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Clinical utility of one versus two faecal immunochemical test samples in the detection of advanced colorectal neoplasia in symptomatic patients

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Abstract

Background: The utility of faecal immunochemical tests (FIT) in assessment of symptomatic patients with lower gastrointestinal symptoms has not been well explored. The aims of this study were to evaluate the diagnostic yield for advanced colorectal neoplasia (ACRN) in symptomatic patients using the first of two FIT samples (FIT/1) and the higher concentration of two FIT samples (FIT/max).
Methods: Samples from two consecutive bowel motions from 208 symptomatic patients who required colonoscopy were analysed using the HM-JACKarc analyser (Kyowa Medex Co., Ltd., Tokyo, Japan). Patients were categorised into two groups: patients with any ACRN and individuals with other diagnoses or normal colonoscopy.
Results: Colonoscopy detected ACRN in 29 patients. In these patients, FIT/1 and FIT/max were significantly higher than in patients with low-risk adenoma (p=0.006 and p=0.024), other findings (p=0.002 and p=0.002) and normal colonoscopy (p<0.001 and p<0.001). The areas under the curves (AUC) of FIT/1 and FIT/max were 0.71 and 0.69, respectively. Undetectable FIT/1 rules out 96.6% of ACRN and the specificity was 10.6%. Increasing the FIT/1 cut-off to 10 μg Hb/g faeces, sensitivity and specificity were 34.5% and 87.2%, respectively. Similar results were obtained using FIT/max with 20 μg Hb/g faeces cut-off, providing a sensitivity and specificity of 34.5% and 85.6%, respectively.
Conclusions: Undetectable FIT is a good strategy to rule-out ACRN in symptomatic patients. The diagnostic yield of collecting two samples for FIT can be achieved with one sample, but a lower faecal haemoglobin concentrations (f-Hb) cut-off is required.

Keywords: advanced colorectal neoplasia; colorectal cancer; diagnostic yield; faecal haemoglobin; faecal immunochemical test; symptomatic patients.

Introduction

In asymptomatic population-based screening programmes for colorectal cancer (CRC), tests for haemoglobin in faeces have been designed to identify occult bleeding from neoplastic lesions before any signs and symptoms become apparent There is considerable evidence from such CRC screening programmes that faecal immunochemical tests (FIT) for haemoglobin have superior analytical and clinical performance characteristics to traditional guaiac-based faecal occult blood tests (gFOBT) [1, 2]. FIT are now well recognised as the non-invasive tests of choice to identify screened individuals for referral to colonoscopy to detect existing neoplasia and pre-malignant colorectal lesions [3–6].

FIT are available in two analytical system formats, qualitative and quantitative. The introduction of quantitative FIT allows different cut-off faecal haemoglobin concentrations (f-Hb) to be selected that, when added to other aspects, such as the frequency of testing, the age range of individuals screened and the number of samples collected for analysis by each individual, facilitates a spectrum of different possible approaches in relation to the screening

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strategy [7–9]. As it is dogma that not all lesions will be detected using one sample, because of the heterogeneous nature of the faecal sample matrix and the likely intermittent bleeding patterns of significant lesions, the diagnostic yield of FIT might be improved by the collection of more than one sample, taken from different bowel motions. However, some studies have concluded that, in using FIT for screening, two or more samples do not improve the diagnostic yield compared to one sample [7–10]. The majority of the studies previously reported have been performed with analytical systems from a single FIT manufacturer, but it has recently been suggested that diagnostic performance may differ between manufacturers [11, 12]. However, this aspect has not been widely studied and it is unknown if, for example the mass of faecal sample collected and buffer volume in the different sample collection devices available also affect diagnostic yield [13]. Other factors than the test characteristics can affect to the variability in FIT positivity and therefore to the diagnostic yield. Some studies suggest the possibility of degradation of haemoglobin in faeces with delayed sample return [14, 15] and ambient temperature also can affect positivity [16, 17].

