A New-Generation Fecal Immunochemical Test (FIT) Is Superior to Quaiac-based Test in Detecting Colorectal Neoplasia Among Colonoscopy Referral Patients

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Abstract. *Aim: To compare a new-generation fecal immunochemical test (FIT) with the leading guaiac-based test in detection of fecal occult blood (FOB) in colonoscopy-referral patients. Patients and Methods: A cohort of 300 patients referred for colonoscopy was examined by two different tests for FOB: ColonView quick test (CV) (FIT test for haemoglobin (Hb) and haemoglobin/haptoglobin (Hb/Hp) complex) and HemoccultSENSA (HS) (quaiac test for Hb). Three fecal samples were tested and all subjects were examined by diagnostic colonoscopy with biopsy verification. The test was interpreted positive if any of the three samples tested positive for Hb (HS test) and either Hb or Hb/Hp complex (CV test). The performance indicators (sensitivity (SE), specificity (SP), positive predictive value (PPV), negative predictive value (NPV) and area under the curve (AUC)) were calculated for both tests using three endpoints (adenoma (A), adenoma/carcinoma (A/AC) and carcinoma (AC)), collectively and were stratified according to tumor site. The two tests were compared regarding their sensitivity/specificity balance (AUC), using the receiver operating characteristics (ROC) comparison test. Results: Colonoscopy (and biopsies) disclosed normal results in 85 (27.2%) subjects, A in 91 cases (30.3%) and AC in 95 (31.7%) patients. For the combined A+AC endpoint, the HS test had SE of 58.3% and SP of 96.5% (AUC=0.774), while the CV test had 97.2% SE and 85.8% SP (AUC=0.916) (p=0.0001). For the A endpoint, the difference between HS and CV was even more significant, AUC=0.637 and AUC=0.898, respectively (p=0.0001). In CV test, the Hb/Hp complex was 15% (93% vs. 78%) and 8% (96% vs. 88%) more sensitive than Hb alone, for the A and A+AC endpoints, respectively. Being more stable than Hb in the feces, the Hb/Hp complex detected 100% of the tumors in the proximal colon, as contrasted to only 41.2% and 52.9% by the Hb of HS and CV test, respectively (p=0.0001). Conclusions: With its 100% SE and 95.3% SP for proximal colon neoplasia, as well as 98.2% SE and 95.3% SP for the distal neoplasia, ColonView is superior to current FIT tests on the market, recently shown to exhibit pooled SE of 79% and pooled SP of 94% for colorectal cancer (CRC) in a comprehensive meta-analysis. With these exceptional performance indicators, ColonView quick test should be the test-of-choice for CRC screening.*

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Colorectal cancer (CRC) continues to be a major global disease burden. In 2012, CRC ranked in the third place among the most common malignancies with over 1.3 million new cases and 700,000 annual deaths worldwide (1). The global age-adjusted incidence and mortality rates of CRC are 17.2/100,000 and 8.3/100,000, respectively (1). In Russia, CRC is the most common cancer with almost 60,000 new cases (both genders) and the second most common cause of cancer mortality (n=39,907) (1).

At least 95% of all CRC cases arise from pre-existing polypoid or flat adenomas (2). The most convincing evidence comes from case-control studies reporting >60% reduction in CRC mortality over 10 years in persons subjected to

Table I. *Key characteristics of patients and their disease.*

Characteristics	Number (n=300)	Per cent
Age (M±SD)*	300	61.8 (11.6; range=21-84)
Sex:		
Women	187	66.7
Men	113	33.3
Type of colorectal lesion:		
Normal mucosa	85	27.2
Benign condition (non-tumor)	22	7.0
Hyperplastic polyp	7	2.2
Adenoma	91	29.1
Carcinoma	95	30.4
No histology**	13	4.1
Three-tier categories:		
Benign	114	38.0
Adenoma	91	30.3
Carcinoma	95	31.7
Localization of lesion:		
Right colon	14	6.5
Transverse colon	6	2.8
Left colon	8	3.7
Sigmoid	65	30.1
Rectum	59	27.3
Multiple sites	64	29.6

