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Immunochemical faecal occult blood tests are superior to guaiac-based tests for the detection of colorectal neoplasms

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ABSTRACT

The aim of this study was to compare the performance of a guaiac-based faecal occult blood test (G-FOBT) with that of an immunochemical faecal occult blood test (I-FOBT). A total of 17,215 average risk individuals aged 50 to 74 enrolled in a population-based organised screening programme and performed a 3-day G-FOBT and a 2-day I-FOBT simultaneously. Among participants, 3.1% were found positive for the G-FOBT and 6.9% for the I-FOBT ($p<10^{-4}$). Among the 1205 participants who tested positive and underwent a colonoscopy, the number of detected cancers and advanced adenomas was respectively 2.6 times higher and 3.5 times higher with the I-FOBT than with the G-FOBT. The positive predictive value of I-FOBT was similar to that of the G-FOBT for cancers (5.9% versus 5.2%) and was higher for advanced adenomas (27.2% versus 17.5%). The I-FOBT was superior to the G-FOBT for the detection of both cancers and advanced adenomas. However, the screen positive rate that staff and financial resources can accommodate has yet to be determined.

1. Introduction

Reducing colorectal cancer (CRC) mortality is a priority in oncology. Population-based controlled studies in Europe and the United States have shown that guaiac faecal occult blood tests (G-FOBT), followed by a colonoscopy in case of positivity to detect the cause of bleeding, can reduce CRC mortality [1–4] and incidence.[5] Thus, screening for CRC, using FOBT, has been included in the European Code Against Cancer [6] and has been endorsed by the European Commission.[7,8] Despite their high specificity, G-FOBT have been criticised for their fairly low sensitivity and because they react with non-human haem in food. For these reasons, attention has been given to alternative FOBT, and in particular, immunochemical FOBT (I-FOBT), which is specific to human haemoglobin.

A number of studies have been performed to evaluate the performance of I-FOBT in detecting colorectal neoplasia. However, they were often small in size or were performed in symptomatic or high-risk groups.[9–15] Only three studies enrolled an asymptomatic average-risk population.[16–18] The performance of I-FOBT is less well established than that of G-FOBT. This is why the European Commission concluded that there was not enough evidence to recommend I-FOBT for population-based CRC screening.[8] In particular, there is a lack of direct comparisons for the G-FOBT and the I-FOBT performed by the same subject in CRC screening programmes. The purpose of this study was to compare, within a population-based screening programme, the performance of a G-FOBT and an I-FOBT done simultaneously on the same stool.

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2. Patients and methods

2.1. Study design

The study was conducted in 2005 in a general population aged 50 to 74 (subjects born between 1931 and 1955) involved in a mass screening programme for CRC in Burgundy (France). Following the French recommendations,[19] subjects with recent digestive symptoms, a previous history of CRC or adenoma, an inflammatory bowel disease, a first-degree relative with an index case aged under 65, a second first-degree relative whatever the age of the index case, a complete colonoscopy during the previous 5 years or a serious illness contraindicating screening were excluded. Included subjects were invited to participate in a biennial screening programme using a G-FOBT. On the occasion of the second screening round, 2 years after the first one, they were asked to perform both a G-FOBT and an I-FOBT. At the beginning of each screening round, the subjects were sent an information letter and a four-page information brochure. During the first 4 months of the screening campaign, GPs (n = 210) offered both types of test free of charge to the eligible patients seen at their office. GPs were thoroughly informed about the research project. For individuals who did not consult their GPs during the 3-months of the medical phase, the coordination centre mailed the two tests. In case of a positive result for either of the two tests, the subject was invited to consult his GP who then organised a full colonoscopy.

2.2. Faecal occult blood tests

The completed tests were sent by mail to a central analysis centre. As in other on-going pilots or national screening programmes for CRC in Europe, no diet or drug restriction was required. On receipt, samples were assayed immediately by trained staff. Each FOBT was read blinded to the other FOBT.

The G-FOBT (Hemoccult II; Beckman Coulter Inc, Fullerton CA, USA) was performed by taking two samples from separate points of three consecutive stools. The test was processed without rehydration according to a standardised procedure using the developer provided by the manufacturer. A second reader independently verified the result. The test was reported as positive if any blue colour was detected within a minute of addition of the developer solution in any test card window.