It is important to note that most of the previous work on analytical and clinical aspects of FIT has been done in the context of CRC screening. Although combinations of symptom and results of FITs were alleged to show good diagnostic performance for CRC, evidence from primary care has said to be lacking [18]. Most primary care providers deal with many patients presenting with lower abdominal symptoms, but the prevalence of significant colorectal disease is low and a tool to aid in triage for colonoscopy would be of considerable value for individuals and for health services. It has been suggested that FIT could provide a very useful investigation in this clinical setting [13, 19, 20]. However, many unexplored variables still exist. Which of the one or two FIT samples is the better investigational approach has never been studied in symptomatic patients and there is controversy over whether FIT should be used as a rule-in or rule-out examination. Thus, the aims of this study were to evaluate the diagnostic yield for advanced colorectal neoplasia (ACRN) in symptomatic patients using one or two FIT samples, to assess differences with respect to the population characteristics and to identify appropriate strategies for use of FIT in this clinical context.

Materials and methods

Study design

We performed a prospective study to assess the diagnostic accuracy of FIT to detect ACRN, comprising CRC and advanced adenoma, comparing the use of one or two FIT samples, and to establish strategies for detection, or exclusion, of ACRN in patients with lower abdominal symptoms.

Study population

The study analysed 208 consecutive patients who attended Hospital Clinic (Barcelona) from December 2013 to March 2014 and required colonoscopy for the investigation of lower abdominal symptoms or colonic polyp surveillance. Patients undergoing CRC screening or with a history of gastrointestinal bleeding, active rectal bleeding, menstruation, haematuria, or known ulcerative colitis were excluded. Patients were asked to begin faecal sampling 5 days before colonoscopy to ensure that two samples were collected before bowel cleansing preparation was initiated. No dietary restriction was undertaken. Medications, such as aspirin and non-steroidal anti-inflammatory drugs (NSAID), were withdrawn 1 week before preparation for colonoscopy. The study was approved by the Hospital Clinic Ethics Committee (2013/8432) and all patients provided written informed consent. All patients received an oral or telephone explanation of the study and were sent written instructions on collecting and storing the faecal sample for FIT with the sample collection devices. In order to assure stability of the haemoglobin in the samples, participants were informed to store the samples at 4 °C before delivery to the laboratory within the following 5 days.

Samples and analysis

Patients were asked to collect samples from two consecutive bowel motions using the sample collection devices provided (Kyowa-Medex Co., Ltd., Tokyo, Japan). The sample collection device collects 2 mg faeces through filling of two small dimples at the end of a round probe attached to the device cap: the probe is then reinserted into the device which contains 2.0 mL buffer. Samples were analysed using the fully automated analyser HM-JACKarc (Kyowa-Medex Co., Ltd.), which employs a latex immunoturbidimetry technology with detection by integrated sphere turbidimetry. The performance characteristics of this recently introduced analytical system have been documented in considerable detail elsewhere [21]. Devices returned were stored at 4 °C and then allowed to warm to room temperature prior to analysis within the next 24 h. f-Hb found were reported as µg Hb/g faeces, as recommended by the Expert Working Group on FIT for Screening, Colorectal Cancer Screening Committee, World Endoscopy Organisation [22]. The method calibration curve is linear for concentrations in the range 7–400 µg Hb/g faeces with a lower analytical detection limit of 0.6 µg Hb/g faeces [21]. Samples >400 µg Hb/g faeces were diluted with buffer from an unused sample collection device. All analysis were carried out by one laboratory technician; the laboratory has a total quality management system and is certified to ISO 9001:2008 standards by AENOR, Asociación Española de Normalización y Certificación (Spain). The analyser was calibrated once every 2 weeks with the calibrators provided (Kyowa-Medex Co., Ltd.). Each analytical run was preceded by analysis of two quality control materials (Kyowa-Medex Co., Ltd.).

