COLORECTAL CANCER SCREENING BY MEANS OF FAECAL IMMUNOCHEMICAL TESTING (FIT)

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Colorectal Cancer Screening by means of Faecal Immunochemical Testing (FIT)

Darmkanker screening met een
immunochemische feces occult bloed test

Proefschrift

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"Our most effective weapon in defeating colorectal cancer is early detection and treatment. Through a regular screening program that includes fecal blood testing, periodic partial or full colon examinations, or both, health professionals can detect and remove pre-cancerous polyps before they turn into cancer. Such cancer screening should become a routine part of preventive health care for anyone over the age of 50, because the risk of developing colorectal cancer increases with age . . ."

"Now, therefore, I, William J. Clinton, President of the United States of America, by virtue of the authority vested in me by the Constitution and laws of the United States, do hereby proclaim March 2000 as National Colorectal Cancer Awareness Month. I encourage health care providers, advocacy groups, policymakers, and concerned citizens across the country to help raise public awareness of the risks and methods of prevention of colorectal cancer and to use the power of our knowledge to defeat this silent disease..."

Bill Clinton, 29th February 2000

Opgedragen aan Roefke

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Chapter 1

General introduction and outline of the thesis

Aafke H.C. van Roon, Monique E. van Leerdam, and Ernst J. Kuipers

Adapted from "Handboek colorectaal carcinoom" 2012 – in press

BACKGROUND OF SCREENING

The term 'screening' is derived from the verb 'to screen' and means 'to guard' or 'to filter'. The aim of a nationwide screening programme is to 'filter' an in principle healthy population in order to detect those with a disease or condition at an early stage, before the occurrence of any signs or symptoms. Actively looking for the early stages of a disease or condition is classified as secondary prevention. Additionally, primary prevention strategies intend to avoid the development of the disease and tertiary prevention aims to reduce the negative impact of established disease by restoring function and reducing disease-related complications.

The development of a malignancy is a multistep process: at some point in time the first cancer cells develop and will start to divide in an uncontrolled way ultimately resulting in a tumour. Growth is local at first but then continues into the surrounding tissues and eventually metastasizes, ultimately leading to the individuals' death. At some stage during this process, the individual generally seeks medical advice for their newly-developed symptoms. Subsequently, further investigations are carried out and the diagnosis of 'cancer' is made. Between the start of the uncontrolled division of the first cancer cells and manifestation of symptoms, there may be a moment at which the tumour is large enough to be detected by a screening test. The aim of screening is therefore to bring forward the time of diagnosis before the stage at which the first signs and symptoms of the disease come to light, the so-called lead time. Detection at an early stage is associated with less intensive treatment and a better outcome. Depending on the disease and test characteristics, screening may in some instances also detect the premalignant lesions that manifest themselves prior to the invasive stage.

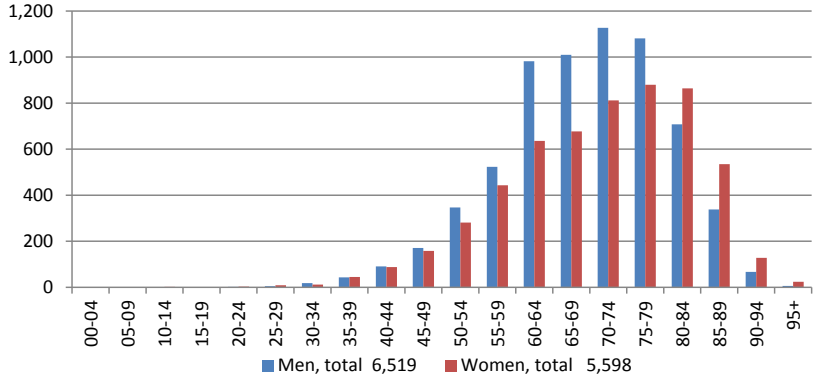
In the 1960s, at request of the commission of the World Health Organization, the Wilson and Jungner criteria for screening were drawn up. These ten classic screening criteria can be summarized as follows:[1-2]

(1) Screening should target a disease which poses a major health problem.
(2) The method of screening should be reliable and valid and should also be generally accepted by the target population who are in principle healthy.
(3) There should be an acceptable form of treatment for people in whom the disease is detected at an early stage. It is essential that this treatment should result in a prognosis which is better than would have been the case without early detection.
(4) The overall benefits of screening should outweigh the potential harm and cost.

Colorectal cancer (CRC) fulfils all of these criteria and can therefore be categorized as a disease that can be traced by means of screening.[3] First, the lifetime risk of developing CRC

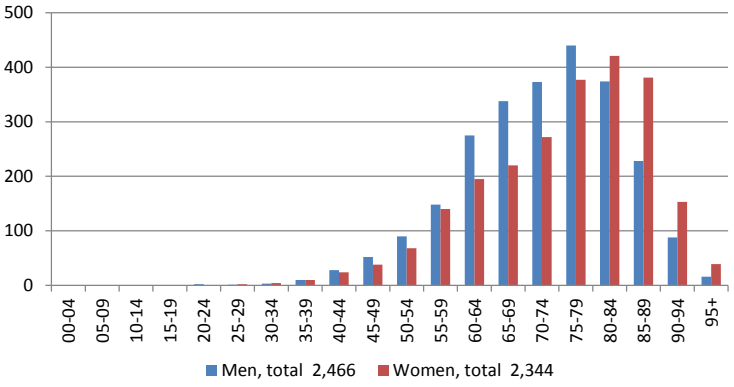
is approximately 5%.[4] This means that this disease is one of the most commonly-occurring forms of cancer in the western world: in men it is the third most commonly diagnosed malignancy after prostate and lung cancer, and in women CRC ranks second after breast cancer.[5] In the Netherlands, a malignant tumour of the large bowel is diagnosed in over 12,000 people every year (www.ikcnet.nl). It is expected that in the future this number will increase by 3% each year, which is mainly attributable to the ageing population in the Netherlands. The incidence of CRC increases with age, the peak being between the ages of 65-74 (**Figure 1**). In the Netherlands, 4,810 patients died from this disease in 2008 (**Figure 2**). For these reasons it can be concluded that CRC is a major health problem.

Figure 1 Incidence of colorectal cancer in the Netherlands (2008)



Source: www.ikcnet.nl

Figure 2 Colorectal cancer mortality rate in the Netherlands (2008)



Source: www.ikcnet.nl

Second, various CRC screening methods are available with differences in test accuracy and screenee acceptability. However, the reported test accuracy results for each screening method differ per study due to differences in CRC prevalence between countries and age limits of the target population, the individuals under investigation (ie, asymptomatic average-risk vs. high-risk individuals), and test variants used. Nevertheless, current CRC screening methods are generally assumed to be sufficiently accurate in detecting early stage disease and to be acceptable to screenees. Third, the chance of being cured of CRC is strongly dependent on the stage at which the disease is discovered. However, in most cases symptoms of CRC only manifest themselves at an advanced stage of the disease and by the time the disease is diagnosed the prognosis is often poor. If tumour growth is limited to the submucosa (stage I), the 5-year survival rate is 94%. However, if the disease is discovered at an advanced stage (stage IV,ie,distant metastases are spread throughout the body), the 5-year survival rate drops to 8% despite intense multi-modality treatment.[6-7] The primary aim of CRC screening is therefore to detect and treat the disease at the earliest possible stage, thereby positively influencing the survival rates of CRC patients. Fourth, based on CRC micro-simulation models and assuming an equally high adherence, four strategies provide comparable cost-effectiveness ratios, namely 10-yearly colonoscopy, annual Hemoccult SENSA or faecal immunochemical test, and 5-yearly flexible sigmoidoscopy in conjunction with Hemoccult SENSA every 2 to 3 years.[8] Furthermore, the various CRC screening methods all have cost-effectiveness ratios which are considerably better than those of other generally accepted screening programmes such as those for cervical cancer and breast cancer.[9] The cost of treatment for advanced CRC is expected to rise in the near future, mainly due to the widespread use of newer and more expensive forms of chemotherapy. Taking this rise in costs into account, most CRC screening strategies have been proven to actually save money.[9] As a consequence, screening is a desirable approach not only to reduce the incidence of CRC and mortality but also to control the costs of CRC treatment.

For all these reasons, in 2003, the European Commission recommended that CRC screening should be offered to all men and women aged 50-75 years.[10] In the Netherlands, such a nationwide screening programme will start in 2013.[3]

METHODS OF SCREENING FOR COLORECTAL CANCER

It is generally accepted that most cancers of the colon and rectum develop from adenomatous polyps.[11] These adenomatous polyps are found in about 25% of people by the age of 50, and prevalence of these polyps increases with age. Indirect evidence to support this adenoma-carcinoma sequence comes from research which showed that endoscopic removal of adenomatous polyps resulted in a lower-than-expected incidence of CRC compared with

a reference population.[12] The probability that an adenomatous polyp will progress to cancer, and the probability that a patient will develop other adenomatous polyps or cancer elsewhere in the colon and rectum, can be estimated by a number of independent factors. The most important risk factors are the presence on index colonoscopy of the following: advanced adenomas, ≥ 3 adenomas, size ≥ 10 mm, age ≥ 60 years, the presence of villous adenomas, high-grade dysplasia, proximal adenomas, and male gender.[13-14] The National Polyp Study Workgroup introduced the concept of an advanced adenoma defined as an adenoma ≥ 10 mm, or an adenoma with more than 25% villous component and/or high-grade dysplasia.[15] CRC, on the other hand, is defined as the invasion of malignant cells through the lamina muscularis mucosa into the submucosa.[16-17] These to definitions combined lead to the designation of advanced neoplasia.

There are a number of screening methods which can be used for the detection and removal of the early stages of advanced neoplasia. These screening strategies vary in the degree of supportive scientific evidence, test-related burden, attendance rate, diagnostic yield and therefore effectiveness (**Table 1**). These screening methods can either be categorized as stool-based tests or as non-invasive or invasive investigations of the colon and rectum.

STOOL-BASED SCREENING TESTS

Faecal Occult Blood Tests

There are several stool-based screening tests, which can be used for CRC screening purposes, but the principle is the same. CRC and its benign precursor lesions (ie, advanced adenomas) can cause microscopic blood loss which can be detected by means of a so-called faecal occult blood test (FOBT). As the bleeding tendency correlates with size and stage of the lesion, FOBT screening primarily aims at early detection of CRC and large polyps.[18-21]

Participants with a positive FOBT are referred for further investigations. A colonoscopy is the most suitable follow-up examination, as during this invasive procedure adenomas can be detected and removed and lesions with a high suspicion for CRC can be biopsied. FOBTs can be categorized as chemical and immunochemical types.

Guaiac-based faecal occult blood tests

Most chemical FOBTs contain a tree extract known as guaiac and for this reason these stool-based screening tests are abbreviated to gFOBT. When guaiac comes into contact with hydroperoxidase, it oxidizes causing a blue colour change on the test card (**Figure 3**). This reaction is catalyzed by haem, a constituent of haemoglobin molecules. Guaiac-based FOBTs are not specific for CRC and advanced adenomas: blood loss caused by other abnormalities or lesions higher up in the gastrointestinal (GI) tract can also give a positive test result.[22] In the

Figure 3 Guaiac-based faecal occult blood test (Hemoccult II; Beckman Coulter, US)

stomach all haemoglobin molecules are broken down into haem and globin. However, only a small amount of haem is absorbed in the upper GI tract. Therefore, in upper GI bleeding, the majority of haem passes into the colon resulting in false-positive test results. In addition, gFOBTs do not react specifically to human haem and can also react if red meat has been consumed by the screenee. Furthermore, false-positive and false-negative test results can occur due to hydroperoxidase reactions (and inhibitors of these) in certain foods, medications, and supplements including high dosages of vitamin C.[23]

Guaiac-based FOBTs are the only stool-based screening tests for which prospective evidence on mortality reduction from CRC exists. Three randomized controlled trials have clearly demonstrated that gFOBT screening can reduce the CRC-related mortality by approximately 16%.[24-27] An American study with a follow-up time of 18-years reported that the incidence of CRC dropped by 17% if gFOBT screening is carried out every two years.[28]

Attendance is an important factor in the effectiveness of a nationwide screening programme. The degree of participation in the first round of gFOBT screening varies between 47-67%.[24, 29-30] These limited numbers are partially due to the more demanding sample collection procedure of gFOBTs. One important requirement for the effectiveness of FOBT-screening in general is that invitees need to be repeatedly screened. One recent Scottish study showed that of all people that participated in the first gFOBT screening round, 85%

also took part in the second round, and that of the invited individuals who participated at least once, 83% also attended the third screening round.[31] Due to the low sensitivity of the test, two stool samples have to be collected from three consecutive bowel movements. Some CRC screening programmes advise participants to restrict their diet and medication prior to gFOBT sampling in an attempt to lower the number of false-positive and false-negative test results.

The reported sensitivity and specificity of gFOBTs varies between studies.[23, 32] This variation is a consequence of differences in test variants used, the a priori risk of CRC in the target population, the utilization of dietary and medication restrictions, the number of samples and method of faecal collection, whether the gFOBT samples are rehydrated or not (this increases sensitivity at the cost of specificity), the number of positive samples that are used as threshold for referral, the accuracy of processing and evaluation of test results, the investigation used as gold standard, and whether the sensitivity and specificity are calculated in the first or a consecutive screening round. Single tests with a standard gFOBT (ie, Hemoccult II - the most common and traditionally used gFOBT in Europe) have sensitivity for CRC of 13-38%.[33-34] However, if a more sensitive gFOBT is used (Hemoccult SENSA), this percentage rises to 64-80%, although this is at the cost of lower specificity.[32]

Due to its low sensitivity for CRC, periodic gFOBT screening is recommended (yearly or two-yearly) in order to achieve better programme sensitivity, estimated as 50-60% in biennial gFOBT screening.[35-40]

Figure 4 Faecal immunochemical test (OC-Sensor Micro; Eiken Chemical Co., Japan)

Faecal immunochemical tests

The concept of applying an immunochemical method to testing stool for microscopic blood loss was first proposed in the 1970s, and commercialization of the technology began in the 1980s.[41-42] These tests are called faecal immunochemical test or FIT and have a number of technical advantages over the gFOBTs (**Figure 4**). The antibodies used specifically target human globin which is incorporated into haemoglobin molecules. FITs are therefore specific for the detection of human blood. For this reason, no dietary or medication restrictions are required for FIT screening. As globin present in blood from the upper GI tract is gradually digested during its passage towards the colon, FITs are more specific to bleedings in the lower GI tract.[43] FITs are able to detect smaller amounts of blood in the faeces than gFOBTs (10 µg Hb/gram faeces which corresponds with 50 ng Hb/mL sample solution, versus 200 µg Hb/gram faeces respectively).[3] Finally, FIT sampling is considerably easier for screenees to carry out.[44]

Both qualitative and quantitative FITs have been developed. The qualitative tests require visual interpretation of the test result and give a positive or negative test outcome at a fixed cut-off level.[45] Quantitative tests are analyzed automatically and the amount of haemoglobin in the faeces is represented as a number. This method of FIT screening has important advantages for quality control. Furthermore, the interpretation of quantitative test results is not open to inter-observer variation, thereby improving reproducibility and allowing for large scale analyses.[20, 46-47] Another advantage of quantitative FIT screening is the possibility to determine the optimal cut-off value for a nationwide screening programme (ie, the amount of haemoglobin above which the test is considered positive and screenees are referred for colonoscopy).[19-20, 48-57] By varying the cut-off level, the positivity rate can be adapted according to the colonoscopy resources available and/or personal risk profile.[58]

Participation rates tend to be 1-13% higher for FIT than for gFOBT screening.[29-30, 59-61] This may be due to perceived comfort, stool sampling method, and the number of faecal samples that need to be collected.[62] The FIT is more user-friendly, mainly due to the modification of the tubes to include a little brush on the inside of the screw top instead of test cards and wooden spatulas in case of gFOBT screening (**Figure 3**).[44] This makes faecal sampling simpler, more user-friendly, more hygienic and more reliable.

The sensitivity and specificity of FITs varies from study to study. Interpretation of all published literature on FIT screening is complicated due to the differences in study design, the variation in type of test (ie, quantitative or qualitative, and FIT brand), the differing number of faecal samples collected, demographic differences in study population, and the cut-off value used to refer a screenee for colonoscopy. A systematic review showed that FITs had an overall higher sensitivity for CRC and advanced neoplasia or large adenomas (61-91% vs. 27-67%) than was reported for the non-rehydrated Hemoccult II test (25-38% vs. 16-31%) although the specificity appeared to be lower (FIT 91-98% vs. gFOBT 98-99%).[32] Recently, two trials have compared gFOBT and FIT screening in a randomized population-based manner.[29-30] In

both studies the degree of participation in a first FIT-based screening round was significantly higher compared with gFOBT screening (60-62% vs. 47-49%, respectively). Positivity rates were on average 2.6% for gFOBT and 8.3% for FIT screening at a cut-off value of 50 ng Hb/mL. Because FITs are able to detect smaller amounts of blood, one FIT sample is of higher diagnostic value than six faecal samples from three consecutive bowel movements in gFOBT screening. Both trials demonstrated that this was not at the cost of the positive predictive value of the test, as this is around 45% for both FOBTs.[52, 57]

Based on technological advances of FIT screening, and the above mentioned evidence in which was clearly shown that FIT outperforms gFOBT, in May 2011 the Dutch Health Council recommended the Minister of Health, Welfare and Sport that a nationwide FIT-based CRC screening programme should be implemented in the Netherlands.[3]

DNA markers

Adenocarcinoma of the large intestine can no longer be considered as one disease but rather a family of diseases with different precursor lesions, different molecular pathways, and different end-stage carcinomas with varying prognoses. The majority of CRCs arise from conventional adenomatous polyps via the suppressor pathway leading to microsatellite stable carcinomas. However, some carcinomas arise along the serrated pathway developing from the precursor lesion known as the sessile serrated adenoma (also referred to as the sessile serrated polyp). The remaining minority arises from conventional adenomas in patients with germ line mutations of mismatch repair genes (such as Lynch syndrome), leading to microsatellite instable carcinomas.[63] During the progression towards an invasive CRC, in each pathway, there is an accumulation of mutations in oncogenes and tumour-suppressor genes.[64-65] DNA marker screening is based on findings that specific mutations are associated with the development of CRC (e.g. mutations in K-ras, p53, APC and BAT-26).[66] These gene mutations can be traced by stool-based DNA marker tests in exfoliated epithelial cells which are continuously shed into the colon and secreted into the faeces. Whereas neoplastic bleeding is intermittent, epithelial shedding is continual which makes DNA marker screening potentially more sensitive to advanced colonic neoplasia.[67]

DNA marker screening requires the analysis of one faecal sample per screening round. Moreover, there is no need for dietary or medication restrictions.[68] However, the currently available DNA marker tests do require the collection of one entire bowel movement, which is frozen in a domestic freezer of the screenee until transportation to the laboratory.[23]

Compared with gFOBT and FIT screening, the use of DNA marker tests has been less extensively described. One of the most widely investigated DNA marker panels involves the measurement of 21 separate mutations, since there is not a single mutation present in all colonic neoplastic cells. The test characteristics of this panel were compared with that of the Hemoccult II test in a large population-based trial involving more than 4,000 asymptomatic average-risk individuals.[33] The main conclusion from this American study was that the DNA

panel displayed a higher sensitivity to CRC than the gFOBT (52% and 13%, respectively) without a reduction in specificity.

Despite their better sensitivity to CRC, a recent cost-effectiveness analysis showed that both the gFOBT and FIT are preferable to DNA marker tests.[69] In addition, the effect of DNA marker screening on lowering the incidence and mortality of CRC will remain limited due to its low sensitivity to advanced adenomas. Furthermore, the interval between consecutive screening rounds is unclear and it is unknown if repeated testing will have any value. Moreover, the most optimum DNA marker panel is not clear yet. A last issue is the meaning and follow-up of positive DNA marker tests in combination with a negative colonoscopy. For all these reasons, CRC screening by means of stool-based DNA marker tests will not be recommended for the time being. When solving the above mentioned issues, more population-based trials are needed to accurately establish the performance characteristics of stool-based DNA marker tests in average-risk individuals since this has only been evaluated by two studies so far.[33, 70]

NON-INVASIVE INVESTIGATIONS OF THE COLON AND RECTUM

CT-colonography

The virtual colonoscopy or CT-colonography (CTC) is a minimally-invasive technique whereby images of the entire colon and rectum are made in order to trace advanced neoplasia. Limited bowel preparation should take place, preferably one day before the investigation. Preparation involves that the screenee follows a low-fibre diet and ingests a small amount of iodine containing contrast. The low-fibre diet ensures that the contrast is well distributed throughout the contents of the bowel which results in significantly less untagged faeces and shows a trend toward better residue homogeneity.[71] CTC screening does not require any sedation or pain medication. If polyps are found, a colonoscopy is necessary in order to confirm the findings and to be able to remove these lesions. At this time, there is consensus that all participants with one or more polyps ≥ 10 mm or three or more polyps ≥ 6 mm should be referred for colonoscopy.[23, 72] The management of patients with fewer polyps in which the largest polyp is 6-9 mm remains controversial. If all patients with polyps 6-9 mm on CTC underwent colonoscopy, the referral rate could increase to 30% which seems unacceptably high. Furthermore, given the screening prevalence of 6-9 mm polyps of about 8% and a frequency of advanced histology in small adenomas of 4%, the overall screening prevalence of small advanced adenomas is approximately 0.3% and the frequency of CRC in small polyps is estimated to be 0.01%.[73] Therefore, the CT Colonography Reporting and Data System C-RADS consensus opinion from the Working Group on Virtual Colonoscopy stated that three-yearly CTC surveillance for patients with one or two 6-9 mm polyps represented a reasonable clinical approach.[74-75]

Because CTC visualizes the whole abdomen and the lower part of the thorax, extra-colonic incidental abnormalities are detected frequently. This is advantageous if these abnormalities are severe and treatable. However, other diseases may also be traced for which it is unclear whether early detection is useful. The rate of all extra-colonic findings varied between 27-69%. Findings of unknown or potential significance reported, varied between 11-18% of patients.[76-78] In 8-16% of them, additional diagnostic investigations or surgical interventions were recommended which resulted in increased total cost.

Generally, CTC is a safe procedure with a low rate of serious complications. The risk of CTC-related perforation in a CRC screening setting was 0.005%.[79] One important side effect of CTC is the potential harm caused by exposure to ionizing radiation which may give rise to cancer later in life.[80] This is considered a major issue in some countries like Germany, where CTC will not be used for screening as long as other methods without exposure to radiation (such as colonoscopy) are available. Because of the large contrast between the colonic wall and the with air- or gas-filled colonic lumen, lower doses of radiation can be used for CTC screening than for routine diagnostic abdominal CT scanning. The screened individual receives a radiation dose of 5 mSv during the CTC. This is similar to annual exposures for airline personnel of which is known that none of these employees has an increased incidence of cancer compared with the general population.[81] At last, it should be pointed out that a negative CTC only needs to be repeated after five years.

Compared with FOBT screening, the use of CTC as a primary screening tool for CRC has been less extensively investigated. In two Australian studies the participation rate varied between 16-28%.[82-83] This corresponds with the findings of a Dutch randomized controlled trial in which all individuals were invited for CRC screening by means of either a colonoscopy or CTC. The attendance rate was significantly higher in the CTC group (32%) compared with individuals who were primary invited for colonoscopy screening (21%; p-value < 0.001).[84]

As yet, little is known about the performance characteristics of CTC in a true screening setting.[85-86] The largest study to date was carried out in an asymptomatic average-risk population (n=2,600), which showed a sensitivity for CRC and large adenomas of 90%; this fell to 78% for lesions with a diameter ≥ 6 mm.[76] In another, non-randomized American study in which referral for colonoscopy was offered for all CTC-detected polyps of at least 6 mm in size, advanced neoplasia were equally as often detected as did direct colonoscopy screening (3.2% vs. 3.4% respectively).[77] In the previously mentioned Dutch randomized controlled trial, a significant difference in detection rate of advanced neoplasia was found in favour of primary colonoscopy screening (5.2% vs. 8.4% respectively).[84] However, in contrast with the American study, only CTC participants with lesions ≥ 10 mm were offered colonoscopy while those with one or more 6-9 mm lesions were offered surveillance CTC.

These data show that CTC screening is almost as reliable as colonoscopy screening in detecting advanced neoplasia of at least 6 mm in size. However, compared with colonoscopy screening, CTC screenees experience their investigation as more burdensome.[87] Further-

more, there are still not enough data on attendance and diagnostic yield in truly population-based CRC screening settings. Additionally, a screenee with a positive CTC result needs to be referred for colonoscopy. At present, there is no international consensus on the referral criteria. Furthermore, no randomized controlled trials on the efficacy of CTC screening for the prevention or mortality reduction of CRC have been performed. As a consequence, studies on CTC screening mainly use the detection rate of advanced neoplasia as a surrogate end-point of efficacy. Moreover, a cost-effectiveness analysis in the Medicare population (US) suggested that the CTC could only be a cost-effective option for CRC screening if the relative adherence to CTC was 25% higher than adherence to other screening tests.[88] Consequently, it is rather doubtful if this screening method will ever be a cost-effective alternative. Therefore, CT-colonography is nowadays only being used in nationwide CRC screening programs if a colonoscopy is incomplete.

INVASIVE INVESTIGATIONS OF THE COLON AND RECTUM

Flexible sigmoidoscopy

Another modality which can be used for nationwide bowel cancer screening is flexible sigmoidoscopy (FS). This procedure entails examination of the rectum, sigmoid and descending colon up to the splenic flexure using an endoscope. An enema which can be administered by the screenees at home is used as bowel preparation in most population-based screening trials.[29, 89-94] The required bowel preparation is less extensive for FS than for colonoscopy. Furthermore, the procedure takes a maximum of 15 minutes and in general no sedative or analgesic is necessary. Taking the shortage of gastroenterologists into account, sigmoidoscopies could be carried out by nurse-endoscopists. A questionnaire completed by Dutch gastroenterologists and gastroenterology fellows showed 89% of them to be in favor of FS screening by nurse-endoscopists.[95] In contrast with FOBT screening, FS enables detection of early neoplastic lesions which can directly be removed. The criteria to refer a participant for colonoscopy are still under debate. However, literature tells us that subjects with three or more adenomas or advanced neoplasia found on sigmoidoscopy have an increased risk of synchronous proximal lesions.[96-98] Most studies therefore consider a FS as positive in case of an advanced adenoma, ≥ 3 adenomas, or a CRC.

