RESEARCH

A prospective study of faecal immunochemical testing following polypectomy in a colorectal cancer screening population

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ABSTRACT

Introduction 52% of faecal immunohistochemistry test (FIT)-positive clients in the Irish National Colorectal Cancer Screening Programme (BowelScreen) have adenomatous polyps identified at colonoscopy in round 1. Although it is known that advanced adenomas and cancers cause an elevated FIT, it is not known if small (<5 mm) adenomas cause a positive FIT.

Aims Determine if removal of small polyps in an FIT-based colorectal cancer (CRC) screening programme is associated with a negative FIT on follow-up.

Methods A single-centre prospective observational study of consecutive participants attending for first round screening colonoscopy who had a positive FIT (>45 µg Hb/g) as part of the Irish Colorectal Cancer Screening Programme. Subjects were consented at the time of colonoscopy and were sent a repeat FIT 4–6 weeks later. Precolonoscopy and postcolonoscopy FITs were compared and correlated with clinical findings and endoscopic intervention.

Results 112 consecutive first round participants were recruited. Eight (7%) had cancer, 75 (67%) adenomatous polyps, 17 (15%) a normal colonoscopy and 12 (11%) other pathology. There was a clear difference in median FIT levels between the four groups ($P=0.006$). Advanced pathology (tumour or adenomatous polyp >1 cm) was associated with higher FIT than non-advanced pathology (median FIT 346 vs 89 $P=0.0003$). 83% (86/104) of subjects completed a follow-up FIT. Follow-up FIT remained positive in 20% (17/86). Polypectomy was associated with a reduction in FIT from a median of 100 to 5 µg Hb/g ($P<0.0001$). Removal of polyps >5 mm was the only factor independently associated

with a negative follow-up FIT on multivariate analysis (OR 3.9 (1.3–11.9, $P=0.04$)).

Conclusion FIT is a sensitive test and levels increase with advanced colonic pathology. Polypectomy of advanced adenomas is associated with a negative follow-up FIT. However, alternative causes for a positive FIT should be considered in patients who have adenomas less than 5 mm detected or a normal colonoscopy.

INTRODUCTION

The Irish National Colorectal Cancer Screening Programme (BowelScreen) commenced in 2013, with initial invitees aged between 60 and 69 years. The programme is based on the faecal immunohistochemistry test (FIT). The current cut-off for a positive FIT leading to invitation for colonoscopy is 45 µg haemoglobin (Hb)/g faeces, equivalent to 225 ng Hb/mL buffer.¹

FIT-based screening is superior to a binary faecal occult blood (FOB) test for detection of significant colonic pathology^{2,3} and data show that FIT-based CRC screening reduces mortality, and has an earlier onset of impact and better compliance than FOB-based screening.^{4,5} One disadvantage of using FIT is its relative instability when stored at room temperature if not measured immediately.⁶ BowelScreen uses an FIT cut-off of 45 µg Hb/g faeces and provisional analysis of outcome data indicates that this is delivering a colonoscopy referral rate of 5.0% (BowelScreen, personal communication, 2016). This cut-off is higher than recommendations for a US consensus group, but in line with the recently changed Dutch

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screening programme who found that by increasing their threshold from 15 to 47 µg Hb/g, their positive predictive value for the detection of advanced pathology changed from 42% to 49%.⁷

One of the challenges of a colorectal cancer (CRC) screening programme is the management of patients with an apparently false-positive screening test. Patients with a normal colonoscopy may have a false-positive test or undetected pathology. A positive FIT may be attributed to small (5–10 mm) or diminutive (<5 mm) polyps. Experience from the English Bowel Cancer Screening programme reports a normal screening colonoscopy in 24% of men and 38% of women, based on over 10 000 FOB-positive patients.⁸ Although FIT levels are known to correlate with both tumour stage⁹ and colonic disease burden,⁹ it is not known if small or diminutive polyps cause a positive FIT or if the removal of these polyps results in a negative FIT.

The aim of this study was to identify if removal of small and diminutive adenomas results in a negative FIT on follow-up testing.

METHODS

This was a prospective single-centre study of consecutive participants attending for index colonoscopy following a positive FIT in the Irish Colorectal Cancer Screening Programme over a 6-month period. All participants undergoing index colonoscopy at our centre in the programme were eligible for inclusion. Prior to their index colonoscopy, participants consented to perform a second FIT test 4–6 weeks after colonoscopy. Subjects diagnosed with CRC at index colonoscopy did not undergo repeat FIT, but their initial FIT results were included in the analysis. All participants received polyethylene glycol electrolyte solution (MoviPrep; Salix Pharmaceuticals, Raleigh, North Carolina, USA) as bowel preparation.