For the I-FOBT (Instant-view, Alpha Scientific Designs, Poway, CA, USA), each participant was instructed to take a faecal sample from the first two consecutive stools. The faecal test sampling device is shaped like a small test tube with the faecal probe inserted into it and sealing it. The probe has a serrated tip which is poked into five different areas of the stool (according to the manufacturer's recommendations) and then pushed back into the tube to seal it. The probe tip with the faecal sample is suspended in a standard volume of haemoglobin-stabilising buffer. After being sealed, the tube has to be shaken vigorously to mix the specimen and the extraction buffer. At the laboratory the ferrule at the extremity of the test tube is snapped off and four drops of the supernatant in the test tube are put into the round well of the testing device. The specimen migrates by capillary action through the test strip. This assay is a one-step lateral flow chromatographic immune assay. The interpretation of the test is made after 5 to 10 min. The appearance of one pink line indicates that the test is completed and that no blood was detected. If two pink lines develop on the test strip then the test is classified as positive. If blood is present in either sample, the concentration of haemoglobin in the specimen is over 50 μg of haemoglobin/g of faeces.

2.3. Colonoscopy and pathology findings

During the evening before the colonoscopy, patients drank 3 L of a polyethylene glycol-based electrolyte solution. Twenty-eight qualified gastroenterologists, practicing either in a public hospital or a private practice, performed the colonoscopies (generally under general anaesthesia). They were blinded to which test(s) proved positive. Standard commercial video colonoscopes were used. Information on diagnosed CRC and polyps was obtained using standardised forms completed by GPs and gastroenterologists. Pathologists provided copies of their reports. The Burgundy cancer registry was also used to ascertain new cases of cancers and adenomas, to check collected information and to identify interval cancers. The number, size, location, histology and dysplasia of any adenomas were recorded. If a patient had more than one adenoma, the most advanced lesion was included in the analysis. Advanced adenoma was defined as an adenoma of 10 mm or more in diameter, or with high-grade dysplasia or a villous component. Cancers were classified according to the TNM classification.[20] Intra mucosal carcinomas were classified as Tis. Hyperplastic polyps were not included as neoplasia. Those who returned a positive test and who decided not to proceed with colonoscopy were excluded from analysis.

2.4. Statistical analysis

We calculated that for a cancer detection rate of 1.5‰ with the G-FOBT and of 3.0‰ with the I-FOBT, with a discordance of 0.5% between the results of the tests, an alpha risk of 0.05 and a power of 0.80, we needed a screen population of 14,000.

Chi-square tests were used to test for statistical significance in comparisons of proportions. Statistical tests were two-sided and a p value of less than 0.05 was considered statistically significant. The screen positive rate was modelled as a function of sex and age at screening using logistic regression. Specificity for colorectal cancer in the screened population was estimated from the false positive rate. The positive predictive value of both tests was determined with its 95% confidence interval (CI). For each diagnosis of interest, test results were compared by paired 2×2 analyses. Differences in the detection rates between the two tests and 95% confidence intervals (CI) were calculated. Data management was performed with MySQL 3.5 and all analyses were conducted using Stata 9.0 (Stat Corp, College Station Texas).

3. Results

A total of 17,215 average risk individuals, aged 50 to 74, performed both a G-FOBT and an I-FOBT. Among them, 64.0%

were invited through their GP and 36.0% via mailing. Overall, 1558 (9.0%) tested positive to either or both tests. The test-specific screen positive rate was 3.1% for the G-FOBT and 6.9% for the I-FOBT ($p < 0.001$). The screen positive rate for the I-FOBT was about twice as high as that for the G-FOBT for both sexes and in all age groups (Table 1). Both tests were more frequently positive in men than in women ($p < 0.0001$) and increased with age. In those aged 50 to 54 and 70 to 74, the screen positive rate for the I-FOBT was respectively 5.4% and 10.0%, and for the G-FOBT 2.7% and 3.6%. Among the participants who screened positive, 1205 (78.2%) underwent a colonoscopy: 76.2% among positive G-FOBT and 79.3% among positive I-FOBT (p=0.15). Among these, the percentage of complete colonoscopy was 95.3%. There was no perforation or bleeding in relation to colonoscopy.

The I-FOBT was superior to the G-FOBT for the detection of both cancers and adenomas. The number of detected cancers was 2.6 times higher with the I-FOBT than with the G-FOBT, and the number of advanced adenomas 3.5 times higher (Table 2). Concordance between the results of the I-FOBT and the G-FOBT was poor (Kappa test: 0.17). Among the 57 participants diagnosed with CRC, 19 tested positive in both tests (33.3%), 36 for the I-FOBT only (63.2%) and two for the G-FOBT only (3.5%). The corresponding percentages among the 275 individuals with advanced adenomas were 17.8%, 74.1% and 8.1%, and among the 149 individuals diagnosed with non-advanced adenomas 5.4%, 70.5% and 24.2%. Differences in detection rates between the two tests was 59.7% for cancers (95% CI 45.2–74.2%) and 65.4% for advanced adenomas (58.0–72.9%).