Complications such as bleeding or perforation occur in FS screening because of the screening procedure itself (0.01-0.03%) or due to the follow-up colonoscopy (0.26-0.55%).[90, 93] The optimal screening interval after a negative sigmoidoscopy has not yet been ascertained. An American study demonstrated no clinical or statistical difference in the incidence of neoplasia in subjects waiting for five years vs. three years after a normal sigmoidoscopy.[99] The evidence from this study supports the safety of the current screening FS interval of five years which has been recommended in most guidelines.[23] However, a British randomized

controlled trial (see below) strongly indicates that this screening interval may be lengthened to at least ten years.[100]

It is expected that endoscopic examinations (ie, sigmoidoscopy and colonoscopy) will cause bowel cancer mortality to fall more than due to FOBT screening, as during these invasive investigations advanced neoplasia can be detected and removed at the earliest possible stage. Recently, the effectiveness of FS screening has been demonstrated.[100-101] Two randomized controlled trials, conducted in Italy and the United Kingdom, have shown that once-only sigmoidoscopy screening can reduce CRC-related mortality by 22-31% in the group of invitees, and by 38-43% in the group who actually participated in CRC screening. In the same populations, the incidence of CRC fell by 18-23% and 31-33% respectively. Incidence of distal CRC (ie, located in rectum and sigmoid colon) was reduced by 50%.[100]

Just as in other screening strategies, the total effect of sigmoidoscopy screening on population level is influenced by the degree of participation. Studies outside the Netherlands have reported attendance rates varying from 10-40%.[3, 102-103] Only Norwegian trials have reported higher participation rates.[92] However, it should be pointed out that in most Scandinavian countries screening often seems to have a remarkably high uptake.[104] In a Dutch randomized controlled trial, carried out in the Rotterdam area, FS screening attendance was 32% - significantly lower than for gFOBT (50%) and FIT screening (62%).[29] Due to this relatively low participation rate, the screening effect on the entire target population is limited. One solution could be to invite non-participants of FS screening to take part in FIT screening. The Rotterdam study mentioned earlier showed that such a two-step recruitment for FS and FIT screening caused overall attendance rate to increase to 45%.[105]

There are many indications that sigmoidoscopy is an effective screening method: in 0.3-0.6% of screenees CRC was diagnosed, and in 3-7% advanced adenomas were found.[29, 90, 92-93] Unfortunately, only few data on the sensitivity of a screening sigmoidoscopy are available. As the technique used for colonoscopy and sigmoidoscopy is the same, test characteristics of FS screening are primarily based on studies of asymptomatic average-risk individuals who underwent a screening colonoscopy.[106-111] In this, advanced neoplasia detected up to the splenic flexure were considered to have been detected by FS screening. Such estimations of sensitivity varied from 58-75% for CRC and 72-86% for advanced neoplasia.[3] However, this approach overestimates the FS sensitivity for several reasons. Firstly, it is assumed that all colonoscopic found lesions between the splenic flexure and rectum would also have been detected with FS screening. This is rather a doubtful assumption because such a "sigmoidoscopy" benefits from the extensive bowel cleansing and probably also from the increased level of experience of the endoscopist. Furthermore, the FS examination is not always completed to the splenic flexure.[112] Secondly, all these studies were based on a very low threshold for referral for a follow-up colonoscopy because all screened subjects with an adenoma, regardless of size and histology, were referred. The previously mentioned Rotterdam study has shown that in the first screening round, the FS detection rate of advanced neoplasia is three times as high

as that of FIT screening and even seven times as high as that of gFOBT screening.[29] It should be pointed out that the yield from FS screening is strongly dependent on the endoscopist and on the reach of the scope.[113]

Given its long-term preventive effect and a higher diagnostic yield of advanced neoplasia compared with FIT sampling, CRC screening by means of flexible sigmoidoscopy is a good alternative to FOBT screening. However, there is still no consensus on the most optimal screening interval and attendance rates remain insufficient. Therefore, this method of screening is not, or not yet, the method of choice in the Netherlands.

Colonoscopy

The technique used for colonoscopy is the same as that of sigmoidoscopy except that the entire colon and rectum are visualized. Colonoscopy can be a primary screening instrument but it is also indicated for secondary screening of subjects with a positive faeces test, sigmoidoscopy or CTC.

The primary aim of colonoscopy screening is the detection of CRC and its benign precursor lesions. An American observational study reported that endoscopic removal of adenomas resulted in a lower-than-expected incidence of CRC.[12] The main advantage of colonoscopy screening is that removal of adenomatous polyps or early CRCs can be performed during the same procedure whereas all other previously mentioned screening tests require colonoscopy for confirmation and removal. Another advantage is that histological assessment of resected polyps and irresectable lesions can directly be obtained which is necessary to determine the surveillance interval or the need for further treatment. Also, a negative colonoscopy only needs to be repeated after ten years.[23, 114] However, there are also indications that screenees with an average-risk profile and in whom no abnormalities are found during a screening colonoscopy do not need to be screened again (ie, once-in-a-lifetime colonoscopy).[115] This may contribute positively towards the problem of capacity, and have a favourable influence on cost-effectiveness and increase the degree of participation. The disadvantages of colonoscopy are the discomfort caused by the extensive bowel preparation and the procedure itself, the complication risk, and its high cost. The required bowel preparation entails oral ingestion of 2-4 litres of laxatives prior to the examination. This is often regarded as being the most burdensome part of the entire colonoscopic procedure.[87, 116] Participants sometimes experience the introduction and advancement of the endoscope as burdensome and painful. For these reasons, most hospitals offer sedation and analgesia during the procedure. Furthermore, a colonoscopic examination is accompanied by a complication risk. Clinically significant complications necessitating hospitalization occur in 0.07-0.3% of screenees, including perforation and bleeding.[117-119] Finally, it should be noted that the test characteristics of colonoscopy screening strongly depend on the endoscopist. This requires major emphasis on quality measures to reduce the polyp miss rate in order to optimize the effectiveness of colonoscopy screening. In relation to this, recent publications have highlighted criteria

for best practice and have selected important quality indicators for colonoscopy.[120-121] High-quality colonoscopy depends on an appropriately trained and experienced endoscopist, obtaining informed consent including a specific conversation about adverse events associated with colonoscopy, in over 95% of colonoscopic procedures a complete examination to the caecum with adequate mucosal visualization and bowel preparation, mean withdrawal time of more than six minutes in a colonoscopy with negative findings performed in patients with intact anatomy[122], adenoma detection rate of ≥ 25% in average-risk men and ≥ 15% in average-risk women aged 50 years or older in a first screening colonoscopy, documentation and appropriate management of adverse events, and recommendations for surveillance or repeat screening based on published guidelines.

To date, there have been no randomized controlled trials assessing the efficacy of colonoscopy screening. However, such trials would be difficult to set up because of the large numbers and the long follow-up period required. Nevertheless, the Nordic-European Initiative on Colorectal Cancer (NordICC) trial is a multicentre collaborative effort in the Nordic countries, the Netherlands, and Poland in which thousands individuals are randomized to either colonoscopy screening or no screening. A fifteen year follow-up is planned and an interim analysis will be performed after ten years. The final results are expected in 2026. A recent Canadian study examined the CRC-related mortality in a database of 2.4 million people who had undergone a colonoscopy for various reasons.[123] This study showed that for every percent increase in complete colonoscopy rate, the hazard of CRC-related mortality decreased by 3%. Another Canadian trial has shown that a successful colonoscopy is strongly associated with a lower mortality rate, in particular left-sided CRCs (Odds ratio (OR) 0.33; 95% confidence interval 0.28-0.39) as no preventive effect on right-sided CRC was observed.[124] Possible explanations for this could be that the colonoscopy was not really complete (ie, no visualization to the base of the appendix), the colon is less clean on the right side, the withdrawal time of the scope from the right colon is too short, and on the right side polyps are more often flat than pedunculated making them more difficult to visualize.[125] These flat adenomas more frequently contain high-grade dysplasia, suggesting a more aggressive pathway in the CRC development.[126-128] It is increasingly believed that from a biological point of view right and left-sided polyps do behave differently.[63, 129]

Results from questionnaires distributed to individuals who have never undergone a colonoscopy have shown that after reading detailed information about this screening method, most of them would prefer FOBT-screening.[130] Studies on colonoscopy screening published to date show that attendance is low, between 3-40%.[45, 82, 103, 131] This corresponds with the findings of the previously mentioned Dutch CRC screening trial in which subjects were randomized for either colonoscopy or CTC. The attendance rate was significantly lower in the colonoscopy group (21%) compared with individuals who were primary invited for CTC screening (32%; p-value < 0.001).[84]

Sensitivity to CRC is ≥ 95%, however this does not necessarily count for advanced adenomas. From studies in which subjects underwent tandem colonoscopies, each carried out by different experienced endoscopists, we know that the sensitivity to large adenomatous polyps (≥ 10 mm) is between 90-98% and 87% for small adenomas with a diameter between 6-9 mm.[132-133] In five European trials, a total of 52,346 participants aged between 50-75 years were included for primary colonoscopy screening. Of this group, 0.5-1.0% were found to have CRC and 5-10% an advanced adenoma.[3, 103, 119, 134] This means that thirteen screenees had to undergo a screening colonoscopy to find one advanced neoplasia. This number is known as the 'number needed to scope'.

In conclusion, colonoscopy is the most sensitive screening method for the detection of CRC and its pre-malignant lesions. However, the participation rate in colonoscopy screening is lower than in other CRC screening strategies. For this reason, the diagnostic yield for a first colonoscopy screening round will probably be lower than for FIT screening for example. Likewise in this context, the cumulative sensitivity of a minimum of five FIT screening rounds (assuming biennial screening) must be contrasted with the yield of one colonoscopy screening round. Future research should provide the answers to these crucial questions.

CONCLUSIONS

In summary, due to its high incidence and mortality rates CRC poses a major health problem. The disease is characterized by a clearly recognizable and treatable precursor lesion, the so-called adenomatous polyp, which can be detected by different screening methods. The high and ever-increasing cost of CRC treatment implies that screening becomes a cost-saving intervention. For these reasons, both the European Union and the Dutch Health Council have recommended implementation of a nationwide CRC screening program.[3, 10] In May 2011 a decision was made by the Dutch Minister of Health, Welfare, and Sports to implement a biennial FIT-based screening program in the very near future for all men and women aged between 55-74. When taking into account the relatively high participation rates in the Dutch CRC screening pilot trials and the two-fold (cut-off 75 ng Hb/mL) higher detection rate of advanced colonic lesions compared with gFOBT testing, FIT screening is currently the most appropriate initial screening method to start with. However, due to the higher detection rate of advanced neoplasia and the very long-term preventative effect, primary flexible sigmoidoscopy or even colonoscopy screening may be a promising alternative of choice.

Table 1 Test characteristics for various colorectal cancer screening methods

	gFOBT (Hemoccult II; 3x2 samples)	**FIT** (1-3 samples)	**DNA markers** (1 sample)	**CT-colonography**	**Flexible sigmoidoscopy**	**Colonoscopy**
Screening interval	Annual / Biennial	Annual / Biennial	?	5-yearly	5-yearly	10-yearly
Sensitivity for CRC (%)	13-38	61-91	52	Unclear: 96	58-75	≥ 95
Sensitivity for advanced adenomas (%)	16-31	27-67	15	46-100 (lesions ≥ 10 mm)	72-86 (including CRC)	90-98 (adenomas ≥ 10 mm)
Specificity for advanced neoplasia (%)	98-99	91-98	94-96	92-93	?	?
Attendance (%)	47-67	62-64	?	Unclear: 16-28	10-40	5-40
Effectiveness	Yes (RCT)	Yes (based on extrapolation of gFOBT results)	No	No	Yes (RCT)	Yes (case-control studies)
CRC-related mortality reduction (%)	11-18	At least 11-18	?	?	31	Unclear: 50

NB. Participants with a positive gFOBT, FIT, DNA markers, CT-colonography, or flexible sigmoidoscopy outcome, will be referred for colonoscopy.

CRC = colorectal cancer
Advanced adenoma = an adenoma ≥ 10 mm, or an adenoma with more than 25% villous component and/or high-grade dysplasia
Advanced neoplasia = a CRC or advanced adenoma
gFOBT = guaiac-based faecal occult blood test
FIT = faecal immunochemical test
RCT = randomized controlled trial

AIM

The general aim of this thesis is to explore various aspects of faecal immunochemical test screening (ie, increasing attendance, determining the stability of stool samples, searching for the best screening strategy in terms of number of FIT samples and screening interval). Most papers are based on important data derived from a large prospective population-based study called the "CORERO" trial (ie,colorectal cancer screening in Rotterdam). This study was conducted in 2006 in which 18,419 individuals aged 50-74 were 1:1:1 randomized for either gFOBT, FIT, or sigmoidoscopy screening. This CORERO-I trial has provided a unique database that formed the basis for the successive CORERO-II trial in which asymptomatic average-risk individuals were invited for FIT-screening. All retrieved CORERO-II data will be presented and discussed in this thesis.

OUTLINE OF THIS THESIS

On May 15, 2006 the Minister of Health, Welfare, and Sports concluded that a nationwide CRC screening programme should be considered seriously in the Netherlands. Following this statement, several pilot trials were initiated to investigate several CRC screening methods as well as the feasibility of such a screening program in the Netherlands. On November 27, 2008 the Minister asked the Dutch Health Council for advice about the desirability and feasibility of introducing a screening program for CRC. Special attention was given to the development of alternative screening methods and how to implement a screening program by keeping the current colonoscopy capacity in mind. On November 17, 2009 the Dutch Health Council presented their recommendations: they concluded that CRC fulfils the criteria for population-based screening. Furthermore, they advised a nationwide screening program based on biennial 1-sample faecal immunochemical testing for all men and women aged between 55-74. On May 25, 2011 the Minister of Health, Welfare, and Sports agreed to start such a screening program in the Netherlands. From 2013 onwards, this screening program will be rolled-out in a stepwise manner. The primary screening method that will be used is the FIT, analysed at a cut-off value of 75 ng Hb/mL. In **chapter 2** of this thesis, the results of a systematic review are presented in order to give a general overview of the available literature concerning different FITs and the strength of evidence regarding their performance characteristics in terms of positivity rate and detection rate of advanced neoplasia.

The effectiveness of FIT screening in decreasing CRC-related mortality has not been studied in large long-term prospective randomized controlled trials. Although the results would be highly valuable, it is questionable whether such studies will ever be conducted. One could argue that this kind of evidence is unnecessary if the FIT is truly more accurate than gFOBT

screening in the same study population. Therefore, it is generally believed that the benefits of screening mainly depend on two parameters; the performance characteristics of a test and the attendance rate. Higher participation rates are associated with greater screening efficacy in terms of mortality reduction and increases cost-effectiveness. Unfortunately, adherence for CRC screening is low in many countries. Factors that are associated with attendance include: (i) knowledge about CRC and CRC screening; (ii) the type of screening test offered; (iii) endorsement by the general practitioner (GP); (iv) distributing and returning FOBTs by mail; (v) using personalized letters signed by the own GP; and (vi) sending reminder letters. The additional value of an advance notification letter with regard to participation rate is unknown. We therefore investigated in a population-based randomized CRC screening trial if the adherence could be raised when the invitation was preceded by an advance notification letter (**chapter 3**).

Although FITs are now one of the recommended screening tools and will be used as CRC screening method in the Netherlands, a lot of important questions remain to be answered. The most important issues pertaining to FIT screening that need clarification are the stability of stool samples, the number of samples necessary for the most favourable sensitivity and specificity, and the optimal interval between two successive screening rounds. All these topics will be investigated and discussed in this thesis. The first question concerning the stability of stool samples will be answered in **chapter 4**. In contrast with gFOBT screening, there are concerns that FITs are vulnerable to a delayed sample return. Firstly, the globin chains in haemoglobin molecules degrade more rapidly than haem. Secondly, the degradation of haemoglobin may occur quite fast in moist samples as used by most FITs, in contrast to the relatively dry smears used on gFOBT sample cards. It has been reported that a delay between faecal sampling at home and arrival at the laboratory impairs the efficacy of FITs. This effect would be a major problem for the yield of FIT-based screening programs and could therefore create a potential obstacle for the implementation and replacement of gFOBT by FIT. However, exact data are lacking and thus recommendations with respect to handling of negative FITs with a prolonged sample return time remain to be determined. We therefore evaluated the effect of sample return time on the performance characteristics of the FIT in a population-based CRC screening trial (**chapter 4**).

Besides pursuing higher participation rates, the detection rate of advanced neoplasia is a factor of similar importance for the effectiveness of population-based CRC screening. Unfortunately, not all advanced colonic lesions will be detected with single stool sampling because they bleed intermittently. Repeated testing probably increases test sensitivity, but it is unknown which effect this will have on attendance, colonoscopy demand, and diagnostic yield. As a result, data on the positive predictive value and cost-effectiveness of repeated FIT testing are also lacking. We therefore determined the attendance, detection rate of advanced

neoplasia, and colonoscopy demand in an average-risk CRC screening naïve population by means of either 1-sample or 2-sample FIT screening in a range of different cut-off values (**chapter 5**). Based on these data, a cost-effectiveness analysis was conducted with the MISCAN-Colon micro-simulation model in order to assess whether the increased effects of a second test (ie, additionally detected advanced neoplasia) outweigh the increased cost (ie, in terms of a higher colonoscopy demand) compared with 1-sample FIT screening (**chapter 6**).

The last question addressed in this thesis concerns the optimal interval between consecutive screening rounds. Repeated screening rounds not only enable to cover a larger proportion of the target population but also help to detect a larger proportion of subjects with advanced colonic lesions, both because of the gradual progression of a proportion of lesions and the intermittent nature of bleeding of advanced neoplasia. As a consequence, successive screening rounds are necessary for an optimal preventive effect in the target population. Based on long-term prospective randomized controlled gFOBT trails on mortality reduction, annual FOBT screening (ie, a high sensitive gFOBT or FIT) has been recommended in international CRC screening guidelines. However, there are no data on the comparison of different intervals for FIT screening and their impact on the attendance and detection rate of advanced neoplasia. We therefore conducted a population-based CRC screening trial in which we compared the attendance and diagnostic yield of repeated FIT testing with screening intervals of various lengths ranging from nil to three years (**chapter 7**).

Finally, in **chapter 8**, the main findings of this thesis and thus the CORERO-II trial are summarized and discussed. In addition, the implications for the future CRC screening program in the Netherlands and directions for further research are highlighted.

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Chapter 2

Faecal immunochemical tests for colorectal cancer screening in average-risk individuals

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Manuscript under preparation

Chapter 3

Advance notification letters increase adherence in colorectal cancer screening: A population-based randomized trial

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ABSTRACT

Objective: The population benefit of screening depends not only on the effectiveness of the test, but also on adherence, which, for colorectal cancer (CRC) screening remains low. An advance notification letter may increase adherence, however, no population-based randomized trials have been conducted to provide evidence of this.

Method: In 2008, a representative sample of the Dutch population (aged 50-74 years) was randomized. All 2,493 invitees in group A were sent an advance notification letter, followed two weeks later by a standard invitation. The 2,507 invitees in group B only received the standard invitation. Non-respondents in both groups were sent a reminder six weeks after the invitation.

Results: The advance notification letters resulted in a significantly higher adherence (64.4% vs. 61.1%, p-value = 0.019). Multivariate logistic regression analysis showed no significant interactions between group and age, sex, or socio-economic status. Cost analysis showed that the incremental cost per additional detected advanced neoplasia due to sending an advance notification letter was €957.

Conclusion: This population-based randomized trial demonstrates that sending an advance notification letter significantly increases adherence by 3.3%. The incremental cost per additional detected advanced neoplasia is acceptable. We therefore recommend that such letters are incorporated within the standard CRC-screening invitation process.

INTRODUCTION

In the United States, colorectal cancer (CRC) is the fourth most commonly diagnosed cancer, and the second leading cause of cancer-related death.[1] CRC is therefore a major health care problem in the Western world.

Faecal occult blood test (FOBT) screening, followed by colonoscopy in case of a positive FOBT, reduces CRC-related mortality by detecting and removing early carcinomas.[2-4]

The benefits of a screening program depend not only on the performance characteristics of a test, but also on adherence. Higher participation is associated with greater screening efficacy in terms of mortality reduction and increases cost-effectiveness.[5] Unfortunately, adherence in CRC-screening is low in many countries.[6-7] Factors that are associated with participation include: (i) knowledge about CRC and CRC-screening;[8] (ii) the type of screening test offered;[9-10] (iii) endorsement by the general practitioner (GP);[11] (iv) distributing and returning FOBTs by mail; (v) using personalized letters signed by the own GP; and (vi) sending reminder letters.[12]

In 2005, a small Australian study suggested that CRC-screening adherence had been raised when the invitation had been preceded by an advance notification letter.[13] We therefore conducted a large population-based randomized trial to assess the effectiveness of such a letter as an intervention to increase adherence.

METHODS

Participants

A total of 5,000 individuals aged 50-74 were randomly selected from municipal population registers and randomized 1:1 using a computer-generated algorithm (Tenalea, Amsterdam, the Netherlands). Further study design details are described elsewhere.[9] The study was approved by the Dutch Ministry of Health (PG/ZP 2.823.158). Recruitment took place between April and December 2008.

Interventions

The 2,493 randomly selected individuals in group A were sent an advance notification letter which contained background information on CRC, the potential benefits of screening, and information about the trial. Two weeks later, a standard invitation was sent which consisted of an invitation letter, an information brochure, one faecal immunochemical test (FIT), an instruction leaflet on how to perform faecal sampling, an informed consent form, and a reply-paid envelope. The invitation letter reinforced the same information mentioned in the advance notification letter.

The 2,507 invitees in group B received only this standard invitation. Six weeks after the invitation, a reminder was sent to all non-respondents.

Faecal immunochemical test

One FIT (OC-Sensor Micro, Eiken Chemical Co., Tokyo, Japan) was sent by mail to collect a single sample of one bowel movement. The FIT was considered positive when the haemoglobin (Hb) concentration in the sample was ≥ 50 ng/mL.

Power calculation

The primary outcome measurement was adherence. To yield an 80% power to discern a 5% difference in adherence between the two groups, the estimated minimum sample size was 1,500 in case of a 5% alpha error, based on a presumed overall adherence of 50%.

Statistical analyses

Adherence was calculated by dividing the number of participants by all eligible subjects (defined as all randomized invitees minus the excluded individuals). Differences in adherence between both groups were calculated using the Pearson-Chi Square test and differences in means were calculated using the Student t-test. Multivariate logistic regression analyses were used to determine whether sending an advance notification letter, age, sex, or socioeconomic status (SES) were associated with adherence. All p-values were two-sided and considered significant if < 0.05.

Cost analysis

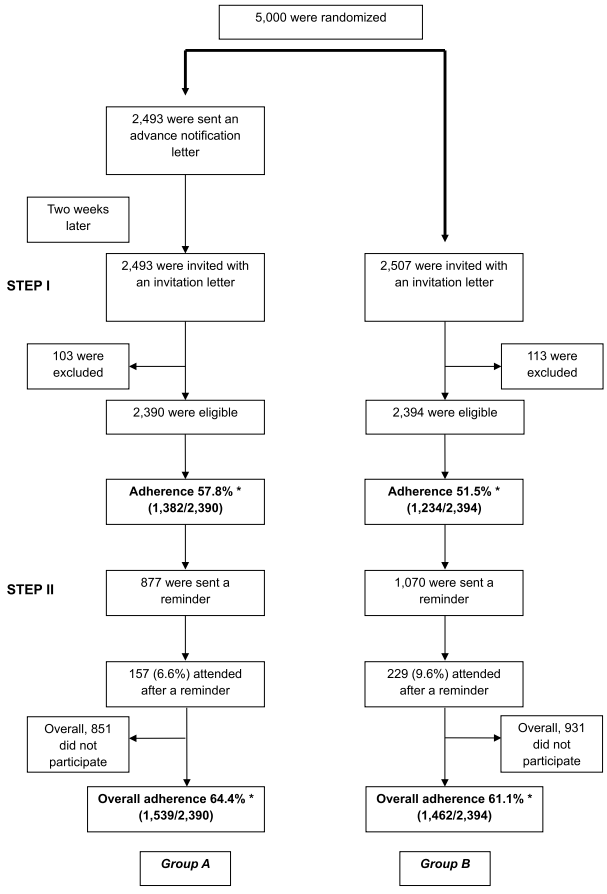
We estimated the incremental cost (including advance notification letters, analyzing extra FITs, and extra colonoscopies) per additional detected advanced neoplasia due to sending an advance notification letter. Per invitee, the additional cost of sending an advance notification letter was €0.48 (€0.06 for the envelope and letter itself, €0.36 for postal charges, and €0.06 for personnel costs). Calculated cost for analyzing one FIT sample was €4.41.[14] Based on an internal study, colonoscopy costs without polypectomy were assumed €303, and €393 in case of polypectomy (data not shown). Based on previous analyses in the same study population, we assumed that in 69% of all performed colonoscopies a polypectomy was carried out.[15] Therefore, total colonoscopy costs were assumed to be €365.10.

RESULTS

Of the 5,000 randomized subjects, 216 (4.3%) were excluded (156 met one of the exclusion criteria, 56 had moved away and 5 had died) (**Figure 1**). The distribution of age, sex and SES was equal between both groups (**Table 1**).

The overall adherence was 62.7% (95% confidence interval (CI): 61.3-64.1%). Independent predictors for non-adherence were age under 60 years (OR 0.8; CI 0.7-0.9), male gender (OR 0.8; CI 0.7-0.9), and low SES (OR 0.7; CI 0.6-0.8). Sending an advance notification letter and invitation was associated with a significantly higher adherence compared to sending an invitation letter alone (57.8% vs. 51.5% respectively; p-value < 0.001) (**Figure 1**, step I). After sending a reminder, this difference was still present (64.4% vs. 61.1% respectively; p-value = 0.019) (**Figure 1**, step II).

Figure 1 Trial profile



* *P* value < 0.05

Table 1 Baseline characteristics

	Group A	Group B	*P* value
Total number of invitees	2,493	2,507	
Eligible subjects (n)	2,390	2,394	0.51
Mean age (SD)	60.4 (7)	60.3 (7)	0.67
Sex (male; n-%)	1,169 (49)	1,180 (49)	0.79
Socio-economic status			0.63
High (n-%)	952 (40)	955 (40)	
Intermediate (n-%)	495 (21)	471 (20)	
Low (n-%)	943 (40)	968 (40)	

In the southwest of the Netherlands, recruitment took place between April and December 2008.

SD = standard deviation
Group A received an advance notification letter followed in 2 weeks by a standard invitation
Group B only received a standard invitation (ie, *no* advance notification letter was sent)

Subgroup analysis

There were no significant interactions between group and age (p-value = 0.84), sex (p-value = 0.92), or SES (p-value = 0.55), indicating that all invitees responded identically after receiving an advance notification letter.

Cost analysis

The additional cost of sending an advance notification letter to 2,493 invitees was €1,197, €340 for the analysis of 77 extra FITs, and €8,032 for the additional colonoscopies. At a cut-off value of 50 ng Hb/mL, 10 additional advanced neoplasia were found in group A. This corresponded with incremental cost of €956.84 per additional detected advanced neoplasia due to sending an advance notification letter in the first screening round.

DISCUSSION

This population-based randomized CRC-screening trial demonstrates that adherence is significantly increased by an advance notification letter. The observed difference of 3.3% may seem small, but when extrapolated to a nationwide CRC-screening program, it represents a large number of subjects.

The positive effect of such a letter may be explained by early gains in awareness, which would then be reinforced by similar information in the invitation and information brochure. This is particularly important in countries where there is low public awareness of CRC and the benefits of screening.[8] To date, little is known about the additional value of advance notification letters. American investigators found that sending such letters did not affect adherence.[16] Others reported a statistically significant rise in adherence after GPs had sent an explanatory letter two weeks before the invitation for screening (46.7% vs. 38.0%).[17] However,

it is not clear whether this positive effect was attributable to the GP involvement, the advance notification letter, or the combination of both. The most promising results came from a small Australian study (n=600 subjects), in which a 48.3% adherence was reported in the advance notification group vs. 39.5% in the control group (p-value = 0.002).[13]

Our results show that an advance notification letter has a greater impact on adherence before a reminder is sent. The higher adherence due to sending an advance notification letter is still present after receiving a reminder, although the reminder diminishes the difference in adherence. For settings in which reminders are sent, further research could focus on the additional value of a second reminder. Australian investigators suggested that adherence increased by 17.8% after the first reminder and by an additional 7.5% after the second.[18] Further studies should therefore compare the relative yield of an advance notification letter versus or combined with repeated reminders.

CONCLUSION

This large population-based randomized trial demonstrates that sending advance notification letters significantly increase adherence in CRC-screening. This simple intervention has low incremental cost per additional detected advanced neoplasia. To increase adherence of CRC-screening programs, we therefore advocate the implementation of an advance notification letter within the standard CRC-screening invitation process.

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Chapter 4

Are faecal immunochemical test characteristics influenced by sample return time? A population-based colorectal cancer screening trial

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ABSTRACT

Background: Faecal immunochemical tests (FIT) are preferred over guaiac-based faecal occult blood testing as colorectal cancer (CRC) screening tool. However, haemoglobin-degradation over time may influence FIT outcome. We therefore evaluated the effect of sample return time on FIT performance characteristics in a population-based CRC screening trial.

Methods: A representative random sample of the Dutch population (n=17,677), aged 50-74 years, was invited for FIT screening (OC-Sensor Micro; cut-off ≥ 50 ng Hb/mL). Sample return time was defined as the interval in days between faecal sampling and FIT laboratory delivery. Additionally, a random sample of positive FITs were selected to be stored at room temperature and re-tested every 3-4 days.

