


# Accuracy of faecal immunochemical testing in patients with symptomatic colorectal cancer

A. A. Khan , M. Klimovskij and R. Harshen

Departments of Surgery, Conquest Hospital, St Leonards-on-Sea, and Eastbourne District General Hospital, Eastbourne, UK

Correspondence to: Mr A. A. Khan, Department of Surgery, Eastbourne District General Hospital, Kings Drive, Eastbourne BN21 2UD, UK (e-mail: aftab.khan5@nhs.net)

**Background:** The aim of this study was to determine the diagnostic accuracy of the faecal immunochemical test (FIT) for detecting colorectal cancer in symptomatic patients.

**Methods:** This was a prospective study of patients with bowel symptoms. Stool samples were collected during rectal examination. The HM-JACKarc assay (Kyowa Medex, Tokyo, Japan) was used to quantify faecal haemoglobin (Hb); positive results were those with at least 10 µg Hb/g faeces. Two-by-two tables and receiver operating characteristic (ROC) curve analysis were used to determine diagnostic accuracy;  $\chi^2$  and Mann–Whitney *U* tests were used to compare other parameters.

**Results:** A total of 928 patients were included (M:F ratio 1:1.5; median age 72 (i.q.r. 64–80) years). The overall prevalence of colorectal cancer was 5.1 per cent. The FIT had sensitivity of 85.1 per cent, specificity of 83.5 per cent, positive predictive value of 22.6 per cent and negative predictive value of 99.0 per cent. ROC analysis of FIT for diagnosing colorectal cancer gave an area under the curve value of 0.89 (95 per cent c.i. 0.84 to 0.94). Significant bowel pathology was detected more frequently in FIT-positive patients (35.1 per cent *versus* 7.1 per cent in FIT-negative patients;  $P < 0.001$ ). There were sex differences in FIT positivity (23.7 per cent in men *versus* 17.4 per cent in women;  $P = 0.019$ ); the sensitivity of FIT for colorectal cancer in women was also low. False-negative FIT results were found mainly in women referred with iron-deficiency anaemia, who were found to have caecal cancer.

**Conclusion:** FIT effectively excluded colorectal cancer in symptomatic patients. Integration of FIT into the diagnostic pathway for colorectal cancer would direct resources appropriately to patients with a greater likelihood of having the disease.

#### Funding information

No funding

Presented in part to the 13th Scientific and Annual Meeting of the European Society of Coloproctology, Nice, France, September 2018; available in abstract form as *Colorectal Dis* 2018; 20(Suppl 4): 13

Paper accepted 23 July 2020

Published online 18 September 2020 in Wiley Online Library (www.bjsopen.com). DOI: 10.1002/bjs5.50346

## Introduction

Early diagnosis of colorectal cancer can be challenging owing to the variability of symptoms. The UK Department of Health has introduced a fast-track referral system (2-week wait) to help diagnose these cancers early. The backbone of referral is based on patient symptoms, as outlined by the National Institute for Health and Care Excellence (NICE)<sup>1</sup>. Most patients who are referred undergo further investigations. Colonoscopy remains the standard investigation for colorectal cancer and is a benchmark for assessing the performance of other colorectal cancer screening tests<sup>2</sup>. The pick-up rate of colorectal

cancer via the 2-week-wait pathway is low (5–12 per cent). Only 27–30 per cent of patients are diagnosed at an early stage, and the subsequent survival rate remains similar to that for patients with colorectal cancer referred through other routes<sup>3</sup>. A large number of patients undergo unnecessary colonoscopies, which are invasive and carry risks such as procedural complications, overdiagnosis and overtreatment. The lack of patient awareness regarding red-flag symptoms delays the seeking of help and, along with inappropriate use of the 2-week referral system in some cases, has been associated with the unsatisfactory outcomes of the current strategy<sup>4,5</sup>.

It is well established that bowel symptoms have a very low positive predictive value (PPV) for bowel cancer. Only high levels of faecal haemoglobin (Hb) are indicative of increased risk of significant bowel pathology<sup>6</sup>. The faecal immunochemical test (FIT) uses antibodies directed against human Hb in small stool samples. It provides a quantitative value for faecal Hb, and has been shown to be more accurate than guaiac-based faecal occult blood tests<sup>7,8</sup>. FIT could be the ideal non-invasive test to aid in the management of these patients.

Studies have shown a negative predictive value (NPV) for FIT of 89–100 per cent<sup>9–11</sup>. FIT results could potentially risk-stratify patients with colorectal cancer. This would direct the use of colonoscopy towards high-risk patients and avoid unnecessary investigations in others. Several trials are underway in the UK to evaluate various aspects of the FIT, including stool collection methods and the type of analyser used.

The primary aim of this study was to establish the diagnostic accuracy (sensitivity, specificity, PPV and NPV) of FIT for detecting colorectal cancer in symptomatic patients referred via the 2-week-wait pathway. Secondary aims were to investigate the diagnostic accuracy of FIT in detecting high-risk polyps and to evaluate the impact on FIT results of using digital rectal examination (DRE) to obtain stool samples.

## Methods

This was a single-centre prospective and blinded study undertaken at East Sussex Healthcare NHS Trust, a pioneering FIT centre in the UK. The study design is shown in *Fig. 1*, and was conducted in accordance to the Standards of Reporting Diagnostic Accuracy Guidelines (STARD)<sup>12</sup>. The target sample size was set at 1000 patients.