Endoscopy

Colonoscopy was carried out to the caecum or up to an obstructing carcinoma if present, without knowledge of f-Hb results. All lesions

were categorised and, if colorectal polyps were detected, the polyp site was recorded and polypectomy performed whenever possible. Histology of all detected lesions was evaluated by expert pathologists devoted to gastrointestinal oncology, following the European Guidelines for Quality Assurance in Colorectal Cancer Screening and Diagnosis [23]. According to the pathology and histology results, patients were categorised into two groups: firstly, those with any ACRN and, second, individuals with other diagnoses (i.e. inflammatory and hyperplastic polyps, inflammatory bowel disease, haemorrhoids, angiodysplasia and diverticulosis) or a normal examination (hereafter referred to as the non-advanced colorectal neoplasia [NACRN] group). ACRN was defined as CRC or high-risk adenoma, which in turn was defined as any advanced adenoma (lesions ≥1 cm in size or with a villous component or high-grade dysplasia) or ≥3 non-advanced adenomas. Tumour staging was established according to the TNM classification system of the UICC [24]. Finally, patients were classified according to the most advanced lesion present.

Statistical analysis

A logarithmic transformation for graphic representation of f-Hb was performed. The Mann-Whitney U-test was used to assess differences between the f-Hb of the two groups, ACRN and NACRN. Receiver operating characteristic (ROC) curves for f-Hb were created as aids to determine clinical performance characteristics and examine f-Hb cut-off ranging 10–40 µg Hb/g faeces. The sensitivity (true positives/[true positives+false negatives]), the specificity (true negatives/[true negatives+false positives]) and the positive (true positives/[true positives+false positives]) and negative (true negatives/[true negatives+false negatives]) predictive values for ACRN at different f-Hb were calculated. ROC curves for the first f-Hb found by FIT (FIT/1) and the higher f-Hb of the two samples (FIT/max) were compared using the Delong method [25] and differences in sensitivity and specificity using FIT/1 or FIT/max at a range of identical f-Hb cut-off were calculated. Statistical analysis was performed using PASW Statistics, Release Version 18.0.0 (SPSS, Inc., Chicago, IL, USA) and GraphPad Prism version 4.00 (GraphPad Software, San Diego, CA, USA). $p<0.05$ was regarded as a statistically significant difference between two data sets.

Results

Colonoscopy findings

Of 208 patients (92 men, 116 women), with a median age of 63 years (range 22–86 years), ACRN detected by colonoscopy in 29 patients (14.0%). These included two CRC at stages II and III (1.0%) and 27 high-risk adenoma (13.0%). Low-risk adenoma were also found in 41 patients (19.7%): other less serious colorectal lesions, including inflammatory and hyperplastic polyps, inflammatory bowel disease, haemorrhoids, angiodysplasia, diverticulosis and minor irrelevant abnormalities, were found in 91 additional patients (43.8%). In 47 patients (22.6%), no unusual findings were detected and the colonoscopy was reported as normal. The prevalence of ACRN in men and women was 18.5% and 10.3%, respectively (p=0.088).

f-Hb

Figure 1 shows the distributions of f-Hb for FIT/1 and FIT/max. The median f-Hb of FIT/1 and FIT/max and interquartile ranges (IQR) were 0.8 (0.3–2.9) µg Hb/g faeces and 0.9 (0.6–7.4) µg Hb/g faeces, respectively. The FIT/max result was not concordant with the FIT/1 result on 39.2% of occasions. As expected, significantly lower f-Hb concentration in FIT/1 as to compared FIT/max was observed ($p<0.001$). The positivity rate at f-Hb cut-offs of 10, 20 and 30 µg Hb/g faeces were 15.8%, 10.5%, 10.0%, for FIT/1 and 23.4%, 17.2% and 16.7% for FIT/max.

