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Faecal haemoglobin: Measurement, applications, and future potential

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ABSTRACT

Faecal hemoglobin concentrations (f-Hb) can be quantitated using faecal immunochemical test for haemoglobin (FIT) analytical systems. FIT are of proven value and widely used in colorectal cancer (CRC) screening. Several factors affect f-Hb including sex, age, deprivation, geographical region, and FIT system. Thus, FIT data may not be transferable. Women are disadvantaged in programmes using a single f-Hb threshold for all participants, but risk scoring or sex stratified thresholds could be used to minimise this problem. In addition, low but detectable f-Hb, below the threshold, implies future risk of CRC. In several countries, where colonoscopy resources are constrained, FIT are now accepted as of added value in assessment of patients presenting in primary or secondary care with symptoms, although some serious colorectal disease is missed. Elevated f-Hb in the absence of any discernible colorectal lesions is common and has been found in several diseases with a systemic inflammatory component, including circulatory, respiratory, digestive, neuropsychological, blood and endocrine diseases, and others. There is growing evidence for the value of f-Hb in post-polypectomy surveillance, potentially saving costs and colonoscopy. There may be a role for FIT systems which have lower limits of detection than currently available methods. The faecal material remaining in FIT specimen collection devices could be used for further studies, including assessment of the microbiome. The estimation of f-Hb is now a mature investigative tool but further research will undoubtedly expand applications of value.

1. Introduction

The estimation of faecal haemoglobin as a biomarker for the presence of blood in the colorectum has become widely used in screening for colorectal cancer (CRC) and in the diagnosis of neoplastic and other lower gastrointestinal (GI) disease in patients presenting with symptoms. There is also growing evidence for other potential applications.

Since the discovery of the property of guaiacum gum of being able to detect blood in the urine and faeces, by making use of the pseudo-peroxidase action of the haem moiety of haemoglobin, which oxidizes guaiac acid to guaiac blue, the guaiac-based faecal occult blood test (gFOBT) has used for the detection of macroscopically invisible (occult) blood in faeces [1]. As a result of studies on the effectiveness of screening for CRC with gFOBT, as described in a systematic review of six trials, the meta-analysis of results from the four randomised controlled trials (RCT) showed that the individuals who participated in screening had a reduction in CRC mortality of 16% and, when adjusted for participation, this was 23% [2]. Subsequently, several pilot evaluations were performed, such as that in the United Kingdom [3], and programmatic CRC screening using gFOBT became widely introduced and was recommended in many CRC screening guidelines, such as those in Europe [4].

However, despite this success in CRC screening using a non-invasive investigation, the demerits of gFOBT became recognised. Indeed, Sherlock Holmes had documented his opinion many years before CRC screening was introduced in saying that: “The old guaiacum test was very clumsy and uncertain” [5]. Dietary or drug restriction was often recommended before the test in attempts to reduce potential interferences leading to false-positive test results, two samples of faeces from each of three bowel motions were required, because of the stability of haem in the GI tract, gFOBT were not specific for colorectal bleeding, and the performance was manual with visual interpretation of results, which makes quality assurance challenging [6]. gFOBT were also widely used in primary and secondary care settings, but their application in these settings was deprecated [7].

Over recent time, gFOBT have been almost ubiquitously replaced in CRC screening with faecal immunochemical tests for haemoglobin, usually termed FIT. FIT have many advantages in that no dietary

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interference is found, only one faecal sample is usually submitted for analysis, and the test is more specific for lower GI tract bleeding; the evolution of gFOBT to FIT has been detailed [8,9]. Indeed, it has been stated that gFOBT are now obsolete in this clinical context [10]. FIT are available in two formats, qualitative tests, based upon immunochromatography, and quantitative tests, generally based on immunoturbidimetry, although a variety of other techniques, including enzyme-linked immunosorbent assays (ELISA), are also available [8,9]. A comparison of the advantages and disadvantages of the formats has been tabulated [11]. Qualitative FIT have some advantages, including potential as point-of-care tests and for opportunistic screening, but have major disadvantages, particularly that they have different analytical limits of detection and thus give very different clinical outcomes [8,9]. Quantitative FIT are much more suitable for programmatic CRC screening and have many advantages [12], especially that numerical estimates of faecal haemoglobin concertation (f-Hb) are obtained, potentially allowing considerable flexibility in programme design as well as deeper knowledge of many aspects of CRC screening. More recently, f-Hb has been recommended to be a very useful first-line investigation of most patients presenting with lower GI symptoms suspected of having CRC [13]. Other applications of f-Hb assessment are potentially useful. The detailed clinical aspects and outcomes of f-Hb measurement have been reviewed [14] will not be documented again in detail here.