Results: In total, 8,958 screenees fulfilled our inclusion criteria. The mean sample return time was three days (± 3). Overall, 792 screenees (8.8%) had a positive test. Between the sample return time groups, the positivity rate (PR) varied between 7.7-9.0%. No statistically significant associations were found between PR or detection rate (DR) and the different sample return time groups (p-values 0.84 and 0.76, respectively). For the laboratory experiment, 71 positive FITs were stored at room temperature and re-tested with standard intervals. The mean daily faecal haemoglobin decrease was 5.88% per day (95% confidence interval 4.78-6.96%). None of the positive FITs became negative before ten days after faecal sampling.

Conclusion: This population-based CRC screening trial demonstrates that both the PR and DR of FITs do not decrease with prolonged sample return times up to ten days. This means that a delay in sending the FIT back to the laboratory, of up to at least one week, does not necessitate repeat sampling in case of a negative test result. These data support the use of FIT-based screening as a reliable tool for nationwide CRC screening programs.

INTRODUCTION

Colorectal cancer (CRC) is a major healthcare problem. Worldwide, CRC is the fourth most occurring malignancy in men and ranks third in women.[1] Furthermore, CRC is the second most frequent cause of cancer-related death in the Western world.[2] For an average-risk individual the life-time risk of developing CRC is around 5%. For these reasons it can be concluded that CRC is a major health problemFour randomized controlled trials showed that screening by means of faecal occult blood tests (FOBT) reduces CRC-related mortality by 15-33%.[3-7] Currently, population-based CRC screening programs using FOBT have been implemented or are studied in feasibility trials in many Western countries. FOBTs fall into two categories based on the detected component of blood: guaiac-based FOBT (gFOBT) and the more recently developed faecal immunochemical tests (FIT). The first type of FOBT detects heme, which is incorporated in haemoglobin (Hb) molecules, using its inherent peroxidase activity. The gFOBTs react to any peroxidase in faeces (eg, plant peroxidases or heme in red meat) and are affected by certain chemicals (eg, high-dose vitamin C supplements), resulting in false-positive and false-negative tests. FIT on the other hand, measures the presence of intact globin chains in Hb molecules by means of specific antihuman antibodies. Therefore, FITs are in contrast with gFOBTs specific for human blood. Furthermore, FITs are more specific for lower gastro-intestinal (GI) bleedings since protease enzymes gradually digest blood from the proximal GI-tract during its passage through the intestine rendering it less likely that globin chains will be recognised by the antibodies used in a FIT.[8-9] Moreover, FITs -at least some, including the one addressed to in this paper- provide a quantitative measurement of microscopic blood loss in stool. This allows selection of an optimal cut-off value for a specific target population and standardization of test outcomes.[10-11] Finally, several trials have demonstrated that faecal immunochemical testing is superior to the traditionally used gFOBT (ie, the non-rehydrated Hemoccult II) in terms of higher attendance and diagnostic yield of advanced neoplasia at the same or even higher specificity.[12-20]

However, in contrast with gFOBT screening, there are concerns that FITs are sensitive to delayed sample return. Firstly, the globin chains in Hb molecules degrade more rapidly than heme.[21-23] Secondly, the degradation of Hb may occur quite fast in moist samples as used by most FITs, in contrast to the relatively dry smears used on gFOBT sample cards.[21] It has been reported that a delay between faecal sampling and arrival at the laboratory impairs the efficacy of FITs.[24] This effect would be a major problem for the yield of FIT-based screening programs and could therefore create a potential obstacle for the implementation and replacement of gFOBT by FIT. However, exact data are lacking and thus recommendations with respect to handling of negative tests with a prolonged sample return time remain to be determined. We therefore evaluated the effect of FIT sample return time on test performance characteristics in a population-based CRC screening trial.

METHODS

Part I: Study population

Between November 2006 and June 2009, a population-based CRC screening trial was conducted in the southwest of the Netherlands with a target population of approximately 350,000 inhabitants. Details of the subsequent trial protocols for 1- and 2-sample FIT screening have been described elsewhere.[12, 25] Briefly, a total of 17,677 individuals between the ages of 50–74 years were randomly obtained from municipal population registers by a computer-generated algorithm (Tenalea, Amsterdam, the Netherlands). Selection was performed per household and occurred before invitation. Since there is no nationwide CRC screening program in the Netherlands, the population used for this trial was screening-naïve. Eventually 14,480 individuals were invited for 1-sample FIT testing, whereas 3,197 individuals were invited to undergo screening with two FITs to be sampled on consecutive days. Exclusion criteria were asked for on the informed consent form which had to be filled in by the screenee itself. Exclusion criteria were a history of CRC; inflammatory bowel disease; a life expectancy of under five years; a colonoscopy, sigmoidoscopy or double-contrast barium enema within the previous three years; and inability to give informed consent.

Interventions

All potential participants were sent an advance notification letter which contained background information on CRC and CRC screening. Two weeks later this letter was followed by a standard invitation, which included an invitation letter, one or two FITs, an instruction leaflet on how to perform faecal sampling, an information brochure, an informed consent form, and a reply-paid envelope. All non-respondents were sent a reminder six weeks after the standard invitation.

Faecal immunochemical test

Each invitee was sent either one or two FITs (OC-Sensor Micro, Eiken Chemical Co., Tokyo, Japan). This quantitative FIT consists of a small test tube with a faecal probe inserted into it. The probe has a serrated tip, which is poked several times in different parts of the stool and then pushed back into the tube, along a scraper, through a membrane, and thereby closing and sealing the test tube. This action removes most of the excess faeces and leaves a semi-standard amount of stool in the probe tip serrations. The tip is then located in the bottom compartment of the tube, which contains a 2 mL haemoglobin stabilizing buffer. Tests do not require dietary restrictions or medication limitations. In case of 2-sample FIT screening, two test tubes were included in the mailing and explicit instructions were given to use them on two bowel movements on consecutive days. All individuals were asked to report the date of faecal sampling on the test tube(s) and instructions were given to return the test(s) as soon as possible. If the test(s) could not be returned immediately after faecal sampling, e.g. in case

of 2-sample FIT screening, storage in a domestic refrigerator was recommended. Participants returned the FIT sample(s) and a signed informed consent form to the Gastroenterology and Hepatology Laboratory at the Erasmus University Medical Centre for analysis using the automatic OC-Sensor μ instrument (OC-Sensor Micro, Eiken Chemical Co., Tokyo, Japan). Samples were collected after arrival at the laboratory and immediately stored at -20°C until test development, which occurred within one week. The manufacturer recommends using a positivity threshold of 100 ng Hb/mL. However, literature as well as data provided by the manufacturer itself show that the test results of the OC-Sensor Micro are also reliable at a lower cut-off to the level of 50 ng Hb/mL.[26] We have previously shown that this low cut-off value remains associated with a substantial positive predictive value.[10] For this trial, FITs were therefore considered positive when the Hb concentration in the sample was ≥ 50 ng/mL (corresponding to 10 μg Hb per gram of faeces).

Test result

In case of a positive test result, the general practitioner (GP) was informed both by telephone and mail within two weeks. The GP informed the participant about the test result and referred the screenee for colonoscopy. Participants with a negative test result were informed by mail within two weeks.

Colonoscopy

All colonoscopies were performed in the regional hospitals by experienced endoscopists. The maximum reach of the endoscope with identification of landmarks, as well as the adequacy of bowel preparation, were recorded. During colonoscopy, characteristics including size, morphology, and location of any polyps, were documented. Location was defined as rectum, sigmoid, descending, transverse, or ascending colon or caecum, and was measured in cm from the anal verge with the endoscope in straightened position. Size of each polyp was estimated using an open biopsy forceps with a span of 7 mm. All removed polyps were evaluated by experienced gastrointestinal pathologists. In accordance with the international classification, CRC was defined as the invasion of malignant cells beyond the lamina muscularis mucosa.[27] Lesions with intramucosal carcinoma or carcinoma in situ were classified as high-grade dysplasia.

Ethical approval

The study was approved by the Dutch National Health Council (PG/ZP 2.727.071 and PG/ZP 2.823.158). The approval included the random selection before invitation design. The study letters and information brochures were approved by the Institutional Review Board at Erasmus University Medical Centre (MEC-2005-264 and MEC-2008-029).

Part II: Laboratory experiment

For this experiment, a total of 71 positive FITs were randomly selected after each series of testing, re-sealed, and stored at room temperature (20°C) without actively keeping laboratory conditions, such as humidity and temperature, at constant levels. With standard intervals of 3-4 days, all selected FIT samples were re-tested under standard laboratory conditions. In the same way, 31 positive FIT samples were selected and stored in a stove at a constant temperature of 30°C. Because it was hypothesized that the Hb degradation would be faster at higher temperatures, a shorter interval of 2-3 days was chosen to re-test all selected FIT samples.

Statistical analysis

Part I: The sample return time was defined as the interval in days between faecal sampling at home, as reported by the screenee itself, and FIT laboratory delivery. We classified all positive screenees based on their sample return time into three subgroups: short (≤ 3 days), average (4-6 days), and prolonged sample return time (≥ 7 days). Uni- and multivariate ordinal logistic regression analyses were used to determine the influence of sex, age, and socio-economic status (SES) on sample return time. In case of 2-sample FIT screening, the positivity rates (PR) of both samples were compared by using the McNemar test, knowing that the first FIT always had been performed at least one day earlier than the second performed test. In order to compare the positive predictive value (PPV) and detection rate (DR), one of both tests was randomly selected for the final analyses. The PR was defined as the proportion of participants having a positive test result. The PPV was defined as the proportion of participants with a positive test result having an advanced neoplasia. This was calculated as the number of screenees with an advanced neoplasia divided by all screenees with a positive test result who underwent a successful colonoscopy. Advanced neoplasia included CRC and advanced adenomas. An advanced adenoma was defined as an adenoma ≥ 10 mm, or with a histology showing either ≥ 25% villous component and/or high-grade dysplasia. The DR was defined as the proportion of participants in whom an advanced neoplasia is found.[10-20] This was calculated as the number of screenees with an advanced neoplasia divided by all screenees with an analyzable screening test. When more than one lesion was present, the screenee was classified according to the most advanced lesion found during the follow-up colonoscopy. The PR, PPV and DR were calculated and described as proportions with 95% confidence intervals (CI). Differences in proportions between the sample return time subgroups were calculated using the Pearson Chi-Square test. Multivariate logistic regression analyses were used to determine the influence of sample return time, sex, age, and SES on the PR, PPV, and DR. Because a recent Italian report demonstrated a 17% lower probability of FITs being positive in summer than in winter, we also included season in the regression analysis.[23] Furthermore, the outside temperatures were based on data of the Royal Netherlands Meteorological Institute (www.knmi.nl), providing average outside temperatures per month. Association between PR

and mean outside temperature was determined. All p-values were two-sided and considered significant if < 0.05. Statistical analysis was performed with SPSS 15.0 for Windows.

Part II: A linear mixed effects model was used to estimate the mean percentage Hb decrease per day.[28] We used the log transformed Hb values as outcome and included time after faecal sampling which was expressed in days, as the only predictor. The faecal sample was included as a random intercept in the model to account for the correlations between the repeated measurements of each individual FIT sample. So, the intercept was allowed to vary from sample to sample but the slope parameter of time was assumed to be equal for all included samples. We used the lmer package in R for the calculations.[29-30]

RESULTS

Part I: Proportion of positive tests

Of the 17,677 subjects who were randomly invited for CRC screening, 8,958 screenees (51%) fulfilled our inclusion criteria as they returned the FIT and wrote down the sampling date on the test tube. **Table 1** shows the baseline characteristics of all included screenees in the various sample return time subgroups. The mean sample return time was three ± 3 days (mean ± SD) and the prolonged sample return time group had a delay which varied between 7-34 days. Screenees who returned their FIT samples within three days were significantly older and more often female (both p-values < 0.05).

Table 1 Baseline characteristics of all included screenees

	Sample return time (days)				
	≤ 3	**4 – 6**	**≥ 7**	**Overall**	***P* value***
Number of included screenees	5,959	2,723	276	**8,958**	
Mean age (SD)	61.0 (6.6)	60.5 (6.6)	60.1 (6.5)	**60.8 (6.6)**	0.001
Sex (male; n-%)	2,750 (46.1)	1,349 (49.5)	136 (49.3)	**4,235 (47.3)**	0.011
SES (n-%)					0.001
Very high	1,291 (21.7)	563 (20.7)	66 (23.9)	**1,920 (21.4)**	
High	1,233 (20.7)	637 (23.4)	83 (30.1)	**1,953 (21.8)**	
Intermediate	1,095 (18.4)	490 (18.0)	48 (17.4)	**1,633 (18.2)**	
Low	1,151 (19.3)	507 (18.6)	31 (11.2)	**1,689 (18.9)**	
Very low	1,189 (20.0)	526 (19.3)	48 (17.4)	**1,763 (19.7)**	

Sample return time = the interval in days between faecal sampling at home and FIT laboratory delivery
SES = socio-economic status
* Pearson Chi-Square test

Overall, 792 screenees (8.8%) had a positive test result at a cut-off value ≥ 50 ng Hb/mL and were therefore referred for colonoscopy. Between the different sample return time groups, the PR varied between 7.7-9.0% (**Table 2**). The results showed a fluctuation of both the PR and mean Hb concentration in relation to the sample return time. There was no statistically significant difference between the mean Hb level and sample return time (p-value = 0.13), although a downward trend was seen from a sample return time of six days onwards (**Figure 1**). When only the PR was taken into account, again no statistically significant difference was observed between the PR and sample return time (p-value = 0.96). Other factors that were associated with PR were in line with previous results.[10] This included higher PRs among men compared to women (odds ratio (OR) 1.71; CI 1.47-1.99), individuals between the ages of 60-64 years (OR 1.27; CI 1.04-1.55) and 65-74 years (OR 1.99; CI 1.68-2.36) compared to screenees aged 50-60, and screenees from a middle (OR 1.29; CI 1.05-1.60) and low SES (OR 1.32; CI 1.12-1.55) compared to those from a high SES. Finally, the PR was significantly higher during winter season compared to the summer (9.7% vs. 8.0% respectively; p-value = 0.006). Furthermore, an odds ratio of 0.974 (CI 0.960-0.990) was found for FITs being positive with each degree Celsius increase in average outside temperature (**Figure 2**).

As mentioned, a separate analysis was carried out for the 2-sample FIT screening group, in which differences in PR between the first and second test were compared. A total of 1,874 individuals participated with 2-sample FIT screening. The first test was positive in 169 screenees (9.0%; cut-off level ≥ 50 ng Hb/mL), compared to a PR of 8.8% with the second test (p-value = 0.74).

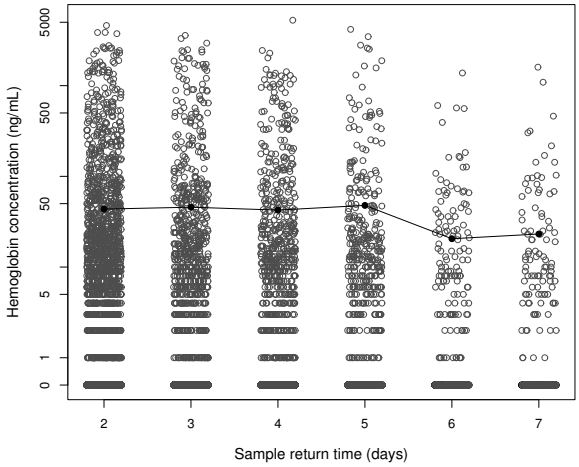
In a multivariate ordinal logistic regression analysis, factors that were associated with a longer sample return time were male gender (OR 1.25; CI 1.15-1.34) and age < 60 years (OR 1.31; CI 1.20-1.43). No correlations were seen between sample return time and SES (p-value = 0.072).

Table 2 Number of included screenees and positive tests in relation to sample return time

Sample return time (days)	Number of screenees	Number of positive FITs (PR: CI) *	Mean haemoglobin concentration (ng/mL) (± SD) #
≤ 2	3,951	352 (8.9: 8.1-9.8)	43.6 (241.9)
3	2,008	180 (9.0: 7.8-10.3)	45.7 (247.1)
4	1,561	141 (9.0: 7.7-10.5)	42.5 (224.2)
5	836	72 (8.6: 6.9-10.7)	47.8 (279.2)
6	326	25 (7.7: 5.3-11.1)	20.5 (98.8)
≥ 7	276	22 (8.0: 5.3-11.8)	23.1 (123.9)
Total	**8,958**	**792 (8.8: 8.2-9.4)**	**42.8 (237.4)**

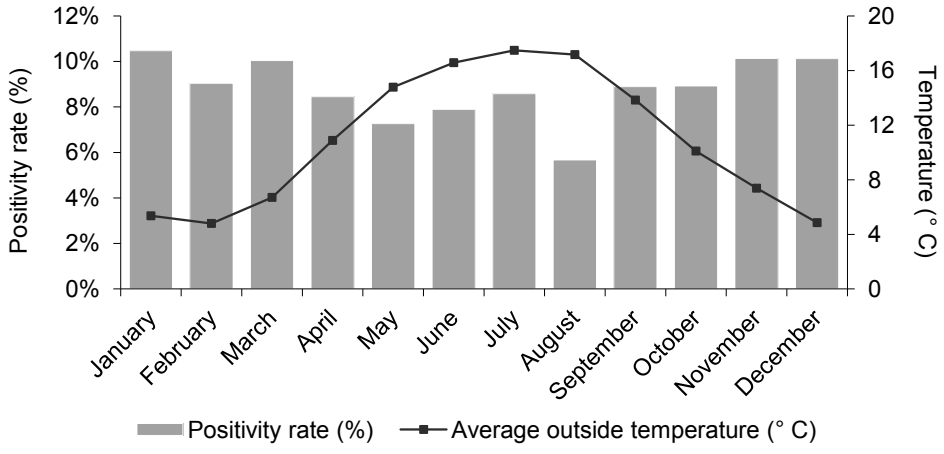
Sample return time = the interval in days between faecal sampling at home and FIT laboratory delivery
FIT = faecal immunochemical test (OC-Sensor Micro; cut-off value ≥ 50 ng Hb/mL)
PR = positivity rate (ie, the proportion of participants having a positive test result)
CI = 95% confidence interval, SD = standard deviation
No statistically significant difference was found between either the PR or mean haemoglobin concentration and sample return time, in which the sample return time group ≤ 2 days was taken as reference.
* Univariate logistic regression analysis: *P* value = 0.96
# ANOVA on the log transformed data: *P* value = 0.13

Figure 1 Haemoglobin concentration of all included FITs for the different sample return time groups



FIT = faecal immunochemical test (OC-Sensor Micro; cut-off value ≥ 50 ng Hb/mL)
Sample return time = the interval in days between faecal sampling at home and FIT laboratory delivery
○ = haemoglobin concentration of one analyzed FIT sample
● = arithmetic mean haemoglobin concentration per sample return time group
No statistically significant difference was found between the mean haemoglobin concentration and sample return time, in which the sample return time group ≤ 2 days was taken as reference. ANOVA was used on the log transformed data: *P* value = 0.13

Figure 2 Positivity rate versus average outside temperature



Follow-up and test performance characteristics

In total, 92% (732/792) of all positive FIT screenees underwent a successful colonoscopy, 294 (40%) of them were diagnosed with an advanced neoplasia (252 advanced adenomas and 42 CRCs). No statistically significant correlation was found between the PPV and sample return time: the PPV was 41% in the sample return time group ≤ 2 days vs. 33% in the group with a sample return time of at least seven days (p-value = 0.66). **Table 3** shows the number of advanced neoplasia, as well as the PPV and DR for the different sample return time groups.

Furthermore, the DR of advanced neoplasia per 100 screenees was calculated. Between the different sample return time groups, the DR varied between 2.5-3.7% with an overall DR of 3.3% (294/8,958). The DR did not significantly decrease when the sample return time was increased (p-value = 0.85). Factors that were associated with higher DRs were in line with previous results.[10] In a multivariate logistic regression analysis, this included in particular higher DR among men compared to women (OR 1.93; CI 1.52-2.46), individuals between the ages of 60-64 years (OR 1.40; CI 1.01-1.94) and 65-74 years (OR 2.31; CI 1.76-3.03) compared to screenees aged 50-60, and screenees from a middle (OR 1.53; CI 1.10-2.13) and low SES (OR 1.40; CI 1.08-1.83) compared to those from a high SES. The DR of advanced neoplasia was significantly higher during winter season (OR 1.30; CI 1.03-1.65) compared to the summer.

Finally, the same conclusions could be drawn for a higher cut-off value of 100 ng Hb/mL: increasing the sample return time did not significantly decrease the PR, PPV, or DR (p-values 0.33, 0.54, and 0.36 respectively).

Table 3 Follow-up results of positive FIT screenees

Sample return time (days)	Number of positive tests	Number of successful colonoscopies (%)	Number of patients with advanced neoplasia (PPV %)	PPV OR (CI)	DR of advanced neoplasia per 100 screenees (%)	DR OR (CI)
≤ 2	352	325 (92)	134 (41)	1	3.4	1
3	180	170 (94)	61 (36)	0.80 (0.54-1.17)	3.0	0.89 (0.66-1.21)
4	141	126 (89)	57 (45)	1.18 (0.78-1.78)	3.7	1.08 (0.79-1.48)
5	72	67 (93)	26 (39)	0.90 (0.53-1.55)	3.1	0.91 (0.60-1.40)
6	25	23 (92)	9 (39)	0.92 (0.39-2.18)	2.8	0.81 (0.41-1.60)
≥ 7	22	21 (95)	7 (33)	0.71 (0.28-1.81)	2.5	0.74 (0.34-1.60)
Total	**792**	**732 (92)**	**294 (40)**		**3.3**	

FIT = faecal immunochemical test (OC-Sensor Micro; cut-off value ≥ 50 ng Hb/mL)
Sample return time = the interval in days between faecal sampling at home and FIT laboratory delivery
Advanced neoplasia = all colorectal cancers and advanced adenomas
Advanced adenoma = an adenoma ≥ 10 mm, or an adenoma ≥ 25% villous component and/or high-grade dysplasia
PPV = positive predictive value
DR = detection rate
OR = Odds ratio
CI = 95% confidence interval

Part II: Laboratory experiment

In total, 71 positive FIT samples were randomly selected, stored at room temperature, and re-tested with standard intervals of three to four over a period of three weeks. In total, 69 (97%) of the screenees from whom these positive FITs had been obtained, underwent a successful colonoscopy. The samples included for this part of the trial had a sample return time of two to seven days. The initial Hb concentration of the selected tests varied between 53-1,894 ng/mL. **Figure 3** shows the Hb concentrations of the repeated measurements on a logarithmic scale, versus the time in days after faecal sampling at home. Furthermore, **Figure 4** demonstrates in more detail all faecal samples with initial Hb concentrations between 50-500 ng/mL on a normal scale. During storage at room temperature, the mean Hb concentration in the faecal samples decreased by 5.88% per day (CI 4.78-6.96%). After correction for sample return time, it was only after ten days that the first Hb concentrations dropped below the 50 ng/mL cut-off level, which resulted in a conversion from a positive test outcome into a negative test result. The corresponding three samples had initial Hb values between 53-58 ng/mL. These three screenees had a negative colonoscopy (ie, two screenees with no lesions, and one screenee with a hyperplastic polyp). The remaining FIT samples became negative by a further lengthening of the interval. Two weeks after faecal sampling, 21/71 samples (30%) became negative. By extending the sample return time towards fourteen days, in total six non-advanced adenomas, five advanced adenomas and one CRC would have been missed.

Figure 3 Laboratory experiment - haemoglobin concentration of repeated FIT measurements (logarithmic scale)

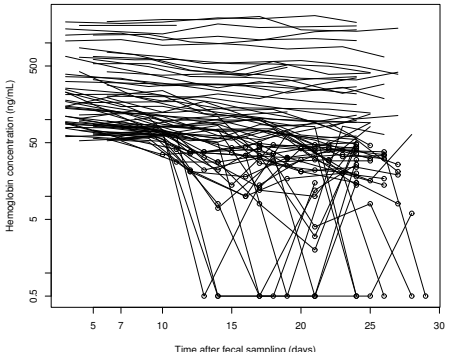
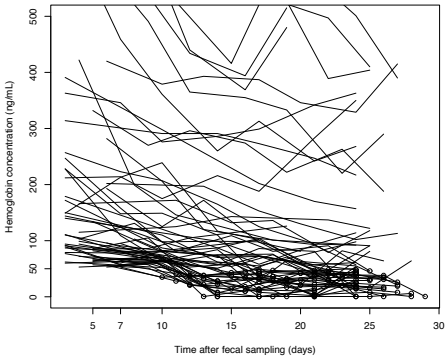


Figure 4 Laboratory experiment - haemoglobin concentration of repeated FIT measurements (normal scale in more detail)



FIT = faecal immunochemical test (OC-Sensor Micro; cut-off value ≥ 50 ng Hb/mL)
○ = haemoglobin concentration < 50 ng/mL (ie, negative test result)

Additionally, another 68 positive FIT samples were stored in a stove at a constant temperature of 30°C and re-tested every two to three days over a period of three weeks. The collected positive tests had a sample return time of two to six days. The initial Hb concentration of the selected faecal samples varied between 52-3,196 ng/mL. When stored in a stove at 30°C, the mean Hb level decreased by 18.07% per day (CI 16.88-19.24%). One week after faecal sampling, 22/68 samples (32%) became negative. Moreover, this percentage increased towards 84% (57/68 samples) when the samples were stored for a period of two weeks.

DISCUSSION

Screening for colorectal cancer by means of a FIT forms an attractive alternative to the most common and traditionally used gFOBT (ie, the non-rehydrated Hemoccult II) because of higher attendance and diagnostic yield of advanced neoplasia.[12-20] Based on modeling of data from various screening trials, annual FIT screening has recently been reported to have an impact on CRC-related mortality which may amount to a similar level as colonoscopy screening.[31] Worldwide, these findings have raised strong interest in FIT testing as a primary screening tool for CRC. In Europe, several countries are considering to switch from gFOBT to FIT screening, while others are preparing to newly introduce CRC screening with FITs. The same applies for certain regions in Canada, while in the US a comparative trial is being prepared between FIT and colonoscopy screening. However, one important obstacle for the implementation of FIT screening is the possible limited stability of the test: due to globin degradation test sensitivity might drop with prolonged intervals between faecal sampling and arrival at the laboratory. However, our results demonstrate that with almost 10,000 FITs analyzed, both the PR as well as the DR of advanced neoplasia do not significantly decrease with sample return times of up to seven days. Moreover, our trial results were confirmed by a laboratory experiment in which 71 positive FIT samples were randomly selected, stored at room temperature, and re-tested with standard intervals of three to four days. Our data show that no clinical significant lesions would have been missed within the first ten days after faecal sampling. It has been shown that non-advanced adenomas have a lower baseline Hb level than advanced adenomas and CRCs.[10, 24, 32] As such, FIT samples from screenees with non-advanced adenomas may sooner convert to negative than samples from patients with advanced neoplasia. Furthermore, our data do show the importance of not further lengthening the sample return time, for instance towards fourteen days. By adapting this strategy, fourteen screenees would have tested false-negative including six with advanced neoplasia.

Our main results confirm the laboratory data reported by Israeli investigators who observed no significant Hb degradation over a period of 21 days when FIT samples were stored at 20°C.[26, 33] However, a fall in the Hb concentration of 3.7% (± 1.8%) per day was observed when tests were kept at ambient summer room temperature (on average 28°C). A first expla-

nation for the discrepancy in main outcome between the Israeli vs. the current study (ie, an interval of 21 vs. ten days respectively for the first tests become negative), is the extreme high initial Hb concentrations found in the Israeli trial, 787-1,032 ng Hb/mL compared to 53-1,894 ng Hb/mL in the present study. These differences can be explained by the fact that the Israeli study was performed among high-risk and symptomatic individuals, whereas our trial only included screenees in an asymptomatic average-risk population and is thus more applicable to general population-based CRC screening. Although different cut-off values were used (100 vs. 50 ng Hb/mL, respectively), it is not surprising that our samples -with initial Hb concentrations close to the cut-off threshold- became negative within a shorter time interval. A second explanation for the somewhat different outcomes with respect to the daily Hb decrease at higher temperatures (ie, 3.7% in the Israeli study vs. 18.1% in the present study), might be the actual temperature at which the positive FIT samples were stored. In contrast with our trial, room temperature was not kept at a constant level in the Israeli study but fluctuated over the day and was, on average, somewhat lower than the constant 30°C in the present study. Nevertheless, the same conclusion can still be drawn from both trials; the Hb degradation process increases at higher outside temperatures.