The f-Hb partitioned by sex, colonoscopy and pathology diagnosis are documented in Table 1. In patients with ACRN, FIT/1 and FIT/max were significantly higher than in patients with low-risk adenoma (p=0.006 and p=0.024), other findings (p=0.002 and p=0.002) and normal colonoscopy ($p<0.001$ and $p<0.001$). FIT/1 was undetectable in one patient (3.4%) with ACRN and FIT/max was detectable (a numerical result >0 µg Hb/g faeces in all patients with ACRN. In men and women with ACRN, no statistically significant differences were observed between FIT/1 and FIT/max (p=0.760 and p=0.378); in contrast, FIT/1

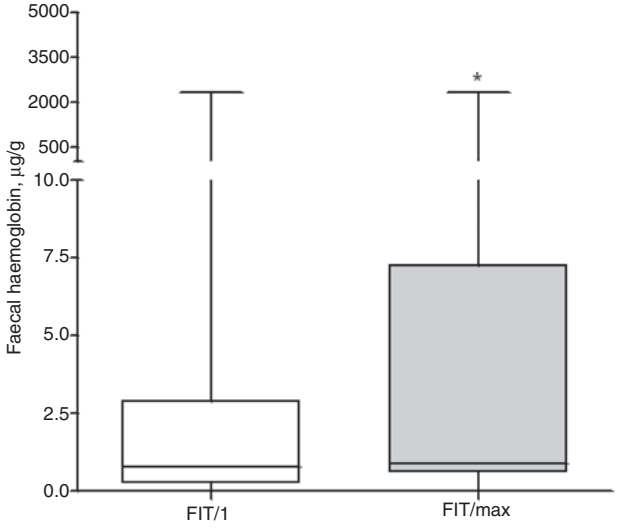

Figure 1: Distributions of faecal haemoglobin concentrations in first sample (FIT/1) and the higher of two samples (FIT/max) in all patients.
*$p<0.05$.

Table 1: Faecal haemoglobin concentrations according to sex, colonoscopy and pathology/histology findings for FIT/1 and FIT/max.

Variable	No.	Faecal haemoglobin concentration, µg Hb/g faeces		
		FIT/1 Median (IQR)	FIT/max Median (IQR)	p-Value
All patients	208	0.8 (0.3–2.9)	0.9 (0.6–7.4)	0.002
Men	92	0.8 (0.3–3.7)	1.2 (0.6–14.4)	0.050
Women	116	0.7 (0.2–2.2)	0.9 (0.6–3.6)	0.013
Advanced colorectal neoplasia	29	3.1 (0.8–43.4)	3.6 (0.9–75.6)	0.423
Men	17	9.2 (0.8–70.3)	9.2 (0.9–80.8)	0.760
Women	12	2.5 (0.8–4.6)	3.0 (1.4–35.1)	0.378
Remaining findings	179	0.7 (0.2–1.7)	0.9 (0.6–3.6)	0.001
Men	75	0.7 (0.3–2.1)	0.9 (0.6–8.0)	0.032
Women	104	0.6 (0.2–1.5)	0.9 (0.5–3.2)	0.016
Low-risk adenoma[a]	41	0.6 (0.3–2.4)	0.8 (0.5–14.0)	0.078
Men	25	0.5 (0.2–2.1)	0.8 (0.4–14.4)	0.080
Women	16	0.7 (0.4–2.0)	0.9 (0.6–7.6)	0.474
Other findings (see text)[a]	91	0.7 (0.3–2.0)	0.9 (0.6–3.7)	0.031
Men	37	0.7 (0.4–2.0)	0.9 (0.6–3.5)	0.330
Women	54	0.6 (0.2–1.8)	1.1 (0.6–5.6)	0.050
Normal colonoscopy[a]	47	0.7 (0.2–1.2)	0.9 (0.4–1.6)	0.117
Men	13	0.8 (0.6–1.2)	1.2 (0.8–13.7)	0.223
Women	34	0.6 (0.1–1.1)	0.7 (0.3–1.2)	0.203

[a]Included in remaining findings.

was significantly lower in men and women with NACRN (p=0.032 and p=0.016) as shown in Figure 2.