*For the patients included in the final cohort; **Patients who failed to attend colonoscopy

polypectomy as compared with matched controls (3). This long preclinical period during, which most CRCs develop from these precursor lesions, makes CRC a suitable target for population-based screening (4, 5), implemented in many countries using fecal occult blood (FOB) tests, flexible sigmoidoscopy (FS) and colonoscopy (6-8). Of these, different quaiac-based FOB tests have become very popular. These tests detect occult blood based on peroxidase activity of hemoglobin (Hb)-derived heme groups. Unfortunately, however, these quaiac-based tests are not specific for human blood as they also detect animal blood derived from food, as well as peroxidases from raw vegetables (6, 7). This inevitably leads to false-positive results and unnecessary referrals to colonoscopy. In addition, these tests are usually not highly sensitive, which can lead to false-negative results (9). Not unexpectedly, the impact of FOB test screening remains controversial (6, 7) and, in a recent meta-analysis, the ongoing FOB test screening trials (n=4) did not show any benefit for CRC mortality (risk ratio (RR)=1.00, 95% confidence interval (CI)=0.99-1.03) (5, 10).

In Japan, the pioneering country in CRC screening, different fecal immunochemical tests (FITs) have been the principal screening method since the early 1990's (11). During the recent past, a number of studies have been published where the performance of guaiac-based FOB tests was compared to the FIT tests (9, 12-17). Despite some controversial results, these data suggest that the sensitivity of the FIT assays is substantially better than that of the FOB tests (6, 7). This was also confirmed in the only randomized controlled trial reported so far implicating that the performance of FIT tests is clearly superior to FOB tests in detecting any type of colorectal neoplasia (16).

Clearly, more studies are mandatory to evaluate the performance of the new-generation FIT tests in comparison to the most sensitive guaiac-based FOB tests. One of these new-generation FIT tests is the Biohit ColonView quick test, based on rapid immunochemical detection of both Hb and haemoglobin/haptoglobin (Hb/Hp) complex in stool samples (15). In the present study, the ColonView test (CV) was compared against the market-leading quaiac-based FOB test (HemoccultSENSA) (HS) in a 100% biopsy-confirmed series of 300 colonoscopy-referral patients.

Patients and Methods

Study design. The present clinical trial is a direct comparison of a new-generation FIT test (Biohit ColonView quick test (thereafter CV) (Biohit, Oyj, Helsinki, Finland) and the market-leading guaiac-based test (HemoccultSENSA; Beckman Coulter, Fullerton, CA, USA) (thereafter HS) in detection of fecal occult blood (FOB) derived from clinically significant colorectal neoplasia. The endpoints in this study include colorectal adenomas (A) and adenocarcinomas (AC), classified by their anatomic site as proximal or distal. As additional endpoints, all non-neoplastic causes of FOB were also recorded, including *e.g.* diverticulosis, vascular malformations or inflammatory bowel disease (IBD) as these conditions can lead to false-positive results in FIT and FOB testing (6, 7, 9, 15).

Patients. This clinical trial was conducted in collaboration with two clinics in St. Petersburg, Russia: the Department of Oncology, City Hospital #9; and the First State Medical University (Pavlov). Enrolment of the study subjects took place among the consecutive patients (with no age limit) referred for colonoscopy at the Department of Oncology, City Hospital #9, with variable clinical indications. Patient enrollment was completed in two steps. First, the potentially eligible patients were identified among the colonoscopy-referral patients, informed about the study details and asked to sign a written consent. Those consenting to participate were given sample delivery boxes (for both tests) containing all necessary material for sampling, as well as instructions for sample collection, handling and mailing. Following the completion of the sampling, its delivery to the laboratory and analysis, all subjects were invited to diagnostic colonoscopy in the clinic. This study was approved by the institutional review board (IRB) of the clinic.

All adults were considered eligible, irrespective whether symptomatic or asymptomatic, who have i) been scheduled for diagnostic colonoscopy in the clinic and ii) given a written consent to participate. The following patients were considered non-eligible: a) Those patients who refused to participate, b) patients in whom the colonoscopic examination remained unsatisfactory

Table II. *Performance indicators of the HemoccultSENSA and ColonView test for different endpoints.*