In this review, the measurement of f-Hb will be considered, factors affecting f-Hb will be documented, the sex inequalities in CRC screening described, strategies to minimise these discussed, and other potentially useful applications of f-Hb addressed. Prior to analysis of numerical f-Hb, FIT usually have easy to use, hygienic, faecal specimen collection devices [15] in which a probe (sometimes termed a stick) attached to the cap of the device is used to collect faeces into dimples or grooves at the end of the probe by either multiple insertions into a single faecal motion or by scraping across the surface of the faecal sample. Then, the probe is reinserted into the device (sometimes termed a bottle or a tube), which contains a volume of buffer. Older data concerning f-Hb were documented using units of ng Hb/mL buffer. Since the different FIT specimen collection devices gather different masses of faeces into different volumes of buffer, the numerical results expressed in these units are not transferable between different FIT. Now, because of efforts by the Expert Working Group on FIT for Screening, CRC Screening Committee, World Endoscopy Organization, significant global harmonisation has occurred with the wide adoption of μg Hb/g faeces units to report f-Hb [16].

2. Measurement of faecal haemoglobin concentration

A plethora of different quantitative FIT analytical systems are currently available. Although these are stated to estimate "faecal haemoglobin concentration," the antibodies against which epitopes on the globin moiety of haemoglobin bind in the immunochemical reaction also bind "early degradation products." In consequence, although all systems are termed FIT, they measure different spectra of molecules and give different numerical results. There are several published comparisons of quantitative FIT systems and the advantages and disadvantages of approaches that can be applied in such studies have been documented in detail [17]. Most published comparisons of qualitative FIT have involved assessment of only two analytical systems. For example, in The Netherlands, it was shown that, in a comparison of two automated FIT systems, the OC-Sensor (Eiken Chemical Co., Ltd, Tokyo, Japan) and FOB-Gold (Sentinel Diagnostics, Milan, Italy) were equally acceptable to a screening population although FOB-Gold was more prone to have specimens submitted that were unsuitable for analysis [18]. Some differences were seen. The positivity differed (7.9% and 6.5% respectively). Interestingly, the diagnostic yield of advanced neoplasia (AN), CRC plus advanced adenoma (AA) sometimes precursors of CRC, and positive predictive value (PPV) were not significantly different when the FIT were assessed at the same positivity instead of the same f-Hb threshold. An analogous study suggested that the acceptability and diagnostic performance of HM-JACKarc (Minaris Medical Co., Ltd, Tokyo, Japan) and of OC-Sensor systems were similar in a screening setting and again described the rationale for comparing FIT systems at the same positivity rather than at the same f-Hb threshold [19]. A further example is provided by the study of Gies et al. which directly compared the sensitivity and specificity values with which nine quantitative (laboratory-based and point-of-care) FIT detected AN in a single CRC screening study [20]. It was suggested that the found differences in diagnostic performance can be overcome to a large extent by adjusting f-Hb thresholds to yield defined levels of specificity or positivity. These studies all support the concept that, instead of adopting the f-Hb thresholds suggested by the manufacturer, CRC screening programmes should choose thresholds based on intended levels of performance for the characteristic selected. An interesting recent study examined the difference in interval cancer proportions (ICP: interval cancers/(interval + screen-detected cancers) found in a FIT pilot evaluation and then in a national programme: for all participants, and women and men, in the FIT-based programme, the ICP were lower than in the pilot: the crucial variable was that two different FIT analytical systems were used, since the f-Hb thresholds were the same at 80 μg Hb/g faeces [21].

As the use of FIT in the assessment of patients presenting in primary (or secondary) care with symptoms potentially of CRC has expanded, a few studies comparing different FIT systems in this clinical context have now been conducted. Chapman et al. showed that there were large variations in f-Hb found using two FIT systems and that the data suggested that system-specific f-Hb thresholds could be applied at lower f-Hb [22]. A more comprehensive recent study of four FIT systems showed that, although the analytical performance characteristics of the four systems were stated all to be acceptable [23], at lower thresholds of the limit of detection and of the recommended [24] and widely used 10 μg Hb/g faeces, differences were observed between both the systems in terms of patients who would be referred for further investigation and their diagnostic accuracies [25].

Overall, the logical conclusion is that differences are observed in the f-Hb generated on different FIT systems, probably due to the lack of standardisation or harmonisation of the analytical methods, although efforts are being made to address this very challenging aspect of FIT [26]. Moreover, manufacturers often change components of the system, such as the calibration strategy or the composition of the buffer in the specimen collection devices, causing further difference in the f-Hb results. In consequence, it is important to realise that data on f-Hb may not be consistent over time as well as analytical system.