Diagnostic yield for ACRN

Figure 3 displays the ROC curves for ACRN detection obtained with FIT/1 and FIT/max. The areas under the curves (AUC) were 0.71 (95% CI 0.59–0.82) and 0.69 (95% CI 0.59–0.80), respectively. No statistical differences were observed between these. However, at specificities ranging from 60% to 85%, sensitivity was higher with FIT/1 as compared to FIT/max. The sensitivity, specificity and positive and negative predictive values for ACRN in relation to sex at different f-Hb cut-off are shown in Table 2. Higher sensitivities at similar specificities were observed

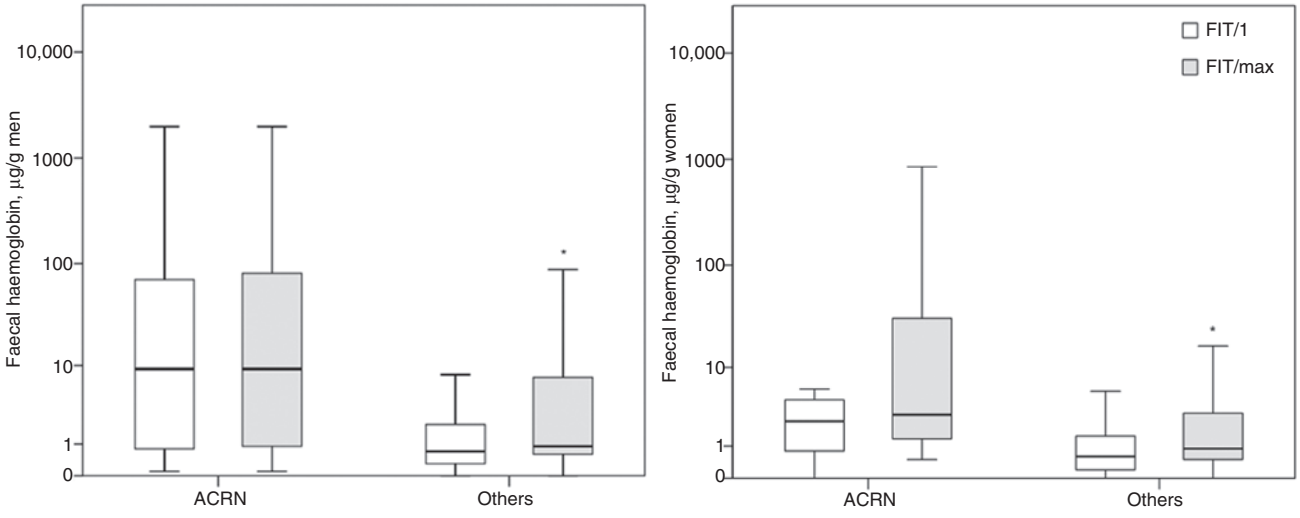

Figure 2: Box and whisker plots showing median and interquartile ranges for faecal haemoglobin concentration (µg Hb/g faeces) in relation to sex and colonoscopy findings for FIT/1 and FIT/max in two groups, advanced colorectal neoplasia (ACRN, colorectal cancer plus high-risk adenoma) and all other findings (others).
*p<0.05.

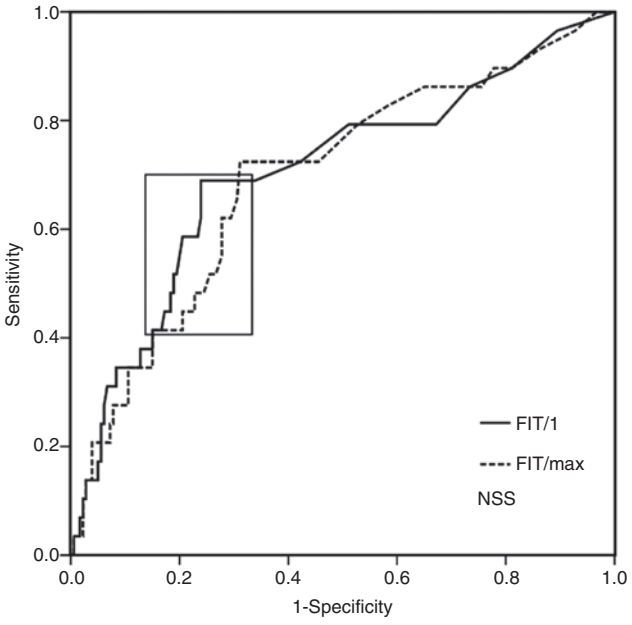

Figure 3: ROC curve for faecal haemoglobin in advanced colorectal neoplasia using first sample (FIT/1) and higher of the two FIT samples (FIT/max).
AUC for FIT/1=0.71 (95% CI 0.59–0.82): AUC for FIT/max=0.69 (95% CI 0.59–0.80). Not statistically significant (NSS).