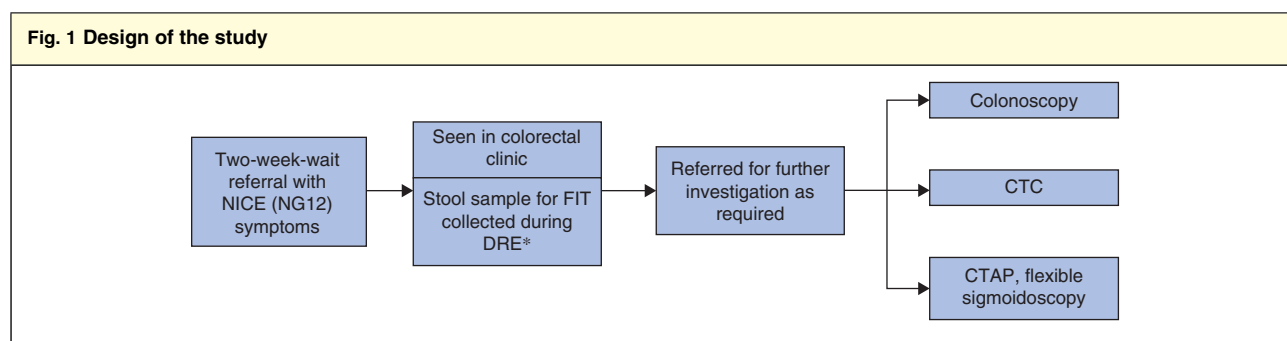
Patients with bowel symptoms, referred via the 2-week-wait colorectal cancer pathway, were recruited over a 1-year period (August 2017 to August 2018).

Patients attending the colorectal clinic on the pathway were given information in advance regarding what to expect during clinical assessment and the investigations that would be undertaken, including FIT. Only patients who had FIT performed and underwent definitive diagnostic investigations were included in the final analyses.

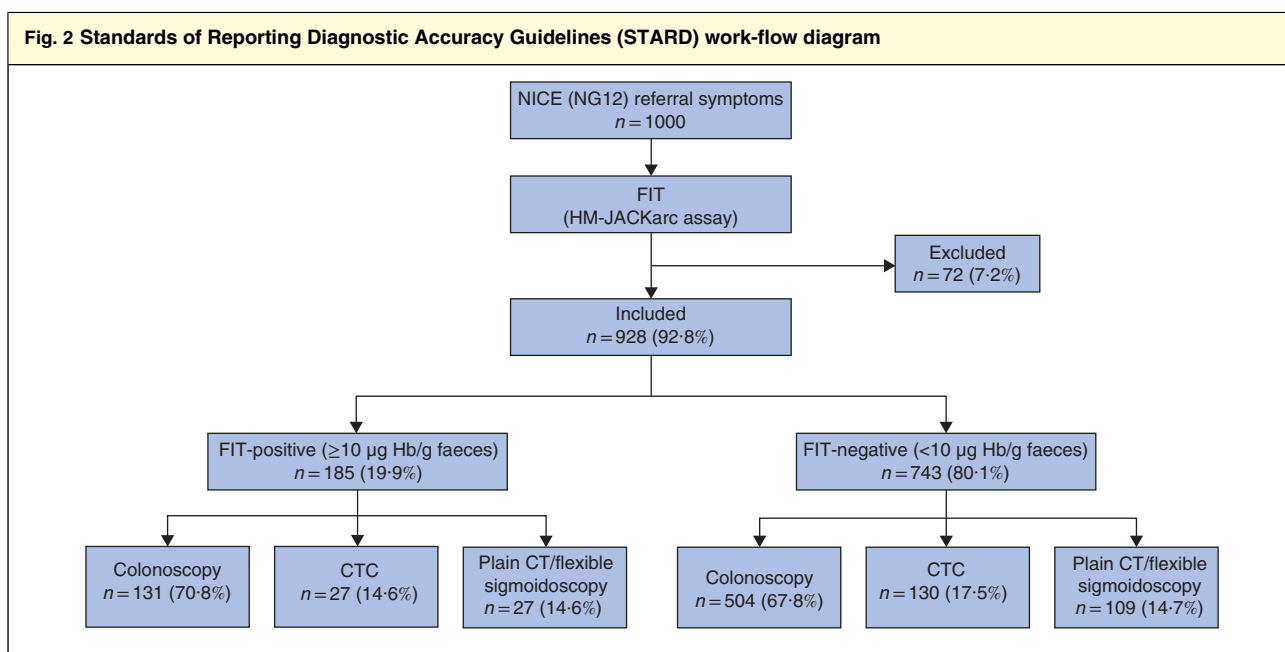
Definitive diagnostic investigations included colonoscopy, CT colonography (CTC) and plain CT, performed on a case-by-case basis depending on the patient's fitness status and their willingness to undergo a particular investigation. Colonoscopy was offered most frequently as the primary investigation. When colonoscopy failed, or a patient refused to have this investigation, CTC was offered. Plain CT was offered only to frail patients deemed unfit for an invasive procedure. Patients with anaemia were investigated according to the British Society of Gastroenterology (BSG) guidelines<sup>13</sup>. Some patients had more than one modality of investigation. Patients re-presenting with similar symptoms, who had had normal findings on comprehensive colonic investigation in the preceding 3 years, were offered focused investigations (for example, flexible sigmoidoscopy for patients re-presenting with rectal bleeding). Clinicians, endoscopists and radiologists were all blinded to the FIT results.