3. Factors affecting faecal haemoglobin concentration distributions

It has been apparent for many years, even using gFOBT, that the positivity in screening was lower in women than in men, increased with age in both sexes, was higher in more deprived populations, and differed from country to country, as well as with stage of screening (prevalent or incident). The reasons for this are clear on examination of the distributions of f-Hb. The first assessment of numerical f-Hb distributions showed that, as inferred from positivity, f-Hb varied with sex and age. At any f-Hb threshold, more men were declared positive than women and more older people were declared positive than younger people. The future risk of neoplasia was higher in men than in women and in older people. It was concluded that more tailored strategies were needed in screening programmes, that f-Hb could be included in individual risk assessment scores, and that examination of distributions should assist in screening programme design [27]. The same database was used to show that deprivation and f-Hb are directly related, which has important implications for selection of f-Hb for thresholds in screening programmes and supports the inclusion of deprivation in risk-scoring systems [28]. This work was followed up by comparisons of f-Hb distributions in Scotland, Florence, and Taiwan [29] and Barcelona [30]

which showed that, in all locations, f-Hb was lower in women than men and increased with age. The relationships between f-Hb and sex, age, and deprivation were confirmed in an Australian study [31]. More recently, a large study further confirmed these findings and showed that, even in a small country like Scotland, f-Hb differed from region to region, possibly due to different levels of deprivation [32]. Interestingly, the f-Hb distributions found in the FIT evaluation in Scotland [27] were different to those found in the Scottish Bowel Screening Programme (SBoSP) [32] again showing that it is likely that different f-Hb results are obtained using different FIT systems. The 95th percentile of distributions in women and men, in four groups of 50–54, 55–59, 60–64 and 60–69 years of age, and in the SBoSP [32] and the Scottish pilot evaluation [27], Florence [29] Taiwan [29], and Barcelona [30] are shown in Fig. 1 confirming visually that f-Hb rises with age and is lower in women than men in all age groups, but differs from country to country, also noting that the SBoSP and Scottish pilot data were determined using different FIT systems and the other four data sets with the same FIT system. Moreover, as expected, f-Hb distributions are different in prevalence and incident screening: as a recent example, it was shown that the distributions of f-Hb in the first and second rounds differed in those who had had a FIT negative result in the first round and in those in whom neoplastic pathology had been found [33]. CRC also affects f-Hb distributions: marked differences were found in f-Hb between women and men who had been diagnosed with CRC: the distributions clearly showed lower f-Hb in women with CRC at all sites and stages although the difference in f-Hb in women and men became less statistically significant as stage advanced from stages I to IV [34]. Finally, in addition to these factors affecting f-Hb distributions, several studies have shown that these can be affected by pre-analytical factors such as sample storage and transport temperature, and buffer formulation [35].

As a result of such findings, it has been proposed that local assessment of f-Hb distributions is vital before the initiation of, and for the ongoing quality assurance of FIT-based screening programmes [14,36].

4. The role of faecal haemoglobin concentration in the sex inequality in CRC screening

The differences between women and men in almost all aspects of CRC screening have been the subject of much attention over the years and there are many publications reviewing these [37–40]. Despite women accepting invitations to screening at higher rates than men and adhering to serial round invitations for screening more than men, women are disadvantaged in CRC screening, particularly in programmes that use FIT with a single f-Hb threshold for all participants followed by colonoscopy or other bowel visualisation techniques. The findings to support this have been described in detail in a recent review [41] and are summarised in Table 1 (modified from Ref. [41] with permission) and will not be detailed again here. Although there is no single cause for all the known disadvantages, many can be attributed to the ubiquitous finding that, as documented above, women have lower f-Hb than men. Moreover, this explains the effects on many other outcome characteristics as shown in Table 1. It is fascinating to conjecture why f-Hb is lower in women and possible reasons include the following. Peripheral venous blood haemoglobin concentrations are lower in women than in men: however, these differences are not apparent after the menopause, when screening is generally initiated [42]. Women have longer gut transit times than men [43], and slower gut emptying, which results in higher levels of degradation of any haemoglobin released into the colon and, thus, lower detection of proximal neoplastic lesions, since faecal

Table 1
Disadvantages experienced by women as compared to men in colorectal cancer (CRC) screening programmes based on faecal immunochemical test(s) (FIT) followed by colonoscopy using a single faecal haemoglobin concentration threshold for all participants.