in men compared to women. Positive predictive values were higher in men, whereas negative predictive values were higher in women. The diagnostic yield for the entire group was calculated at a range of f-Hb cut-offs from 10 to 40 µg Hb/g faeces (Table 3).

Discussion

We have provided here a detailed evaluation of the utility of one vs. two samples for f-Hb measurement using a new automated FIT analytical system in the detection or exclusion of ACRN among symptomatic men and women. It is widely recognised that f-Hb are higher in men than women, and also increase as age increases [12, 26, 27]: these are important considerations to take into account, not only when an analytical system is evaluated, but also in the use of FIT in screening, surveillance and diagnosis. We have confirmed that higher f-Hb are found in men with lower abdominal symptoms compared with women, consistent with other studies [26, 27]. Similarly, a higher clinical sensitivity for detecting ACRN was observed in men than in women, as

Table 2: Sensitivity, specificity and positive and negative predictive values for advanced colorectal neoplasia of FIT/1 and FIT/max at different cut-off faecal haemoglobin concentrations in relation to sex.

Cut-off f-Hb, µg Hb/g faeces	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
Men				
FIT/1$^{>0}$	100% (81.6%–100%)	8.0% (3.7%–16.4%)	19.8% (12.7%–29.4%)	100% (60.9%–100%)
FIT/max$^{>0}$	100% (81.6%–100%)	2.7% (0.7%–9.2%)	18.9% (12.1%–28.2)	100% (34.2%–100%)
FIT/1^{10}	47.1% (26.2%–69.0%)	86.7% (77.2%–92.6%)	44.4% (24.6%–66.3%)	87.8% (78.5%–93.5%)
FIT/max^{10}	47.1% (26.2%–69.0%)	76.0% (65.2%–84.3%)	30.8% (16.5%–50.0%)	86.4% (76.1%–92.7%)
FIT/1^{20}	41.2% (21.6%–64.0%)	92.0% (83.6%–96.3%)	53.8% (29.1%–76.8%)	87.3% (78.2%–93.0%)
FIT/max^{20}	41.2% (21.6%–64.0%)	82.7% (72.6%–89.6%)	35.0% (18.1%–56.7%)	86.1% (76.3%–92.3%)
FIT/1^{30}	41.2% (21.6%–64.0%)	92.0% (83.6%–96.3%)	53.8% (29.1%–76.8%)	87.3% (78.2%–93.0%)
FIT/max^{30}	41.2% (21.6%–64.0%)	82.7% (72.6%–89.6%)	35.0% (18.1%–56.7%)	86.1% (76.3%–92.3%)
FIT/1^{40}	41.2% (21.6%–64.0%)	92.0% (83.6%–96.3%)	53.8% (29.1%–76.8%)	87.3% (78.2%–93.0%)
FIT/max^{40}	41.2% (21.6%–64.0%)	84.0% (74.1%–94.6%)	36.8% (19.2%–59.0%)	86.3% (76.6%–92.4%)
Women				
FIT/1$^{>0}$	91.7% (64.6%–98.5%)	12.4% (7.4%–20.0%)	10.7% (6.1%–18.1%)	92.9% (68.5%–98.7%)
FIT/max$^{>0}$	100% (75.5%–100%)	3.8% (1.5%–9.4%)	10.6% (6.2%–17.7%)	100% (51.0%–100%)
FIT/1^{10}	16.7% (4.7%–44.8%)	87.6% (79.8%–92.6%)	13.3% (3.7%–37.9%)	90.2% (82.7%–94.5%)
FIT/max^{10}	33.3% (13.8%–60.9%)	81.9% (73.2%–88.0%)	17.4% (7.0%–37.1%)	91.5% (83.9%–95.6%)
FIT/1^{20}	16.7% (4.7%–44.8%)	93.3% (86.8%–96.7%)	22.2% (6.3%–54.7%)	90.7% (83.6%–94.8%)
FIT/max^{20}	25.0% (8.9%–53.2%)	87.6% (79.8%–92.6%)	18.8% (6.6%–43.0%)	91.1% (83.8%–95.2%)
FIT/1^{30}	16.7% (4.7%–44.8%)	94.3% (88.0%–97.3%)	25.0% (7.2%–59.1%)	90.8% (83.8%–94.9%)
FIT/max^{30}	25.0% (8.9%–53.2%)	88.6% (80.9%–93.3%)	20.0% (7.1%–45.2%)	91.2% (83.9%–95.2%)
FIT/1^{40}	8.3% (1.5%–35.4%)	95.2% (89.3%–97.9%)	16.7% (3.0%–56.3%)	90.1% (83.1%–94.4%)
FIT/max^{40}	25.0% (8.9%–53.2%)	91.4% (84.5%–95.4%)	25.0% (8.9%–53.2%)	91.4% (84.5%–95.4%)