In a recent Italian report, it was demonstrated that the Hb concentrations measured during summer were significantly lower than those during winter.[23] An increase in temperature of 1°C resulted in a 0.7% reduced probability of FITs being positive. Our results confirmed a significantly reduced PR and DR during summer time with an odds of 0.974 (CI 0.960-0.990) for FITs being positive with each degree Celsius increase in average outside temperature.

In contrast with our results, another Dutch study found that the PR significantly decreased with each extra day of delay with an OR of 0.9 (CI 0.8-1.0).[24] In this trial individuals from the same age were recruited from an asymptomatic average-risk population and identical FITs were used (OC-Sensor Micro; cut-off value ≥ 50 ng Hb/mL). However, the number of included subjects in that study was considerably smaller (3,767 vs. 8,958 screenees in our trial), only allowing for calculations with rather wide confidence intervals. Second, the PRs were remarkably different for the average sample return time group (6.0% vs. 8.3% in our study, respectively), and prolonged sample return time group (4.1% vs. 8.0%). The only likely explanation for these differences was the storage conditions used at the laboratory. In the previous Dutch trial, all included samples were stored in a laboratory refrigerator at 4°C, compared to storage at -20°C in our trial. The previously mentioned Israeli trial also reported a drop in FIT results below the 100 ng Hb/mL threshold after prolonged storage at 4°C.[26, 33]

The present study had some limitations. Although the number of participants was high, the number of screened individuals with a sample return time of six days or more was relatively small which limited power of the study. These relatively low number of screenees with a strongly delayed sample return time, in turn resulted in relatively even lower numbers of screened individuals with an advanced neoplasm. Therefore, a type II error, that is, ruling out an actual difference between the different sample return time groups, could not be excluded

and larger series are necessary to confirm our observations. Secondly, advice was given to store the FITs in a domestic refrigerator if the test(s) could not be returned instantly after faecal sampling. However, we were not able to verify if screenees obeyed these instructions. Therefore, the home conditions could have been a potential bias in our results, because keeping the FIT samples refrigerated would have postponed the Hb degradation process. On the other hand, the organization of this trial mimics the reality and we therefore believe that our results are still applicable for a nationwide FIT-based CRC screening program. Thirdly, the FIT performance characteristics for different sample return times only pertain to screenees who had a positive test result (faecal Hb concentrations ≥ 50 ng/mL) and subsequently underwent a follow-up colonoscopy. These results can therefore not be used to evaluate the FIT sensitivity for advanced neoplasia subdivided per sample return time. Fourthly, only a limited number of FIT samples were used for the laboratory experiment. However, we used the repeated measurements only to create more insight in the Hb degradation process and we did not use these results to compare the mean Hb decrease percentage for different subgroups (ie, CRC, advanced adenomas, and non-advanced adenomas). Fifthly, the Hb stabilizing buffer only consists of 2 mL, which is sufficient for a maximum of ten repeated measurements. Based on the promising laboratory results by Vilkin et al, we wanted to spread all re-tests over a period of at least three weeks and we were therefore not able to perform the re-tests every day.

CONCLUSION

This population-based CRC screening trial demonstrates that, with almost 10,000 FITs analyzed, both the positivity rate and detection rate do not decrease with prolonged sample return times up to ten days. This means that a delay in sending the FITs back to the laboratory, of up to at least one week, does not necessitate repeat testing in case of a negative test result. Our data support the use of FIT-based screening as a reliable tool for nationwide CRC screening programs.

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Chapter 5

Diagnostic yield improves with collection of 2 samples in faecal immunochemical test screening without affecting attendance

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ABSTRACT

Background & Aims: The faecal immunochemical test (FIT) is superior to the guaiac-based faecal occult blood test in detecting neoplasia. There is not much data on the optimal number of FITs to collect. We conducted a population-based trial to determine attendance and diagnostic yield of 1- and 2-sample FIT screening.

Methods: The study included two randomly selected groups of subjects aged 50–74 (1-sample FIT n=5,007; 2-sample FIT n=3,197). The 2-sample group was instructed to collect faecal samples on two consecutive days. Subjects were referred for colonoscopy when at least one sample was positive (≥ 50 ng Hb/mL).

Results: Attendance was 61.5% in the 1-sample group (2,979/4,845; 95% confidence interval (CI): 60.1-62.9%) and 61.3% in the 2-sample group (1,875/3,061; CI: 59.6–63.0%; p-value = 0.84). In the 1-sample group 8.1% had a positive test, and in the 2-sample group 12.8% had at least one positive test and 5.0% had two positive tests (p-value < 0.05). When the mean from both test results in the 2-sample group was used, 10.1% had a positive test (p-value < 0.05). The detection rate for advanced neoplasia was 3.1% in the 1-sample group, and 4.1% in the 2-sample group with at least one positive test, 2.5% when both test results were positive, and 3.7% when concentrating on subjects with the mean from both test results being positive (p-value = n.s.).

Conclusions: There is no difference in attendance for subjects offered 1- or 2-sample FIT screening. The results allow developing efficient FIT screening strategies adapted to local colonoscopy capacity beyond the range of varying the cut-off value in a 1-sample strategy.

INTRODUCTION

Colorectal cancer (CRC) is a public health issue of high importance in Western countries, due to its high incidence and mortality rates.[1] Screening of average-risk individuals can result in an early detection of CRC and will therefore improve prognosis considerably.[2] Furthermore, most CRCs develop from benign adenomatous polyps and slowly progress over many years, providing a window of opportunity for detecting and removing precancerous polyps and early-stage cancers. Endoscopic removal of adenomas will result in a lower than expected incidence of CRC, compared to reference populations.[3] Therefore, based on the characteristics of CRC, screening is of considerable value.

Colonoscopy is the most accurate test for detecting neoplasia and for the removal of adenomas. However, colonoscopy is associated with discomfort both related to the bowel preparation and the examination itself, and the procedure carries a small but distinct complication risk. Other limitations are the availability of qualified endoscopists and costs. For these reasons, other strategies have been proposed for nationwide CRC screening. There is considerable evidence that screening of asymptomatic average-risk individuals using guaiac-based faecal occult blood tests (gFOBT) can detect cancers at an early and curable stage, resulting in a reduction of CRC-related death of 15-33%.[4] Recently more evidence has become available that the faecal immunochemical test (FIT) is superior to gFOBT screening, both with respect to attendance and detection of advanced neoplasia.[5-10] Unfortunately, even bleeding advanced neoplasia may be missed with single stool sampling because they bleed intermittently. Repeated testing probably increases test sensitivity, but it is unknown which effect this will have on attendance, colonoscopy demand and diagnostic yield.

Therefore, the aim of our study was to compare the attendance and diagnostic yield of 1- vs. 2-sample FIT screening in a range of different cut-off values.

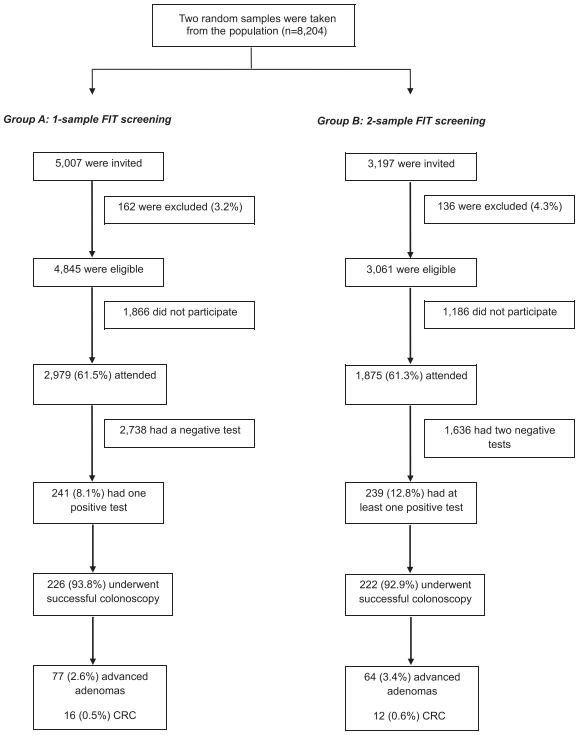
METHODS

Study population

Demographic data of all individuals between the ages of 50–74 years in the southwest of the Netherlands were obtained from municipal population registers. Two random samples were taken from the target population by a computer-generated algorithm (Tenalea, Amsterdam, the Netherlands). Selection occurred before invitation. Both groups were stratified for socioeconomic status (SES) into group A (1-sample FIT screening, n=5,007) or group B (2-sample FIT screening, n=3,197) (**Figure 1**). Since there is no nationwide CRC screening program in the Netherlands, the population used for this trial was screening-naïve. The SES was based on the data of Statistics Netherlands (www.cbs.nl), providing average SES per postal code area, each representing small neighborhoods. Exclusion criteria were asked for on the informed

consent form which had to be filled in by the screenee itself. Exclusion criteria were a history of CRC; inflammatory bowel disease; a life expectancy of less than 5 years; a colonoscopy, sigmoidoscopy or double-contrast barium enema within the previous 3 years; and inability to give informed consent. Recruitment took place between November 2006 and December 2007 for the 1-sample FIT group, and between October 2008 and June 2009 for the 2-sample FIT group.

Figure 1 Trial profile



Group A: 1-sample FIT screening

One FIT (OC-Sensor Micro, Eiken Chemical Co., Tokyo, Japan) was sent by mail to collect a single sample of one bowel movement. The test was considered positive when the haemoglobin (Hb) concentration in the FIT sample was ≥ 50 ng/mL (1-sample FIT50). Details about the study design are extensively described elsewhere.[6]

Group B: 2-sample FIT screening

All subjects who were randomly selected for this group were sent two FITs. Explicit instructions were given to take one sample per FIT of two bowel movements on consecutive days, and to write down the sampling date on both test tubes. When both tests were performed on

the same day, one additional FIT was sent to the screenee to make sure that of each individual two different stool samples were available. The test result was considered positive when the haemoglobin concentration in at least one FIT sample was ≥ 50 ng/mL (2-sample FIT50).

Test result

In case of a positive test result, a colonoscopy was scheduled within four weeks. All colonoscopies were performed by experienced endoscopists. The maximum reach of the endoscope, adequacy of bowel preparation, characteristics and location of all polyps were recorded. In accordance with the international classification, all removed polyps were evaluated by experienced gastrointestinal pathologists.[11]

Ethical approval

The study was approved by the Dutch Ministry of Health (PG/ZP 2.727.071 and PG/ZP 2.823.158). The study letters and information brochures were approved by the Institutional Review Board at Erasmus University Medical Centre (MEC-2005-264 and MEC-2008-029).

Power calculation

Assuming an attendance rate of 60% based on a previous CRC screening trial with FITs (1-sample) in the same region,[6] 3,200 invited individuals were needed to provide an 80% power for demonstrating a 1% difference in diagnostic yield, with a standard error for the difference of 0.5%.

Statistical analysis

Differences in proportions between screening strategies were calculated using a χ^2 test. Differences in mean between screening strategies were calculated using a Student's t-test. All p-values were two-sided and considered significant if < 0.05. The attendance rate was calculated by dividing the number of participants by all eligible subjects (defined as all invitees minus the excluded subjects). The positivity rate (PR) was defined as the proportion of participants having a positive test result. The detection rate (DR) was defined as the proportion of participants having advanced neoplasia. This was calculated as the number of screenees with an advanced neoplasia divided by all screenees with an analyzable screening test. Advanced neoplasia included CRC and advanced adenomas. An advanced adenoma was defined as an adenoma ≥ 10 mm, or an adenoma ≥ 25% villous component and/or high-grade dysplasia. When more than one lesion was present, the screenee was classified according to the most advanced lesion. Attendance, PR, positive predictive value (PPV), and DR were calculated and described as proportions with 95% confidence intervals (95% CI).

All test characteristics were separately calculated for both 1- and 2-sample FIT screening for cut-off levels varying from 50-200 ng Hb/mL in steps of 25. For the 2-sample FIT group,

separate analyses were done for at least one test being positive, both tests being positive, and the mean from both test results being positive.

For all different screening strategies, a graph was made in which the PR at the different cut-off values were plotted against the DR of advanced neoplasia per 100 screenees. The line that connects the most efficient screening strategies is called the efficient frontier.

RESULTS

Attendance rate

Of the 5,007 subjects invited for 1-sample FIT screening, 162 individuals (3.2%) were excluded from analyses (142 subjects met one of the exclusion criteria, thirteen had moved away, and seven had died). In total, 61.5% (2,979/4,845; 95% CI: 60.1-62.9) attended 1-sample FIT screening. The FIT was analyzable in 2,975 individuals.

The 2-sample FIT group consisted of 3,197 invitees of whom 136 individuals (4.3%) were excluded from analyses (132 subjects met one of the exclusion criteria, one had moved away, and three had died). A total of 1,875 out of 3,061 eligible invitees (61.3%; CI: 59.6-63.0) responded to the 2-sample FIT invitation. The participation rate in both groups did not significantly differ (61.5% vs. 61.3%, p-value = 0.837 (**Figure 1**). In total, 2 FIT samples were analyzable in 1,874 screenees.

Baseline characteristics of all randomly selected invitees did not differ between both screening strategies (**Table 1**).

Table 1 Baseline characteristics of the two screening strategies

	1-sample FIT screening	2-sample FIT screening
Total number of invitees	5,007	3,197
Subjects included (n)	4,845	3,061
Sex (male; n-%)	2,508 (50)	1,593 (50)
Mean age (SD)	61 (7)	62 (7)
Socio-economic status (SES)		
Low (n-%)	2,011 (40)	1,277 (40)
Intermediate (n-%)	975 (20)	638 (20)
High (n-%)	2,021 (40)	1,282 (40)

Proportion of positive tests

At a cut-off value of 50 ng Hb/mL, the positivity rate (PR) of the 1-sample FIT group was 8.1% (95% CI: 7.2-9.1). At the same cut-off level, the PR of the 2-sample FIT group was 12.8% (95% CI: 11.4-14.4) when taking any positive test into account, 10.1% (95% CI: 8.8-11.5) when using the mean from both test results and 5.0% (95% CI: 4.1-6.1) when taking two positive tests into account (**Table 2**). The PR of 1-sample FIT screening was statistically significantly lower than for the 2-sample FIT group with at least one positive test (p-value < 0.001), and with the mean from both test results (p-value = 0.036). In contrast, the PR of 1-sample FIT screening was statistically significantly higher than the 2-sample FIT group when requiring both tests positive (p-value < 0.001). The same comparisons were made for the other cut-off values (see Supplementary material).

Follow-up per screening strategy

In the group of 1-sample FIT screening, 77 advanced adenomas and sixteen CRCs were found (**Figure 1**). Overall, 81% of the detected advanced neoplasia was located in the distal colon (ie, defined as descending colon, sigmoid and rectum). In the 2-sample FIT group, 64 advanced adenomas and twelve CRCs were found. In total, 83% of all detected advanced neoplasia was located in the distal colon which was not significantly different compared to the 1-sample FIT group (p-value = 0.707).

Test characteristics

Between the 1-sample and 2-sample FIT group, no statistically significant differences could be observed with respect to the PPV (**Table 2**; cut-off value 50 ng Hb/mL), although there was a trend for a higher PPV for the 2-sample FIT group with both positive tests compared to 1-sample FIT screening (52% vs. 41%, respectively; p-value = 0.075).

Two sample FIT screening with at least one positive test detected more advanced neoplasia than 1-sample FIT screening (1-sample FIT50: 3.1%; 95% CI 2.5-3.8%; 1-sample FIT200: 2.0%; 95% CI 1.6-2.6%; 2-sample FIT50: 4.1%; 95% CI 3.3-5.1%; 2-sample FIT200: 2.7%; 95% CI 2.1-3.5%). An increased DR for advanced neoplasia was also seen for the mean from both test results at any cut-off range (see Supplementary material). At a cut-off value of 50 ng Hb/mL, none of the observed differences in DR in the 2-sample FIT group compared to 1-sample FIT screening reached the level of statistical significance. However, a statistically significant difference in DR was found between 2-sample FIT screening with at least one positive test compared to the 1-sample FIT group at cut-off levels of 75, 100 and 125 ng Hb/mL (p-value = 0.017, 0.032 and 0.039, respectively).

Table 2 Test characteristics of different FIT screening strategies (cut-off value 50 ng Hb/mL)

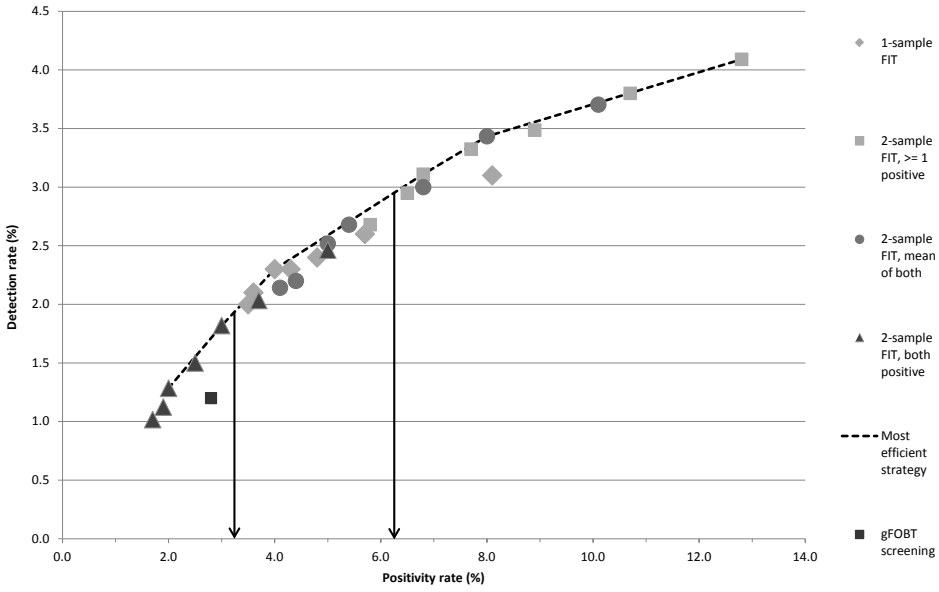
Screening strategy	Positivity rate		Positive predictive value			NNScope		Detection rate				NNScreen	
			Advanced neoplasia	CRC	Advanced adenoma	Advanced neoplasia	CRC	Advanced neoplasia		CRC		Advanced neoplasia	CRC
	n	% (95%CI)	% (95%CI)	% (95%CI)	% (95%CI)	n	n	n	% (95%CI)	n	% (95%CI)	n	n
1-sample FIT screening	241	8.1 (7.2-9.1)	41 (35-48)	7 (4-11)	34 (28-40)	2.4	14.1	93	3.1 (2.5-3.8)	16	0.5 (0.3-0.8)	32	186
2-sample FIT (≥1 positive)	239	12.8 (11.4-14.4) *	34 (28-40)	5 (3-9)	29 (23-35)	2.9	18.5	76	4.1 (3.3-5.1)	12	0.6 (0.3-1.1)	25	156
2-sample FIT (mean of both tests)	190	10.1 (8.8-11.5) *	39 (32-46)	7 (2-7)	32 (26-39)	2.6	14.8	69	3.7 (2.9-4.7)	12	0.6 (0.3-1.1)	27	156
2-sample FIT (both positive)	94	5.0 (4.1-6.1) *	52 (42-62)	10 (5-18)	42 (32-53)	1.9	9.8	46	2.5 (1.9-3.3)	9	0.5 (0.3-1.0)	41	208

FIT = faecal immunochemical test
Hb = haemoglobin
NNScope = number of colonoscopies that needs to be performed to find one screenee with an advanced neoplasia
NNScreen = number of individuals that needs to be screened to find one individual with an advanced neoplasia
Advanced neoplasia = CRC and advanced adenoma
CRC = colorectal cancer
Advanced adenoma = adenoma ≥ 10 mm, or an adenoma ≥ 25% villous component and/or high-grade dysplasia
* *P* value < 0.05 compared with 1-sample FIT screening

Positivity rate versus detection rate for advanced neoplasia

The PR of the different screening strategies was plotted at different cut-off values in the range of 50-200 ng Hb/mL against the DR for advanced neoplasia per 100 screenees (**Figure 2**). In terms of number of colonoscopies per detected advanced neoplasia, the results can be subdivided in three parts along the PR-axis. At the low end, up to a PR of 3.2% the most efficient screening strategy is provided by 2-sample FIT screening with both FITs being positive at a cut-off value ≥ 100 ng Hb/mL. With lower cut-off levels, the PR of 2-sample FIT screening with both positive tests exceeds 3.2%, at which this strategy is outperformed by 1-sample FIT screening (**Figure 2**). Two-sample FIT screening with both positive tests generates a similar PR as gFOBT screening,[7] however with a higher DR for advanced neoplasia (**Figure 2**, lower left part of the graph). At the high end, at a PR equal to or above 6.2% the most efficient screening strategy is 2-sample FIT screening using either the mean from both test results, or at least one positive test (between cut-off values of 50-175 ng Hb/mL). At the cost of high PRs and thus high colonoscopy demands, these strategies provide the highest DRs for advanced neoplasia (**Figure 2**). For the intermediate PR levels between 3.2-6.2%, the different screening strategies lie all very close to the efficient frontier.

Figure 2 Positivity rate versus detection rate for advanced neoplasia (at different cut-off values)



Per screening strategy, the data points represent the results at cut-off values in the range of 50-200 ng Hb/mL, increasing in steps of 25 ng. For each screening strategy, a higher cut-off level is associated with a lower detection rate,ie,the data points at the left end represent the results at a cut-off value of 200 ng Hb/mL, where as the data point at the right end represents the results at a cut-off value of 50 ng Hb/mL. The arrows at positivity rates of 3.2 and 6.2% define zones in which either 1- or 2-sample FIT screening forms the most efficient strategy (see text).

Comparison of individual FITs in 2-sample FIT group

The laboratory test results generated for the 2-sample FIT group can be used to achieve more insight in the bleeding pattern of advanced adenomas (**Table 3**) and CRCs (**Table 4**), as well as to determine the additional value of a second test. At a cut-off value of 50 ng Hb/mL, in 27/64 screenees (42%) with an advanced adenoma, a discrepancy was seen between the first and last performed test. This means that in 42% of advanced adenoma cases, one of both tests was negative and the other one was positive (≥ 50 ng Hb/mL). For CRC, this discrepancy was 25% (3/12).

When we take the average of the first and the second test in the 2-sample FIT group as reference, the PPV of a single test was 37%, with a DR for advanced neoplasia of 3.3%. This means that 31 individuals will need to perform one test (ie, NNScreen), and 3 screenees will need to be referred for colonoscopy to find one advanced neoplasia (ie, NNScope). These results are quite comparable to those of the 1-sample FIT group (see **Table 2**). When the same data of the two tests were used to determine the added value of a second test, on average, fifteen extra advanced neoplasms were found in 1,875 participants. The PPV and DR of an additional second FIT were respectively 21% and 0.8%. In other words, to find one extra advanced neoplasia by means of a second test, 125 additional individuals need to be screened and five additional colonoscopies need to be performed.

Table 3 Comparison of first (vertical axis) vs. last performed test (horizontal axis) in 64 screenees with an advanced adenoma in the 2-sample FIT group

Haemoglobin concentration (ng Hb/mL)

Haemoglobin concentration (ng Hb/mL)	0 - 49	50 - 74	75 - 99	100 -124	125 - 149	150 - 174	175 - 199	200 - 224	225 - 249	> 250	Total
0 - 49		1	4		1		1	1	1	1	10
50 - 74	3			1		1				1	6
75 - 99	1	1			1						3
100 - 124	1										1
125 - 149					1		1			2	4
150 - 174	2						1			1	4
175 - 199	1		1						1		3
200 - 224	2		1	1							4
225 - 249											0
> 250	7	3	1	4		1				13	29
Total	17	5	7	6	3	2	3	1	2	18	64

Table 4 Comparison of first (vertical axis) vs. last performed test (horizontal axis) in 12 screenees with a CRC in the 2-sample FIT group

Haemoglobin concentration (ng Hb/mL)		Haemoglobin concentration (ng Hb/mL)										
		0 - 49	**50 - 74**	**75 - 99**	**100 -124**	**125 - 149**	**150 - 174**	**175 - 199**	**200 - 224**	**225 - 249**	**> 250**	**Total**
	0 - 49				1							1
	50 - 74			1								1
	75 - 99											0
	100 - 124											0
	125 - 149	1										1
	150 - 174											0
	175 - 199										1	1
	200 - 224											0
	225 - 249											0
	> 250	1				1					6	8
	Total	2	0	1	1	1	0	0	0	0	7	12

DISCUSSION

The efficacy of screening for CRC is determined by the attendance and diagnostic yield of a certain screening strategy. Several studies have shown that FIT screening outperforms guaiac-based faecal occult blood testing on both parameters.[5-10] However, the optimal number of FITs to be used per screening round has not been elucidated. This trial demonstrates no differences in attendance between 1-sample and 2-sample FIT screening. This observation is in accordance with an Italian study, which also showed no difference in participation between 1-sample and 2-sample FIT screening (mean attendance rate 56%).[12] Therefore, the decision on the optimal number of FITs to be used for a nationwide screening program can be based on differences in test characteristics. Our results provide important new insights in strategies tailored to local situations, in particular colonoscopy capacity. In areas with limited access to colonoscopy the best way to get to a low PR is to use 2-sample FIT screening with referral for colonoscopy only when both tests are positive. This strategy yields more advanced neoplasia at the same or even lower colonoscopy demand compared to gFOBT screening, which guarantees optimal use of limited colonoscopy resources. The other extreme portrays a nationwide screening program in which colonoscopy capacity is not a limiting factor. In that setting, the strategy of 2-sample FIT screening with referral for colonoscopy in case of at least one positive test is associated with a significantly higher detection rate of advanced neoplasia than 1-sample FIT screening. For that reason, the optimal FIT screening strategy in regions with wider colonoscopy capacity is 2-sample FIT screening, whereby the positivity and detection rate can be tailored to meet colonoscopy availability and budgets by choice of the cut-off value (**Figure 2**). This starts using 2-sample FIT screening with relatively high cut-off levels (100-200 ng Hb/mL). In case of even higher colonoscopy capacities, the most attractive option is to decrease the cut-off value of 2-sample FIT screening below 100 ng Hb/mL. In this range, the extra diagnostic yield per additional colonoscopy only slightly levels off

(**Figure 2**). A full cost-effectiveness analysis should determine whether 2-sample FIT screening with such high PRs is still cost-effective. In between these two extremes, in the PR range of 3.2-6.2%, all screening strategies tested are very close to the efficient frontier (**Figure 2**). However, given the same attendance, lower burden to the screenees and lower costs for one test, 1-sample FIT screening should be advised in those situations.

Until now, limited data were available regarding the most optimal number of FITs to be used. Most data published used the highest haemoglobin concentration of multiple samples (ie, at least one test positive) and therefore valuable analyses about both positive tests or the mean of both FITs were missing.[13-14] Literature also lacks comparative trials of 1-sample vs. 2-sample FIT screening with regard to attendance and diagnostic yield. Available studies compared the results of 2- or 3-sample FIT screening with either a gFOBT or "an internal control group".[9, 14-16] In comparison with two Italian studies evaluating the number of FITs, we observed higher PR, PPV and DR for advanced neoplasia (cut-off value 100 ng Hb/mL).[12, 17] Potential explanations for these differences included the younger Italian population (aged 50-69 years vs. 50-74 years), and the higher proportion of female screenees (53.8% vs. 49.9%).

With respect to sensitivity, it is worth noting that different screening strategies vary more in their impact on DR of advanced adenomas than of cancer.[10] It is thought that CRCs have a more permanent bleeding pattern than advanced adenomas, which are believed to bleed more intermittently. Therefore it could be hypothesized that with one additional faecal sample (ie, 2-sample FIT screening), especially more advanced adenomas will be detected. Based on our findings, it can be concluded that 25% of all detected patients with CRC in the 2-sample FIT group had only one positive test. In other words, about 12.5% of CRC cases would have been missed by using 1-sample FIT screening because of intermittent bleeding. When the same calculations are made for the advanced adenomas, 42% of them had just one positive test result. This suggests that 2-sample FIT screening has a larger impact on the detection of extra advanced adenomas than on detecting more CRCs. On the other hand, the extra CRCs could be more important because of the greater urgency to detect them. Furthermore, we demonstrated that five screenees would need to be referred for colonoscopy to find one extra advanced neoplasia by means of a second test. Whether this is an acceptable number needed to scope, depends on local situations with respect to colonoscopy capacity and on further cost-effectiveness analyses.