TEST/ENDPOINT	SE (95%CI)	SP(95%CI)	PPV(95%CI)	NPV(95%CI)	AUC(95%CI)
HemoccultSENSA*					
Adenoma	30.8% (21.5-41.3)	96.5% (91.3-99.0)	87.5% (71.0-96.5)	63.6% (55.9-70.8)	0.636 (0.586-0.687)[1]
Adenoma+Carcinoma	58.6% (51.2-65.8)	96.5% (91.3-99.0)	96.5% (91.2-99.0)	58.8% (51.4-66.0)	0.775 (0.736-0.815)[2]
Carcinoma	85.3% (76.5-91.7)	96.5% (91.3-99.0)	95.3% (88.4-98.7)	88.7% (81.8-93.7)	0.909 (0.869-0.948)[3]
ColonView VR**					
Adenoma	94.5% (87.6-98.2)	85.1% (77.2-91.1)	83.5% (74.9-90.1)	95.1% (88.9-98.4)	0.898 (0.858-0.938)[1]
Adenoma+Carcinoma	97.3% (93.9-99.1)	85.1% (77.2-91.1)	91.4% (86.6.2-94.9)	95.1% (88.9-98.4)	0.912 (0.877-0.947)[2]
Carcinoma	100% (96.2-100)	85.1% (77.2-91.1)	84.8% (76.8-90.9)	100% (96.3-100)	0.925 (0.893-0.958)[3]
ColonView AR***					
Adenoma	94.7% (85.4-98.9)	79.8% (70.8-87.0)	72% (60.4-81.8)	96.5% (90.1-99.3)	0.873 (0.824-0.921)[11]
Adenoma+Carcinoma	97.2% (92.2-99.4)	79.8% (70.8-87.0)	83.5% (75.8-89.5)	96.5% (90.1-99.3)	0.885 (0.844-0.927)[21]
Carcinoma	100% (93.2-100)	79.8% (70.8-87.0)	71.2% (59.4-81.2)	100% (95.7-100)	0.899 (0.860-0.938)[31]

SE, Sensitivity; SP, specificity, PPV, positive predictive value; NPV, negative predictive value; CI, confidence interval; AUC, area under ROC curve; *Three consecutive samples from each patient, any sample positive; **Three consecutive samples from each patient, any single test positive for Hb or Hb/Hp (VR, visual reading); ***Three consecutive samples from each patient, any single test positive for Hb or Hb/Hp (AR, automate reading: n=226); [1,2,etc]Differences in AUC values (HemoccultSENSA=reference): [1]p=0.0001; [2]p=0.0001; [3]p=0.480; [11]p=0.0001; [21]p=0.0001; [31]p=0.718.

(judged by the endoscopist), as well as c) all patients who reported visible blood in the stools.

Altogether, 313 patients returned the stool samples for CV and HS testing. Of those who returned the fecal sample, 13 failed to attend the diagnostic colonoscopy and had to be excluded from the final analysis because of no histological confirmation, thus resulting in the total cohort of 300 subjects (187 women and 113 men) with complete data. The mean age of these patients is 61.8 years (range=21-84 years).

Patient instructions. As a FIT test, the CV quick test does not necessitate any preparatory steps of the patient or compliance with any restrictions in her/his daily dietary habits or daily medication. In contrast, reliable results from the HS test necessitate preparatory measures of the patients. To make the comparison of these two different tests as unbiased as possible, the clinics preserved the option for any patient who felt unsecure or clearly non-compliant with the instructions of HS sampling to refrain from the stool sampling for this test and only submit the sample for CV testing.

Sample collection for CV and HS tests. All sample collection for these two tests was done by the patients at home following the detailed instructions delivered to each patient as a part of the sample delivery box. After completion of the sample collection in three consecutive days, the patients delivered the sample box without delay to the test laboratory following the instructions of the manufacturers.

Sample processing and interpretation of the results. Due to the basically different principles of the CV quick test and HS test, the sample processing in the laboratory is different. For the HS, testing, only Hb is interpreted by visual reading as positive or negative. The analytical sensitivity of HS is reported to be 0.3 mg Hb/g feces.

Because the CV quick test consists of two components (Hb and Hb/Hp complex), the test result has four options: both components negative, both components positive, either Hb or Hb/Hp complex positive. The results were interpreted both by visual reading (VR) and by automatic reading (AR) using an opTrilyzer Lateral flow reader (opTricon GmbH, Berlin, Germany). For both tests, three stool samples were tested and the result was interpreted positive if any of the three samples tested positive. For CV, any sample positive for either Hb or Hb/Hp complex was classified as a positive test. The analytical sensitivity for Hb is 15 ng/ml and for the Hb/Hp complex, 4 ng/ml.

Colonoscopy and biopsy procedures. All 300 patients were examined by colonoscopy, thus providing the histological confirmation used as the gold standard in calculating the performance indicators of the two tests. If colonoscopy was completely normal, biopsies were not considered necessary and, in such a case, normal colonoscopy was used as an indicator of a negative result regarding the study endpoints.