The initial FIT result was recorded in addition to detailed clinical information including a record of antiplatelet and anticoagulant medications at the time of colonoscopy. Enrolled subjects were mailed a follow-up FIT and returned the samples to the sole laboratory performing BowelScreen FIT (MedLab Pathology, Dublin, Ireland). The second FIT was recorded only for the purpose of the study and did not impact on the patient's pathway through BowelScreen.

Precolonoscopy and postcolonoscopy FIT levels were recorded prospectively. Precolonoscopy FIT levels were correlated with endoscopic and pathological findings and compared with postcolonoscopy FIT. The same reference level for a positive test used in BowelScreen was used for this study (FIT >45 µg Hb/g faeces), and qualitative (positive or negative) and quantitative comparisons were made. Polyp number, size and cumulative polyp burden were recorded. Cumulative polyp burden was calculated as the sum of the size of each adenoma detected at an individual index colonoscopy.

Table 1 Baseline characteristics of faecal immunohistochemistry test (FIT)-positive subjects from the first round of a national colorectal cancer screening programme

Characteristic	n (%)
Number of initial patients	112
Gender	
Male	63 (56)
Female	49 (44)
Age (median)	66.6
(IQR)	(65.9–67.2)
Time from colonoscopy to repeat FIT (days)	43 (41–46)
Antiplatelet/anticoagulation use	
Warfarin/Novel Oral Anticoagulant	9 (8)
Aspirin	37 (33)
Polyp pathology	
Adenoma detected	75 (67)
Median polyp number	2 (1–4)
Median cumulative polyp load (mm)	13 (6–19)
Size of largest adenoma	
1–5 mm	26 (35)
6–10 mm	25 (33)
11–15 mm	16 (21)
>15 mm	8 (11)
Polyp cancer (pT1)	3
Non-adenomatous pathology	
Malignancy	8 (7)
Diverticulitis	1 (1)
Angiodysplasia	3 (3)
Colitis/proctitis	8 (7)
Haemorrhoids	20 (18)
Hyperplastic polyp	16 (14)

STATISTICAL ANALYSIS

Continuous data are presented as medians and IQRs. Differences between two groups were assessed by the Mann-Whitney U test, and between multiple groups by Kruskal-Wallis test. Categorical variables were analysed by Fisher's exact test or the χ^2 test as appropriate. Multivariate analyses were performed using a Cox proportional hazards model. Original raw continuous and categorical variables were used in all multivariate analyses. Calculations were performed using Statistical Package for the Social Sciences (SPSS V.20.0), and graphical analysis was performed by GraphPad PRISM V.5 (GraphPad, La Jolla, California, USA). P values less than 0.05 were considered statistically significant.

RESULTS

One hundred and twelve consecutive first round participants were enrolled. Demographics are shown in [table 1](#).

The median (IQR) age was 66.6 (66–67.2) years. Fifty-six per cent were male. Thirty-three per cent were taking aspirin and 8% anticoagulants (warfarin or novel oral anticoagulant) at the time of inclusion.

Figure 1 Boxplot graph comparing the size of largest adenomatous polyp removed and quantitative faecal immunohistochemistry test (FIT) level. (y axis; logarithmic scale). Red line indicates 45 $\mu\text{g Hb/g faeces}$. (A) Precolonoscopy; (B) Postcolonoscopy.

Initial FIT levels ranged between 46 and 9623 $\mu\text{g Hb/g faeces}$, with a median (IQR) of 111 (67–348) $\mu\text{g Hb/g faeces}$. CRC was detected in 8 cases (7%), while 17 participants (15%) had a normal colonoscopy. Adenomatous polyps were detected and removed in 75 cases (67%) with a median (IQR) size of 8 mm (5–12 mm). Three participants had tumour within a polyp (pT1). Median (IQR) cumulative polyp burden was 13 (6–19) mm. All identified adenomas were removed at the index colonoscopy.

Initial FIT levels are shown in [figure 1A](#). The highest levels were observed in participants with CRC (647 $\mu\text{g Hb/g faeces}$) and polyps >15 mm (1004 $\mu\text{g Hb/g faeces}$), with lowest median levels in patients with normal colonoscopy (74 $\mu\text{g Hb/g faeces}$). Participants with adenomatous polyps had median FIT levels of 100 $\mu\text{g Hb/g faeces}$, while those with other pathology (angiodysplasia, colitis or haemorrhoids) had a median of 143 $\mu\text{g Hb/g faeces}$. There was a positive and linear correlation between polyp size and FIT level ($P < 0.001$) ([figure 1A](#)). The median FIT level was 73 $\mu\text{g Hb/g faeces}$ for polyps 1–5 mm, 94 $\mu\text{g Hb/g faeces}$ for polyps 6–10 mm, 195 $\mu\text{g Hb/g faeces}$ for 11–15 mm and 1004 $\mu\text{g Hb/g faeces}$ for polyps >15 mm.