FIT positivity is lower in women than for men: thus, a lower proportion of women are invited for further investigation as compared to men.
Although the uptake of, and adherence to, FIT-based screening is consistently higher in women than that in men, the cancer detection rate and yield of neoplastic pathology are lower in women.
FIT-based screening programmes are associated with a lower reduction in CRC incidence and mortality in women than in men.
FIT clinical sensitivity is consistently lower, and specificity is consistently higher, for women as compared to men.
The interval cancer proportion, which is proportion of CRC detected after the finding of a FIT result below the threshold before the next invitation, is higher in women.
CRC location is different between women and men, with women showing more adenomas located in the proximal colon so that any haemoglobin released may be degraded.
Sessile serrated lesion detection is significantly higher in women compared with men: such lesions are not well detected by FIT.
The faecal haemoglobin concentrations in women found to have CRC in FIT screening are lower than in men.
Women have a significantly higher risk of false positive FIT results leading to unnecessary colonoscopy.
Post colonoscopy CRC (PCCRC) rates are higher in women than in men.

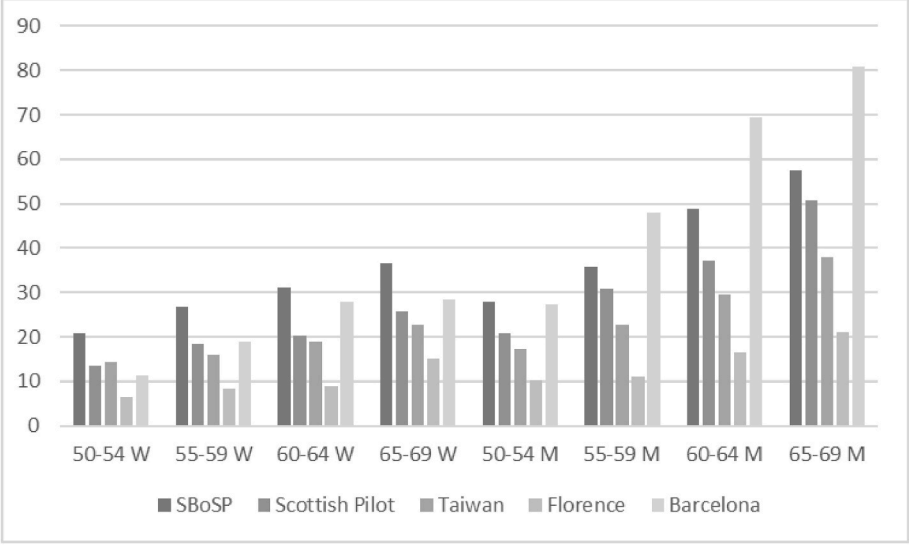

Fig. 1. Faecal haemoglobin concentrations (95th percentile in μg Hb/g faeces) in women (W) and men (M) in four age groups of 50–54, 55–59, 60–64 and 64–69 years in the Scottish Bowel Screening Programme (SBoSP), the Scottish pilot of FIT, Taiwan, Florence, and Barcelona.

haemoglobin degrades in vivo [9]. Further, women have more constipation than men, again leading to higher degradation of any haemoglobin present in the colon [44]. Women have lengthier colons than men, again possibly leading to greater degradation of any haemoglobin present in the colons of women as compared to men [45]. Generally, women probably have healthier lifestyles than men and, in consequence, they have less chronic disease which has been shown to be associated with higher f-Hb without findings of rectal bleeding on colonoscopy [46].

5. Strategies using faecal haemoglobin concentration to reduce sex inequality in CRC screening

A laudable aim for the future of CRC screening would be to develop means whereby screening could be tailored to the individual participant, the precision medicine approach. One approach could be risk-scoring in which variables known, or thought, to affect CRC risk, including f-Hb, are taken together in an algorithm to create a risk score. Many, such as the QCancer®(15yr,colorectal) risk calculator [47] are available to all on the Internet and, while some include previous test results for the presence of blood in faeces, none use f-Hb, to the knowledge of the author: these have been considered elsewhere [41]. Many risk-scoring approaches have been generated and these have been documented in a comprehensive recent review in which 102 unique studies were examined. Overall, it was shown that risk-stratified CRC screening programmes perhaps could improve diagnostic performance, but a large quantum of information did not exist on the longer-term outcomes achieved with such strategies. In addition, it was stated that, despite over 20 years of studies and increasing demands for the routine introduction of risk stratification strategies, only a limited number of studies have investigated such approaches further. Several studies have involved examination of the efficacy of the risk-based screening approaches in comparison, and/or in combination, with FIT results but the review concluded that it is difficult to draw definitive conclusions about these since the results were mixed [48].