Colonoscopy was performed according to the usual practice with a detailed record of all findings in the colonoscopy report. This applies to all study endpoints ((adenomas (A), adenocarcinomas (AC)) and other potential causes of occult blood (confounders), including their number, size and locations. The lesion site (caecum, ascending-, transverse-, descending colon, sigma, recto-sigmoid, rectum) was used as dichotomized variable (proximal and distal colon) in the final analyses.

All colonoscopy biopsies were examined by the expert pathologists at both clinics. The diagnoses were reported using the standard WHO classification of colorectal neoplasia. In addition to their size, all polypoid lesions were classified as hyperplastic polyps or adenomas, with the latter being further classified according to their histological pattern as tubular, tubulo-villous, villous or serrate adenomas.

Statistical analyses. For statistical analyses, three software were utilized (for special purposes): the SPSS 22.0.0.1 for Windows (IBM, New York, NY, USA), the STATA/SE 13.1 (STATA Corp., Texas, TX, USA) and the MedCalc 14.12.0 (MedCalc Software, Ostend, Belgium). The descriptive statistics were conducted according to routine procedures. Performance indicators (sensitivity (SE), specificity (SP), positive predictive value (PPV), negative

Table III. *Performance indicators of Hb and the Hb/Hp complex detection by ColonView* for different endpoints.*

TEST/ENDPOINT	SE (95%CI)	SP(95%CI)	PPV(95%CI)	NPV(95%CI)	AUC(95%CI)
COLONVIEW: VR Hb					
Adenoma	78.0% (68.1-86.0)	90.4% (83.4-95.1)	86.6% (77.3-93.1)	83.7% (76.0- 89.8)	0.842 (0.791-0.893)[1]
Adenoma+Carcinoma	88.2% (82.7-92.5)	90.3% (83.2- 95.0)	93.8% (89.1-96.8)	82.3% (74.4-88.5)	0.893 (0.857-0.928)[2]
Carcinoma	98.9% (94.3-100)	90.4% (83.4-95.1)	89.5% (82.0-94.7)	99.0% (94.8-100)	0.946 (0.917-0.976)[3]
COLONVIEW: VR Hb/Hp					
Adenoma	93.4% (86.2-97.5)	83.3% (75.2-89.7)	81.7% (72.9-88.6)	94.1% (87.5-97.8)	0.884 (0.841-0.927)[1]
Adenoma+Carcinoma	96.8% (93.1-98.8)	84.1% (76.0-90.3)	91% (86.1-94.6)	94.1% (87.5-97.8)	0.904 (0.868-0.940)[2]
Carcinoma	100% (96.2-100)	83.3% (75.2-89.7)	83.3% (75.2-89.7)	100% (96.2-100)	0.917 (0.882-0.951)[3]
COLONVIEW: AR Hb					
Adenoma	84.2% (72.1-92.5)	85.6% (77.3-91.7)	76.2% (63.8-86.0)	90.8% (83.3-95.7)	0.849 (0.790-0.908)[11]
Adenoma+Carcinoma	90.9% (83.9-95.6)	85.4% (77.1-91.6)	87.0% (79.4-92.5)	89.8% (82.0-95.0)	0.882 (0.838-0.925)[22]
Carcinoma	100% (93.2-100)	85.6% (77.3-91.7)	77.6% (65.8-86.9)	100% (95.9-100)	0.928 (0.894-0.962)[33]
COLONVIEW: AR Hb/Hp					
Adenoma	93.0% (83.0-98.1)	80.8% (71.9-87.8)	72.6% (60.9-82.4)	95.5% (88.8-98.7)	0.869 (0.818-0.919)[11]
Adenoma+Carcinoma	96.4% (91.0-99.0)	81.6% (72.7-88.5)	84.8% (77.3-90.6)	95.5% (88.8-98.7)	0.890 (0.848-0.931)[22]
Carcinoma	100% (93.2-100)	80.8% (71.9-87.8)	72.2% (60.4-82.1)	100% (95.7-100)	0.904 (0.866-0.942)[33]

SE, Sensitivity; SP, specificity, PPV, positive predictive value; NPV, negative predictive value; CI, confidence interval; AUC, area under ROC curve; *Pooled results for Lot 1 and Lot2; VR, visual reading; AR, automatic reading; [1,2,etc]Differences in AUC values: [1]p=0.111; [2]p=0.478; [3]p=0.023; [11]p=0.444; [22]p=0.649; [33]p=0.055.

predictive value (NPV) and their 95% confidence interval (CI)) of the two tests were calculated separately for each study endpoint using the "diagti" algorithm (in STATA) introduced by Seed *et al.* (2001) (18). This algorithm also calculates the area under receiver operating characteristics (ROC) called area under the curve (AUC) ((SE+SP)/2). For the CV test, all these indicators were calculated separately for Hb and the Hb/Hp complex. Significance of the difference between AUC values was estimated using the ROC comparison test (MedCalc) with 95%CI.