## Stool sample collection for faecal immunochemical testing

Before sampling and FIT, face-to-face counselling was undertaken to explain the process and obtain informed consent. DRE is a usual part of clinical examination during colorectal assessment; a stool sample for FIT was obtained at the same time. For DRE, patients were positioned in the left lateral decubitus position with hips and knees flexed at 90° angles. Disposable, non-latex, non-sterile gloves were used. Water-based lubricant (Optilube®; Optimum Medical, Leeds, UK) was used for lubrication.



\*Patients with obvious blood or no stool on digital rectal examination (DRE) did not have FIT performed. NICE, National Institute for Health and Care Excellence; FIT, faecal immunochemical test; CTC, CT colonography; CTAP, CT of abdomen and pelvis.



NICE, National Institute for Health and Care Excellence; FIT, faecal immunochemical test; Hb, haemoglobin; CTC, CT colonography.

After assessment of the anorectum for pathology, a small amount of faeces was collected and smeared on to the collection picker (EXTEL HEMO-AUTO MC device; Kyowa Medex, Tokyo, Japan). The sample was stored according to manufacturer guidelines and transferred immediately to the local laboratory for analysis. Patients with absent stool or obvious blood on DRE were excluded.

### Faecal haemoglobin measurements

Faecal samples were analysed using the fully automated HM-JACKarc analyser system (Kyowa Medex and Alpha Laboratories, Eastleigh, UK). This system uses latex immunoturbidimetry technology with detection by integrated sphere turbidimetry<sup>14</sup>. In the laboratory, the system is calibrated and checked for quality control every day before samples are run, once daily. The faecal Hb measurements were reported as micrograms of Hb per gram of faeces, as recommended by the Expert Working Group of the Colorectal Cancer Screening Committee (FIT for Screening) of the World Endoscopy Organization<sup>15</sup>. Minimum and maximum reported values were 0.0 and more than 450 µg Hb/g faeces respectively. The NICE<sup>1</sup> recommended threshold value of at least 10 µg Hb/g faeces was set as a positive result. The analysis was carried out by a registered biomedical scientist, and the results were authorized by two consultant clinical scientists before results were issued. The laboratory has a comprehensive quality

management system and is accredited to ISO 15189-based standards.

### Bowel investigations

Colonoscopy and CTC were performed at either Conquest Hospital or Eastbourne District General Hospital. All colonoscopies were performed by an endoscopist approved by the Joint Accreditation Group on Gastrointestinal Endoscopy and reported using a standard template. The CTC protocol was based on guidelines developed by the International Collaboration for CT Colonography Standards<sup>16</sup>. All CTC findings were reported by a consultant radiologist expert in gastrointestinal radiology. Patients with suspicious lesions on CTC underwent optical colonoscopy, if able. Polyps were excised where possible, and histopathological findings reported by a consultant pathologist, based on standards set by the Royal College of Pathologists<sup>17</sup>. All patients with colorectal cancer were staged according to the TNM system and Dukes' staging. Management was discussed and planned by the local colorectal multidisciplinary team (MDT).

### Definition of terms

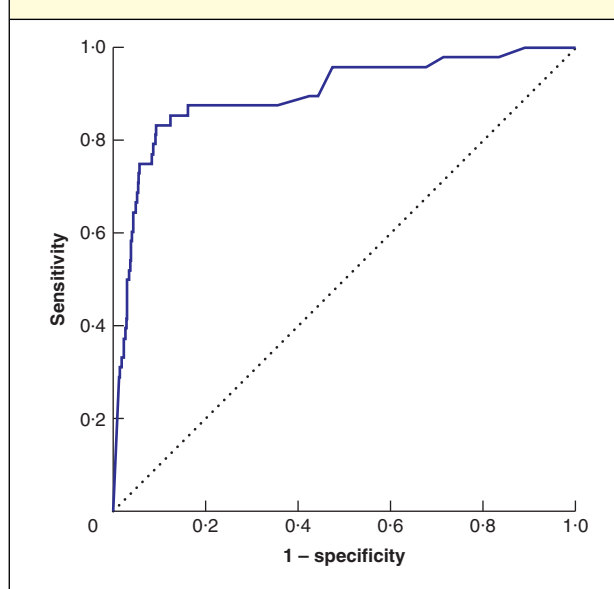
The term colorectal cancer was used for all histological types of malignant colonic neoplasm, including adenocarcinoma, neuroendocrine tumours and lymphoma. The term high-risk polyp included polyps of 10 mm or

**Table 1 Patient demographics, referral signs and symptoms, and completed investigations**

	No. of patients* (n = 928)
<b>Patient demographics</b>	
Age (years)†	72 (64–80)
Sex ratio (M:F)	376:552
<b>Referral signs and symptoms‡</b>	
Change in bowel habit	609 (65.6)
Anaemia	189 (20.4)
Intermittent rectal bleeding	94 (10.1)
Weight loss	70 (7.5)
Abdominal pain	69 (7.4)
Abdominal mass	29 (3.1)
Rectal mass	21 (2.3)
FOB test-positive	2 (0.2)
<b>FIT result</b>	
Positive	185 (19.9)
Negative	743 (80.1)
<b>Investigations performed</b>	
Colonoscopy	635 (68.4)
CTC	157 (16.9)
CTAP, CTCAP or flexible sigmoidoscopy	136 (14.7)