Innovations such as faecal DNA analysis along with FIT [49], and genomic profiling [50], have attracted considerable attention in recent years. Further, polygenic risk scores (PRS) are increasing proposed as providing added value for risk stratification in CRC screening; however, even in combination with FIT, using PRS did not improve diagnostic accuracy of FIT-based screening in a large asymptomatic CRC screening population [51]. Further recent research has investigated the value of estimating PRS: for example, in a recent study a PRS was determined, based on the number of risk alleles in 140 single nucleotide polymorphisms and it was shown that PPV and number needed to screen (NNS) of FIT varied widely across people with high and low genetic risk score. It was recommended that further research should evaluate the relevance of these differences for personalized CRC screening [52]. Other approaches have used microRNA: for example, an algorithm based on two faecal miRNA and f-Hb, age, and sex differentiated patients with CRC from those with non-advanced adenomas or normal colonoscopy with an area under the receiver operating characteristic curve value of 90% and avoided 34% of colonoscopies [53].

One of the disadvantages of certain proposed risk scoring approaches is that knowledge of a large number of variables is required, for example, in a recent study [54], two algorithms were developed, a predefined algorithm based on clinically available biomarkers: f-Hb, age, carcinoembryonic antigen (CEA), high sensitivity c-reactive protein (hs-CRP) and ferritin, and an exploratory algorithm adding additional biomarkers, TIMP-1, Pepsinogen-2, HE4, CyFra21-1, Galectin-3, B2M and sex to the predefined algorithm. Although it was concluded that a screening algorithm including a combination of FIT result, blood-based biomarkers, and demographics outperforms FIT in discriminating subjects with or without CRC, it would be interesting to see cost analysis on these, and many of the other proposed complex algorithms, as compared to f-Hb alone or f-Hb with simple addition of sex and age as has been advocated in screening [55]. Interestingly, a rather more mathematical model was established to aid in the diagnosis of patients presenting with lower GI symptoms in a collaboration between Spain and Scotland creating the **F**aeces, **A**ge and **S**ex Test (FAST) score [56], the evidence on which would suggest that further study and refinement are warranted [57,58]: this might be a model for a future risk-scoring approach for use in screening, a concept supported in a recent publication, which also includes screening history as well as sex, age, and f-Hb [59].

Since, as documented above, it is evident that f-Hb is related to sex and age as well as other variables, it has been suggested that use of sex and/or age stratified f-Hb thresholds would be of advantage and might be an approach to attain individualised CRC screening. The FIT-based CRC screening programme in Stockholm-Gotland, Sweden, invited individuals aged 60–69 years, with f-Hb thresholds for further investigation of 40 and 80 μg Hb/g faeces for women and men, respectively. The positivity was 2.6% in women and 2.5% in men [60]. A group in The Netherlands examined individualised f-Hb thresholds for the detection of AN after creating thresholds by sex and age for an overall specificity of 96.9%, equalising the specificity of FIT at a cut-off of 20 μg Hb/g faeces. At this threshold, age and sex adjusted f-Hb thresholds ranged from 36.9 μg Hb/g faeces for 50-year-old women to 9.5 μg Hb/g faeces for 75-year-old men. Thus, f-Hb thresholds at this specificity could vary four-fold between screening participants and it was suggested that use of a spectrum of f-Hb thresholds might benefit CRC screening [61]. It is interesting to note that, in the Swedish CRC screening programme with the stratified f-Hb thresholds for women and men as described above, the interval cancer proportions (ICP) were 25.2% for women and 38.0% for men [62]. In consequence, since the interval cancer rate higher in women, and thus the test sensitivity lower, it was suggested that it might be appropriate to lower the f-Hb threshold in men [63], but in the opinion of this author, this would clearly disadvantage men in such a modified screening programme. Similarly, in Denmark, in a study aimed at finding sex and age specific f-Hb thresholds that could improve population-based CRC screening, it was concluded that, in a FIT-based programme, it is possible to decrease the number of colonoscopies required while at the same time increasing overall sensitivity and specificity and detect more CRC and adenomas by using different f-Hb thresholds for different women and men age groups. This did, however, increase inequality in sensitivity [64]. A report from Finland showed that, use of f-Hb thresholds of 25 μg Hb/g faeces for women and 70 μg Hb/g faeces for men gave similar CRC detection rates in both sexes (0.16% for women and 0.18% for men) and similar positive predictive values (PPV) for CRC (6.4% for women and 6.6% for men) [65].