Before the start of enrolment, power analysis was conducted to estimate the necessary cohort size needed for adequate statistical power. The original calculations ending up with a cohort size of 500 were based on conservative estimates of 10-15% prevalence of FOB test positivity among these colonoscopy referral patients. This proved to be a clear underestimate in the current cohort in which, however, the true test positivity was markedly higher: 37.4% for the HS test and 59.1% for the CV test. This translates to true disease prevalence of 30.3% for As and 31.7% of ACs (Table I). With the true effect size difference between HS and CV tests (ranging from 0.14 up to 0.64 for ACs and As, respectively) (Table I), the present cohort of 300 subjects gives 100% power (even at alpha level 0.0001) to demonstrate the true difference between the two tests for all three study endpoints used: A (n=91), A/AC (n=186), AC (n=95).

Results

The key characteristics of the patients and their colorectal lesions are summarized in Table I. The mean age of the remaining patients (n=300) is 61.8 years (range=21-84 years). There is a clear preponderance of women in this series (66.7%). Out of the 300 subjects, 85 presented with completely normal mucosa on colonoscopy and histology. There were 22 cases where a non-neoplastic origin of occult blood was disclosed. The two main categories of lesions diagnosed include AC (n=94) and A (n=91). The single most frequent site of the lesion was sigma (n=65), followed by multiple-site lesions (n=64). Altogether, 20 lesions were located in the proximal colon (Table I).

Table II depicts the results of the two tests in detecting the different study endpoints. For each study endpoint, the CV test is clearly superior in performance to the HS test, as shown by the statistics comparing the AUC values. For both A and A/AC combined endpoint, this difference is highly significant (p=0.0001), but for AC, there was no difference. For all three endpoints, CV is up to 60% more sensitive, whereas HS is more specific (by 15-20%), which is not enough to compensate the poor sensitivity in the AUC comparison.

To assess the difference between Hb and the Hb/Hp complex detection, the CV test was stratified to its components and analyzed separately for all study endpoints (Table III). For both Hb and Hb/Hp complex detection, the VR and AR mode give practically identical results (AUC comparison, p=N.S.). The Hb/Hp complex detection is markedly more sensitive than Hb alone in detecting all three endpoints; however, the lower specificity precludes AUC comparison reaching statistical significance. In the VR mode, Hb/Hp detection is 15% more sensitive than Hb alone for the A endpoint and >8% more sensitive for the A/AC endpoint. This is counterbalanced by up to 7% higher specificity of Hb detection as compared with the Hb/Hp complex.

Table IV compares the two tests regarding their capacity of detecting proximal and distal neoplasias using the combined A/AC endpoint. The HS test performs particularly

Table IV. *Performance indicators of HemoccultSENSA and ColonView* in detection of neoplasia in the proximal and distal colon**.*