95% CI, 95% confidence interval; f-Hb, faecal haemoglobin concentration with cut-off shown as superscript; NPV, negative predictive value; PPV, positive predictive value.

Table 3: Diagnostic yield for advanced colorectal neoplasia (ACRN) of FIT/1 and FIT/max at different cut-off faecal haemoglobin concentrations (f-Hb).

Cut-off f-Hb, µg Hb/g faeces	Sensitivity	Specificity	PPV	NPV	Positivity	Detection rate	NNS	NNSC
FIT/1$^{>0}$	96.6% (82.8%–93.4%)	10.6% (6.9%–15.9%)	14.8% (10.4%–20.6%)	95% (76.4%–99.1%)	90.4%	13.4%	7.5	6.8
FIT/max$^{>0}$	100% (88.3%–100%)	3.3% (1.5%–7.1%)	14.3% (10.1%–19.8%)	100% (60.9%–100%)	97.1%	13.9%	7.2	7.0
FIT/1^{10}	34.5% (19.9%–52.7%)	87.2% (81.6%–91.3%)	30.3% (17.4%–47.3%)	89.2% (83.7%–92.9%)	15.8%	4.8%	20.9	3.3
FIT/max^{10}	41.4% (25.5%–59.3%)	79.4% (73.0%–84.7%)	24.5% (14.6%–38.1%)	89.4% (83.6%–93.3%)	23.4%	5.7%	17.4	4.1
FIT/1^{20}	31.0% (17.3%–49.2%)	92.8% (88.0%–95.7%)	40.9% (23.3%–61.3%)	89.3% (84.1%–92.9%)	10.5%	4.3%	23.2	2.4
FIT/max^{20}	34.5% (19.9%–52.7%)	85.6% (83.5%–92.9%)	27.8% (15.8%–44.0%)	89.0% (83.5%–92.9%)	17.2%	4.8%	20.9	3.6
FIT/1^{30}	31.0% (17.3%–49.2%)	93.3% (88.7%–96.1%)	42.9% (24.5%–63.5%)	89.4% (84.1%–93.0%)	10.0%	4.3%	23.2	2.3
FIT/max^{30}	34.5% (19.9%–52.7%)	86.1% (83.6%–92.9%)	28.6% (16.3%–45.1%)	89.1% (83.6%–93.0%)	16.7%	4.8%	20.9	3.5
FIT/1^{40}	27.6% (14.7%–45.7%)	93.9% (89.4%–96.6%)	42.1% (23.1%–63.7%)	88.9% (83.7%–92.7%)	9.1%	3.8%	26.1	2.4
FIT/max^{40}	34.5% (19.9%–52.7%)	88.3% (82.8%–92.2%)	32.3% (18.6%–49.7%)	89.3% (83.9%–93.1%)	14.8%	4.8%	20.9	3.1