Thus, stratifying f-Hb thresholds for women and men in different geographical locations can clearly have different effects and the data might not be transferable between programmes, perhaps because, as explained above, f-Hb have different distributions in different countries [29] and different FIT analytical systems give different numerical results [25,26]. Further, it is apparent that the stratified f-Hb thresholds selected to eliminate or minimise sex inequality depend on the variable selected to make equal, for example, positivity, ICP, sensitivity, specificity, or another key performance indicator. This is a vital consideration since the f-Hb thresholds chosen for women and men will differ depending on the characteristic chosen for sex equalisation. For, example, in Scotland, the f-Hb threshold currently used for all participants is 80 μg Hb/g faeces and, to have equal positivity, the f-Hb threshold for women would have to be 50 μg Hb/g faeces if, an important consideration, men were not to be disadvantaged as compared to the current Scottish Bowel Screening Programme [66]. In contrast, should it be considered that ICP should be equal in women and men, then the f-Hb threshold for women would have to be 40 μg Hb/g faeces [67]. However, it would be difficult to objectively assess the effect of introducing sex stratified f-Hb thresholds in a timely fashion. except for positivity. The “elephant in the room” in advocating sex stratified f-Hb thresholds is, of course, the additional colonoscopy requirement, a real problem in many countries.

6. Other potential uses of faecal haemoglobin concentration estimates

6.1. Faecal haemoglobin concentration in chronic diseases with a systemic inflammatory component

Both in CRC programmatic screening and in assessment of patients presenting in primary (or secondary) care with lower GI symptoms using FIT, a sizeable percentage of the participants or patients have detectable f-Hb but are found to have no lesions on bowel visualisation. One factor that might cause such "false positive" results is the longer-term use of medicines, particularly anti-thrombotic drugs, which undoubtedly lead to lower PPV for colorectal neoplasia, as shown in recent studies [68, 69]. Haug nicely summarised the data documented in the Danish study [70] as, among people receiving any antithrombotic treatment, 11.8% tested positive, as compared with 6% among people not receiving antithrombotic treatment. The PPV, assessed based on those with a positive FIT result undergoing follow-up colonoscopy, was lower in users of antithrombotic drugs compared with those without treatment. Therefore, users of these drugs were more likely to have a false-positive test. However, in practice, only a small number of participants in CRC screening programmes take such medicines.

In contrast, the role of f-Hb in diseases with a systemic inflammatory component has become of considerable interest as a major cause of allegedly false positive FIT results. Using FIT, a study from Taiwan demonstrated the impact of an incremental increase in f-Hb on the risk for death from CRC and all-cause death, both suggesting that f-Hb might not only facilitate individually tailored screening for CRC but also could be used as a significant predictor for life expectancy [71]. Although this was assessed using gFOBT and not FIT, Scottish [72] and Danish studies [73] demonstrated a relationship between f-Hb, both all-cause mortality and causes of death unrelated to CRC in screening programmes, A recent review documented the now significant quantum of evidence that elevated f-Hb, determined by FIT, is associated with increased all-cause and cause-specific mortality and with many longer-term conditions with a systemic inflammatory component, including diabetes, hypertension, cardiovascular disease, and psoriasis, and with probable intake of particulate matter into the lungs [46]. Since the publication of this review, further studies have confirmed the association. A Korean study investigated the association between positive FIT test results and the incidence of dementia using a nationwide database. FIT result positivity was correlated with an increased risk especially in participants under 65 years of age. It was proposed that the results of the study suggested that dementia could be considered when participants with positive FIT results failed to show any neoplasia [74]. A Danish study investigated the association between f-Hb and both all-cause mortality and cause of death in a population-wide cohort of screening participants. The findings support the hypothesis that f-Hb may indicate an elevated risk of having chronic conditions if causes for the bleeding have not been identified. It was postulated that the mechanisms still need to be established, but f-Hb may be a potential biomarker for several non-CRC disease [75]. A further Danish study showed that the risk of CRC mortality increased with the increasing f-Hb even for f-Hb considered negative in all current European screening programmes. The risk of all-cause mortality was also increased for individuals with detectable blood in the faeces. For CRC specific mortality and all-cause mortality, the risk was increased at f-Hb as low as 4–9 μg Hb/g faeces, that is, at the limit of detection or quantitation [76]. Thus, the now mature body of evidence suggests that elevated f-Hb has considerable potential to identify individuals at risk of, or who already have, early stage undiagnosed chronic disease. As stated in the recent review [46], if f-Hb does prove to be an effective biomarker for chronic disease and multimorbidity, individuals with detectable f-Hb, but without an obvious source of GI blood loss, might benefit from early further assessment and, possibly, early intervention. It was also proposed that, to test this hypothesis rigorously, perhaps longitudinal data-linkage methodology was required linking CRC screening data, and data on patients presenting with lower GI symptoms, with other routinely collected individual health information.