LESION SITE/TEST	SE (95%CI)	SP(95%CI)	PPV(95%CI)	NPV(95%CI)	AUC(95%CI)	
PROXIMAL COLON:						
HemoccultSENSA	41.2% (18.4-67.1)	100% (95.8-100)	100% (59.0-100)	89.6% (81.7-94.9)	0.706	(0.585-0.826)
ColonView Hb (VR)	52.9% (27.8-77.0)	97.7% (91.9-99.7)	81.8% (48.2-97.7)	91.3% (83.6-96.2)	0.753	(0.630-0.876)[1]
ColonView Hb/Hp (VR)	100% (80.5-100)	95.3% (88.5-98.7)	81.0% (58.1-94.6)	100% (95.6-100)	0.977	(0.954-0.999)[2]
ColonView Hb or Hb/Hp(VR)	100% (80.5-100)	95.3% (88.5-98.7)	81.0% (58.1-94.6)	100% (95.6-100)	0.977	(0.954-0.999)[3]
ColonView Hb (AR)	70.0% (34.8-93.3)	93.7% (85.8-97.9)	58.3% (27.7-84.8)	96.1% (89.0-99.2)	0.818	(0.666-0.970)[4]
ColonView Hb/Hp (AR)	100% (69.2-100)	92.4% (84.2-97.2)	62.5% (35.4-84.8)	100% (95.1-100)	0.962	(0.933-0.991)[5]
ColonView Hb or Hb/Hp(AR)	100% (69.2-100)	91.1% (82.6-96.4)	58.8% (32.9-81.6)	100% (95.0-100)	0.956	(0.924-0.987)[6]
DISTAL COLON:						
HemoccultSENSA	65.5% (56.0-74.2)	100% (95.8-100)	100% (95.1-100)	68.8% (59.9-76.8)	0.827	(0.783-0.871)
ColonView Hb (VR)	92.0% (85.4-96.3)	97.7% (91.9-99.7)	98.1% (93.4-99.8)	90.3% (82.4-95.5)	0.949	(0.919-0.978)[11]
ColonView Hb/Hp (VR)	96.5% (91.2-99.0)	95.3% (88.5-98.7)	96.5% (91.2-99.0)	95.3% (88.5-98.7)	0.959	(0.931-0.987)[21]
ColonView Hb or Hb/Hp(VR)	98.2% (93.8-99.8)	95.3% (88.5-98.7)	96.5% (91.3-99.0)	97.6% (91.7-99.7)	0.968	(0.942-0.993)[31]
ColonView Hb (AR)	93.2% (83.5-98.1)	93.7% (85.8-97.9)	91.7% (81.6-97.2)	94.9% (87.4-98.6)	0.934	(0.892-0.977)[41]
ColonView Hb/Hp (AR)	93.2% (83.5-98.1)	92.4% (84.2-97.2)	90.2% (79.8-96.3)	94.8% (87.2-98.6)	0.928	(0.884-0.972)[51]
ColonView Hb or Hb/Hp(AR)	94.9% (85.9-98.9)	91.1% (82.6-96.4)	88.9% (78.4-95.4)	96.0% (88.8-99.2)	0.930	(0.888-0.973)[61]

SE, Sensitivity; SP, specificity, PPV, positive predictive value; NPV, negative predictive value; CI, confidence interval; AUC, area under ROC curve; *Pooled results for Lot 1 and Lot2; VR, visual reading; AR, automatic reading; **Includes A and AC cases; Proximal (Cecum, ascending colon, transverse colon), Distal (descending colon, sigma, rectum); ^{1,2,etc}Differences in AUC values (HemoccultSENSA=reference): ^{1}p=0.426; ^{2}p=0.0001; ^{3}p=0.0001; ^{4}p=0.258; ^{5}p=0.0001; ^{6}p=0.0001; ^{11}p=0.0001; ^{21}p=0.0001; ^{31}p=0.0001; ^{41}p=0.0006; ^{51}p=0.0014; ^{61}p=0.0010. All non-neoplastic causes of FOB (n=29) were excluded.

poorly in detecting neoplasia in the proximal colon, with 41.2% SE only (AUC=0.706). The difference to the CV test Hb detection in VR- and AR-mode is not significant, however, with p=0.426 and p=0.258 for AUC comparison, respectively. Using the Hb/Hp complex of the CV test, however, the detection of proximal lesions increases significantly (p=0.0001) reaching 100% SE and over 95% (VR) and 91% (AR) SP.

As to the A/AC in the distal colon, the HS test performs better than for proximal lesions: AUC=0.827 and AUC=0.706, respectively (p=0.0646). The SE (65.5%) of HS is far inferior to that of CV, irrespective whether Hb detection alone (92.0% *vs*. 93.2% for VR and AR, respectively) or the Hb/Hp complex (96.5% *vs*. 93.2%, respectively, for VR and AR) is used. These figures are further improved for the complete CV test that records either Hb or the Hb/Hp complex positivity (AUC=0.968 and AUC=0.930, VR- and AR-mode).

Discussion

In principle, screening of CRC can provide possibilities for both i) the primary prevention (*i.e*., finding pre-cancerous adenomas that could later undergo malignant transformation) and ii) the secondary prevention (detecting early cancers that can be more effectively treated) (6-9). Until now, however, the impact of CRC screening by the conventional quaiac-based FOB tests (19, 20) has been doubtful. Indeed, a recent meta-analysis of the ongoing FOB test screening trials indicated no benefit for all-cause mortality (RR=1.00, 95%CI=0.99-1.03) (5, 10), strongly challenging the efficacy of CRC screening by conventional FOB testing (6, 7). In this respect, the new FIT tests seem more promising. When compared to, the quaiac tests in head-to-head settings (12-15, 17) and in a randomized controlled trial (RCT) (16), FIT tests proved to be superior to the quaiac tests in detecting any type of colorectal neoplasia (6, 7).