The positive predictive value of the I-FOBT was similar to that of the G-FOBT for cancers (5.9% versus 5.2%) and was higher for advanced adenomas (26.9% versus 17.5%) (Table 3). However, the relative specificity of the I-FOBT for detecting cancer (93.2%) was lower than that of the G-FOBT (96.9%).

Table 4 shows the results of FOBT according to stage at diagnosis of cancers. The distribution of diagnosed cancers with both the I-FOBT and the G-FOBT was similar. About half of them were Tis or T1N0M0. The I-FOBT detected 97% of the 31 early cancers (Tis and T1N0M0) compared to 32% for the G-FOBT ($p< 0.001$).

4. Discussion

The objective of mass screening for CRC is to detect the 50 prevalent CRC among 10,000 subjects over 50 years of age. Given the large size of the population at risk, the World Health Organisation (WHO) has clearly defined the main requirements of the screening test:[21] the test must be easy to perform, acceptable, safe, cheap and with proven effectiveness. For this reason, screening for CRC, with the present state of knowledge, needs to include two tests: a selection test which would be performed by a large part of the population, then a diagnostic test which would only be proposed to subjects with a positive screening test. Currently, the simplest screening method for CRC is periodic stool testing for occult blood. The most extensively evaluated test is a guaiac-based test, the Hemoccult II. It has been shown to be effective in decreasing CRC mortality [1–4] and, in one study, CRC incidence.[5] Other faecal occult blood tests, particularly immunochemical tests, have been developed. The aim of this paper was to compare the performance of the reference G-FOBT with that of an I-FOBT in an average-risk population who performed the two tests simultaneously. It is also the largest study of its kind published to date. The fact that a significant proportion of individuals with a positive screening test did not undergo colonoscopy was a limitation of this study. However, since the proportion of people who did not undergo colonoscopy was the same whether one or both screening tests were positive, it did not produce bias in the comparison between tests.

The results of this study clearly show that I-FOBT is significantly better than the G-FOBT at detecting cancers and adenomas. It confirms the results of previous reports comparing the two types of test.[9–15,18,19,21] Various studies have used different cut-off values for the amount of faecal blood that is required for a positive result, but whatever the chosen cut off, the I-FOBT has been shown to be more sensitive than the G-FOBT.[18] Most studies, in particular those including a colonoscopy to determine the sensitivity of the screening tests, were small in size or included symptomatic and high-risk patients, thus overestimating the sensitivity of the test. The interest of this study lies in the fact that it was conducted within an organised population-based CRC screening programme. However, because of the size of the population, it was not possible to perform a colonoscopy in those who screened negative. It was thus impossible to determine the true sensitivity and specificity of the test. The best evaluation of the performance of an I-FOBT was provided by a large Japanese study in an asymptomatic population that compared a one-time I-FOBT (with a cut-off point of 20 ng /mL) to the findings of

Table 1 – Faecal occult blood test positivity by sex and age

	n	% Positive (95% CI)		Odds ratio (95% CI)	
		I-FOBT	G-FOBT	I-FOBT	G-FOBT
Men	7868	9.0 (8.6–9.4)	3.9 (3.6–4.2)	1	1
Women	9347	5.1 (4.8–5.4)	2.5 (2.3–2.7)	0.55 (0.48–0.61)	0.63 (0.54–0.76)
50 – 54	3633	5.4 (5.1–5.7)	2.7 (2.5–2.9)	1	1
55 – 59	4276	5.7 (5.4–6.0)	2.7 (2.5–2.9)	1.08 (0.89–1.31)	1.01(0.77–1.33)
60 – 64	3105	6.5 (6.1–6.9)	3.3 (3.0–3.6)	1.22 (0.99–1.49)	1.24 (0.93–1.64)
65 – 69	3284	7.8 (7.4–8.2)	3.5 (3.2–3.8)	1.51 (1.25–1.83)	1.32 (1.0–1.74)
70 – 74	2917	10.0 (9.6–10.4)	3.6 (3.3–3.9)	1.97 (1.63–2.39)	1.36 (1.03–1.90)

G-FOBT = guaiac-based faecal occult blood test; I-FOBT = immunochemical faecal occult blood test; 95% CI = 95% confidence interval.

Table 2 – Results of paired comparisons of guaiac and immunochemical faecal occult blood tests

		Cancer G-FOBT		Advanced adenomas G-FOBT		Other adenomas G-FOBT	
		+	-	+	-	+	-
I-FOBT	+	19	36	47	204	8	105
	–	2		24		36	

Table 3 – Positive predictive values of the guaiac and immunochemical faecal occult blood tests

	I-FOBT (932 colonoscopies)	G-FOBT (406 colonoscopies)	*p*
Cancer	55	21	
PPV	5.9% (4.4–7.4)	5.2% (3.0–7.3)	0.596
Advanced adenoma	251	71	
PPV	26.9% (24.1–29.8)	17.5% (13.8–21.2)	0.0001
Non-advanced adenoma	113	44	
PPV	12.1%(10.0–14.2)	10.8%(7.8–13.9)	0.501

PPV = positive predictive value; G-FOBT = guaiac-based faecal occult blood test; I-FOBT = immunochemical faecal occult blood test.