\*With percentages in parentheses unless indicated otherwise; †values are median (i.q.r.). ‡Many patients presented with more than one symptom. FOB, faecal occult blood; FIT, faecal immunochemical test; CTC, CT colonography (virtual colonoscopy); CTAP, CT of abdomen and pelvis; CTCAP, CT of chest, abdomen and pelvis.

larger, high-grade dysplasia or the presence of multiple polyps (more than 5), according to BSG and Association of Coloproctology of Great Britain and Ireland guidelines<sup>18</sup>.

**Fig. 3 Receiver operating characteristic (ROC) curve analysis of faecal immunochemical testing for detecting colorectal cancer**

The cut-off value was 10 µg haemoglobin/g faeces.

### Statistical analysis

Data collection included general patient demographics, referral signs and symptoms, use of antiplatelets and/or anticoagulants, faecal Hb measurements, results of endoscopy or CT, histopathology reports of polyps and colorectal cancers, and MDT outcomes of patients with colorectal cancer. The distribution of FIT measurements

**Table 2 Prevalence of bowel disease and distribution of faecal haemoglobin measurements and faecal immunochemical test results**

	No. of patients (n = 928)	FIT measurement (µg Hb/g faeces)*	FIT-positive (> 10 µg Hb/g faeces) (n = 185)	FIT-negative (≥ 10 µg Hb/g faeces) (n = 743)	P§
<b>Colorectal cancer</b>	47 (5.1)	151.9 (66.2, 208.9)	40 (21.6)	7 (0.9)	< 0.001
Adenocarcinoma	45 (4.8)	158.9 (87.0, 291.7)	39 (21.1)	6 (0.8)	< 0.001
Rectal GIST	1 (0.1)	34.2	1 (0.5)	0 (0)	–
Lymphoma	1 (0.1)	9.9	0 (0)	1 (0.1)	–
<b>Polyps</b>	166 (17.9)	1.8 (1.2, 2.0)	32 (17.3)	134 (18.0)	0.823
Low risk†	136 (14.7)	1.8 (1.1, 1.9)	22 (11.9)	114 (15.3)	0.234
High risk‡	30 (3.2)	1.8 (1.2, 10.7)	10 (5.4)	20 (2.7)	0.061
<b>Colitis</b>	41 (4.4)	1.6 (0.7, 11.4)	15 (8.1)	26 (3.5)	0.006
<b>Diverticulosis</b>	237 (25.5)	1.1 (0.9, 1.5)	36 (19.5)	201 (27.1)	0.034
<b>Haemorrhoids</b>	29 (3.1)	1.7 (0.7, 4.2)	6 (3.2)	23 (3.1)	0.920
<b>Other</b>	109 (11.7)	1.8 (1.1, 2.4)	19 (10.2)	90 (12.1)	0.483
<b>Normal finding</b>	299 (32.2)	0.9 (0.7, 1.1)	37 (20.0)	262 (35.3)	< 0.001

Values in parentheses are percentages unless indicated otherwise; \*values are median (95 per cent c.i.). †Polyps 9 mm or less, or low-grade dysplasia. ‡Polyps 10 mm or above, high-grade dysplasia or multiple polyps (more than 5). FIT, faecal immunochemical test; Hb, haemoglobin; GIST, gastrointestinal stromal tumour. § $\chi^2$  test (FIT-positive versus FIT-negative).

**Table 3** Prevalence of bowel disease and subgroup analysis of faecal haemoglobin measurements according to sex

	Total (n = 928)	Men (n = 376)	Women (n = 552)	P§
<b>Colorectal cancer</b>				
n	47 (5.1)	22 (5.9)	25 (4.5)	0.365
Faecal Hb†		184.1 (114.9, 450.0)	76.2 (34.2, 197.7)	0.049¶
<b>Adenocarcinoma</b>				
n	45 (4.8)	22 (5.9)	23 (4.2)	0.276
Faecal Hb*		184.1 (114.9, 450.0)	87 (35.3, 207.8)	0.097¶
<b>GIST</b>				
n	1 (0.1)	0 (0)	1 (0.2)	–
Faecal Hb*		–	34.2	–
<b>Lymphoma</b>				
n	1 (0.1)	0 (0)	1 (0.2)	–
Faecal Hb*			9.9	–
<b>Polyps</b>				
n	166 (17.9)	79 (21.0)	87 (15.8)	0.045
Faecal Hb*		2.0 (1.4, 4.1)	1.5 (0.8, 1.8)	0.063¶
<b>Low risk†</b>				
n	136 (14.7)	62 (16.5)	74 (13.4)	0.219
Faecal Hb*		1.8 (0.9, 4.0)	1.5 (0.8, 1.8)	0.266¶
<b>High risk‡</b>				
n	30 (3.2)	17 (4.5)	13 (2.4)	0.088
Faecal Hb*		4.3 (1.8 – 11.3)	1.2 (0.2, 45.7)	0.121¶
<b>Colitis</b>				
n	41 (4.4)	17 (4.5)	24 (4.3)	1.000
Faecal Hb*		1.6 (0.8, 22.0)	1.3 (0.5, 11.7)	0.434¶
<b>Diverticulosis</b>				
n	237 (25.5)	92 (24.5)	145 (26.3)	0.592
Faecal Hb*		1.4 (1.0, 2.1)	1.0 (0.8, 1.4)	0.072¶
<b>Haemorrhoids</b>				
n	29 (3.1)	9 (2.4)	20 (3.6)	0.340
Faecal Hb*		4.0 (1.6, 37.4)	1.2 (0.6, 2.9)	0.137
<b>Other</b>				
n	109 (11.7)	46 (12.3)	63 (11.4)	0.756
Faecal Hb*		1.8 (1.7, 5.7)	1.8 (0.9, 3.7)	0.499¶
<b>Normal finding</b>				
n	299 (32.2)	111 (29.5)	188 (34.1)	0.153
Faecal Hb*		1.1 (0.8, 1.7)	0.8 (0.6, 1.0)	0.055¶