The accumulated literature on FIT tests was recently subjected to meta-analysis by Lee *at al*. (21). Out of the 53 available studies, 18 were found to be eligible for this formal meta-analysis, fulfilling all the inclusion criteria (9, 22-38). Accordingly, the pooled SE, SP, positive likelihood ratio and negative likelihood ratio of FITs for CRC detection were: 0.79 (95%CI=0.69-0.86), 0.94 (CI=0.92-0.95), 13.10 (CI=10.49-16.35) and 0.23 (CI=0.15-0.33), respectively, with an overall diagnostic accuracy of 95% (95%CI=93%-97%). There was a substantial heterogeneity between the studies in both the pooled SE and SP estimates (21). Interestingly, a single-sample FIT had similar SE and SP as multiple-sample testing, independent of the FIT brand. Unfortunately, the authors decided to conduct their meta-analysis using the CRC as the only study endpoint, mainly because of the substantial heterogeneity in the reporting practices of adenomas in different studies (21). Biohit ColonView quick test, as a newcomer (in 2014) in the field, was not among the FIT brands included in this meta-analysis.

The present study reports a direct (head-to-head) comparison of the new ColonView quick test and the leading FOB test (HemoccultSENSA) in the colonoscopy referral setting. Importantly, we preserved the option for any patient who felt unsecure or clearly non-compliant with the instructions of HemoccultSENSA sampling to refrain from sampling for this test and only submit the ColonView sample. Although potentially compromising the patient compliance, this approach offers an advantage for comparing these two tests without bias caused by the non-compliant dietary and medication restrictions inherent to all guaiac-based FOB tests (6, 7, 15, 16, 19, 20).

For all three study endpoints, CV is a more sensitive test, while HS is a more specific one (Table II). The small superiority in SP does not compensate the marked inferiority in SE of HS, however, which makes CV a clearly superior test in detecting A and A/AC endpoints (p=0.0001). The same applies to both the visual and automatic reading of the CV test, which give practically identical results. This difference in clinical performance of the two tests is clearly due to their different analytical sensitivity. CV, as a more sensitive test, detects practically all cases of occult blood (>95%), while HS, with a lower analytical sensitivity, misses 70% of the adenomas, >40% of the A/AC combined endpoint and 15% of AC cases. On the other hand, HS is more specific to all these neoplastic causes of FOB as these calculations (Table II) include all causes of bleeding and most of the non-neoplastic causes remain below the threshold of HS, although ranking (false-) positive with the CV test. Due to this very same reason, the PPV of HS to all neoplastic causes of FOB is higher than that of CV, which predicts occult blood due to any cause. In CRC screening, a sensitive test is preferred, however, because all occult blood is abnormal and important to detect. On the other hand, a test with low SE is a disadvantage because of the real danger of missing a substantial proportion of clinically important colorectal neoplasia (6, 7, 9, 12-17).

There is published evidence that detection of the Hb/Hp complex displays a significantly increased sensitivity in recognition of colorectal neoplasia, compared to the Hb detection (15, 39). This observation has a solid foundation in the known metabolism of the Hb molecule in any extra-vascular blood, FOB included. The Hb molecule consists of 2 pairs of peptide (α- and β-globins) chains and 4 heme groups, each with one atom of iron. Free Hb may separate into α-β molecules, which are bound to a protein called haptoglobin (Hp). This Hb/Hp complex plays an important role in the retrieval of Hb from the lysed erythrocytes and, importantly, this complex is very stable resisting acid and proteolytic degradation. This means that the Hb/Hp complex can be detected even after longer passage through the bowel, thus increasing the chance that also the blood derived from proximal adenomas and carcinomas (and even from the stomach) can be detected in the stool sample (15, 39). Out of the two tests compared here, HS is based on Hb detection alone, while CV detects both Hb and Hb/Hp complex. Thus, the mere demonstration of a significantly higher SE of the CV test (Table II) provides indirect confirmatory evidence to substantiate this observation of Sieg *et al*. (15) and Lüthgens *et al*. (39).