Table 4 – Stage of colorectal cancer for the guaiac and immunochemical faecal occult blood tests[a]

	Number of cancers	I-FOBT positive (*n*=55)	G-FOBT positive (*n*=21)
Tis	9	9 (15.8%)	3 (14.3%)
Stage I	22	21 (38.6%)	7 (33.3%)
Stage II	12	11 (21.0%)	6 (28.6%)
Stage III	12	12 (21.0%)	4 (19.0%)
Stage IV	2	2 (3.5%)	1 (4.8%)

G-FOBT = guaiac-based faecal occult blood test; I-FOBT = immunochemical faecal occult blood test.
a According to the TNM classification.

colonoscopy.[19] The sensitivity was 66% for CRC and 27% for advanced adenomas.

The main drawback with I-FOBT, however, is the high screen positive rate reported in all studies. Thus, the false positive rate with I-FOBT is higher than that with G-FOBT, which explains the lower specificity. Colonoscopy is not without risks and is an expensive procedure. The number of colonoscopies generated by CRC screening can be a problem. Furthermore, available results are difficult to interpret: the chosen cut-off is given in different units (μg of haemoglobin / g of faeces, or ng of haemoglobin / ml). It is difficult to compare tests as they require different amounts of faeces and use different buffers and different antibodies to detect the globin portion of human haemoglobin. This, together with the discrepancies between studies, explains the absence of consensus on the number of bowel movements on which the test must be performed and on the optimal threshold for detecting CRC and advanced adenomas. The problem is to determine a limit of analytical detection that is not too low in order to achieve an acceptable frequency of positive tests. Furthermore, the stability of the test in everyday practice is not well established. An average decrease of 2.2% per day in the concentration of haemoglobin has been reported at an ambient temperature of 20 °C; this rises to 3.7% at 28 °C.[22] This problem needs to be evaluated more carefully. In addition, no studies have compared the performance of the available I-FOBT; hence the need for more data. The screen positive rate that staff and financial resources can accommodate whilst retaining good sensitivity is still not clear. In the UK, a sensitive G-FOBT has to be repeated if between one and four windows out of six are positive; this is because the initial screen positive rate is too high given the current colonoscopy resources.[23] Taking into account the large size of the average-risk population and the experience gathered from pilot studies, a manageable overall screen positive rate in France could fall in the range of 3–4% given the number of available endoscopists. Choosing a higher screen positive rate would reduce test specificity and result in an increase in the number of false-positive results and low-yield colonoscopy referrals.

Although the I-FOBT had a higher screen positive rate than the G-FOBT, an important finding is that we did not observe a difference in the positive predictive value for cancers compared with the positive predictive value for the G-FOBT. The higher proportion of colonoscopy referrals with the I-FOBT compared with the G-FOBT resulted in the detection of a larger number of cancers and advanced adenomas as indicated by our study. The stage distribution of CRC detected by G-FOBT and I-FOBT was similar. As expected in a screening study, more than 3/4 of diagnosed cancers were at an early stage (up to stage II).

Accurate interpretation of G-FOBT is not easy to achieve and requires well-run centralised laboratories. The positive predictive value of the G-FOBT in this study was lower than that anticipated from our previous studies.[1] The broad range

of variation in the screen positive rates of G-FOBT tests illustrates the problem of quality control related to development and reading of G-FOBT. Qualitative I-FOBT has the advantage of being easier to interpret, and is suitable for use at a small-scale, by GPs, for example. However, automated quantitative I-FOBT is better suited to the analysis of a large number of tests, and is able to quantify faecal haemoglobin. This allows a screen positive rate to be chosen. I-FOBT minimises human error in test development thus making it a more objective laboratory test with excellent quality control.

There are also data suggesting that the presentation of the test combined with the lower number of samples can improve participation.[15,24] However, conflicting results are available. This is a key point; the effectiveness of the screening program depends on both the performance of the test and compliance.

In conclusion, I-FOBT appears to be a potentially important test for CRC screening. However, policy makers need more data before determining the optimal use of I-FOBT. It is too early to make European recommendations. Several planned or on-going studies performed in large average-risk populations using quantitative I-FOBT will be available soon and will provide the missing data.

Conflict of interest statement

None declared.

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