillance guidelines after removal of colorectal adenomatous polyps. *Gut* 2002;**51**(Suppl v):v6–vv9.
12 **Cole SR**, Young GP, Esterman A, *et al*. A randomized trial of the impact of new faecal haemoglobin test technologies on population participation in screening for colorectal cancer *J Med Screen* 2003;**10**:117–22.
13 **UK Colorectal Cancer Screening Pilot Group**. Results of the first round of a demonstration pilot of colorectal cancer screening in the United Kingdom. *BMJ* 2004;**329**:133–5.
14 **Ransohoff DF**. Lessons from the UK sigmoidoscopy screening trial [editorial]. *Lancet* 2002;**359**:1266–7.
15 **Eide T**. Risk of colorectal cancer in adenoma bearing individuals within a defined population. *Int J Cancer* 1986;**38**:173–6.
16 **Scottish Executive Department of Health**. Bowel Screening Programme. HDL (2006)3. http://www.sehd.scot.nhs.uk/mels/HDL2006_03.pdf (accessed 21 Mar 2007).
17 **Anon**. Immunochemical versus guaiac fecal occult blood tests. *Technol Eval Cent Assess Program* 2004;**19**:1–28.