This study had some limitations. First, the population under investigation was not invited at the same time. It could be hypothesized that a discrepancy in attendance rate between the different screening strategies could not be observed due to a balance between on the one hand a difference in intervention (either 1- or 2-sample FIT screening) and on the other hand a difference in time period and thus maybe more awareness about CRC and CRC screening in general. However, two random samples were taken from exactly the same target population in the southwest of the Netherlands. Since 2006, we have been approaching newly invited individuals for their first CRC screening round and differences in attendance rate were rather

small. Therefore, we believe that the main conclusions drawn from this trial are still applicable. Second, this trial only describes results of the first CRC screening rounds with either 1 or 2 FIT samples in a screening-naïve population. Data on attendance and diagnostic yield of successive CRC screening rounds are needed to provide more insight in the long-term (cost-) effectiveness of a population-based screening programme and the most optimal FIT screening strategy to be used. It could be hypothesized that 2-sample FIT screening may require less screening rounds to be as effective as more frequently 1-sample FIT screening when the cumulative sensitivity of several screening rounds, as well as the number of interval cancers found, are compared with each other. In collaboration with the Dutch Comprehensive Cancer Centre, we have started to collect information about interval cancers in screenees testing negative by FIT. When these data are completely available, it remains to be shown to what extent the higher diagnostic yield of 2-sample FIT screening reduces the incidence of interval CRCs and therefore might allow longer screening intervals. Third, we only made a comparison between 1-sample and 2-sample FIT screening. We thus do not have any information about the effect of 3-sample FIT screening on attendance and diagnostic yield. A Japanese study reported no additional value of a third sample compared to 2-sample FIT screening.[18] The same conclusion was drawn from a study conducted in Israel.[13] However, the Israeli trial only included patients who were referred for colonoscopy (ie, both asymptomatic but at increased risk for colorectal neoplasia and symptomatic). Therefore, these data can not be generalized to an asymptomatic average-risk population.

CONCLUSION

This comparative population-based CRC-screening trial demonstrates a similar attendance of 1- and 2-sample FIT screening. Two sample FIT screening using at least one positive test as cut-off, provides a higher detection rate for advanced neoplasia than 1-sample FIT screening. However, this is at the expense of higher positivity rates and thus the need for more colonoscopies. In case of limited colonoscopy capacity, 2-sample FIT screening with the demand for two positive tests has the highest diagnostic yield. In between these two extremes, 1-sample FIT screening is equally effective as 2-sample FIT screening. These results can be used for optimal screening strategy planning, tailored to a range of local needs and colonoscopy capacities that is even wider when also considering 2-sample FIT strategies.

Supplementary material

To access the supplementary material accompanying this article, visit the online version of Clinical Gastroenterology and Hepatology at www.cghjournal.org.

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Chapter 6

Cost-effectiveness of one versus two sample faecal immunochemical testing for colorectal cancer screening

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ABSTRACT

Objective: The sensitivity and specificity of a single faecal immunochemical test (FIT) are limited. The performance of FIT screening can be improved by increasing the screening frequency or by providing more than one sample in each screening round. We aimed to evaluate if 2-sample FIT screening is cost-effective compared to 1-sample FIT.

Method: The MISCAN-colon micro-simulation model was used to estimate costs and benefits of strategies with either 1- or 2-sample FIT screening. The FIT cut-off level varied between 50-200 ng haemoglobin per mL, and the screening schedule was varied with respect to age range and interval. In addition, different definitions for positivity of the 2-sample FIT were considered: a) at least one positive sample, b) two positive samples, or c) the mean of both samples being positive.

Results: Within an exemplary screening strategy, biennial FIT from age 55-75 years, 1-sample FIT provided 76.0-97.0 life years gained (LYG) per 1,000 individuals, at a cost of €259,000-€264,000 (range reflects different FIT cut-off levels). Two sample FIT screening with at least one sample being positive provided 7.3-12.4 additional LYG compared to 1-sample FIT at an extra cost of €50,000-€59,000. However, when all screening intervals and age ranges were considered, intensifying screening with 1-sample FIT provided equal or more LYG at lower costs compared to 2-sample FIT.

Conclusion: If attendance to screening does not differ between strategies it is recommended to increase the number of screening rounds with 1-sample FIT screening, before considering to increase the number of FIT samples provided per screening round.

INTRODUCTION

In industrialized countries colorectal cancer (CRC) is the third most commonly diagnosed malignancy in men and ranks second in women.[1] The majority of CRC cases are diagnosed later in life. Because life expectancy increases in many countries and the costs of CRC treatment rapidly rise, it is expected that CRC will place an increasing burden on national healthcare systems.

Screening for CRC and its premalignant lesions (ie, adenomatous polyps) can detect the disease at an earlier and more curable stage. Faecal occult blood tests (FOBTs) have been developed to detect microscopic bleeding from colorectal neoplasms before there are any clinical signs or symptoms. At least three randomized controlled trials proved the effectiveness FOBT screening, demonstrating a mortality reduction of 15-33%.[2-4] Subsequently, several screening trials have confirmed the superiority of faecal immunochemical test (FIT) screening over the more traditionally used guaiac-based FOBTs (ie, non-rehydrated Hemoccult-II test) both with respect to attendance as well as detection rate of advanced neoplasia.[5-11] Most of these trials used screening strategies with a single FIT sample.

Since not all advanced neoplasia will be detected by means of 1-sample FIT screening, providing two FIT samples collected on consecutive days could increase the effectiveness of a screening program. On the one hand, referring a screenee for a diagnostic colonoscopy when at least one sample is positive, increases sensitivity since some colorectal neoplasms bleed intermittently and can therefore be missed with 1-sample FIT screening.[12] On the other hand, referring a screened individual when both samples are positive can increase specificity since only colonic lesions with a more consistent bleeding pattern will be detected which will lead to less false positive test results. However, in either way, providing two FIT samples within one screening round will also increase screening costs because twice the number of samples needs to be analyzed.

The aim of this study was to evaluate the cost-effectiveness of 1-sample and 2-sample FIT screening strategies with variable intervals, age ranges and cut-off levels in order to assess if the increased performance of a second FIT sample outweighs the increased costs compared to 1-sample FIT screening.

METHODS

We used the MISCAN-Colon micro-simulation model to estimate the additional life-years gained and costs of 2-sample FIT screening over 1-sample FIT for the screening strategy of biennial FIT from age 55-75. This screening strategy has intermediate screening intensity and was previously found to be cost-effective.[13] Additional life-years gained can also be achieved by increasing the intensity of 1-sample FIT screening instead of adding a second sample. We

therefore also compared the costs and life-years gained of 1-sample FIT screening with that of 2-sample FIT for a range of screening strategies.

MISCAN-colon micro-simulation model

The MISCAN-colon model and the data sources that inform the quantifications of the model are described in detail in previous publications,[14-18] and in a standardised model profile available online.[19] In brief, the MISCAN-colon model simulates the relevant life histories of a large population of individuals from birth to death. CRC arises in this population according to the adenoma-carcinoma sequence.[20-21] More than one adenoma can occur in an individual and each adenoma can independently develop into a CRC. Adenomas progress in size from small (≤ 5 mm) to medium (6–9 mm) to large (≥ 10 mm). Although most adenomas will never turn into cancer, some will eventually become malignant, transforming to stage I CRC and some may even progress into stage IV. In every stage, there is a probability of the CRC being diagnosed due to the development of symptoms versus symptomless progressing into the next stage. If CRC has developed, the survival rate after clinical diagnosis depends on the stage in which the cancer was detected. The 5-year survival rate is on average 90% if the disease is diagnosed while still localized, 68% for regional disease, and less than 10% for disseminated disease. At any time during the development of the disease, the process may be interrupted because a person dies of other causes.

With FIT screening lesions can be detected before clinical diagnosis; a screened individual with a positive test result will be referred for a colonoscopy for detection and removal of adenomas and early-stage cancers. In this way, CRC incidence and/or CRC-related mortality can be reduced. The life years gained by screening are calculated as the difference in model-predicted life years lived in the population with and without CRC screening.

Study population

In this study we modelled the age distribution of the Dutch population in 2005 (Statistics Netherlands, www.cbs.nl) and all individuals were followed until death. The CRC incidence rate was based on the observed incidence rate in the Netherlands in 1999-2003, which was before the onset of opportunistic screening (Comprehensive Cancer Centre (CCC), www.ikcnet.nl). Survival rates after clinical diagnosis of CRC was based on relative survival data from 1985-2004 from the South of the Netherlands,[22] since nationwide data were not available. The survival for individuals aged 75 years and older was adjusted to fit the observed age-increasing mortality/incidence ratio (CCC).

Screening strategies

CRC screening was simulated in the population starting in 2010. Individuals were offered FIT screening according to different screening schedules varying by:

- Age to start screening at respectively 45, 50, 55, and 60 years
- Age to stop screening at respectively 70, 75, and 80 years
- Screening interval with respectively 1, 1.5, 2, and 3 years

Separate simulations were performed in which individuals were invited for a) 1-sample FIT screening; b) 2-sample FIT screening with referral if at least one sample tested positive; c) 2-sample FIT screening with referral only if both samples tested positive; or d) 2-sample FIT screening with referral if the mean of both samples was positive. The cut-off level for a positive test result varied between 50, 75, 100, 150, and 200 ng Hb/ml. These different screening schedules with varying start and stop ages, intervals, cut-off levels and samples resulted in a total of 960 different screening strategies.

After a positive test result, individuals were referred for colonoscopy. If no adenomas were found during the procedure, the individual was assumed to be at low-risk for CRC and did not return to the screening program until after ten years. If one or more adenomas were found, they were removed and the individual entered a surveillance program according to the Dutch guidelines for follow-up after polypectomy;[23] a colonoscopy after six years in case of one or two adenomas and after three years in case of three or more adenomas. We assumed that surveillance colonoscopies would be performed until the stop age for screening.

Attendance rates

We modelled attendance rates in the first screening round as observed in two Dutch population-based CRC screening trials;[9, 11-12] 60% for both 1- and 2-sample FIT screening, and we assumed these rates to remain stable over time. For subsequent screening rounds, we assumed that 80% of the individuals that attended the previous screening round would attend again.[24-25] Furthermore, we assumed that 10% of the individuals never attended FIT screening[26] and that these never-attendees had a higher risk of CRC than the general population (RR=1.15).[2] Attendance to diagnostic colonoscopies following a positive FIT and subsequent surveillance colonoscopies was assumed to be 85% and 80%, respectively.[27]

Test characteristics

Test characteristics of the 1-sample and 2-sample FIT tests were fitted to the positivity rates (PR) and detection rates (DR) of advanced neoplasia observed in the first screening round of two Dutch randomised trials (**Table 1**).[9-12, 28] Advanced neoplasia included CRC and advanced adenomas, of which the latter was defined as adenomas ≥ 10 mm in size, with ≥ 25% villous component, and/or high-grade dysplasia.

Table 1 Test characteristics of 1-sample and 2-sample FIT used in the model

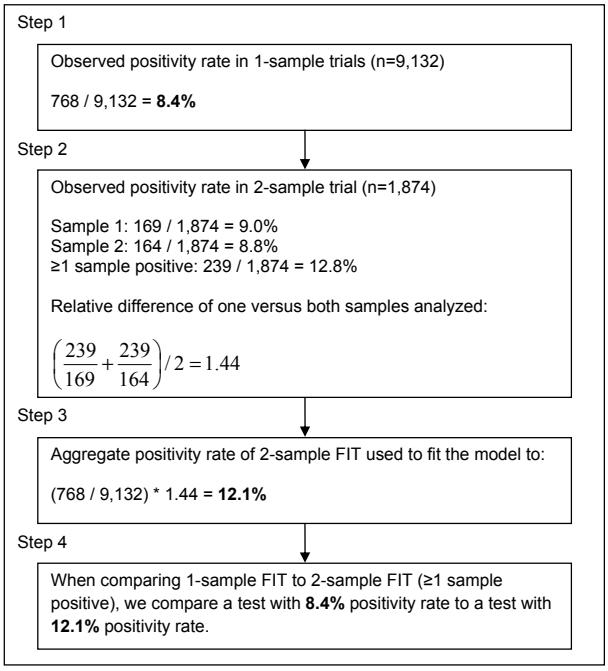
Cut-off level	Specificity	Sensitivity (per lesion, %)[1]				
(ng Hb/mL)	(per person, %)	≤ 5 mm	6-9 mm	≥ 10 mm	CRC early preclinical[2]	CRC late preclinical[2]
1-sample FIT						
50	95.79	0.0	9.6	16.1	65.0	90.0
75	97.05	0.0	5.7	14.4	58.5	87.0
100	97.76	0.0	4.4	13.1	52.0	83.5
150	98.34	0.0	2.9	12.3	50.5	83.0
200	98.70	0.0	2.5	10.3	50.0	82.5
2-sample FIT, at least one sample positive						
50	93.01	0.0	14.2	16.7	75.0	93.5
75	94.90	0.0	8.4	15.5	71.0	92.0
100	96.03	0.0	6.9	14.4	66.0	90.0
150	97.03	0.0	5.2	14.3	66.0	90.0
200	97.65	0.0	4.9	12.5	66.0	90.0
2-sample FIT, mean of both samples positive						
50	95.51	0.0	12.6	17.0	67.0	90.0
75	96.90	0.0	7.5	15.1	61.0	87.5
100	97.66	0.0	5.4	13.8	54.0	84.0
150	98.31	0.0	3.3	12.8	51.0	83.0
200	98.63	0.0	2.1	10.7	49.0	81.5
2-sample FIT, both samples positive						
50	98.40	0.0	3.8	12.0	34.0	70.0
75	98.94	0.0	1.8	10.0	29.0	65.0
100	99.21	0.0	0.9	8.8	24.0	59.0
150	99.43	0.0	0.1	7.1	20.0	53.0
200	99.49	0.0	0.0	5.2	16.0	47.5

[1] Excluding the probability that an adenoma or cancer is found due to a lack of specificity.

[2] It was assumed that the probability a CRC bleeds and thus the sensitivity of FIT for CRC depends on the time until clinical diagnosis, in concordance with findings for gFOBT, which were based on a prior calibration of the MISCAN-Colon model to three FOBT trials.[16] This result is to be expected when cancers that bleed do so increasingly over time, starting "occultly" and ending as clinically visible. This interpretation also holds for FIT. The test characteristics used in the model were fitted to the PR and DR of advanced neoplasia and CRC from two Dutch randomised controlled trials.[9-12,28] Sensitivity for adenomas smaller than 5 mm was assumed to be 0% for all tests, at any cut-off level.

To estimate the 2-sample FIT test characteristics the following approach was applied; we used the average PR and DR of the first and second performed test from the 2-sample FIT group as reference and calculated the relative difference in performance when both samples were evaluated. Subsequently, we added this relative difference to the PR and DR derived from the original 1-sample FIT trials. An example of this method of calculation is presented in **Figure 1**. The main reasons for this approach were: 1) the larger sample size of the 1-sample FIT group provides more statistical power for the estimates of test sensitivity and specificity; 2) to avoid possible bias caused by the fact that the PR and DR of the 1-sample and 2-sample FIT groups were calculated from different cohorts that were not 1:1 randomised before invita-

Figure 1 Example of calculation of the added performance of 2-sample FIT compared with 1-sample FIT screening.



This example provides the calculation of the positivity rate of 2-sample FIT with at least one sample positive at a cut-off level of 50 ng Hb/ml. The method of calculation is similar for both positivity rate and detection rate, as well as for the different 2-sample FIT positivity criteria (ie, at least one sample positive, both samples positive and the mean of both samples positive).

tion;[10, 12] and 3) in this way we used paired observations, which gives a better estimate of the additional performance of a second FIT sample.

The sensitivity of diagnostic colonoscopies was assumed to be 75% for adenomas 1-5 mm, 85% for adenomas 6-9 mm and 95% for adenomas ≥ 10 mm and CRC.[29]

Costs

In the base case analyses, we included screening and treatment costs as presented in **Table 2**. Base case organisational costs for 1-sample FIT screening were based on the Dutch cervical cancer screening program, adjusted for differences with FIT screening. Costs for the test kits were based on prices from the manufacturer. Costs for analysis of the tests included material and personnel needed during the process of registration, analysis and authorization of returned tests.[30] The additional costs associated with 2-sample FIT screening included double costs for FIT test kits and packaging material, and double costs for materials needed during the analysis of returned samples. Although double the number of FIT samples would need to be analysed, the costs of personnel needed for the analysis only increased by a factor

of 1.5 since some tasks (e.g. patient registration) do not require double the amount of work compared to analyzing samples with 1-sample FIT screening. Colonoscopy costs were based on an internal six months study at the Erasmus MC (data not shown). Costs for complications after colonoscopy were based on DBC-rates (Diagnosis Treatment Combination), derived from the Dutch Health Care Authority (http://ctg.bit-ic.nl/Nzatarieven/top.do).

Table 2 Summary of model assumptions of the base case and sensitivity analyses

Variable	Base case analysis	Sensitivity analyses	
Quality of life loss			
Colonoscopy	-	1 day lost per colonoscopy	
CRC from diagnosis onwards[2] *(1-utility)*	-	Initial treatment:[34] - Stage I: 0.26 during first year - Stage II: 0.3 during first year - Stage III: 0.4 during first year - Stage IV: 0.75 during first year Continuous care: 0.15 in years between initial and terminal phase[35] Terminal care death by CRC: 0.75 in last year before dying of CRC Terminal care death by other cause: 0.35 in last year before dying of other causes	
Adherence to:			
- Screening tests	60%	100% adherence to all tests	
- Diagnostic tests	85%		
- Surveillance tests	80%		
Correlation of FOBT results	-	74% of the large adenomas (≥10 mm) that are not detected, will not be detected in the next screening round36	
Colonoscopy capacity	Not limited	Limited to either 40, 20, 10 and 5 colonoscopies per 1,000 individuals per year	
		Low value	**High value**
Fatal complications after colonoscopy	1 per 10,000 colonoscopies	No fatal complications	- 1 per 1,000 colonoscopies with polypectomy - 1 per 10,000 colonoscopies without polypectomy
Relative increase in test performance between 1-sample and 2-sample FIT	Average of the first and second sample used as comparator	Relative increase in test performance 50% smaller	Relative increase in test performance 50% greater

Table 2 (continued)

Variable	Base case analysis				Sensitivity analyses	
FIT costs	*1-sample FIT*		*2-sample FIT*			
Costs per invitation (organization and test kit)	€15.51		€17.76		Difference between 1- and 2-sample FIT 50% smaller	Difference between 1- and 2-sample FIT 200% greater
Costs per attendee (personnel and materials for analysis)	€4.37		€8.19			
Colonoscopy costs						
Without polypectomy	€303				50%	200%
With polypectomy	€393					
Costs complications after colonoscopy[1]	€1,250				50%	200%
Treatment costs [2]	*Initial treatment*	*Continuous care*	*Terminal care death CRC*	*Terminal care death other causes*		
Stage I	€12,100	€340	€17,500	€4,400	50%	200%
Stage II	€16,600	€340	€17,500	€4,000		
Stage III	€20,600	€340	€18,500	€5,200		
Stage IV	€24,600	€340	€25,000	€14,000		

[1] The assumed complication rate is 2.4 per 1,000 colonoscopies.

[2] CRC treatments were divided into three clinically relevant phases - initial, continuous and terminal care. The initial phase was defined as the first 12 months following diagnosis, the terminal phase was defined as the final 12 months of life, and the continuous phase was defined as all months between the initial and terminal phase. For patients surviving less than 24 months, the final 12 months were allocated to the terminal phase. The remaining months of observation were allocated to the initial phase.

Costs for treatment of CRC were divided into three clinically relevant phases of care: initial treatment, continuous care and terminal care. Initial treatment costs were based on DBC-rates, except for oxaliplatin. The costs for oxaliplatin were derived from the Dutch Health Care Insurance Board (www.medicijnkosten.nl). We assumed that during the continuous care phase, individuals would follow the Dutch CRC treatment guidelines (www.oncoline.nl) and costs for periodic control were based on DBC-rates. Terminal care costs were based on a Dutch last year of life cost analysis. These were estimated at €19,700 for patients that ultimately died from CRC.[31] We assumed that these costs increased with stage at diagnosis, at a rate observed for US patients.[32-33] Dutch terminal care costs for individuals that died from CRC were approximately 40% of the US costs. We assumed that terminal care costs of CRC patients that die from other causes were also 40% of the US costs.

Cost-effectiveness analyses

For all screening strategies we used the MISCAN-colon model to estimate costs and number of life years gained due to screening to the situation without screening. Costs and life years gained were discounted by 3% per year.[37] Strategies that were more costly and less effective than other strategies were ruled out by simple dominance. Strategies that were more costly and less effective than a mix of other strategies were ruled out by extended dominance. The remaining strategies are not dominated and are known as "efficient". On a plot of life years gained versus costs, the line that connects the efficient strategies is called the efficient frontier, which implies that all dominated strategies lie below this line. The incremental cost-effectiveness ratio (ICER) of an efficient strategy was determined by comparing its additional costs and effects to those of the next less costly and less effective efficient strategy.

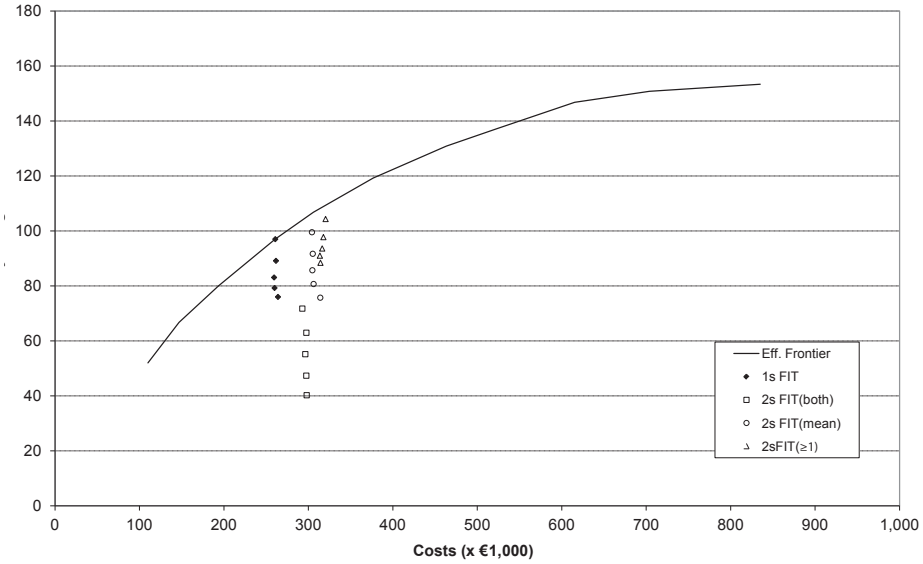
Sensitivity analyses

We performed several one way sensitivity analyses on different parameters, which are summarized in **Table 2**. We started with sensitivity analyses with respect to the additional performance and costs of 2-sample FIT over 1-sample FIT. Furthermore, we adjusted for reduced quality of life due to screening as well as CRC treatment. Correlated FIT test results were assumed because individuals with a false negative test result are likely to have a higher than average probability to have another false negative test result at a successive screening round. We used the results of a population-based CRC screening program in Italy to estimate the correlation between false negative FIT results for cancers and advanced adenomas in subsequent screening rounds.[36] Effects of limited colonoscopy capacity were evaluated by only considering strategies in which colonoscopy demand did not exceed 40, 20, 10, or 5 colonoscopies per 1,000 individuals per year. In order to assess the cost-effectiveness of the different strategies for individuals who adhere to the CRC screening guidelines, we simulated all screening strategies with 100% attendance to screening, diagnostic and surveillance colonoscopies. In addition, we performed sensitivity analyses on lower and higher values than the base case analysis for fatal complication rates with colonoscopy and for unit costs of FIT, colonoscopy, complications and treatment.

RESULTS

The strategy of biennial 1-sample FIT screening from age 55-75 years yielded 76.0-97.0 life years gained (LYG) per 1,000 individuals aged 45 years and older, compared to no screening (the range in life years gained reflects different FIT cut-off levels). The associated costs ranged from €259,000-€264,000 per 1,000 individuals, corresponding with €2,690-€3,473 per LYG compared to no screening (**Figure 2**). The 2-sample FIT screening strategies with the mean of both test results being positive and at least one test result being positive provided

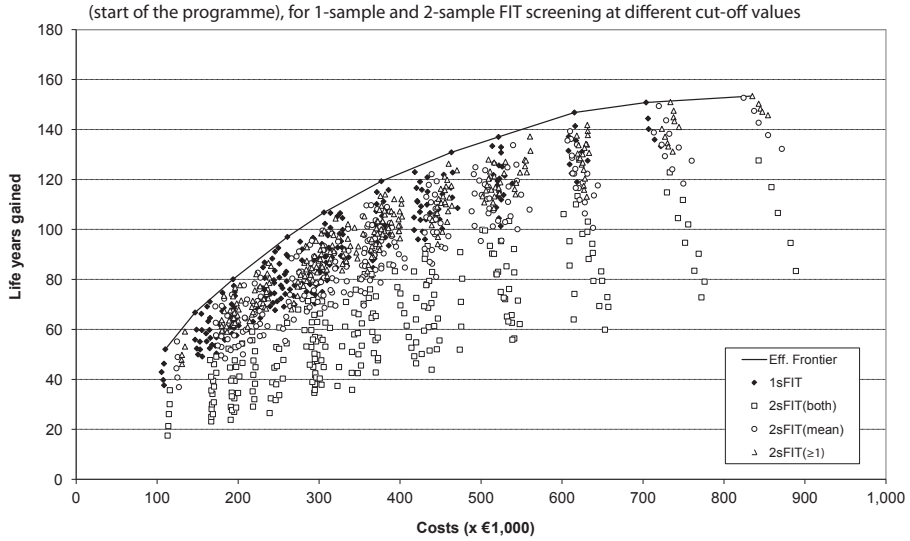
Figure 2 Costs and life years gained compared to no screening per 1,000 individuals aged 45 years and older in 2005 (start of the programme), for 1-sample and 2-sample FIT screening at different cut-off values



The data points represent biennial FIT screening from age 55 to 75.
1s FIT = 1-sample FIT; 2s FIT(both) = 2-sample FIT, both samples positive; 2s FIT(mean) = 2-sample FIT, mean of both samples positive; 2sFIT(≥1) = 2-sample FIT, at least one sample positive; The efficient strategies are connected by the efficient frontier (Eff. frontier).
Per screening test, the data points represent the results at cut-off values of 50, 75, 100, 150 and 200 ng Hb/ml. For each test, a higher cut-off level is associated with fewer life years gained,ie,the data point at the bottom represents the result at a cut-off value of 200 ng Hb/ml, whereas the data point at the top represents the result at a cut-off value of 50 ng Hb/ml.
The screening interventions were modelled from the year 2005, all individuals were invited for screening until they reached the end age for screening, and health care costs for each individual were calculated until death. Costs and life years gained were discounted at an annual rate of 3%.

respectively between -0.3-2.6 and 7.3-12.4 more LYG than 1-sample FIT screening at additional costs of respectively €43,000-€50,000 and €50,000-€59,000 per 1,000 individuals. The corresponding incremental cost-effectiveness ratios (ICERs) ranged from €16,818-€31,930 and €4,024-€8,041 per additional LYG. The 2-sample FIT screening strategies with two positive outcomes were less effective (ie, less LYG per 1,000 individuals) and more costly than 1-sample FIT screening and were therefore dominated from a cost-effectiveness standpoint .

Figure 3 Costs and life years gained compared to no screening per 1,000 individuals aged 45 years and older in 2005 (start of the programme), for 1-sample and 2-sample FIT screening at different cut-off values



The data represents all simulated screening strategies, which include various sampling strategies, cut-off levels, screening age ranges, and intervals.

