

# 8

**Summary, general discussion  
and future perspectives**

## SUMMARY

The first section of this thesis deals with the history of fecal occult blood testing. Screening by means of a guaiac based FOBT (g-FOBT) followed by a colonoscopy in case of positivity, has been showed to reduce CRC mortality and incidence.<sup>1-4</sup> However, g-FOBT has been criticized for its fairly low sensitivity and for being non-specific to human hemoglobin. Consequently, as an alternative to g-FOBT, a test specific to human hemoglobin was sought and the Fecal Immunochemical Test (FIT) was introduced.

In **chapter 2** sensitivity and specificity of g-FOBT (Hemoccult-II©) and FIT (OC-sensor©) in advanced adenomas and different stages of CRC was assessed in eligible subjects who underwent complete colonoscopy. G-FOBT was thereafter considered an unreliable test of the past as FIT was found to be more sensitive for CRC and advanced adenomas compared to g-FOBT. In a sub analysis of the neoplastic lesions found in the study cohort, sensitivity of FIT for the screen relevant neoplasia (SRN) as well as for the early stage cancers and advanced adenomas, was proven to be significantly higher compared to g-FOBT. In this direct comparison the more current FIT proved to be superior.

Chapters 3 to 6 form the second section of this thesis and concern different strategies for the use of FIT for the detection of CRC. One of the major advantages of FIT is the non-dichotomous outcome. By adjusting the cut-off value, the test positivity rate can be influenced. The influence of using higher cut-off values for a FIT on the detection rates of SRN was assessed in **chapter 3**. It was found that higher FIT cut-off levels substantially decrease test positivity rates with only limited effects on detection rates of early-stage CRCs. Adjusting the threshold for positivity of quantitative fecal immunochemical tests (FIT) will allow for controlling the number of initial colonoscopies in a screening program.

**Chapter 4** focuses on repeated FIT sampling as another strategy, aimed at improving test sensitivity of FIT for detection of early stage CRC and its precursors, defined as SRN. Test positivity and sensitivity of double FIT sampling was evaluated for three different strategies at several cut-off values, “one of two FITs positive” when at least one out of two measurements exceeded the cut-off value, 2) “both FITs positive” when both measurements exceeded the cut-off value, and 3) “the average of two FITs positive” when the geometric mean of two FITs exceeded the cut-off value. Regardless of the cut-off that was used, “two of two FITs+” resulted in the lowest and “one of two FITs+” in the highest sensitivity for SRN (range 35–44% and 42%–54% respectively). Receiver Operator Curves (ROCs) of double FIT sampling were similar to those of single FIT sampling. However,

at fixed levels of specificity of 85/90/95%, sensitivity of any double FIT sampling strategy did not significantly differ from single FIT (p-values 0.07–1.00). In conclusion, none of the double FIT strategies had a superior combination of sensitivity and specificity over single FIT.

In **chapter 5** the focus lies on false positive FITs. In a screening setting, false positive results will result in futile colonoscopies. In this chapter, the contribution of hemorrhoids on the frequency of false positive FITs was determined. In only 9 individuals, out of a cohort of 2855 patients, who had a FP FIT (4.1%; 95% CI 1.4–6.8), hemorrhoids were the only abnormality found. In univariate unadjusted analysis, subjects with hemorrhoids as single abnormality did not have more positive results (9/134; 6.7%) compared with subjects without any abnormalities (43/886; 4.9%;  $p=0.396$ ). Logistic regression identified hemorrhoids, non advanced polyps and a group of miscellaneous abnormalities (for instance angioectasia) all of significantly influence on false positivity. Still, hemorrhoids detected at colonoscopy remain an infrequent cause of false positive fecal immunochemical tests and therefore, the influence of hemorrhoids on the effectiveness of an FIT-based screening program is likely to be limited.

Recently, FITs were found to have a higher sensitivity and lower specificity for advanced colorectal neoplasia in males compared to females. The aim of **chapter 6** is to compare males and females with respect to the sensitivity and specificity of FIT, at different cut-off values. Outcomes were CRC and advanced adenomas. We studied whether location in the large bowel, number and size of neoplastic lesions were potential explanatory variables in the relation between sex and FIT characteristics.

Using cut-off values between 50–100 ng/ml, a large but non-significant difference of 13%, was found for sensitivity for CRC in favor of men. FIT was significantly more specific for CRC in females than in males. By choosing a lower cut-off value for women, corresponding test characteristics could be reached. For advanced adenomas no sex-specific differences of any clinical relevance were observed. Additionally, FIT proved to be more sensitive for left-sided lesions than for right-sided lesions. However, a difference in distribution of lesions between the sexes proved to not be responsible for the observed gender disparities. Gender specific screening guidelines could be considered in order to optimize or balance the effectiveness of a screening program in males and females. However, since sensitivity for advanced adenomas did not differ between men and women and compliance to screening programs is known to be higher in women, the observed gender difference will probably hold no consequences for the design of a screening program.