95% CI, 95% confidence interval; f-Hb, faecal haemoglobin concentration with cut-off shown as superscript; NNS, number needed to screen to detect one ACRN; NNSC, number needed to scope to detect one ACRN; NPV, negative predictive value; PPV, positive predictive value.

previously suggested by others [26, 28]. The positive predictive value was higher and the negative predictive value was lower in men when compared with women, again in agreement with previous studies [11, 12, 26, 27]. It is interesting to note that, unsurprisingly, higher sensitivity is found in women, when FIT/max is used. This aspect may be due to differences between men and women, such as colonic transit time and differences in colorectal lesions among men and women [14, 29–31], but may also be associated with certain analytical characteristics of systems from different manufacturers, such as faecal mass collected, buffer volume and, possibly most importantly, the specificity of the antibodies against human haemoglobin and its early degradation products that is employed. We support, at least in part, a suggestion that a design in the collection device standardisation is needed [13], although there are drawbacks in that this concept might inhibit flair and imagination in design of sample collection devices for FIT at this comparatively early stage of the evolution of this technology.

Irrespective of the previously documented sex differences, when FIT/1 and FIT/max were compared at identical f-Hb cut-off, an increase in certain diagnostic yield variables was observed in FIT/max (higher sensitivity and higher detection rate for ACRN and a decrease in the number of individuals needed to screen to detect an advanced neoplastic lesion); in contrast, other variables become less advantageous (increase in positivity rate – and therefore colonoscopy demand, the number of individuals needed to scope to detect an advanced neoplastic lesion and lower specificity). These results are consistent with previous studies [10, 32] and suggest that the diagnostic yield of collecting two samples for FIT can be achieved with a one sample strategy, but using a lower f-Hb cut-off. Such considerations have not as yet been incorporated into the routine use of FIT in either screening or assessment of the symptomatic.

The strengths of this study are the availability of high quality colonoscopy and histology results in all patients, and the opportunity to evaluate the HM-JACKarc analyser (Kyowa-Medex Co., Ltd.), an automated FIT analytical system that has only recently become available in a laboratory accredited to internationally accepted standards. To our knowledge, this is the first study that has assessed the diagnostic yield performance in a symptomatic population using this analyser. Some limitations are also clear, such as the small number of patients and the low number of CRC detected. However, this study was undertaken as a pilot for future more extensive investigations using this particular FIT system.

In conclusion, we strongly advocate that better strategies are needed in the use of f-Hb, Different f-Hb cut-off in relation to sex may confer benefit to women. Moreover, the

diagnostic yield of collecting two samples for FIT (using a fHb cut-off of 20 μg Hb/g faeces) can be achieved with one sample, albeit using a lower f-Hb cut-off (10 μg Hb/g faeces). We have studied the diagnostic yield and in this study have concentrated on the use of FIT as a diagnostic rule-in test for ACRN. However, irrespective of the f-Hb cut-off and the number of samples analysed, the clinical sensitivity is low, as is the positive predictive value and the AUC. This implies that this investigation is far less than ideal at detecting significant colorectal disease, with many false positives being found: all positives are referred for colonoscopy and so much of the workload will be directed to those who may not truly warrant this expensive, time-consuming and not without risk invasive investigation. In contrast, the specificity is high at all f-Hb cut-offs and, with both one and two samples, there are few false negative test results. In addition, the negative predictive values are similarly high throughout and do not depend on the f-Hb cut-off. This suggests that those with an undetectable f-Hb will be unlikely to have significant colorectal disease, although a few cases would be missed. In our opinion, FIT users must consider choosing the cut-off based on the clinical needs and in relation to different clinical and demographic aspects. It has been advocated that FIT should be used as a rule-out test in the symptomatic [20], although some have also examined FIT as a rule-in test [19]. Clearly, there are merits and disadvantages to both approaches and we believe that this requires considerable further research, including whether adding age and sex into the interpretation, as we have done in asymptomatic population screening [33], would have significant benefits for individual patient care.

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