1sFIT = 1-sample FIT; 2sFIT(both) = 2-sample FIT, both samples positive; 2sFIT(mean) = 2-sample FIT, mean of both samples positive; 2sFIT(≥1) = 2-sample FIT, at least one sample positive; The efficient strategies are connected by the efficient frontier (Eff. frontier).

Strategies with the least intensive screening schedule (ie, small age range, and long screening interval) are located at the bottom left of the graph, whereas strategies with the most intensive screening schedule (ie, large age range and short screening interval) are located at the top right of the graph. The screening interventions were modelled from the year 2005, all individuals were invited for screening until they reached the end age for that particular screening strategy, and health care costs for each individual were calculated until death. Costs and life years gained were discounted at an annual rate of 3%.

When all simulated screening strategies were considered (ie, by varying not only the cut-off level, but also the screening age range and interval), the number of LYG compared to no screening ranged between 17.5-153.4 per 1,000 individuals, and costs ranged between €105,000-€889,000 per 1,000 individuals (**Figure 3**). The LYG and costs of the strategies on the efficient frontier are presented in **Table 3**. Although the ICER of 2-sample FIT screening (mean of both samples being positive, or at least one sample being positive) compared to 1-sample FIT seemed reasonable, **Figure 3** shows that 2-sample FIT strategies are not cost-effective. The reason for this is illustrated in **Figure 2**. When comparing the additional effect of providing two samples per screening round to the effect of providing 1-sample FIT more frequently (ie, with a larger age range and/or shorter interval), the latter provided more LYG at equal or less costs than any of the 2-sample FIT strategies. The 2-sample FIT screening strategies with the mean from both test results being positive or at least one positive test outcome were therefore ruled out by extended dominance and were considered not cost-effective compared to 1-sample FIT screening. Although **Figure 2** demonstrates this effect for biennial screening, the principle applies to all screening intervals, including annual screening.

Table 3 Costs per life-years gained compared with no screening and incremental cost-effectiveness ratio of the cost-effective screening strategies, in a population with realistic attendance[1] to the screening program

Test (cut-off)	Start age (yrs)	Stop age (yrs)	Interval (yrs)	LYG (yrs)	Costs (€)	Costs / LYG (€)	ICER[2] (€)
1s FIT (50)	60	69	3	52	110,000	2,115	2,115
1s FIT (50)	60	70	2	67	147,000	2,200	2,500
1s FIT (50)	60	74	2	80	194,000	2,420	3,524
1s FIT (50)	55	75	2	97	261,000	2,688	3,956
1s FIT (50)	55	74.5	1.5	107	306,000	2,865	4,613
1s FIT (50)	55	79	1.5	119	377,000	3,159	5,678
1s FIT (50)	50	80	1.5	131	463,000	3,541	7,480
1s FIT (50)	55	80	1	137	522,000	3,806	9,427
1s FIT (50)	50	80	1	147	615,000	4,191	9,590
1s FIT (50)	45	80	1	151	704,000	4,667	22,099
2s FIT ≥1s pos. (50)	45	80	1	153	835,000	5,444	51,336

[1] Attendance rate was 60% for screening, 85% for diagnostic colonoscopies, and 80% for surveillance colonoscopies.
[2] The ICER of an efficient strategy is determined by comparing its additional costs and effects to those of the next less costly and less effective efficient strategy.
Costs and life-years gained are expressed per 1,000 individuals aged 45 years and older in 2005. The strategies are in ascending order from least to most costly. LYG = Life-years gained; ICER = Incremental cost-effectiveness ratio. The screening interventions were modelled from the year 2005, all individuals were invited for screening until they reached the end age for that particular screening strategy, and health care costs for each individual were calculated until death. Costs and life years gained were discounted at an annual rate of 3%.

Sensitivity analyses

The higher cost-effectiveness of more frequent 1-sample FIT screening compared to 2-sample FIT strategies was robust to alterations in our model assumptions. However, decreasing the cost difference between 1-sample and 2-sample FIT by 50% resulted in multiple 2-sample FIT strategies to become efficient next to 1-sample FIT. In addition, limited colonoscopy capacity did not affect the preference of 1-sample FIT over 2-sample FIT strategies, with the exception of the most stringent scenario. In case the colonoscopy demand was not allowed to exceed five colonoscopies per 1,000 individuals per year, 2-sample FIT strategies with both samples being positive were preferred over 1-sample FIT.

DISCUSSION

Our analysis demonstrates that given a screening schedule (ie, age range and screening interval), 2-sample FIT strategies with the mean from both test results being positive or at least one positive test outcome provide more LYG at acceptable costs than 1-sample FIT screening. However, when all simulated screening strategies are considered (ie, including varying age ranges and screening intervals), increasing the screening intensity of 1-sample FIT testing (ie, greater age range and/or shorter screening interval) is more cost-effective than providing two FITs within one screening round.

This study was based on data from a randomized trial in which the attendance and diagnostic yield of 1- and 2-sample FIT were compared.[12] Considering only the relation between positivity rate and detection rate of advanced adenomas it seems to be recommendable to choose for FIT screening with either one or two samples based on the available colonoscopy capacity. However, the current analysis demonstrates that including the costs for screening and treatment of CRC over multiple screening rounds, affects the relation between 1- and 2-sample FIT. Although a number of 2-sample FIT screening strategies (e.g. with at least one sample, or the mean of both samples being positive) are close to the cost-efficiency frontier, increasing the number of 1-sample FIT screening rounds was found to be a more cost-effective way of gaining health benefits.

Other cost-effectiveness analyses determining the optimal number of FIT samples are limited. Two Japanese studies compared the costs of FIT screening with either one, two or three FITs, per cancer detected in a single screening round.[38-39] In all three sampling strategies individuals were referred for diagnostic colonoscopy if at least one sample was positive. In both studies it was concluded that 2-sample FIT screening with at least one test being positive would be the most desirable strategy from a diagnostic accuracy and cost-effectiveness stand-point. A more recent French study did include multiple screening rounds in their cost-effectiveness model and also evaluated the effect of different cut-off levels.[40] The authors concluded that 3-sample FIT screening with a cut-off level of 50 ng Hb/ml was the most cost-effective strategy to be preferred. The results of our current analysis do agree with these studies about the added value of multiple FIT sampling within a given screening schedule. More than one FIT sample can provide additional health benefits at acceptable costs. Unfortunately, these studies do not provide information comparing the added effect of multiple FIT samples per screening round to the effect of increasing screening intensity with 1-sample FIT.

Several limitations need to be acknowledged. Firstly, we based our analysis on data from one screening round. Therefore we could not estimate the correlation of test outcomes between successive screening rounds. Individuals with a false negative test result in one screening round may have a higher than average probability to have another false negative test result at a successive screening round. Therefore, we performed a sensitivity analysis based on Italian results in which correlation of systematic false negative test outcomes was assumed for advanced adenomas and CRCs.[36] The analysis showed that the cost-effectiveness of 2-sample FIT decreased less than the cost-effectiveness of 1-sample FIT strategies, but 1-sample FIT screening remained dominant. Nevertheless, we need further data from repeat screening rounds in the Netherlands to get a good estimate of systematic false negative rates in the population we modelled. Secondly, we assumed the screening attendance rate to be independent of screening intensity and number of FIT samples performed. In the first screening round of one of the Dutch trials,[10-12] screening attendance rate was not significantly different between the 2-sample FIT and 1-sample FIT study arm (61.3% vs. 61.5%; p-value = 0.837).

However, it could be hypothesized that, e.g. adherence in case of a more intense screening schedule with 1-sample FIT would decrease compared to less intense screening schedules with 2-sample screening. This would negatively affect the cost-effectiveness of more intensive screening strategies relative to 2-sample testing and might alter our conclusions. Thirdly, we based our analyses on a screening naïve population. Depending on the amount of prior screening, CRC incidence in the population and the resulting cost-effectiveness could be lower. However, this would affect the strategies we compared in a similar way. If any, the effect of prior screening would make 1-sample FIT screening more preferable, since a lower CRC incidence would reduce the added value of a second FIT sample. Finally, we did not perform a probabilistic sensitivity analysis. Given the large number of strategies that has to be evaluated for each draw, such an analysis would require a huge computational effort. We believe that simulating the range of varying strategies is one of the strengths of this analysis, because we were primarily interested in the comparison of different FIT screening strategies with varying numbers of samples provided, FIT cut-off levels, screening intervals and age ranges. Regardless, data on the probability distributions of most of the parameter values are lacking, which makes the interpretation of a probabilistic sensitivity analysis difficult and the outcome of limited added value. One of the most uncertain assumptions of the model is that all CRCs arise from adenoma precursors. For FIT screening, this assumption will have limited impact because FIT has a low sensitivity for adenomas, and the assumption of non-bleeding and therefore for FIT undetectable adenomas was evaluated in the sensitivity analysis by assuming correlation between false-negative results.

CONCLUSION

Our analysis provides new insights for decision makers; in a situation where attendance to screening does not differ between strategies, intensifying screening with 1-sample FIT was found to be more cost-effective than providing two FIT samples within one screening round. It is therefore recommended to increase the number of screening rounds with 1-sample FIT screening, before considering to increase the number of FIT samples provided per screening round.

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Chapter 7

Random comparison of repeated faecal immunochemical testing at different intervals for population-based colorectal cancer screening

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ABSTRACT

Objective: Colorectal cancer screening by means of faecal immunochemical tests (FITs) requires successive screening rounds for an optimal preventive effect. However, data on the influence of screening interval length on participation and diagnostic yield are lacking. We therefore performed repeated FIT screening in a population-based trial comparing various repeated intervals.

Method: A total of 7,501 Dutch individuals aged 50-74 years were randomly selected and invited for two 1-sample FIT screening rounds (haemoglobin (Hb) concentration ≥ 50 ng/mL, corresponding to 10 µg Hb/g faeces) with intervals of one (group I), two (II), or three years (III), respectively.

Results: In group I, participation was 64.7% in the first and 63.2% in the second screening round. The corresponding percentages for groups II and III were 61.0% vs. 62.5%, and 62.0% vs. 64.0%. Triennial screening resulted in a higher participation to the second screening round compared with individuals who were invited every year (p-value = 0.04). The overall positivity rate in the second screening round was significantly lower compared with the first round (6.0% vs. 8.4%, OR 0.69; 95% CI, 0.58-0.82) and did not depend on interval length (p-value = 0.23). Similarly, the overall detection rate of advanced neoplasia was significantly lower in the second round compared with the first screening round (1.9% vs. 3.3%, OR 0.57; 95% CI, 0.43-0.76) and did also not depend on interval length (p-value = 0.62). The positive predictive value of the FIT did not significantly change over time (41% vs. 33%; p-value = 0.07).

Conclusion: The total number of advanced neoplasia found at repeated FIT screening is not influenced by the interval length within a one to three years range. Furthermore, this trial shows a stable and acceptably high participation to the second screening round. This implies that screening intervals can be tailored to local resources.

INTRODUCTION

Colorectal cancer (CRC) is a major health problem in the Western world which fulfils the conditions for population-based screening.[1] There is considerable evidence that annual to biennial screening of asymptomatic average-risk individuals using a guaiac-based faecal occult blood test (gFOBT) can detect cancers at an early, curable stage, which results in a 15-33% reduction of CRC-related deaths.[2-5] Based on these results, repeated FOBT screening has been advocated in international guidelines.[6-8] Recent studies have indicated that faecal immunochemical testing (FIT) is superior to gFOBT screening both with respect to participation and diagnostic yield.[9-11] Introduction of FIT-based screening is therefore widely considered and implemented in the US, Canada, and many countries throughout Europe. Unfortunately, a single FIT test is insufficient for the detection of all advanced neoplasia (ie, all patients with CRC or an advanced adenoma, usually defined as an adenoma of 10 mm or larger, an adenoma with 25% or more villous histology, or with high-grade dysplasia) due to a suboptimal sensitivity for such lesions.[12] This necessitates successive screening rounds, which may result in a similar preventive effect as a screening strategy with an invasive, highly sensitive test such as colonoscopy.[13] However, there are no data on the comparison of different intervals for FIT screening and their impact on participation and detection of advanced neoplasia, two factors which both highly determine the efficacy of a screening programme.

The aim of this study was therefore to compare the participation and diagnostic yield of repeated FIT testing with screening intervals of various lengths ranging from one to three years in a population-based colorectal cancer screening trial.

METHODS

Study population

Details about the design of our ongoing population-based CRC screening programme have been described.[9, 14-15] In short, demographic data of all individuals between 50–74 years living in the southwest of the Netherlands were obtained from municipal population registers. Random samples were taken from the target population by a computer-generated algorithm (Tenalea, Amsterdam, the Netherlands). Selection was performed per household and occurred before invitation. Since there is no CRC screening programme in the Netherlands, the target population invited for this trial was screening-naïve when first approached. Exclusion criteria were asked for on the informed consent form that had to be completed by the screenee. Exclusion criteria were a history of CRC; inflammatory bowel disease; an estimated life expectancy of less than five years; a colonoscopy, sigmoidoscopy or double-contrast barium enema within the previous three years; and inability to give informed consent. Recruitment took place between November 2006 and December 2010.

Interventions

With each screening round, one FIT (OC-Sensor Micro, Eiken Chemical Co., Tokyo, Japan) was sent by mail to collect a single sample of one bowel movement. The test was considered positive when the haemoglobin (Hb) concentration in the FIT sample was ≥ 50 ng/mL, which corresponds to 10 µg Hb/g faeces. Details about the study design have been described elsewhere. [9, 14-16] All study subjects were divided over three groups to undergo repeated FIT testing at various screening intervals. The groups were designated in relation to the interval length, expressed in years, between the consecutive FITs.

Study groups

Groups I-III: Repeated 1-sample FIT screening

Subjects assigned to groups I-III were offered repeated 1-sample FIT screening at intervals of respectively one, two, or three years (**Figure 1**). In order to complete the repeated FIT screening trial, we started with recruitment of subjects who were scheduled for a longer interval. Recruitment for groups II and III took place between November 2006 and December 2007. Individuals selected for group I received their first invitation between May and November 2008. In each group, invitees who fulfilled the exclusion criteria after the first invitation, those who tested positive during the first screening round, individuals who had become 75 years of age or older, and those who had moved out of the region or had died were not approached for the second screening round.

Reference group 0: Once only 2-sample FIT screening

Subjects assigned to Reference group 0 were offered once only 2-sample FIT screening (**Figure 1**). All subjects who were randomly selected for this group simultaneously received two FIT kits. Explicit instructions were given to obtain a single stool sample per FIT and use both FITs on two consecutive days while noting the sampling date on both test tubes. Recruitment took place between October 2008 and June 2009. Results concerning this once only 2-sample FIT group have been published before.[15] Only those data relevant for the current comparison with repeated FIT testing with longer screening intervals are presented in this paper.

Follow-up evaluation

Subjects with a positive FIT were scheduled for colonoscopy within four weeks. All colonoscopies were performed by experienced endoscopists. The maximum reach of the endoscope, adequacy of bowel preparation, as well as characteristics and location of any polyps were recorded. All removed polyps were evaluated by experienced gastrointestinal pathologists.[17-18] Patients with a positive colonoscopy entered a surveillance programme, whereas

patients with a negative colonoscopy were referred back to the screening programme but were considered not to require FIT screening for ten years.[6, 19]

Screen-detected and interval carcinomas

Except for individuals who moved out of the Netherlands, all recruited participants were followed for the development of CRC. Screen-detected cancers were defined as cancers identified at colonoscopy performed after a positive test result. Interval cancers were defined as colorectal cancers diagnosed within the time period between two consecutive screening rounds. Interval cancers were identified through record linkage with the Dutch Comprehensive Cancer Centre (www.iknl.nl).

Power calculation

The primary outcome measurement was the participation rate for each screening strategy. The sample size was chosen based on a presumed 50-60% participation rate to yield an 80% power to determine second round participation rates for each group with a confidence interval of ± 2.5%.

Statistical analysis

Differences in proportions between the screening interval groups were tested using the χ^2 test. Differences in means between the various groups were tested using the Student t-test. The participation rate was calculated by dividing the number of participants by all eligible subjects (defined as all invitees minus the individuals who fulfilled the exclusion criteria). The positivity rate (PR) was defined as the proportion of participants having a positive test result, the positive predictive value (PPV) as the proportion of participants with a positive test result having advanced neoplasia, and the detection rate (DR) as the proportion of participants having an advanced neoplasia. Participants with more than one lesion were classified according to the most advanced lesion found.

A logistic regression model was fitted to the data to determine differences in second round participation between the three interval groups (ie, groups I-III). In a subgroup analysis, we extended this model by adding (non-)participation in the first screening round as a separate parameter. In a subsequent multivariate logistic regression model, the variables age, sex, and socio-economic status (SES) were added. A second logistic regression model was fitted to the data to determine differences in PR, PPV and DR between groups I-III. Because participants with a positive screening test followed by colonoscopy during the first round were not invited for the second screening round, participants could only have one positive FIT result. This allowed us to combine the test outcomes from both rounds in a simple logistic regression analysis without using multi-level techniques. A third logistic regression model was used to determine the differences in second round PR and DR (subdivided into (non)-participant of the first screening round) between the three interval groups. All p-values were

two-sided and considered significant if < 0.05. Statistical analysis was performed with SPSS 15.0 for Windows. Finally, we performed an analysis in which the once only 2-sample FIT group was considered to be a 1-sample group which was re-invited for a second screening round after an interval of zero years (ie, reference group 0). The 2-sample FIT data presented under the subheading 'First sample / Screening round I' were obtained when the average of the PR and DR of the first and second performed test was taken as reference. The data presented as 'Second sample / Screening round II' were acquired when the same data of both performed tests were used to determine the added value of a second test. Additionally, for these analyses only individuals who participated twice were considered appropriate. This comparison is presented in **Table 3**.

RESULTS

Participation rate

During the first screening round of groups I-III, a total of 7,501 asymptomatic average-risk subjects were invited (**Table 1**) of which 272 (3.6%) were excluded from analyses after the invitation had been sent (223 individuals met one of the exclusion criteria, 41 had moved away, and eight had died) (**Figure 1**). From the remaining, a total of 4,523 subjects responded to the first round invitation: the participation rate in group I was 64.7% (95% CI, 62.8-66.6), in group II 61.0% (95% CI, 59.0-62.9), and in group III 62.0% (95% CI, 60.1-64.0). A total of 1,021 (13.6%) individuals were not re-invited for the second screening round (380 subjects had tested positive during the first screening, 342 individuals had become 75 years of age or older, 88 individuals had died, and the remaining 211 subjects had moved out of the region). Therefore, 6,208 individuals were approached for the second screening round of which 97 (1.6%) invitees fulfilled the exclusion criteria (**Figure 1**). In group I, the participation rate in the second round slightly decreased to 63.2% (95% CI, 61.1-65.3). For the biennial and triennial screening groups, participation increased towards 62.5% (95% CI, 60.4-64.6) and 64.0% (95% CI, 61.9-66.0), respectively. In a multivariate analysis, in which we corrected for participation in the first screening round, the interval length was associated with second round participation (p-value = 0.04). Higher second round participation was achieved with biennial screening (odds ratio (OR) 1.18; 95% CI, 0.98-1.43) and triennial screening (OR 1.26; 95% CI, 1.04-1.52) compared with annual screening.

Of first round participants, 89.8% (1,166/1,299; 95% CI, 88.0-91.3) also attended the second screening round after an interval of one year, 90.9% (1,123/1,235; 95% CI, 89.2-92.4) after an interval of two years, and 91.3% (1,138/1,247; 95% CI, 89.6-92.7) participated again after a triennial screening interval (**Table 2**). The same calculations were made for the non-participants of the first screening round: the proportion of eligible previous non-participants attending the second screening round was respectively 16.3% (120/735; 95% CI, 13.8-19.2),

19.3% (157/813; 95% CI, 16.7-22.2), and 20.5% (160/782; 95% CI, 17.8-23.4), for groups I, II and III. No interaction was found between the parameters 'first round participation' and 'interval length' (p-value = 0.86), indicating that the differences in second round participation for participants and non-participants in the first screening round (expressed in ORs) were the same in the three interval groups.

Finally, a separate analysis was made for the cumulative participation rate after two 1-sample FIT screening rounds. In the group with an interval of one year, 69.7% (1,663/2,385; 95% CI, 67.9-71.5) of all eligible subjects participated at least once. This was 67.5% (1,638/2,428; 95% CI, 65.6-69.3) in the biennial screening group and 68.7% (1,659/2,416; 95% CI, 66.8-70.5) in the triennial screening group. The interval length was not associated with the cumulative participation rate after two successive screening rounds (p-value = 0.24).

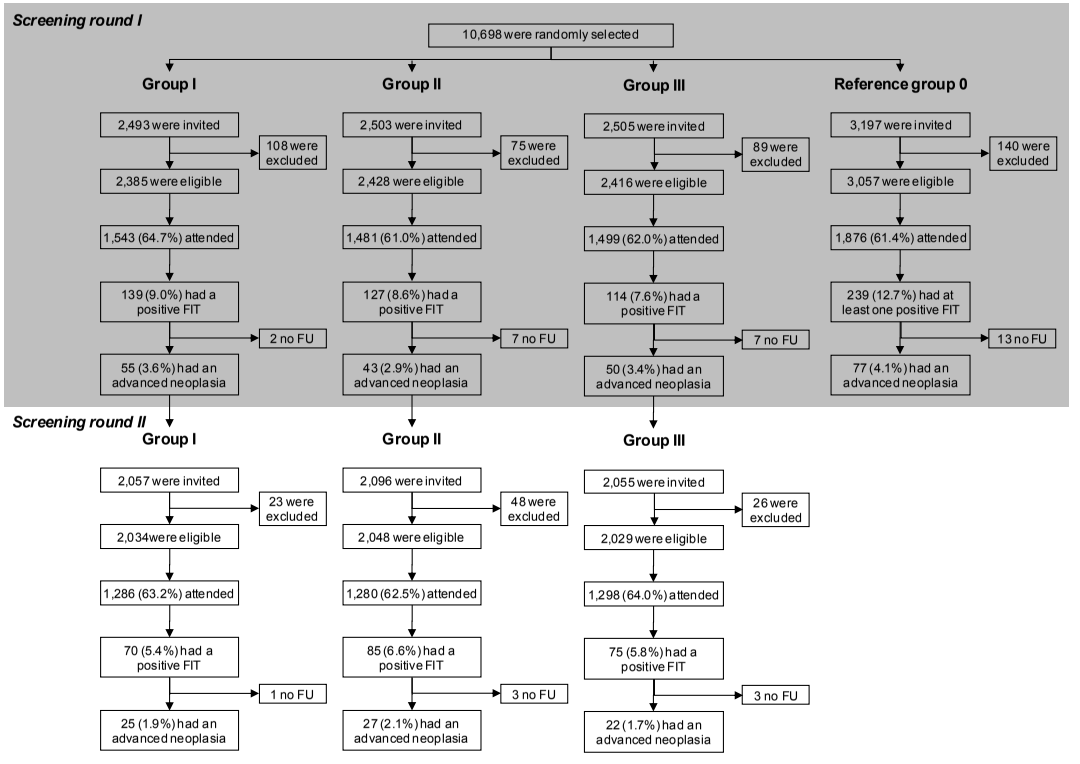
Table 1 Baseline characteristics (first screening round)

	Repeated 1-sample FIT screening			Once only 2-sample FIT screening	*P* value
	Group I	Group II	Group III	Reference group 0	
Invited subjects (n)	2,493	2,503	2,505	3,197	
Median age (yrs-IQR)	60.0 (55.0-66.0)	60.0 (55.0-66.0)	60.0 (55.0-65.5)	62.0 (56.0-68.0)	0.001
Sex (male; n-%)	1,223 (49.1)	1,254 (50.1)	1,254 (50.1)	1,593 (49.8)	0.87
SES (n-%)					0.99
High	993 (39.8)	1,019 (40.7)	1,019 (40.7)	1,280 (40.0)	
Intermediate	509 (20.4)	503 (20.1)	503 (20.1)	640 (20.0)	
Low	991 (39.8)	981 (39.2)	983 (39.2)	1,277 (39.9)	

Group I: Individuals were invited for two 1-sample FIT screening rounds after an interval of 1 year; **Group II**: Individuals were invited for two 1-sample FIT screening rounds after an interval of 2 years; **Group III**: Individuals were invited for two 1-sample FIT screening rounds after an interval of 3 years; **Reference group 0**: Individuals were invited for one 2-sample FIT screening round.

IQR = interquartile range; SES = socio-economic status, which was based on the data of Statistics Netherlands (www.cbs.nl), providing average SES per postal code area, each representing small neighborhoods.

Figure 1 Trial profile



Group I: Invitees were invited for a second 1-sample FIT screening round after 1 year; **Group II:** Invitees were invited for a second 1-sample FIT screening round after 2 years; **Group III:** Invitees were invited for a second 1-sample FIT screening round after 3 years; **Reference group 0:** Invitees were invited for their first 2-sample FIT screening round. Screenees with a positive test result in the first screening round, subjects who fulfilled the exclusion criteria of the first round, individuals who had moved out of the region, had died, or turned over 75 years were not invited for a second FIT-based screening round. FIT = faecal immunochemical test (OC-Sensor Micro), cut-off value 50 ng Hb/mL; FU = follow-up after a positive test result (ie, colonoscopy); Advanced neoplasia was defined as a colorectal cancer and an adenoma 10 mm or larger, or an adenoma with 25% or more villous component, and/or high-grade dysplasia.

Proportion of positive tests

At a Hb concentration ≥ 50 ng/mL, a total of 380/4,523 (8.4%, 95% CI, 7.6-9.2) first round participants tested positive.

In the second screening round, a total of 230/3,864 (6.0%, 95% CI, 5.2-6.7) screened individuals tested positive. In a multivariate model, the overall PR was significantly lower in the second round compared with the first screening round (OR 0.69; 95% CI, 0.58-0.82). Among subjects who had tested negative during the first screening, the PRs in the second screening round were not significantly different between the three interval groups, being 5.1% (95% CI, 4.0-6.6) for group I, 6.8% (95% CI, 5.4-8.4) for group II and 5.6% (95% CI, 4.4-7.1) for group III (p-value = 0.23; **Table 2**).

Table 2 Overview of participation and FIT performance characteristics per screening round

	Group I	Group II	Group III	*P* value
Screening round I				
Eligible invitees (n)	2,385	2,428	2,416	
Participation rate (n-%)	1,543 (64.7)	1,481 (61.0)	1,499 (62.0)	
Positivity rate (n-%)	139 (9.0)	127 (8.6)	114 (7.6)	
Detection rate of				
Advanced neoplasia (n-%)	55 (3.6)	43 (2.9)	50 (3.4)	
Advanced adenoma (n-%)	51 (3.3)	33 (2.2)	42 (2.8)	
Colorectal cancer (n-%)	4 (0.3)	10 (0.7)	8 (0.5)	
Screening round II				
Eligible invitees (n)	2,034	2,048	2,029	
Participation rate (n-%)	1,286 (63.2)	1,280 (62.5)	1,298 (64.0)	0.04
Participant round I (n-%)	*1,166 (89.8)*	*1,123 (90.9)*	*1,138 (91.3)*	
Non-participant round I (n-%)	*120 (16.3)*	*157 (19.3)*	*160 (20.5)*	
Positivity rate (n-%)	70 (5.4)	85 (6.6)	75 (5.8)	0.40
Participant round I (n-%)	*60 (5.1)*	*76 (6.8)*	*64 (5.6)*	
Non-participant round I (n-%)	*10 (8.3)*	*9 (5.7)*	*11 (6.9)*	
Detection rate of				
Advanced neoplasia (n-%)	25 (1.9)	27 (2.1)	22 (1.7)	0.77
Advanced adenoma (n-%)	24 (1.9)	23 (1.8)	20 (1.5)	
Colorectal cancer (n-%)	1 (0.1)	4 (0.3)	2 (0.2)	
Detection rate of				
Advanced neoplasia (n-%)	25 (1.9)	27 (2.1)	22 (1.7)	0.77
Participant round I (n-%)	*19 (1.6)*	*23 (2.1)*	*18 (1.6)*	
Non-participant round I (n-%)	*6 (5.0)*	*4 (2.5)*	*4 (2.5)*	

Group I: Individuals were invited for two 1-sample FIT screening rounds after an interval of 1 year; **Group II**: Individuals were invited for two 1-sample FIT screening rounds after an interval of 2 years; **Group III**: Individuals were invited for two 1-sample FIT screening rounds after an interval of 3 years.
Screenees with a positive test result in the first screening round, subjects who fulfilled the exclusion criteria of the first round, individuals who had moved out of the region, had died, or turned over 75 years were *not invited* for a second FIT-based screening round. FIT = faecal immunochemical test (OC-Sensor Micro), haemoglobin concentration ≥ 50 ng/mL; Advanced neoplasia was defined as a colorectal cancer and an adenoma 10 mm or larger, or an adenoma with 25% or more villous component, and/or high-grade dysplasia.