A follow-up FIT was returned by 86 of 104 participants (83%) ([figure 2](#)). The median time from index colonoscopy to return of repeat FIT was 43 days (IQR 41–45 days). [Figure 1B](#) highlights the changes in FIT following

Figure 2 Endoscopic findings in the subjects who completed a postcolonoscopy faecal immunohistochemistry test (FIT).

colonoscopy. Eighty per cent (69/86) of patients had a negative FIT after colonoscopy. Twenty per cent (17/86) had a positive follow-up FIT. Forty-seven per cent (8/17) of these had ‘other pathology’ detected at their index colonoscopy, 24% (4/17) had a normal index colonoscopy, 12% (2/17) had diminutive polyps (1–5 mm) and 17% (3/17) had polyps >5 mm removed.

Patients with large adenomas removed had the most significant FIT reduction. Patients with a normal colonoscopy or diminutive polyps (1–5 mm) removed ($n=37$) recorded a smaller change in median FIT level (74 $\mu\text{g Hb/g faeces}$ precolonoscopy to 17 $\mu\text{g Hb/g faeces}$ postcolonoscopy), while in those with polyps >5 mm ($n=44$), median FIT dropped from 142 to 2 $\mu\text{g Hb/g faeces}$ after polypectomy ([figure 3](#)).

Figure 3 Postcolonoscopy faecal immunohistochemistry test (FIT) level in patients with a normal colonoscopy or diminutive (<5 mm) polypectomy ($n=37$) (median 17 $\mu\text{g Hb/g faeces}$ (IQR 2–56)) compared with a polypectomy of a polyp greater than 5 mm ($n=44$) (median 2 $\mu\text{g Hb/g faeces}$ (IQR 1–11)).

Table 2 Univariate analysis of factors associated with persistently positive faecal immunohistochemistry test postcolonoscopy

Clinical variable	Relative risk (95% CI)	P value†
Aspirin	1.49 (0.50 to 4.45)	0.47
Novel Oral Anticoagulant/ warfarin	2.74 (0.59 to 12.84)	0.2
Polypectomy	0.43 (0.13 to 1.36)	0.15
Polypectomy <5 mm	1.81 (0.57 to 5.66)	0.31
Polypectomy >5 mm	0.22 (0.07 to 0.75)	0.016*
Colitis/proctitis	10.3 (1.7 to 32.25)	0.011*
Removal of hyperplastic polyp	2.05 (0.55 to 7.69)	0.29
Normal colonoscopy	2.05 (0.55 to 7.69)	0.29

*P<0.05.

†Log rank test.

Ninety-three per cent (41/44) of those who had a polyp >5 mm had a negative follow-up FIT. Overall, polypectomy was associated with a significant reduction in FIT for all polyps removed (decrease from a median of 100 to 5 µg Hb/g faeces) (P<0.0001). Univariate and multivariate analyses were performed (tables 2 and 3) to identify factors independently and significantly associated with a negative follow-up FIT.

Removal of polyps >5 mm was the only factor independently associated with a negative follow-up FIT on multivariate analysis (OR 3.9 (1.3–11.9) P=0.04).

DISCUSSION

FIT correlates well with progression of colonic lesions in the adenoma-carcinoma sequence. An increased FIT level is associated with increased risk of advanced tumour.⁹ In addition, studies have suggested that large adenomas cause a positive FIT and the FIT level correlates to polyp size.⁹ Our data confirm these studies, with a clear increase in median FIT level with increasing polyp size. Some of our patients with polyps >15 mm had FIT levels greater than 6000 µg Hb/g faeces and the median FIT level is greater in patients with adenomas >15 mm than those with CRC, perhaps because large, mobile pedunculated polyps are more likely to bleed than a fixed tumour. This is supported by the fact that large thick-stalked pedunculated polyps have a rich blood supply.¹⁰ Data correlating diminutive adenomas (<5 mm) and FIT levels are more limited. Using FIT, higher false-negative

rates exist for detection of non-advanced adenomas compared with advanced adenomas (using a cut-off of 15 mm to define advanced adenomas).¹¹ No study has conclusively determined the size of adenoma that causes a positive FIT, but it is assumed that adenomas greater than 10 mm do.

In this study, we demonstrate a significant drop in median FIT levels following colonoscopy and polypectomy. In addition, there is a correlation between the size of the largest adenoma removed and the median postcolonoscopy FIT, with lower median FIT levels observed following larger polypectomy, confirming the association between adenoma size and FIT level. Our study suggests that when polyps are detected, those greater than 5 mm are likely the source of a positive FIT, and when removed, the FIT becomes negative. Patients with a normal colonoscopy or a colonoscopy that demonstrated pathology other than polyps showed a smaller reduction in FIT than those that had polyps removed.