6.2. Post-polypectomy surveillance using faecal haemoglobin concentrations

Polypectomy may be performed at colonoscopy and then subsequent surveillance undertaken. There is now growing evidence that f-Hb might be useful in this clinical setting since surveillance colonoscopy is expensive, can be uncomfortable for patients, and has a small risk of complications such as perforation. In a study in England, FIT were offered at one, two, and three years post polypectomy. Participants with FIT positive results (with a f-Hb threshold of 40 μg Hb/g faeces) for any of the FIT were referred for colonoscopy and not offered further FIT [77]. Participants with FIT negative results were offered colonoscopy at three years post polypectomy. It was concluded that annual FIT with colonoscopy for those with FIT positive results achieved high sensitivity for CRC and would be cost saving compared with colonoscopy every three years, although some CRC would be missed. A further study concerned consecutive patients enrolled in colonoscopy surveillance who were approached at hospitals in Tayside and London. A specimen for FIT was provided before colonoscopy and, ideally after three weeks, a second FIT sample was collected from those who had polypectomy. Of 593 patients who had a f-Hb result and completed colonoscopy, AN was found in 41 (6.9%); four CRC: 0.7% and 37 AA: 6.3%, and a further 127 (21.4%) had non-advanced adenoma (NAA). Interestingly, the median f-Hb was significantly greater in AA as compared to NAA: f-Hb in patients with AA did fall post-polypectomy but did not change from the pre-colonoscopy low f-Hb in those with NAA [78]. This might have been expected since f-Hb is related to the severity of colorectal disease [79] and it is unlikely that NAA bleed [80]. A very recent Australian investigation involved a retrospective cohort study on surveillance intervals in individuals who had completed a two-sample FIT between colonoscopies, from one to four rounds at one to two yearly intervals, each with a negative result (<20 μg Hb/g faeces). This demonstrated that there was a low risk of AN after multiple rounds of FIT negative results in above average risk individuals undergoing surveillance and who had no neoplasia or non-advanced adenoma at prior colonoscopy and the finding supported the use of interval FIT to personalise surveillance by lengthening colonoscopy intervals following serial FIT negative results [81]. Thus, quantitative f-Hb estimates generated in patients in post-polypectomy surveillance programmes could reduce colonoscopy requirements and thereby also reduce the potential risk to patients of this invasive investigation. A large-scale study proposed in Spain could add significant information to use of FIT in this clinical setting [82].

6.3. Application of low faecal haemoglobin concentrations

There has been considerable recent interest in the use of f-Hb lower than the widely applied threshold (20 μg Hb/g faeces) in screening and the f-Hb threshold recommended and most widely used in assisting the diagnosis of patients presenting with lower GI symptoms (10 μg Hb/g faeces}. Examples of the use of such f-Hb in screening all show that participants with f-Hb above the limit of detection had an increased risk of AN in subsequent screening [83–86]. Similar studies on the value of low f-Hb in the diagnostic setting show that the yield of patients with significant colorectal disease is greater [87,88], although additional colonoscopy resources are required. However, problems are apparent regarding the reporting of low f-Hb concentrations and seemingly considerable misunderstanding of the metrological aspects of analyses of f-Hb at low concentrations. These might be ameliorated if the terminology of detectability characteristics of f-Hb concentration examinations, namely, the limit of blank (LoB), limit of detection (LoD), and limit of quantitation (LoQ) was used [89]. The LoB is the highest measured result likely to be observed (typically at 95% certainty) for a

sample containing no f-Hb. The LoD is the lowest concentration at which f-Hb can be detected 95% of the time. LoD is determined by first determining LoB and then performing replicate studies on samples of faeces containing a very low f-Hb concentration and is calculated as LoD = LoB + (1.645 × SD of samples with low f-Hb concentration). The LoQ is the lowest f-Hb concentration that can be determined when some predefined analytical performance specifications (APS) are satisfied. It is recommended that these detectability characteristics are generated, validated, and used in reporting systems exactly as recommended in the EP17-A2 guideline of the Clinical Laboratory Standards Institute [90]. As mentioned earlier, using available FIT systems, many participants in screening programmes and in participants in diagnostic endeavours have f-Hb less than the LoD. However, dogma has it that everyone has blood in their faeces. The germane question is would it be of value to have FIT systems that could quantitate f-Hb below current LoD.