Follow-up and test performance characteristics

Of the 380 screenees in groups I-III who tested positive during the first screening round (**Table 2**), 364 (96%) underwent a successful colonoscopy. The remaining sixteen subjects either refused a colonoscopy or turned out to have too severe co-morbidity to benefit from an invasive endoscopic procedure. Colonoscopy resulted in the detection of advanced lesions in 148 (PPV 41%; 95% CI, 35.7-45.8) patients, consisting of 126 advanced adenomas and 22 CRCs of which seventeen (77%) were classified as early stage (Stage I: 14; Stage II: 3) and five (23%) as advanced (Stage III: 5). In the second screening round, 223 (97%) of the 230 positive screenees underwent colonoscopy, revealing advanced lesions in 74 (PPV 33%; 95% CI, 27.3-39.6) patients, consisting of 67 advanced adenomas and seven CRCs of which six were early stage (Stage I: 5; Stage II: 1) and one was Stage III. The difference in PPV between the first and second round of FIT screening was not statistically significant (p-value = 0.07).

Overall, 148 of 4,523 participants in the first screening round were diagnosed with an advanced neoplasia, corresponding with a DR of 3.3% (95% CI, 2.8-3.8), without significant differences between the three groups (p-value = 0.60; **Table 2**). In the second screening round, the overall DR of advanced colonic lesions dropped to 1.9% (95% CI, 1.5-2.4), significantly lower than in the first round (OR 0.57; 95% CI, 0.43-0.76). In addition, significantly fewer CRCs were found during the second screening (0.18%; OR 0.37; 95% CI, 0.16-0.86) compared with the first screening round (0.49%). Among first round participants, the overall DR with a second FIT was 1.8% (95% CI, 1.4-2.3; **Table 3**, Second sample / Screening round II), without significant differences between the three groups, being 1.6% (95% CI, 1.0-2.5) in group I, 2.1% (95% CI, 1.4-3.1) in group II, and 1.6% (95% CI, 1.0-2.5) in group III (p-value = 0.62; **Table 2**). In contrast, among non-participants in the first screening round, the second round DR was 3.2% (95% CI,1.9-5.3) which is as expected similar to the 3.3% among the participants in the first screening round, and significantly higher than the second round DR among those who had participated in the first screening round (p-value = 0.02).

Looking at the once only 2-sample FIT group, the DR of advanced neoplasia of a single test was 3.3% (95% CI, 2.6-4.2) (**Table 3**, First sample / Screening round I). The additional second FIT sample enabled detection of 16 additional advanced neoplasia in 1,876 participants, corresponding with an additional DR of 0.9% (95% CI, 0.5-1.4) (**Table 3**, Second sample / Screening round II) and thus an overall DR of 4.1% (95% CI, 3.3-5.1).

Table 3 Overview of positivity rate and detection rate per screening round for either 1-sample FIT screening (ie, Groups I-III) or 2-sample FIT screening (ie, Reference group 0)

	Groups I-III	Reference group 0
First sample / Screening round I		
Screened individuals (n)	4,523	1,876
Positivity rate (n-%)	380 (8.4)	167 (8.9)
Detection rate of		
Advanced neoplasia (n-%)	148 (3.3)	62 (3.3)
Advanced adenoma (n-%)	126 (2.8)	51 (2.7)
Colorectal cancer (n-%)	22 (0.5)	11 (0.6)
Second sample / Screening round II		
Screened individuals (n)	3,427	1,876
Positivity rate (n-%)	200 (5.8)	73 (3.9)
Detection rate of		
Advanced neoplasia (n-%)	60 (1.8)	16 (0.9)
Advanced adenoma (n-%)	54 (1.6)	14 (0.8)
Colorectal cancer (n-%)	6 (0.2)	2 (0.1)

Individuals were invited for two 1-sample FIT screening rounds after an interval of one (group I), two (group II), or three years (group III). However, since no statistically significant differences were found between the three groups, corresponding data were pooled (ie, **Groups I-III**). For the 'Second sample / Screening round II' comparison *only individuals who participated twice* were included. Furthermore, for this comparison the 2-sample FIT group was considered to be a 1-sample FIT group which was re-invited for a second screening after a virtual interval of zero years (ie, **Reference group 0**). The 2-sample FIT data presented under the subheading 'First sample / Screening round I' were obtained when the average of the first and second performed test was taken as reference. The data presented as 'Second sample / Screening round II' were acquired when the same data of both performed tests were used to determine the added value of one extra test.

Interval carcinomas

After record linkage with the Dutch Comprehensive Cancer Centre, 32 CRCs were found in the total study population. Twenty-nine CRCs (90.6%) were screen-detected tumours (**Table 2**), of which 22 (76%) were detected during first and seven (24%) during second round screening. The other three (9.4%) were interval cancers. Two of those were detected in the 4,143 first round participants with a negative test: one Stage III tumour (FIT result at baseline, 24 ng Hb/mL) was detected nine months after baseline screening, and one Stage II cancer (7 ng Hb/mL) was discovered two years and five months after stool sampling. The third and last CRC was diagnosed at Stage I in one of 117 subjects with a positive first round test (960 ng Hb/mL) but negative follow-up colonoscopy. The tumour was located at 50 cm of the anal verge. Reassessment of the original colonoscopy report and pictures revealed no explanation for missing this lesion.

These results imply that in the first screening round 0% (0/4) of all CRCs diagnosed in group I were interval cancers. The corresponding percentages for interval cancers were 9.1% (1/11) for the biennial screening and 20.0% (2/10) for the triennial screening group, respectively.

DISCUSSION

The effectiveness of FIT-based screening in decreasing colorectal cancer-related mortality has not been studied in large long-term prospective randomized controlled trials. Although such trials would be highly valuable, they may never be conducted. CRC screening programmes using FITs are therefore based on evidence from prospective randomized controlled trials showing that annual or biennial gFOBT screening led to a 15-33% reduction in CRC mortality,[2-5] combined with observations from other randomized trials that FIT screening compared with gFOBT is associated with higher participation and diagnostic yield.[9, 11] This forms the basis for the assumption that repeated FIT screening will eventually have a larger impact on CRC-related mortality than gFOBT screening. This is further supported by modelling results.[13, 20] The effectiveness of a FIT-based screening programme is however highly dependent on adherence to repeat testing. This trial demonstrates that participation slightly increases with second round screening when performed with biennial or triennial intervals. This increased participation was seen both among first round participants as well as first round non-participants, in particular in the triennial screening group. This underlines the importance of re-inviting previous non-participants to increase the effectiveness of screening. Unfortunately, this is not routinely applied in CRC screening programmes.[21] Optimising participation rates must be a priority in any screening programme and requires scrutiny of health promotion campaigns, invitation techniques, the test kit, and involvement of general practitioners.[14, 22-24]

Besides pursuing high participation to repeated screening, the detection rate of advanced neoplasia is of similar importance for the effectiveness of screening. Repeated screening rounds enable to cover a larger proportion of the population and help to detect more subjects with advanced lesions, both because of the gradual progression and the intermittent bleeding pattern of advanced neoplasia.[15] As a consequence, CRC screening requires successive screening rounds for an optimal preventive effect. This trial first demonstrates that repeated FIT screening enables a higher population coverage and a higher detection rate of advanced neoplasia, even when compared with single round 2-sample FIT screening.[15] The cumulative coverage of the target population was 67.5-69.7% in the repeated 1-sample FIT screening groups compared with 61.4% in the once only 2-sample FIT group, and the cumulative DR of advanced neoplasia ranged from 5.3-5.7% in the repeated 1-sample FIT screening groups compared with 4.1% in the once only 2-sample FIT group. Second, our study demonstrates that second round FIT screening yields fewer advanced neoplasia compared with baseline screening. This finding confirms that FIT screening has a considerable yield of advanced neoplasia already with single round screening.[10, 25] Third, our study shows that there is no association between the interval length within a one to three years range and the DR of advanced neoplasia at the second screening round. This finding was, to some extent, against our assumption that a longer screening interval would result in more newly bleeding

advanced neoplasia at the second screening round. Our current findings support the concept of slow progression of sporadic colorectal neoplasia. Finally, these findings could also be an expression that non-bleeding advanced neoplasia persist in not bleeding for a long time. This issue needs further We performed additional analyses for the positivity rate and detection rate, including only participants who attended both screening rounds (**Table 3**). Since the DRs in the three interval groups did not differ, corresponding data were pooled (ie, Groups I-III) and compared with 2-sample FIT screening where the second test was performed after a virtual interval of zero years. The pooled data showed that 1.8 advanced neoplasia per 100 participants were detected during the second screening of the 1-3 yearly screening interval groups, versus 0.9 after an interval of zero years (ie, the second test of the once only 2-sample FIT screening on two consecutive days). These figures imply that 50% of detected advanced neoplasia with second round screening could have been detected at baseline, but were -at that time- not bleeding (consistently) enough to be detected by one FIT. Moreover, the fact that the second round DRs did not differ between groups I-III suggests that even a triennial screening interval might be too short to detect genuine newly developed or at least newly bleeding advanced neoplasia. This is consistent with the long so-called polyp dwell time; the average time for transformation from a small adenoma to an invasive CRC which is estimated to be on average at least ten years.[1] In this respect, it is important to note that the sensitivity of FIT for the detection of low concentrations of blood in stool samples, in particular at a low cut-off value which was used in this trial, leads to considerably higher detection of advanced neoplasia than screening with gFOBT. For instance, in our previous randomized comparative trial, gFOBT and FIT screening led to the detection of respectively six vs. twenty subjects with an advanced neoplasia per 1,000 screenees invited.[10] The majority of these subjects had advanced adenomas, not cancer. This learns that adenomas can bleed prior to becoming an invasive cancer, and single FIT sampling at a low cut-off detects part of these lesions. Therefore, while current international CRC screening guidelines recommend that FOBT screening should apply fixed one year intervals with a single test,[6-8] our data suggest that FIT screening may progress to faecal sampling with longer intervals. This strategy may be further improved by using two FIT samples in every screening round, with optimization of the number of days or bowel movements between FIT sampling.[15] If this is true, such a multiple sample strategy with longer screening intervals could become more advantageous than a one sample FIT strategy with a shorter interval.

To our knowledge, this is the first study to evaluate the second round participation and diagnostic yield of a FIT-based CRC screening trial comparing different interval lengths between successive screening rounds. Moreover, in screening for CRC comparatively little is known about the outcome measures of the first vs. subsequent screening rounds. Most available studies were conducted with the gFOBT, which has been used for more than forty years.[26-30] Additionally, the majority of FIT-related data that have been published so far have not been tabulated by screening round and therefore do not allow analysis of participation

and diagnostic yield per screening round.[31-35] One exception is an Italian study in which all individuals were invited for biennial 1-sample FIT screening.[36] Our main results concerning second round participation and diagnostic yield are in line with these Italian results. However, when the same Hb concentration threshold was used (ie, 100 ng/mL), we observed a lower first round PR and a higher DR of colorectal cancer. Potential explanations for the lower number of detected cancers in the Italian study included the younger population (aged 50–69 vs. 50–74) and the lower proportion of positive screenees undergoing follow-up colonoscopy (86% vs. 96% respectively). It is difficult to explain differences in PR since the brand name of the used FIT kit was not provided, neither were additional baseline characteristics of the target population given.

This study had some limitations. First, the invitations for the first screening round were not sent at the same time. Since the recruitment of all groups took place in the same screening-naïve population, more awareness about CRC and CRC screening could have been obtained over time. This implies that the participation rate of group I at first screening and group III at second screening could have been affected the most by this potential bias as these were invited later in time. This increased awareness about CRC screening would then explain the higher first round participation seen in the annual FIT screening group compared with groups II and III, although this contrasts with the lower second round participation in this same group. Second, this trial was powered on participation and therefore lacks power to detect small differences in second round PRs and DRs between the different interval length groups. Additionally, although no significant differences were found in the total number and stage of advanced neoplasia between the three interval groups, this has to be confirmed with further studies.

CONCLUSION

This comparative population-based CRC screening trial demonstrates that the association, if any, between longer screening intervals and larger numbers of advanced neoplasia detected at repeated FIT screening is limited. Furthermore, this trial shows a stable and acceptably high participation to the second screening round within a one to three years range. This implies that screening intervals can be tailored to local resources.

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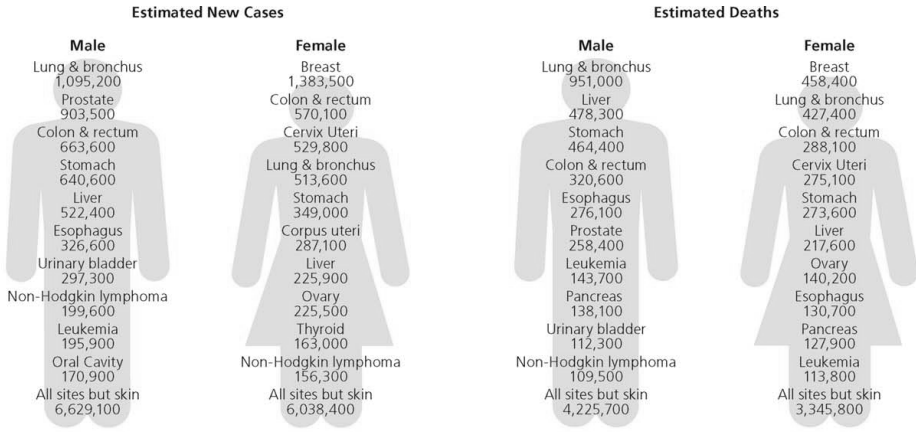
Chapter 8

Summary and general discussion

INTRODUCTION

Cancer is the leading cause of death in economically developed countries and the second leading cause of death in developing countries.[1] Colorectal cancer (CRC) is the third most commonly diagnosed cancer in males and the second in females, with over 1.2 million new cancer cases and 608,700 deaths estimated to have occurred worldwide in 2008 (**Figure 1**). At current rates, a person at the age of 50 has a 5% cumulative lifetime risk of being diagnosed with cancer of the colon or rectum and a 2.5% chance of dying from it.[2-4]

Figure 1 Estimated new cancer cases and deaths worldwide for leading cancer sites in 2008.[5]



CRC is strongly associated with a Western lifestyle. In the past several decades, much has been learned about the dietary, lifestyle, and medication risk factors for this malignancy. Modifiable risk factors for CRC include smoking, physical inactivity, overweight and obesity, red and processed meat consumption, and excessive alcohol consumption.[6-9] Modifications in diet and lifestyle (ie, primary prevention) can substantially reduce the risk of CRC and can complement screening in reducing the incidence of CRC. Screening, on the other hand, is an example of secondary prevention in which members of a defined population, who do not perceive that they are at risk for or are already affected by a disease, are offered a test for early detection of this condition or its precursor lesion. The aim of screening is therefore to bring forward the time of diagnosis before the stage at which the first signs and symptoms of the disease come to light, thereby improving the prognosis considerably. There are several methods that can be used for CRC screening purposes, one of which is the faecal immunochemical test (FIT).

FAECAL IMMUNOCHEMICAL TESTING

The concept of applying an immunochemical method to examine stool for occult blood was first proposed in the 1970s.[10] Commercialization of the technology began in the 1980s. The FIT measures the presence of globin chains of haemoglobin molecules in stool samples by means of human specific anti-globin antibodies. It is a non-invasive test that is collected in a patient's home, without a need for dietary or medication restrictions. Individuals are instructed to put a small stool sample onto a test card or poke a probe into different places of the stool and seal the test tube. In both cases, the faecal sample will be returned to a laboratory for further analysis. A positive FIT result requires a diagnostic work-up with a colonoscopy which is considered to be the gold standard for detecting colonic lesions.

Based on the currently available evidence,[11-19] nationwide FIT-based screening programmes are widely being considered and implemented in many countries. Since there are many FITs available and different strategies to adopt (ie, single or multiple sample FIT screening, or in case of quantitative FIT screening, selected cut-off value), it is difficult for policy makers to decide which FIT and strategy should be implemented. In **chapter 2**, we therefore aimed to provide an overview of all published data concerning FIT screening in asymptomatic average-risk populations with regard to the positivity rate (PR), positive predictive value and detection rate (DR) of advanced neoplasia. In total, 50 references met the inclusion criteria of this systematic review: 25 of which evaluated the performance characteristics of fourteen qualitative FITs, and another 25 references evaluated five quantitative FITs. Overall, a large variation was seen between FITs and number of samples performed in both the PR (3.7-35.0%) and DR of CRC (0.1-1.6%) and advanced adenomas (0.5-5.5%). None of the investigated FITs dominated others with regard to the ratio between PR and DR of CRC and advanced adenomas.

When looking at the optimal number of stool samples performed per screening round, there seemed no additional value of 2-sample FIT screening compared with 1-sample screening for the detection of CRC. However, a trend was seen for a higher DR of advanced adenomas when a 2-sample strategy was adopted (ie, FOB Gold, Magstream, and OC-Sensor Micro). An explanation for the finding that 2-sample FIT screening only increases the DR of advanced adenomas and not of CRC, may be the fact that CRCs are believed to have a more constant bleeding pattern while advanced adenomas are believed to bleed more intermittently. Therefore, it could be hypothesized that when extending the number of performed stool samples especially more advanced adenomas will be detected. Therefore, in summary, 2-sample FIT screening seems of no additional value for CRC but might be beneficial for the detection of advanced adenomas.

Conclusions and future research

Although a lot of studies have been published about the performance characteristics of FIT screening, overall evaluation of a superior FIT is hindered by too little studies investigating the same test or too small numbers of participating individuals. Furthermore, the heterogeneity in study design, used definitions, target population, CRC prevalence rates, and screening round complicate fair comparisons. In order to make an optimal comparison between different FITs, there is a need for directly comparative trials in which individuals perform several FITs on the same bowel movement. Such trials are unfortunately scarce at this moment. Further recommendations for future research and reporting concerning FIT-based screening in asymptomatic average-risk populations are given in **chapter 2**.

OPTIMIZING ATTENDANCE RATE

Attendance is of fundamental concern in evaluating the effect of CRC screening, as the survival advantage of the screened group is offset by the presentation of late-stage disease and, consequently, poor prognosis among non-responders. Non-compliance with FOBT screening is a very important factor limiting the impact of screening on CRC-related mortality, since it is well known that non-responders are those at greater risk of death from CRC.[20-23] Factors that are associated with participation include knowledge about CRC and CRC screening; whether screening is recommended by the general practitioner (GP); sending potential participants an invitation letter signed by their own GP; the type of screening test offered; whether FOBT kits are posted with an invitation letter rather than provided by the GP or screening organization; if FOBT samples can be returned by mail rather than being hand-delivered; and including reminder letters in the invitation process.[24-30] In 2005, a small Australian study (n=600 subjects) suggested that an advance notification letter increased attendance to CRC screening.[31] We therefore conducted a large population-based randomized trial to assess the effectiveness of such a letter as an intervention to increase this attendance (**chapter 3**). We demonstrated that sending an advance notification letter resulted in a significantly higher participation rate (64.4% vs. 61.1%, p-value = 0.019) to CRC screening. The positive effect of such a letter may be explained by early gains in awareness, which would then be reinforced by similar information in the invitation and information brochure. This is particularly important in countries where there is low public awareness of CRC and the benefits of CRC screening.

Conclusions and future research

The observed difference of 3.3% may seem small but when extrapolated to a nationwide CRC screening programme it represents a large number of subjects. For instance, the Dutch population has 4.5 million individuals aged between 50-74 years. If advance notification letters are included in the invitation procedure approximately 155,000 extra individuals

might attend. Furthermore, this simple intervention has low incremental cost per additional detected advanced neoplasia due to sending an advance notification letter in the first screening round. Based on our results, we advocate the implementation of an advance notification letter within the standard CRC screening invitation process to increase adherence of CRC screening programmes. The results are based on Dutch data derived from a CRC screening naïve population in which the public awareness of CRC and its risk factors was among the lowest in Europe.[32] It therefore remains to be seen whether the observed effect of an advance notification letter will persist over subsequent screening rounds or whether this effect will diminish. It could be hypothesized that sending an advance notification letter during consecutive screening rounds does not have a significant effect and that sending a (second) reminder would be a better alternative.[27] Further studies should therefore compare the relative yield of an advance notification letter versus or combined with (repeated) reminder letters in subsequent screening rounds.

Future research should also focus on improving uptake among groups suffering from disparities (particularly ethnic minorities and low-income populations). Retrospective studies have clearly demonstrated that individuals living in areas of low socio-economic status (SES) were at a significantly increased risk for late-stage CRC diagnosis and therefore decreased survival rates compared with those living in higher SES areas.[33-34] This underscores the need to continue our efforts to evaluate interventions that can possibly remove specific language, attitudes, and cultural barriers in low-uptake groups in order to increase CRC screening attendance rates.

STABILITY OF STOOL SAMPLES

In contrast with gFOBT screening, there are concerns that faecal immunochemical tests are sensitive to a delayed sample return. FITs measure the presence of intact globin chains in haemoglobin molecules by means of human specific anti-globin antibodies. These globin chains degrade more rapidly than haem,[35-37] the component that is searched for by means of gFOBT screening. Moreover, the degradation of haemoglobin may occur quite fast in moist samples as used by most FITs, in contrast to the relatively dry smears used on gFOBT sample cards.[35] Taken these facts together, it has been suggested that a prolonged interval between faecal sampling and arrival at the laboratory impairs the efficacy of FITs.[38] This effect would be a major problem for the yield of FIT-based screening programmes and could therefore create a potential obstacle for the implementation and replacement of gFOBT by FIT. However, until now exact data were lacking and so were recommendations with respect to handling of negative tests with a prolonged sample return time. We therefore evaluated the effects of postal delays on FIT performance characteristics in an ongoing population-based CRC screening trial (**chapter 4**).

A total of 17,677 individuals between the ages of 50–74 years were randomly selected from municipal population registers in the southwest of the Netherlands. In **chapter 4** we demonstrated that with almost 10,000 FITs analyzed, both the positivity rate and detection rate did not decrease with prolonged sample return times of up to seven days. These trial results were confirmed by a laboratory experiment in which positive FIT samples were randomly selected, stored at room temperature, and re-tested with standard intervals. This experiment showed that no clinical significant lesions would have been missed within the first ten days after faecal sampling. The results presented in **chapter 4** confirm the laboratory data reported by Israeli investigators who observed no significant haemoglobin degradation over a period of 21 days when FIT samples were stored at 20°C.[39-40] The difference in interval between the Israeli vs. our study (ie, a period of respectively 21 and ten days before the first FIT samples became negative) lies in the extreme high initial haemoglobin concentrations found in the Israeli trial, 787-1,032 ng Hb/mL compared with 53-1,894 ng Hb/mL in our study. Although different cut-off values were used (100 vs. 50 ng Hb/mL, respectively), it is not surprising that our samples -with initial haemoglobin concentrations close to the cut-off value- became negative within a shorter time period. Additionally, we investigated the influence of (higher) temperature on the haemoglobin degradation process. Interestingly, when positive FIT samples were stored in a stove at a constant temperature of 30°C, the mean haemoglobin level decreased by 18.1% per day compared with 5.9% at room temperature. This is in line with a recently published Italian report, in which the authors concluded that accuracy of the FIT depends on seasonal variations.[37] The authors demonstrated that the haemoglobin concentrations measured during summer were significantly lower than those during winter.

Conclusions and future research

Our results imply that a delay in sending the FITs back to the laboratory, of up to at least one week, does not necessitate repeat testing in case of a negative test result. Our data support the use of FIT-based screening as a reliable tool for nationwide CRC screening programmes. However, the stability of FIT samples must be considered a critical point, particularly in countries with periods of high temperatures. New CRC screening programmes in such countries should therefore determine their performance characteristics prior to roll-out. Future research should focus on improving the quality of (haemoglobin-stabilizing) buffers used in the test tubes and packaging of returned FIT samples. The processing of a temperature-protecting aluminium return envelope, which has been used in a CRC screening trial conducted in Israel, seems promising.[41] Moreover, in some countries, it has been suggested not to invite potential participants during the hottest months of the year or to modify the period of invitation to either 1.5 or 2.5 years so that a subject invited in summer for the first test would be invited during winter for the subsequent test. This issue needs further research.

SEARCHING FOR THE BEST SCREENING STRATEGY

There is considerable evidence that screening of asymptomatic average-risk individuals using the gFOBT can detect cancers at an early and curable stage which results in a reduction of CRC-related deaths.[42] In one study with a follow-up time of eighteen years, the cumulative CRC-related mortality was 33% lower in the annual screening group than in the control group, and the biennial screening group had a 21% lower CRC mortality rate than the control group.[43] Based on these results, annual FOBT screening has been advocated.[44-46] The effectiveness of FIT-based screening in decreasing CRC-related mortality has not been studied in similar large long-term prospective randomized controlled trials. Population-based CRC screening programmes using FITs are therefore based on evidence from the previously mentioned randomized controlled gFOBT trials, combined with observations from other randomized trials that FIT screening in comparison with gFOBT is associated with higher attendance and diagnostic yield.[11-19] However, not all advanced neoplasia will be detected with single stool sampling. This is not so surprising since only bleeding colonic lesions can be detected by means of faecal testing. Unfortunately, colonic lesions may start bleeding late in their development, and even then, in particular adenomas, may still be missed due to an intermittent bleeding pattern. Repeated testing (ie, either by means of multiple FIT sampling per round or by successive screening rounds) increases the effectiveness of CRC screening.

Number of FIT samples (attendance and diagnostic yield)

Until now, limited data were available regarding the most optimal number of FITs to be used. Most data published used the highest haemoglobin concentration of multiple samples (ie, at least one test positive) and therefore valuable analyses about either positive tests or the mean of both FITs were missing.[17, 47] The literature also lacked comparative trials of 1-sample vs. 2-sample FIT screening with regard to attendance and diagnostic yield.

In **chapter 5** we demonstrated no differences in attendance rate between 1-sample and 2-sample FIT screening (61.5% vs. 61.3%, respectively). This observation is in accordance with an Italian study that also showed no difference in participation between 1-sample and 2-sample FIT screening (mean attendance rate, 56%).[48] Therefore, the decision on the optimal number of FITs to be used for a nationwide CRC screening programme can be based on differences in test characteristics. Since colonoscopy capacity will always play a crucial role in determining which FIT screening strategy should be preferred and could be implemented nationwide, a graph was made which provided important new insights into strategies tailored to local situations (**chapter 5**). Per screening strategy, we varied the cut-off values in the range of 50–200 ng Hb/mL, increasing in steps of 25 ng. This study demonstrated that in areas with limited access to colonoscopy, the best way to get to a low positivity rate was to use 2-sample FIT screening with referral for colonoscopy only when both tests were positive. This strategy yielded more advanced neoplasia at the same or even lower colonoscopy

demand compared with gFOBT screening, which guarantees optimal use of limited colonoscopy resources. The other extreme portrayed a nationwide screening programme in which colonoscopy capacity was not a limiting factor. In that setting, the strategy of 2-sample FIT screening with referral for colonoscopy in case of at least one positive test was associated with a significantly higher detection rate of advanced neoplasia than 1-sample FIT screening. For that reason, we concluded that the optimal FIT screening strategy in regions with wider colonoscopy capacity should be 2-sample FIT screening, whereby the positivity rate and detection rate can be tailored to meet colonoscopy availability and budgets by choice of the cut-off value. However, a full cost-effectiveness analysis should determine whether 2-sample FIT screening with such high positivity rates is still cost effective. Between these two extremes, all tested screening strategies resulted in more or less the same positivity rates and detection rates.