Occult gastrointestinal blood loss is unspecific to colonic cancers and large adenomas, while there are several other possible causes.<sup>5</sup> Blood loss from other sources like, for instance, colonic angioectasia will lead to false positive FIT results. Furthermore, false negative results can occur because blood loss from neoplastic lesions is intermittent.<sup>6</sup> False negative FIT's can be a serious problem for the credibility of nationwide screening program. In **chapter 7** the combination of FIT with a methylation marker, in order to improve the FIT for future use, is assessed. Phosphatase and Actin Regulator 3 (PHACTR3) was identified from a pool of potential candidates using a bio-informatics based strategy, as a novel hypermethylated gene that could serve as a biomarker for early detection of colorectal cancer in stool. Subsequently, the complementary value to a Fecal Immunochemical Test (FIT) was evaluated in two series of whole stool samples, which were specifically collected for this purpose. It was found that adding PHACTR3 methylation to FIT increased its sensitivity for CRC up to 15%. This new hypermethylated gene in CRC has a good performance in stool DNA testing and was found to have an additional value to FIT.

## GENERAL DISCUSSION AND FUTURE PERSPECTIVES

Prevention of colorectal cancer (CRC) is the ultimate goal of CRC screening. In order to achieve the maximum benefit in terms of reducing morbidity and mortality, a primary CRC screening tool must be able to effectively detect advanced precursor lesions and cancers at a curable stage throughout the colon.

In September 2013, the much anticipated national screening program for CRC in the Netherlands will finally be initiated.<sup>7</sup> Adequate knowledge on test performance of the primary screening tool is crucial for making estimations of the logistic demands for such a program and is therefore essential for projecting the needed capacity to meet the needs for a nationwide screening program.

Nationwide screening programs are well on their way in several (other) countries. At the time of the initiation of the research reported in this thesis, g-FOBT was the test most used in screening programs. At present, g-FOBT is being replaced by FIT.

The research described in this thesis focuses on the diagnostic accuracy of FITs. Studies of diagnostic test accuracy require the true disease status of each individual to be known. In our population this was determined by colonoscopy, which was considered the gold standard. Here, sensitivity and specificity were calculated in a large cohort of individuals

who were referred for colonoscopy. To avoid bias this gold standard was conducted and interpreted while the endoscopists were blinded to the FIT result.

A limitation of our FIT-cohort is that it is a referral population, partially containing high risk individuals, rather than a true screening population. In a referral population the prevalence of CRC is higher when compared to a true screening population, which consist of asymptomatic individuals. Therefore, test characteristics that depend on the prevalence of disease, like the positive and negative predictive values, could not be generalized from this population. Sensitivity and specificity however, are test characteristics not influenced by the prevalence of the disease.<sup>8</sup> Yet due to work-up bias, in this cohort of referred individuals, sensitivity could still have been overestimated and specificity underestimated.<sup>9</sup> Work-up bias can influence results, as a subset of the individuals has signs or symptoms which increase the likelihood of having both a positive FIT and CRC.<sup>9</sup> Nevertheless, it can be questioned whether a screening population could indeed be free of work-up bias, as it was previously shown that almost half of the subjects with screen detected CRC experienced hematochezia.<sup>10</sup> Similar results are found when comparing sensitivity and specificity of the FIT described in this thesis with results of some of the large screening trials using a FIT.<sup>11,12</sup>

Identifying the most suitable FIT for a screening program and, consequently, reaching the maximum benefit from FIT screening, requires comparability of results between different FITs. FITs new on the market should be evaluated properly and should be compared to the current standard.<sup>13</sup> In order to facilitate adequate comparison of FITs in the future, the concentrations of hemoglobin that one FIT measures should be comparable with the result of a FIT from another manufacturer. For this purpose, uniformity in reporting is required. This analytical comparability could be reached by expressing the quantity of hemoglobin present in the amount of feces in the FIT tube and not in the amount of buffer solution in the test tube.<sup>14</sup>

Compliance to screening and accuracy of the screening tests are the two major determinants of the effectiveness of a screening program.<sup>15,16</sup> With compliance rates around 50%, participation remains poor in population screening programs carried out in Europe. Criticizing the test to be used in a screening program could bear the risk of undermining the faith the general public has in the test and thereby reduce adherence, even before the initiation of the Dutch population screening program. The current debate among professionals concerning the preferred FIT for screening could therefore lead to a reduced uptake of CRC screening.

To date, the Fecal Immunochemical Test seems to be the best available primary screening test for CRC screening as it meets some of the major requirements: it can detect both advanced adenomas and early cancers, it has high specificity to keep the costs of screening low and minimize risks to healthy patients, and it is user-friendly, affordable, and widely available.<sup>17</sup>

## **FUTURE PERSPECTIVES**

Knowledge on test performance and acceptance is required for selection of the FIT best suited to meet pre-specified clinical and logistical requirements of a screening program.

FITs detect blood in stool. Occult blood loss in the colon is non-specific to neoplastic disease and consequently hemoglobin is not an ideal marker for the presence of colonic adenomas and cancers. Additionally, it is unknown whether the adenomas most likely to bleed are indeed those adenomas most likely to progress to malignant disease. As a result, FIT is a rather unspecific tool for detecting (pre)cancerous colonic lesions. Therefore, although it seems to be the best available option at this moment, FIT might not maintain this status in the future.

In order to improve the uptake of a CRC screening program, markers specifically targeted at those adenomatous lesions most likely to progress are needed. These markers could be tumor derived DNA products in stool. However, the ultimate panel of markers for a stool DNA test still has to be developed.<sup>18</sup>

In the Netherlands, the upcoming screening program will create the opportunity to assess the new FITs and other stool markers and compare them to the current standard. This will hopefully facilitate translation of basic research into screening tools ready for implementation into screening programs.

This thesis touched on the past and present use of FITs in CRC screening. As new options will arise in the upcoming years, FIT will probably not hold up as a single marker test in screening programs. The question therefore remains for how long the “FI-test” will survive?

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