Values in parentheses are percentages unless indicated otherwise; \*values are median (95 per cent c.i.) ( $\mu\text{g}$  haemoglobin (Hb)/g faeces). †Polyps 9 mm or less, or low-grade dysplasia. ‡Polyps 10 mm or above, high-grade dysplasia or multiple polyps (more than 5). GIST, gastrointestinal stromal tumour. § $\chi^2$  test, except ¶Mann–Whitney *U* test.

was checked for normality with the Shapiro–Wilk test and Q–Q plots, and showed non-parametric distribution. The Mann–Whitney *U* test was used to compare quantitative variables, and the  $\chi^2$  test for categorical variables.

Receiver operating characteristic (ROC) curve analysis and area under the curve (AUC) estimates were used to determine the diagnostic accuracy of FIT in detecting CRC. The two-by-two table comparing FIT against definitive diagnostic investigation was used to calculate sensitivity, specificity, PPV and NPV with 95 per cent confidence

intervals.  $P < 0.050$  was considered statistically significant. Data maintenance and analysis were performed using Excel® 2013 (Microsoft, Redmond, Washington, USA) and SPSS® version 25 (IBM, Armonk, New York, USA).

## Results

The STARD work-flow diagram is shown in *Fig. 2* and demographic data in *Table 1*. A total of 928 patients (M:F ratio 1:1.5; median age 72 (i.q.r. 64–80 years)) were

included in the final analysis; 72 patients were excluded. Of the excluded patients, 45 (63 per cent) were deemed unfit for further investigation, 17 (24 per cent) declined further investigation, nine (13 per cent) had not completed investigation at the time of analysis, and one (1 per cent) had no stool for analysis on DRE. The most common referral symptoms were change in bowel habit, anaemia and rectal bleeding, and the majority of patients had colonoscopy as the definitive diagnostic investigation. The overall prevalence of colorectal cancer was 5.1 per cent, and that for high-risk polyps was 3.1 per cent.

The diagnostic accuracy analysis of FIT for colorectal cancer showed a sensitivity of 85.1 (95 per cent c.i. 71.0 to 93.3) per cent, specificity of 83.5 (80.8 to 85.8) per cent, PPV of 22.6 (16.0 to 28.3) per cent, and NPV of 99.0 (97.9 to 99.5) per cent. For high-risk polyps, FIT showed sensitivity of 34.4 (18.5 to 54.3) per cent, specificity of 83.5 (75.9 to 89.2) per cent, PPV of 31.2 (16.7 to 50.1) per cent, and NPV of 85.4 (78.0 to 90.8) per cent. ROC curve analysis of FIT estimated the AUC as 0.89 (95 per cent c.i. 0.84 to 0.94) for colorectal cancer and 0.60 (0.50 to 0.70) for high-risk polyps (Fig. 3).

Details of faecal Hb measurements grouped according to final diagnosis are shown in Table 2. In general, significant bowel pathology (colorectal cancer, high-risk polyps and colitis) was detected more frequently in FIT-positive than in FIT-negative patients (35.1 versus 7.1 per cent;  $P < 0.001$ ).

Sex differences in faecal Hb measurements and final diagnoses are shown in Table 3. In general, there were sex differences in FIT positivity between men (90 of 376, 23.9 per cent) and women (95 of 552, 17.2 per cent) ( $P = 0.015$ ). In the subgroup analysis, the diagnostic accuracy of FIT for colorectal cancer showed similar NPV in men and women: 99.6 (95 per cent c.i. 97.7 to 99.9) and 98.6 (97.0 to 99.4) per cent respectively. The other diagnostic parameters were better for men (sensitivity 95.4 (75.1 to 99.7) per cent; specificity 80.5 (75.9 to 84.4) per cent); PPV 23.0 (15.3 to 33.6) per cent) compared with values in women (sensitivity 76.1 (54.5 to 89.8) per cent; specificity 85.5 (82.2 to 88.4) per cent; PPV 20.0 (12.7 to 29.4) per cent).