What about small polyps? Our data, in addition to already published studies, have shown that large polyps cause a positive FIT and the FIT level is reduced following their removal. Van Doorn *et al*⁹ reported that FIT-positive individuals with non-advanced adenomas (<10 mm) had similar FIT levels compared with individuals without colorectal lesions. The authors concluded that smaller polyps were incidental, rather than causative of a positive FIT. This study was a retrospective analysis of lesions detected in an FIT-based screening pilot in the Netherlands and used a slightly different FIT cut-off than that currently used in BowelScreen (50 µg Hb/g faeces vs 45 µg Hb/g faeces). Our data suggest the threshold at which a polyp may be considered incidental is lower (5 mm rather than 10 mm) as the removal of polyps between 6 mm and 10 mm was associated with a subsequent negative FIT. Over 25% of our patients who had removal of a polyp <5 mm still had a positive FIT following polypectomy, and the median postcolonoscopy FIT level in these patients was almost identical to those who had a normal colonoscopy.

This study confirms that polyp removal in FIT-positive individuals results in a negative follow-up FIT for polyps >5 mm in size. This is reassuring for FIT-based CRC screening programmes as it confirms that these polyps are detected by FIT and that their removal results in the FIT becoming negative. The results suggest possible benefit of a follow-up FIT following colonoscopy in a screening programme. Colonoscopy is not a perfect test and even with optimal performance, cancers are missed, with rates ranging between 2% and 6% dependent on tumour location.¹² A negative FIT provides reassurance and a potential baseline for future FIT-based surveillance. Repeat colonoscopies of patients enrolled in screening programme places a huge burden on endoscopy units. Sequential postcolonoscopy FIT testing may have a role in reducing this demand, but this requires formal study.

Table 3 Cox proportional hazards model of independent clinical variables associated with persistently positive faecal immunohistochemistry test

Clinical variable	Beta	SE	Relative risk (95% CI)	P value†
Polypectomy >5 mm	-1.318	0.641	0.27 (0.07 to 0.93)	0.04*
Colitis/proctitis	2.015	0.95	7.49 (1.16 to 28.26)	0.034*

*P<0.05.

†Log rank test.

The FIT-positive subjects with a normal colonoscopy provide a further discussion point. These individuals may have a transiently positive FIT, which becomes negative with no endoscopic intervention. This requires formal study with validation of a negative colonoscopy necessary to confirm transient FIT positivity. A double-FIT screening strategy where clients undergo colonoscopy after two rather than one positive FIT may address this issue but this would have a potential negative impact on screening uptake. It is of course possible that these individuals have undetected pathology that may become apparent at a later date and it is important that those participating in a CRC screening programme understand that the screening does not seek to diagnose pathology other than colorectal polyps and cancers. This is supported by recent data, which shows that in subjects with a positive FIT and a negative colonoscopy, a higher baseline FIT is associated with increased risk of developing interval cancer in subsequent years.¹³

This study answers a simple question and is strengthened by its prospective design and high uptake among invited participants (over 80%). The inclusion of FIT-negative patients as a control group may have strengthened our findings, but as this was an observational study of a national screening programme, performing endoscopy on the FIT-negative patients was not possible.

We confirm that removal of polyps greater than 5 mm results in a reduction in FIT levels and subsequent FIT negativity. Patients with polyps less than 5 mm have similar baseline FIT levels to those with a normal colonoscopy and do not demonstrate a significant decrease

in FIT level following polypectomy. This suggests that diminutive polyps do not cause a positive FIT.

Contributors DJG, AS, GAD, HEM and GC were involved in study concept and design. DJG, DOD, KS, GAD, AS and GC were involved in drafting and revising of manuscript. DJG, HEM and GC were involved in statistical analysis. DJG, BN, JR, GH and MB were involved in acquisition of data and patient recruitment.

Competing interests None declared.

Ethics approval St. Vincent's Healthcare Group Ethics and Medical Research Committee.

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Significant of this study

What is already known on this topic

- ▶ High rates of adenoma detection are observed in faecal immunohistochemistry test (FIT)-based screening programmes.
- ▶ Advanced adenomas and colorectal cancer are associated with a positive FIT, and there is a correlation between increasing adenoma size and increasing quantitative FIT level.

What this study adds

- ▶ We confirm that removal of advanced adenomas results in a negative follow-up FIT.
- ▶ However, where only diminutive adenomas (<5 mm) are detected, this is unlikely to be the source of a positive FIT.

How might it impact on clinical practice in the foreseeable future

- ▶ Alternative sources of a positive FIT should be explored when only diminutive adenomas are detected at index colonoscopy.