6.4. Other potential uses of faecal haemoglobin concentration estimates

There has now been significant research done in assessment of the use of FIT in the diagnosis and monitoring of inflammatory bowel disease (IBD), particularly ulcerative colitis [91,92]. The role in this clinical setting requires further elucidation particularly possible integration between the uses in CRC screening, detection, and surveillance with similar applications in the field of IBD.

It has been suggested that the gut microbiome could play a significant role in the development and progression of CRC since changes in the microbiome occur during different stages of colorectal neoplasia, from adenomas to early stage cancer, to metastatic disease. This supports supporting an etiologic and diagnostic role for the microbiome. Several studies have indicated that FIT specimen collection devices from CRC screening programmes are appropriate for gut microbiome analysis, for example, a recent study from Austria documents previous relevant work in detail [93]. It remains to be seen if this an appropriate method to collect faecal samples for gut-based microbiome profiling of value in CRC detection. Further, it might be that it is possible to find DNA from cancer cells in the sample collection devices used to collect faeces for CRC screening. If successful, this technique could potentially be used to improve screening and diagnosis of CRC. A Scottish project aims to assess whether DNA that contains changes known to be associated with CRC can be detected [94].

7. Conclusions

A recent editorial suggested that quantitative f-Hb analyses have come of age, but further maturation seems desirable [95]. CRC screening programmes using a two-step strategy, now usually use f-Hb. However, because of limited colonoscopy capacity, some countries have high f-Hb thresholds, and it would benefit neoplasia detection if these were lowered. Further, some countries do not screen from age 45 years or even 50 years and lowering the age of first invitation would have undoubted benefits. Many of the clear disadvantages experienced by women in CRC screening are because, for several reasons, women have lower f-Hb than men. These disadvantages should be addressed by either risk-scoring or, with advantages, the simple expedient of lowering the f-Hb threshold for women while retaining that for men so that they are not disadvantaged. Further use should be made of low f-Hb in screening, to predict future risk and perhaps introduce a range of screening intervals. The use of f-Hb in the triage of patients with lower GI systems is now used extensively in several countries and the recent professional body guidelines [13], with recommendations for rational application and proposals for future research to clarify the remaining issues, provide a very valuable resource. Again, use of lower than commonly applied f-Hb thresholds might have advantages. Evidence that f-Hb is associated with diseases with a systemic inflammation component is now convincing and further studios on the application of this finding in real clinical practice would be advantageous. The use of f-Hb in post-polypectomy is growing and again further real practice studies would have benefit. The application of very low f-Hb thresholds in screening have potential applications in assessment of future risk and, perhaps, screening intervals, and have benefits in the diagnosis of serious colorectal disease (AN plus IBD) in patients with symptoms and it might be of value to have FIT systems which can measure f-Hb below current LoD. Other applications such as using the contents of the FIT specimen collection devices in assessment of the microbiome and faecal DNA seem more than possible.

The ideal tumour marker [96] would demonstrate high clinical sensitivity and a low rate of false negative results, high clinical specificity and a low rate of false positive results and would show a positive correlation with both tumour size and stage. The removal of neoplasia would cause a fall in the concentration and a subsequent rise would imply the presence of further neoplasia. The marker would have several applications in a spectrum of clinical settings. The clinical usefulness would have been verified by clinical trials. Analytically, the ideal marker would be quantitative, non-invasive, inexpensive, simple, and able to be automated. It is suggested that f-Hb could attain this ideal but further refinements are necessary to attain this laudable goal.

Practice points

- FIT are of proven value in CRC screening and in the diagnosis of patients presenting with symptoms, but the numerical results differ from FIT system to system and data might not be transferable
- Faecal haemoglobin concentration (f-Hb) is affected by several factors including sex, age, deprivation region, and round of screening.
- Use of a single f-Hb threshold in screening disadvantages women in several clinical outcomes including, positivity, CRC and neoplasia detection rates, interval cancer proportion, and reduction in incidence and mortality
- A f-Hb lower than the threshold but still detectable does imply future risk of CRC
- The finding of a f-Hb higher than the threshold but with no significant colorectal disease requires further consideration and assessment of possible further interventions

Research agenda

- Comparative assessment of strategies to reduce sex inequality in CRC screening, including risk-scoring and stratified f-Hb haemoglobin thresholds
- Further studies on the use of FIT in assessment of individuals with possible chronic disease with a systemic inflammation component and in inflammatory bowel disease
- Use of FIT in post-polypectomy surveillance
- Assessment of the value of FIT analytical systems with limits of detection lower than those currently available
- Practical applications of faecal materials available in FIT specimen collection devices

Declaration of competing interest

None.

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