A total of 185 patients (19.9 per cent) tested positive on FIT analysis. Amongst the FIT-positive patients, 40 (21.6 per cent) were diagnosed with colorectal cancer, 10 (5.4 per cent) had high-risk polyps, 15 (8.1 per cent) had colitis, 83 (44.9 per cent) had other diagnoses, and 37 (20.0 per cent) had a normal finding. Patients with colorectal cancer had the highest FIT values (151.9 (95 per cent c.i. 66.2 to 208.9)  $\mu\text{g Hb/g faeces}$ ) (Table 2). The colorectal cancers diagnosed included 39 adenocarcinomas (98 per cent) and one gastrointestinal stromal tumour (GIST) (3 per cent).

Details of the 40 patients with FIT-positive colorectal cancer are given in Table S1 (supporting information).

A total of 743 patients (80.1 per cent) tested negative on FIT analysis. Of these patients, seven (0.9 per cent) were diagnosed with colorectal cancer, 19 (2.6 per cent) had high-risk polyps, 26 (3.5 per cent) had colitis, 414 (55.7 per cent) had other diagnoses and 277 (37.3 per cent) had normal findings. The seven colorectal cancers diagnosed included six adenocarcinomas (86 per cent) and one (14 per cent) lymphoma. The median faecal Hb measurement for FIT-negative adenocarcinoma was 1.5 (95 per cent c.i. 0.5 to 9.9)  $\mu\text{g Hb/g faeces}$ . Details of the FIT-negative colorectal cancers are presented in Table S2 (supporting information).

## Discussion

In patients with bowel symptoms referred on the UK NHS 2-week-wait pathway, FIT was highly accurate in diagnosing colorectal cancer with high AUC (0.89), sensitivity (85.1 per cent), specificity (83.5 per cent) and NPV (99.0 per cent). Only 19.9 per cent of patients tested positive on FIT analysis and, of these, one-third had significant bowel pathology. FIT-negative patients had predominantly normal findings on further investigation.

Only a handful of previous studies<sup>9–11</sup> have used the HM-JACKarc analyser. Widlak and colleagues<sup>11</sup> reported similar results to those in the present study, with a FIT threshold of at least 7  $\mu\text{g Hb/g faeces}$  (the minimum detectable limit for the analyser used). In a sample of 430 patients, 24 colorectal cancers and one high-grade dysplasia were found, and combined for the purpose of analysis. An AUC of 0.94 was reported, with sensitivity of 84 per cent, specificity of 93 per cent and NPV of 99 per cent<sup>11</sup>. There were three FIT-negative colorectal cancers (two descending colon, one hepatic flexure). Godber *et al.*<sup>10</sup> reported a NPV of 100 per cent at a FIT threshold of at least 10  $\mu\text{g Hb/g faeces}$ , but with a lower than average prevalence of colorectal cancer of 2.2 per cent in a cohort of 507 patients. As the analysis of accuracy is dependent on disease prevalence, this could account for the higher NPV estimate. On the other hand, Auge and co-workers<sup>9</sup>, in a study of 208 patients, combined two patients with colorectal cancer and 27 with high-risk polyps into a single group of 'advanced colorectal neoplasia'. Unsurprisingly, the reported NPV of 89.4 per cent was lower than that in other studies.

Studies<sup>6,19,20</sup> using FIT OC-Sensor<sup>TM</sup> analysers (Eiken Chemical, Tokyo, Japan), at a FIT-positive threshold of at least 10  $\mu\text{g Hb/g faeces}$ , have reported similar data on accuracy to those in the present study for detection of colorectal cancer. In general, studies quoting a low AUC often have a

low prevalence of colorectal cancer, and have usually combined colorectal cancer with high-risk polyps and colitis to generate accuracy data<sup>6,9,10</sup>. Only a few studies<sup>11,19,20</sup> have reflected the true prevalence of colorectal cancer. The high NPV (99.0 per cent) in the present study provides further evidence for the efficacy of FIT as a rule-out test for colorectal cancer in symptomatic patients<sup>21</sup>.

The FIT-positive colorectal cancers included adenocarcinomas and one malignant rectal GIST. The median faecal Hb value was significantly higher for adenocarcinoma (158.9 (95 per cent c.i. 87.0 to 291.7) µg Hb/g faeces) than for malignant rectal GIST (34.2 µg Hb/g faeces). The M:F ratio was equal at 1:1, and the distribution of these cancers by anatomical site was similar to that for the overall incidence of bowel cancer in England<sup>22</sup>.

Patients in the FIT-negative colorectal cancer group were predominantly women (6 of 7), with a main referral symptom of iron deficiency anaemia. Most patients had caecal adenocarcinoma, and one had a colonic lymphoma. The median faecal Hb measurement for FIT-negative adenocarcinoma was 1.5 (95 per cent c.i. 0.5 to 9.9) µg Hb/g faeces, and lowering the threshold to at least 7 µg Hb/g faeces, as in other studies, would not have improved the accuracy in the present cohort. There are some plausible explanations for these findings. A series of FIT studies<sup>10,19,23</sup> have shown that women have a lower concentration of faecal Hb than men. In the present study, women had a lower median faecal Hb level, a difference that increased in the presence of colorectal cancer and high-risk polyps. These sex differences in faecal Hb were reflected in the diagnostic accuracy of FIT.