Number of FIT samples (cost-effectiveness analysis)

Before a government can make a thorough decision about the implementation of a CRC screening programme and the preferred screening strategy, information about cost-effectiveness is of paramount importance. We therefore performed a cost-effectiveness analysis comparing either 1-sample or 2-sample FIT screening based on the data presented in **chapter 5**. For this study we used the MISCAN-Colon micro-simulation model to assess under which conditions the increased performance of 2-sample FIT screening outweighs the increased costs compared with 1-sample FIT screening. Screening strategies in the model varied with respect to cut-off value (ie, 50, 75, 100, 150, and 200 ng Hb/mL), age to start and stop screening, and interval between successive screening rounds. In addition, different definitions for positivity of the 2-sample FIT group were tested (ie, at least one positive test outcome, two positive test outcomes, or using the mean from both test results). The presented data in **chapter 6** showed that within a given screening schedule 2-sample FIT screening is a cost-effective alternative for screening with only one sample; 2-sample FIT screening resulted in more life-years gained compared with screening by means of one FIT. Biennial 1-sample FIT screening (cut-off value 50 ng Hb/mL) between the ages of 55-75 years resulted in a cost-effectiveness ratio of €2,607 per life-years gained. The corresponding ratio for the 2-sample FIT group was €2,948 per life-years gained when using the mean from both test results, versus €3,150 when taking any positive test into account. However, when all age ranges and intervals between successive rounds were taken into consideration, increasing the screening intensity with 1-sample FIT screening consistently provided equal or even more life-years gained at lower cost compared with the 2-sample FIT screening strategies.

Unfortunately, randomized controlled trails in which the optimal FIT-based screening interval is evaluated (ie, in terms of CRC-related mortality reduction) are not available, nor are there any data on subsequent 2-sample FIT screening rounds. Moreover, assumptions were made for the attendance rate in subsequent screening rounds, since the data presented in

chapter 7 were not available when this cost-effectiveness analysis was performed. Therefore, for the model we made assumptions based on gFOBT trial observations.[49-50]

Screening interval length

Since the effectiveness of a screening programme in reducing the CRC-related mortality is highly dependent on participants' willingness to repeat testing at regular intervals, adherence to consecutive screening rounds is important. However, the detection rate of advanced neoplasia is a factor of similar importance. Repeated screening rounds not only enable to cover a larger proportion of the population, but also help to detect a larger proportion of subjects with advanced colonic lesions, both because of the gradual progression of a proportion of lesions and the intermittent nature of bleeding of advanced neoplasia. As a consequence, successive screening rounds are necessary for an optimal preventive effect in the target population. Unfortunately, we have limited knowledge on outcome parameters of the first vs. subsequent CRC screening rounds. Most available studies were conducted with the gFOBT that has been used for more than forty years now.[49-53] We therefore conducted a comparative study in which the attendance and diagnostic yield of repeated FIT testing, with intervals of various lengths, were determined in a population-based CRC screening trial.

In **chapter 7** we demonstrated that the attendance to a second screening round, within a one to three years range, is stable and acceptably high. Moreover, we demonstrated that repeated FIT screening enables a higher detection rate of repeated vs. single round screening (ie, the cumulative detection rate of advanced neoplasia ranged from 5.3-5.7% in the repeated 1-sample FIT screening groups compared with 3.3% in the first round of screening). Furthermore, it was shown that the association, if any, between longer screening intervals and larger numbers of advanced neoplasia detected at repeated FIT screening, is limited. A close to stable detection rate with increasing intervals can partly be explained due to the limited sensitivity of FIT for adenomas, which leaves many adenomas to be detected in a second screening round. In addition, it supports the concept of very slow progression of sporadic colorectal neoplasia. At last, these findings could also be an expression that non-bleeding advanced neoplasia persist in not bleeding for a long time.

In the same chapter, we performed an additional analysis in which a comparison was made between participants who attended both 1-sample FIT screening rounds vs. the once only 2-sample FIT screenees (described in **chapter 5**) who sampled the second test after a virtual interval of zero years. This comparison suggested that 50% of the detected advanced neoplasia in the second screening round could have been detected at baseline screening, but were not bleeding (consistently) enough to be detected by one FIT. These findings, in combination with the fact that no statistically significant differences could be observed between the different interval length groups for second round detection rates, suggests that FIT screening may progress to (initial) multiple faecal sampling in combination with a longer screening interval. In addition, this multiple testing strategy could possibly be further im-

proved by optimization of the number of days or bowel movements between FIT sampling. This issue needs further research.

Conclusions and future research

Given the fact that no large differences in attendance rate were observed between either 1-sample or 2-sample FIT screening or to the second screening round within a one to three years range, the decision for the most optimal FIT screening strategy can be based on differences in test characteristics. The results presented in this thesis can therefore be used for optimal screening strategy planning, tailored to a range of local characteristics such as colonoscopy capacity.

From 2013 onwards, a national bowel cancer screening programme will be introduced in the Netherlands. With more than 70 Dutch vacancies for gastroenterologists on a total of 354, this screening programme will be rolled-out in a stepwise manner. Not only in the Netherlands but in many other countries the current colonoscopy capacity is limited and waiting times for a colonoscopic procedure of up to eighteen weeks have been reported.[54-56] Colonoscopy capacity cannot be increased at once and thus screening programmes should be adjusted to the available capacity. There are several strategies available to do so: one way is to screen individuals less frequently by starting to screen at older ages, stopping at younger ages, or by increasing the screening interval. Another option could be to elevate the haemoglobin cut-off level for referral to colonoscopy in case of quantitative FIT screening. Finally, reduction of colonoscopy demand can be achieved by more selective referral of individuals to surveillance colonoscopy after removal of adenomas. In a recently published cost-effectiveness analysis based on the CORERO-I data, it was demonstrated that a 1-sample FIT screening strategy with higher cut-off values was most effective when there is limited colonoscopy capacity.[57] In addition to this adaptation, the age ranges of the invited subjects could be narrowed. With these results in mind, it is not surprising that the Dutch Health Council have recommended starting CRC screening by means of a 1-sample FIT strategy with a cut-off value of 75 ng Hb/mL, and only inviting individuals who are aged between 55-75 years. It is expected that within a period of 6 years this FIT-based screening programme will be rolled-out over the entire Dutch target population (**Figure 2**).

However, it should be mentioned that FIT screening can become considerably more effective if the colonoscopy capacity is expanded. With a stepwise introduction of the Dutch colorectal cancer screening programme, efforts should be undertaken to achieve an increased colonoscopy capacity to be able to screen more effectively in the future. There are several established ways to adapt the screening strategy when colonoscopy capacity is extended. Subsequently lowering the cut-off value for referral to colonoscopy (ie, towards 50 ng Hb/mL) is probably the easiest way to implement in an ongoing screening programme. Adding age groups by beginning screening earlier and stopping later in life (ie, adjusting the age range to 50-80 years) is also feasible. Furthermore, the results presented in this thesis

Figure 2 Stepwise roll-out of Dutch colorectal cancer screening programme[58]

	Gefaseerde invoering in jaren					Alle leeftijds-categorieën geïncludeerd	Doelgroep minimaal eenmaal uitgenodigd
	1	2	3	4	5	6	7
	2013	2014	2015	2016	2017	2018	2019
Geboortejaar	**Leeftijd bij oproep voor het bevolkingsonderzoek**						
1964							55
1963						55	
1962							57
1961						57	
1960					57		59
1959						59	
1958					59		61
1957				59		61	
1956					61		63
1955				61		63	
1954			61		63		65
1953				63		65	
1952			63		65		67
1951		63		65		67	
1950			65		67		69
1949		65		67		69	
1948	65		67		69		71
1947		67		69		71	
1946			69		71		73
1945				71		73	
1944					73		75
1943						75	
1942					75		
1941				75			
1940			75				
1939		75					
1938	75						
Aantal uitnodigingen (*1000)	338	762	1.195	1.538	1.990	2.218	2.260

opgeroepen leeftijdscategorie

give another alternative: 1-sample FIT screening with a shorter interval between consecutive rounds (ie, annual screening). Alternatively, a two or more sample FIT strategy with a longer screening interval could become more advantageous than a 1-sample FIT strategy with a shorter interval. Further research on this comparison, together with subsequent 2-sample FIT screening rounds is required.

Future research should also focus on personalized screening. Individuals with a personal history of CRC, adenomas, or inflammatory bowel disease, subjects with a family history of CRC, or a genetic predisposition (e.g. familial adenomatous polyposis and Lynch syndrome) are at increased risk for CRC and should therefore enter specialised screening or separate surveillance programmes.[44, 59-66] Differences in CRC risk exist even within the average-risk population. To date, screening guidelines have not been tailored across different subgroups of the average-risk population. The detection rates of advanced adenomas and CRC are significantly higher in African Americans and men compared with whites and women, respectively.[67] This is probably a result of the higher CRC incidence rates in these subgroups of individuals.[68-69] Due to this higher pre-test probabilities for advanced neoplasia, the American College of Gastroenterology has advocated that screening should start earlier in African Americans.[70] Moreover, several studies have suggested to develop gender specific recommendations for CRC screening.[71-72] A differentiated approach taking gender and potentially age into account would be relatively easy with FIT screening. One could argue to use different cut-off values for men and women to achieve a similar number needed to scope which would result in a considerable higher cut-off value for women than for men.[67] On the other hand, one should realize that personalization of CRC screening recommendations is complex and it might confuse invitees to the point of decreasing attendance. Logically, a decrease in participation rate would easily offset the gains from personalization.

CONCLUSION

Based on data obtained from the CRC screening feasibility trials, conducted in the Rotterdam and Amsterdam/Nijmegen region, the Dutch Health Council concluded that colorectal cancer fulfils the criteria for population-based screening. The results of both CORERO trials, which were partly described in this thesis, helped to form the basis for the implementation of a nationwide FIT-based colorectal cancer screening programme in the Netherlands. Moreover, these results are being used for similar processes in other countries.

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Nederlandse samenvatting

In de westerse wereld komt dikke darmkanker veel voor. In Nederland werd in 2008 bij ruim 12.000 mensen dikke darmkanker vastgesteld. De ziekte is bij mannen na prostaat- en longkanker en bij vrouwen na borstkanker de meest voorkomende maligniteit. Naar verwachting zal de incidentie van dikke darmkanker met drie procent per jaar toenemen. Dit wordt voornamelijk toegeschreven aan de vergrijzing van de Nederlandse bevolking. In 2008 overleden 4.810 patiënten aan de gevolgen van deze ziekte. De diagnose dikke darmkanker wordt veelal laat gesteld, als de ziekte zich al in een vergevorderd stadium bevindt. Symptomen, zoals bloed bij de ontlasting of een veranderd defecatie patroon, worden meestal pas opgemerkt als de tumor groot is of de darm obstrueert. Hierdoor zijn er op het moment dat de diagnose gesteld wordt vaak al uitzaaiingen in de regionale lymfeklieren en/of op afstand aanwezig. De prognose van darmkanker patiënten hangt af van de uitgebreidheid van de ziekte. Wanneer de tumorgroei nog beperkt is tot de darmwand is de 5-jaarsoverleving 94%. Echter, wanneer er aanwijzingen zijn voor uitzaaiingen op afstand daalt de 5-jaarsoverleving naar 8%.

Dikke darmkanker heeft een langdurig en goed herkenbaar voorstadium, de zgn. adenomateuze poliep of adenoom. Dankzij screening zijn de (hoog-risico) adenomen betrekkelijk eenvoudig op te sporen en endoscopisch te verwijderen, wat volgens een Amerikaanse studie resulteerde in een lager dan verwachtte incidentie van dikke darmkanker. Het *primaire doel* van darmkanker screening is echter om tumoren in een zo vroeg mogelijk stadium te detecteren én te behandelen om zo een gunstige invloed uit te oefenen op de overleving van darmkanker patiënten.

Vanaf 2013 zal in Nederland een landelijk bevolkingsonderzoek naar darmkanker worden ingevoerd. Door middel van een gefaseerde implementatie zullen alle mannen en vrouwen tussen de 55 en 75 jaar elke twee jaar worden uitgenodigd om hun ontlasting te laten onderzoeken op (onzichtbare) sporen bloed. In dit proefschrift worden verschillende aspecten van deze zgn. immunochemische ontlastingstest (afgekort tot FIT) belicht.

In **hoofdstuk 1** wordt een overzicht gegeven van de verschillende technieken die kunnen worden ingezet voor de vroege opsporing van darmkanker. Hierbij wordt een onderscheid gemaakt tussen ontlastingstesten, uitwendig afbeeldend onderzoek en invasieve onderzoeken van de dikke darm. Tevens worden de algemene doelstellingen van dit proefschrift beschreven.

Hoofdstuk 2 beschrijft een systematische beschouwing over de beschikbare literatuur betreffende FIT screening. In dit hoofdstuk wordt *per FIT merk*, uitgesplitst naar het aantal uitgevoerde testjes per screeningsronde, een overzicht gegeven van het positiviteitspercentage en de detectiegraad van darmkanker en hoog-risico neoplasieën. In totaal werden 50 internationale artikelen geïncludeerd, waarin veertien qualitatieve en vijf quantitatieve FIT merken werden belicht. Op basis van deze literatuur kan geconcludeerd worden dat geen

enkele FIT significant beter is dan andere FIT merken. Dit komt waarschijnlijk door het kleine aantal opgezette studies en/of het lage totaal aantal deelnemers per FIT merk. Gezien de grote verscheidenheid in onderzoeksopzet, gebruikte definities voor 'advanced adenomas', verschillen in leeftijdscategorieën en man/vrouw verhouding, en of de gepresenteerde test karakteristieken betrekken hebben op een eerste versus vervolg screeningsronde, maakt een eerlijke vergelijking tussen de huidige FIT merken lastig. Direct vergelijkend onderzoek, waarin individuen verschillende FITs uitvoeren op dezelfde stoelgang, zijn daarom noodzakelijk om een valide uitspraak te kunnen doen over de beste FIT. Verder worden in dit hoofdstuk aanbevelingen gedaan voor toekomstig onderzoek om vergelijkingen tussen verschillende immunochemische ontlastingstesten verder te optimaliseren.

In meerdere gerandomiseerde, gecontroleerde studies is de effectiviteit van guaiac-gebaseerde feces occult bloed test (gFOBT) screening op de aan darmkanker gerelateerde mortaliteitsreductie aangetoond. Tot op heden ontbreken dergelijk trials voor FIT screening. Recente gerandomiseerde studies laten zien dat FIT screening in vergelijking met gFOBT resulteert in een hogere opkomst en opbrengst van hoog-risico neoplasieën. Derhalve wordt in het algemeen aangenomen dat herhaalde FIT screening een minstens zo grote impact zal hebben op de mortaliteitsreductie van darmkanker als beschreven voor gFOBT screening. De effectiviteit van een FIT screeningsprogramma is voornamelijk afhankelijk van twee parameters: de test karakteristieken van de FIT en de deelnamegraad binnen de te screenen populatie. Een hogere opkomst wordt geassocieerd met een grotere effectiviteit in termen van mortaliteitsreductie en een betere kosteneffectiviteit. Helaas is de participatie voor dikke darmkanker screening in veel landen laag. In **hoofdstuk 3** wordt een gerandomiseerde studie beschreven (n=5.000) die is opgezet om de invloed te bepalen van een zgn. vooraankondi gingsbrief op de deelnamegraad voor darmkanker screening. De interventie groep ontving twee weken voor de daadwerkelijke uitnodiging, een vooraankondiginsbrief met daarin aanvullende informatie over dikke darmkanker en darmkanker screening. De controle groep ontving een dergelijke brief niet en werd direct benaderd middels een standaard uitnodi ging. In beide groepen werd zes weken na de uitnodigingsbrief een herinnering verzonden aan alle niet-respondenten. De vooraankondigingsbrief zorgde voor een significant hogere opkomst binnen de interventie groep (64,4% vs. 61,1%, p-waarde = 0,019). Deze studie toont aan dat het in een screeningsnaïeve populatie zinvol is om in de eerste screeningsronde een vooraankondigingsbrief te versturen, om zo de bewustwording en kennis over darmkanker screening te vergroten.

De immunochemische ontlastingstest die voor het Nederlandse bevolkingsonderzoek naar darmkanker gebruikt zal gaan worden is een kwantitatieve FIT. Binnen ons proef-bevolkings-onderzoek is aan alle deelnemers gevraagd om de datum van uitvoering op het testbuisje te noteren. De ervaring leert dat niet alle ontlastingstesten onmiddellijk na uitvoering worden

geretourneerd naar het laboratorium. Wij vroegen ons daarom af of het vertraagd retourneren van invloed zou kunnen zijn op de test karakteristieken van de FIT. Om deze vraag te beantwoorden zijn in **hoofdstuk 4** alle test karakteristieken van de tot dan toe geanalyseerde ontlastingstesten (n=8.958) retrospectief bekeken en uitgezet tegen de terugstuur tijd, uitgedrukt in dagen. Bij een terugstuur tijd van zeven dagen kon geen significante daling worden geobserveerd m.b.t. het aantal positieve testen, dan wel het aantal gedetecteerde hoog-risico neoplasieën. Op basis van deze resultaten kan geconcludeerd worden, dat negatieve ontlastingstesten die tot een week na uitvoering binnenkomen op het laboratorium niet herhaald hoeven te worden door de deelnemer. Deze bevinding kan gunstige implicaties hebben voor het op handen zijnde Nederlandse bevolkingsonderzoek naar darmkanker. Tenslotte is in dit hoofdstuk verder onderzoek verricht naar de temperatuursinvloed op het afbraakproces van hemoglobine (Hb). Wanneer positieve FIT monsters werden opgeslagen bij een constante omgevingstemperatuur van 30°C, daalde de gemiddelde Hb concentratie met 18,1% per dag, dit in tegenstelling tot 5,9% wanneer de monsters werden opgeslagen bij kamertemperatuur. Een hoge omgevingstemperatuur zou dus nadelige gevolgen kunnen hebben voor de test karakteristieken van de FIT.

Zoals eerder beschreven hangt de effectiviteit van een screeningsprogramma niet alleen af van de opkomst, maar is de detectiegraad van hoog-risico neoplasieën minstens zo be langrijk. Helaas kunnen niet alle hoog-risico neoplasieën met een éénmalige ontlastingstest worden opgespoord, omdat deze laesies (met name de hoog-risico adenomen) onregelmatig bloeden. In **hoofdstuk 5** worden twee groepen met elkaar vergeleken waarbij de invloed van het aantal ontlastingstesten wordt bepaald op de opkomst, colonoscopie belasting en de detectiegraad van hoog-risico neoplasieën (n=8.204). In vergelijking met de groep die gevraagd werd één ontlastingstest uit te voeren, werden in de groep met twee ontlastingstesten significant meer hoog-risico neoplasieën gedetecteerd zonder dat dit ten koste ging van de opkomst. Afhankelijk van de locale colonoscopie capaciteit kan behoudens de variatie in de verwijsdrempel, nu dus ook gekozen worden tussen het aantal uit te voeren ontlas tingstesten. In geval van een beperkte colonoscopie capaciteit kan gekozen worden voor screening met twee ontlastingstesten waarbij beide FITs een positieve testuitslag moeten hebben alvorens men wordt doorverwezen voor verder onderzoek. Deze strategie levert meer hoog-risico neoplasieën op, tegen een gelijke of zelfs lagere colonoscopie belasting, t.o.v. gFOBT screening. Aan de andere kant, wanneer de colonoscopie capaciteit geen belemmerende factor is, valt wederom screening met twee ontlastingstesten tot de mogelijkheden. In dergelijke gevallen zou gekozen kunnen worden voor doorverwijzing wanneer één van beide ontlastingstesten positief uitvalt, omdat dit significant meer hoog-risico neoplasieën oplevert dan screening met één ontlastingstest. Deze strategie kan uiteraard nog verder geoptimaliseerd worden door de verwijsdrempel aan te passen op de lokale beschikbaarheid van endoscopie en budgets. Voordat de overheid een beslissing kan nemen over het

door te voeren aantal ontlastingstesten dient ook een gedegen kosteneffectiviteitanalyse te worden uitgevoerd. Uit een dergelijke analyse zal namelijk moeten blijken of het aantal extra gedetecteerde hoog-risico neoplasieën ook daadwerkelijk opweegt tegen de extra uit te voeren colonoscopieën. In **hoofdstuk 6** wordt een dergelijke kosteneffectiviteitanalyse uitgevoerd door middel van het gevalideerde MISCAN-Colon microsimulatie model. Deze analyse toont aan dat binnen een gegeven schema, screening met twee ontlastingstesten een kosteneffectief alternatief is naast screening met één ontlastingstest. Screening met twee ontlastingstesten kan namelijk meer gewonnen levensjaren opleveren dan screening met één ontlastingstest. De kosten per gewonnen levensjaar voor tweejaarlijks screenen in de leeftijdscategorie van 55 en 75 jaar middels één ontlastingstest (verwijsdrempel 50 ng Hb/mL) waren € 2.607. Dit in tegenstelling tot € 2.948 voor screening met twee ontlastingstesten waarbij de gemiddelde Hb concentratie boven de verwijsdrempel ligt, versus € 3.150 voor screening met twee ontlastingstesten waarbij tenminste één FIT een positieve testuitslag heeft. Echter, wanneer de colonoscopie capaciteit het toelaat is intensivering van 1-sample FIT screening (door een korter interval en/of het vergroten van de leeftijdsgrenzen) meer kosteneffectief dan de 2-sample FIT screening alternatieven.

De laatste vraagstelling die in dit proefschrift wordt onderzocht, betreft het meest optimale interval tussen twee opeenvolgende FIT screeningsronden. Herhaalde screening zorgt niet alleen voor een grotere dekking van de doelgroep, maar zorgt er ook voor dat meer mensen met hoog-risico neoplasieën gedetecteerd kunnen worden. Dit laatste hangt samen met de geleidelijke progressie van een deel van de adenomen tot hoog-risico poliepen en het intermitterende bloedingspatroon van deze hoog-risico neoplasieën. Dit heeft als consequentie dat opeenvolgende screeningsronden noodzakelijk zijn om daadwerkelijk een preventief effect binnen de doelgroep te bewerkstelligen. Tot op heden zijn er echter geen data bekend over de invloed van interval lengte tussen twee opeenvolgende FIT screeningsronden en de invloed van die lengte op de opkomst en diagnostische opbrengst. In **hoofdstuk 7** worden drie groepen beschreven, die middels een aselecte steekproef uit de regio Groot-Rijnmond zijn geselecteerd. Elke groep bestaat uit 50 tot en met 74-jarige mensen met een gemiddeld risicoprofiel voor darmkanker (n=7.501). Na een interval van respectievelijk 1, 2 of 3 jaar werden zij opnieuw benaderd voor dikke darmkanker screening met één ontlastingstest. Binnen onze studie resulteerde screening om de drie jaar niet in significant meer hoog-risico neoplasieën in vergelijking met een jaarlijkse screening. Verder toonde deze studie aan dat de opkomst voor een tweede FIT screeningsronde stabiel en acceptabel hoog is wanneer een interval van 1, 2 of 3 jaar wordt toegepast. Deze studie laat daarmee zien dat de keuze voor een bepaalde FIT screeningsstrategie volledig bepaald kan worden door de locale situatie in een land, waaronder de colonoscopie capaciteit.

De belangrijkste bevindingen uit dit proefschrift en aanbevelingen voor toekomstig onderzoek worden tenslotte beschreven in **hoofdstuk 8**.

CONCLUSIE

De verzamelde gegevens uit de regionale proef-bevolkingsonderzoeken naar darmkanker hebben er mede voor gezorgd dat de Gezondheidsraad constateerde dat er voldoende bewijs voorhanden was om in Nederland te starten met een bevolkingsonderzoek naar darmkanker. Daarmee kan worden geconcludeerd dat de resultaten van de twee CORERO trials, die deels in dit proefschrift werden beschreven, ertoe hebben bijgedragen dat de minister van Volksgezondheid, Welzijn en Sport in mei 2011 besloten heeft tot de landelijke invoering van een bevolkingsonderzoek naar darmkanker. Tevens zullen de in dit proefschrift gepresenteerde studie resultaten bruikbaar zijn voor gelijksoortige besluitvormingen in andere landen.

Dankwoord

PhD portfolio

ORAL PRESENTATIONS

2012 Ins en outs van de FIT test, verschillen per seizoen?
Nationaal Symposium, "Invoering van colonscreening; wat betekent dit voor ons in de praktijk?", Zeist, the Netherlands

2011 Attendance and diagnostic yield of repeated faecal immunochemical test screening with intervals of 1, 2, or 3 years: a comparative population-based colorectal cancer screening trial
Dutch Society of Gastroenterology, Veldhoven, the Netherlands

2010 Attendance and diagnostic yield of 1 versus 2-sample faecal immunochemical test (FIT) screening: a comparative population-based colorectal cancer trial
Digestive Disease Week, New Orleans, United States

Faecal immunochemical test (FIT) characteristics by sample return time in a population-based colorectal cancer screening trial
Digestive Disease Week, New Orleans, United States

2008 MicroRNA expression profiling of colorectal cancer and its precancerous lesions using LNATM oligonucleotide arrays: a pilot study
United European Gastroenterology Week, Vienna, Austria

POSTER PRESENTATIONS

2011 Attendance and diagnostic yield of repeated faecal immunochemical test screening with intervals of 1, 2, or 3 years: a comparative population-based colorectal cancer screening trial
Digestive Disease Week, Chicago, United States

2010 Costs of guaiac versus immunochemical faecal occult blood testing within a randomized population-based colorectal cancer screening trial
Digestive Disease Week, New Orleans, United States

2009 An advance notification letter increases attendance in colorectal cancer screening: a population-based randomised trial.
United European Gastroenterology Week, London, the United Kingdom

ATTENDED SEMINARS AND WORKSHOPS

2010 Cochrane Systematic Reviews of Diagnostic Test Accuracy
Academic Medical Centre, Amsterdam, the Netherlands

Developing a Cochrane Systematic Review
Academic Medical Centre, Amsterdam, the Netherlands

Advanced English course
Language Institute Regina Coeli BV, Vught, the Netherlands

2009 Erasmus Winter Program:
Biostatistics for Clinicians
Regression Analysis for Clinicians
Netherlands Institute for Health Sciences (NIHES), Rotterdam, the Netherlands

English Biomedical Writing and Communication
Erasmus University Medical Centre, Rotterdam, the Netherlands

MS Access database, Basis course
Erasmus University Medical Centre, Rotterdam, the Netherlands

2008 Young Investigator Workshop
United European Gastroenterology Week, Vienna, Austria

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Curriculum vitae

Aafke H.C. van Roon werd geboren op 29 december 1981 te Vlaardingen. In 2000 behaalde zij haar VWO examen aan 't Groen van Prinsterer Lyceum te Vlaardingen. In afwachting van de studie Geneeskunde, studeerde zij Biomedische Wetenschappen aan de Universiteit Leiden. In 2001 ging haar lang gekoesterde wens in vervulling en kon zij beginnen met de studie Geneeskunde aan de Erasmus Universiteit Rotterdam, waarna zij in 2002 haar propedeuse cum laude behaalde. Gedurende de doctoraalfase was zij actief in de jaarvertegenwoordiging en hield zij zich bezig met de verbetering van het nieuwe curriculum '*Erasmus-arts 2007*'. Haar wetenschappelijke stage werd verricht aan de Flinders University, Department of Surgery, Bedford Park, South Australia onder leiding van prof.dr. D.I. Watson en dr. B.P.L. Wijnhoven. Tijdens deze stage verrichtte zij onderzoek naar de verschillende mucine expressie profielen in de slokdarm ten gevolge van gastro-oesofageale reflux. De daarop volgende co-schappen werden verricht in het St. Elisabeth Ziekenhuis te Tilburg. Haar oudste co-schap doorliep zij op de afdeling Maag-, Darm- en Leverziekten van het Erasmus MC te Rotterdam, waarna zij in 2008 haar arts-examen behaalde. In mei 2008 startte zij met promotie-onderzoek op het gebied van dikke darmkanker screening onder leiding van haar promotoren prof.dr. E.J. Kuipers en prof.dr. J.D.F. Habbema. In november 2011 is zij met veel plezier gestart in het Sint Franciscus Gasthuis te Rotterdam (opleider: drs. A.P. Rietveld) aan haar opleiding tot Maag-, Darm- en Leverarts (opleider Erasmus MC: dr. R.A. de Man).