There has been only one previous study<sup>24</sup> that investigated the efficacy of FIT in patients with anaemia. Although the authors reported a higher frequency of endoscopic lesions in the FIT-positive group (79.2 per cent *versus* 27.2 per cent in the FIT-negative group;  $P < 0.001$ ), on subgroup analysis a considerable frequency of lesions (8.7 per cent) were also seen in FIT-negative patients at colonoscopy, highlighting that FIT should be interpreted cautiously in anaemic patients. There is conflicting evidence about the impact of tumour location on FIT results, with some authors<sup>11,25,26</sup> finding left-sided lesions to be associated with a higher faecal Hb level, and therefore improved FIT sensitivity, whereas others<sup>8,27,28</sup> have found no association between colorectal cancer location and FIT results.

There were only 30 patients with high-risk polyps in the present study, and none had high-grade dysplasia on histopathological analysis. Although a significant proportion of patients with high-risk polyps were FIT-positive, the diagnostic accuracy of FIT for high-risk polyps was

poor and thus use of FIT for detection of high-risk polyps is not supported.

Median faecal Hb measurements were considerably higher in patients with colorectal cancer than in those with other diagnoses, supporting the theory that faecal Hb increases with severity of disease<sup>28</sup>. Previous studies<sup>6,9–11,28,29</sup> have reported similar findings of high median faecal Hb measurements in patients with significant bowel pathology, independent of the FIT analyser used.

One-third of patients had normal findings and one-quarter had diverticulosis; in both of these groups there was a significantly higher proportion of FIT-negative patients, as has been seen in previous studies<sup>6,9–11</sup>. This suggests that the integration of FIT into the colorectal investigative pathway could prevent unnecessary invasive investigations in a large number of patients.

A new approach was taken to stool collection in this study. In current practice, the FIT kit is posted to patients for sample collection. With this method, the response rate is generally poor, as reported in previous studies<sup>6,9–11,28</sup>. Other issues found include challenging sample collection and sample labelling difficulties for patients. In this study, samples were collected during DRE in the colorectal clinic. Rectal examination is a point-of-care test and, in this study, improved issues with compliance as well as errors or delays in sampling (such as collection, storage and transfer to the laboratory).

The observational study design is a potential limitation of this study, and there may have been selection bias as the study sample included only symptomatic patients who visited their general practitioner for assessment; however, this is representative of the population referred through the 2-week-wait pathway. The large sample size is a strength of this study, as it allowed separate analysis of the accuracy of FIT for colorectal cancer and high-risk polyps.

The results of this study support the integration of FIT into the colorectal cancer diagnostic pathway to direct resources appropriately to patients with a higher likelihood of colorectal cancer. FIT is not a diagnostic test, but an aid to diagnosis, and it is suitable for use in the primary care setting to guide specialist referral. Clinical judgement is always warranted, particularly in FIT-negative patients.

## Disclosure

The authors declare no conflict of interest.

## References

- 1 NICE. *Suspected Cancer: Recognition and Referral*. NICE Guideline [NG12]. <https://www.nice.org.uk/guidance/ng12/>

- chapter/1-Recommendations-organised-by-site-of-cancer#lower-gastrointestinal-tract-cancers [accessed 7 December 2019].
- 2 Lin JS, Piper MA, Perdue LA, Rutter CM, Webber EM, O'Connor E *et al.* Screening for colorectal cancer. *JAMA* 2016; **315**: 2576–2594.
  - 3 Zafar A, Mak T, Whinnie S, Chapman MAS. The 2-week wait referral system does not improve 5-year colorectal cancer survival. *Colorectal Dis* 2012; **14**: 177–180.
  - 4 Redaniel MT, Ridd M, Martin RM, Coxon F, Jeffreys M, Wade J. Rapid diagnostic pathways for suspected colorectal cancer: views of primary and secondary care clinicians on challenges and their potential solutions. *BMJ Open* 2015; **5**: e008577.
  - 5 Mozdiak E, Tsertsvadze A, McFarlane M, Widlak M, Tabuso M, Dunlop A *et al.* The effect of the 2-week wait referral system on the detection of and mortality from colorectal cancer: protocol of a systematic review and meta-analysis. *Syst Rev BioMed Central* 2016; **5**: 182.
  - 6 McDonald PJ, Digby J, Innes C, Strachan JA, Carey FA, Steele RJC *et al.* Low faecal haemoglobin concentration potentially rules out significant colorectal disease. *Colorectal Dis* 2013; **15**: e151–e159.
  - 7 Brenner H, Tao S. Superior diagnostic performance of faecal immunochemical tests for haemoglobin in a head-to-head comparison with guaiac based faecal occult blood test among 2235 participants of screening colonoscopy. *Eur J Cancer* 2013; **49**: 3049–3054.
  - 8 Cubiella J, Salve M, Díaz-Ondina M, Vega P, Alves MT, Iglesias F *et al.* Diagnostic accuracy of the faecal immunochemical test for colorectal cancer in symptomatic patients: comparison with NICE and SIGN referral criteria. *Colorectal Dis* 2014; **16**: O273–O282.
  - 9 Auge JM, Fraser CG, Rodríguez C, Roset A, Lopez-Ceron M, Grau J *et al.* Clinical utility of one *versus* two faecal immunochemical test samples in the detection of advanced colorectal neoplasia in symptomatic patients. *Clin Chem Lab Med* 2016; **54**: 125–132.
  - 10 Godber IM, Todd LM, Fraser CG, MacDonald LR, Younes HB. Use of a faecal immunochemical test for haemoglobin can aid in the investigation of patients with lower abdominal symptoms. *Clin Chem Lab Med* 2016; **54**: 595–602.
  - 11 Widlak MM, Thomas CL, Thomas MG, Tomkins C, Smith S, O'Connell N *et al.* Diagnostic accuracy of faecal biomarkers in detecting colorectal cancer and adenoma in symptomatic patients. *Aliment Pharmacol Ther* 2017; **45**: 354–363.
  - 12 Bossuyt PM, Reitsma JB, Bruns DE, Gatsonis CA, Glasziou PP, Irwig LM *et al.* Towards complete and accurate reporting of studies of diagnostic accuracy: the STARD initiative. Standards for reporting of diagnostic accuracy. *Clin Chem* 2003; **49**: 1–6.
  - 13 Goddard AF, James MW, Mcintyre AS, Scott BB; British Society of Gastroenterology. Guidelines for the management of iron deficiency anaemia. *Gut* 2011; **60**: 1309–1316.
  - 14 Carroll M, Piggott C, Pearson S, Seaman H, Bruce H, Halloran S. PWE-019 an evaluation of quantitative faecal immunochemical tests for haemoglobin. *Gut* 2014; **63**: A129–A130.
  - 15 Fraser CG, Allison JE, Young GP, Halloran SP, Seaman HE. Improving the reporting of evaluations of faecal immunochemical tests for haemoglobin. *Eur J Cancer Prev* 2015; **24**: 24–26.
  - 16 Burling D; International Collaboration for CT Colonography Standards. CT colonography standards. *Clin Radiol* 2010; **65**: 474–480.
  - 17 Loughrey MB, Quirke P, Shepherd NA. *Standards and Datasets for Reporting Cancers: Dataset for Histopathological Reporting of Colorectal Cancer*. Royal College of Pathologists: London, 2017.
  - 18 Atkin WS, Saunders BP; British Society for Gastroenterology; Association of Coloproctology for Great Britain and Ireland. Surveillance guidelines after removal of colorectal adenomatous polyps. *Gut* 2002; **51**: V6–V9.
  - 19 Mowat C, Digby J, Strachan JA, Wilson R, Carey FA, Fraser CG *et al.* Faecal haemoglobin and faecal calprotectin as indicators of bowel disease in patients presenting to primary care with bowel symptoms. *Gut* 2016; **65**: 1463–1469.
  - 20 Rodríguez-Alonso L, Rodríguez-Moranta F, Ruiz-Cerulla A, Lobatón T, Arajol C, Binefa G *et al.* An urgent referral strategy for symptomatic patients with suspected colorectal cancer based on a quantitative immunochemical faecal occult blood test. *Dig Liver Dis* 2015; **47**: 797–804.
  - 21 Westwood M, Lang S, Armstrong N, Van Turenhout S, Cubiella J, Stirk L *et al.* Faecal immunochemical tests (FIT) can help to rule out colorectal cancer in patients presenting in primary care with lower abdominal symptoms: a systematic review conducted to inform new NICE DG30 diagnostic guidance. *BMC Med* 2017; **15**: 189.
  - 22 Cancer Research UK. *Bowel Cancer Incidence Statistics*. <https://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/bowel-cancer/incidence#heading=Four> [accessed 7 December 2019].
  - 23 McDonald PJ, Strachan JA, Digby J, Steele RJC, Fraser CG. Faecal haemoglobin concentrations by gender and age: implications for population-based screening for colorectal cancer. *Clin Chem Lab Med* 2011; **50**: 935–940.
  - 24 Cilona A, Zullo A, Hassan C, Ridola L, Annese M. Is faecal-immunochemical test useful in patients with iron deficiency anaemia and without overt bleeding? *Dig Liver Dis* 2011; **43**: 1022–1024.
  - 25 Haug U, Kuntz KM, Knudsen AB, Hundt S, Brenner H. Sensitivity of immunochemical faecal occult blood testing for detecting left- vs right-sided colorectal neoplasia. *Br J Cancer* 2011; **104**: 1779–1785.



- 26 Wong MCS, Ching JYL, Chan VCW, Lam TYT, Shum JP, Luk AKC *et al.* Diagnostic accuracy of a qualitative fecal immunochemical test varies with location of neoplasia but not number of specimens. *Clin Gastroenterol Hepatol* 2015; **13**: 1472–1479.
- 27 Quintero E, Castells A, Bujanda L, Cubiella J, Salas D, Lanasa Á *et al.* Colonoscopy *versus* fecal immunochemical testing in colorectal-cancer screening. *N Engl J Med* 2012; **366**: 697–706.
- 28 Digby J, Fraser CG, Carey FA, McDonald PJ, Strachan JA, Diament RH *et al.* Faecal haemoglobin concentration is related to severity of colorectal neoplasia. *J Clin Pathol* 2013; **66**: 415–419.
- 29 Quyn AJ, Steele RJC, Digby J, Strachan JA, Mowat C, McDonald PJ *et al.* Application of NICE guideline NG12 to the initial assessment of patients with lower gastrointestinal symptoms: not FIT for purpose? *Ann Clin Biochem* 2018; **55**: 69–76.

### Supporting information

Additional supporting information can be found online in the Supporting Information section at the end of the article.