To provide direct evidence that detection of the Hb/Hp complex is superior to Hb alone, the performance indicators of our CV test were analyzed separately for its Hb and Hb/Hp components (Table III). The results are straightforward. For the adenoma endpoint, the Hb/Hp complex is 15% more SE than Hb alone and this difference is still 8% for the A/AC endpoint, but disappears for the AC endpoint. This is most likely explained by a more profuse leading from the malignant lesions, being detectable equally by Hb and Hb/Hp complex. This superior sensitivity of the Hb/Hp complex -as compared to Hb alone- becomes more accentuated when the lesions are stratified into proximal and distal tumors (A/AC endpoint) (Table IV). Exactly as suggested (15, 39), the Hb/Hp complex remains stable over the entire course of the large bowel in contrast to Hb, which is degraded on the way and remains undetectable both by HS and the Hb component of the CV test. Indeed, HS and Hb component of the CV test perform equally poorly in detection of proximal colon tumors, with no statistical significance in the AUC comparison test (p=0.426 and p=0.258 for VR- and AR-mode, respectively) (Table IV). The difference is dramatic when Hb/Hp detection is used; all proximal colon tumors are invariably detected (SE=100%). In these calculations, all non-neoplastic causes of FOB were excluded, to test the detection accuracy for the neoplastic lesions. This is in sharp contrast to the Hb testing alone, showing SE of 41.2% and 52.9% in the HS and CV test, respectively. These observations imply that, to be highly effective, a FIT test can be based on detection of the Hb/Hp complex alone, with no need to include the Hb component in the test.

Not unexpectedly, this difference between Hb and Hb/Hp complex is less dramatic for the distal colon tumors. Even HS performs markedly better in distal than in proximal tumors (AUC comparison, p=0.0646) but the difference to the CV test remains still highly significant (p=0.0001 for almost all settings). This includes also the comparison between HS and the Hb component of CV, with the latter being almost 30% more sensitive for distal tumors (65.5% *vs*. 92.0%). In this setting, where all non-neoplastic causes of FOB were excluded, also the differences in test specificity (shown in Table II) between HS and CV disappear completely confirming the above notions to explain the reason for this higher specificity of HS in settings where all causes of FOB are included. Thus, the only reason for the inferior sensitivity of HS -as compared to CV in both proximal and distal colon tumors- must reside in the lower analytical sensitivity of the former, leading to a significant proportion (almost 60% at worst) of false-negative results. Missing almost 60% of the

proximal colon tumors (A/AC) and even 35% of those in the distal colon, should invalidate the use of HemoccultSENSA as an acceptable CRC screening test (6, 7, 19, 20).

Taken together, the present study reports a direct (head-to-head) comparison of the new-generation FIT test and the leading FOB test in 100% biopsy-confirmed colonoscopy-referral setting adequately powered to permit unbiased demonstration of the true differences between the two tests. The ColonView quick test is superior in performance to HemoccultSENSA in detecting fecal occult blood derived from colorectal neoplasia. This difference is most significant for adenomas followed by the combined adenoma/carcinoma endpoint, but disappears for carcinomas. Similarly, the assay testing the Hb/Hp complex is 15% to 8% more sensitive than the detection of Hb-alone, pending on the study endpoints. This difference is most significant in the detection of proximal neoplasia, out of which almost 60% and 50% are missed by HS and the Hb component of CV, respectively, as contrasted to 100% detection by the Hb/Hp complex. These results clearly confirm the recent meta-analytical data on the superiority of FIT test over conventional quaiac-based FOB tests (6, 7, 9, 12-16).

Similarly, the Biohit ColonView quick test is clearly superior to the currently available FIT tests on the market (22-38) shown in a recent meta-analysis by Lee *et al*. 2014 (21) to exhibit a pooled SE of 79% and pooled SP of 94% for CRC. In the present study, ColonView showed 100% SE and 95.3% SP (AUC=0.977) for proximal colon neoplasia and 98.2% SE and 95.3% SP (AUC=0.968) for the distal neoplasia; both are exceptional performance indicators that advocate the prompt adoption of this new FIT assay as the test-of-choice for CRC screening.

References

1 Ferlay J, Soerjomataram I, Ervik M, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D and Bray, F: GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11 (Internet). Lyon, France: International Agency for Research on Cancer; 2013. Available from: http://globocan.iarc.fr, accessed on 13/January/2015.

2 Chen CD, Yen MF, Wang WM, Wong JM and Chen TH: A case-cohort study for the disease natural history of adenoma-carcinoma and de novo carcinoma and surveillance of colon and rectum after polypectomy: implication for efficacy of colonoscopy. Br J Cancer *88*: 1866-1873, 2003.

3 Selby JV, Friedman GD, Quesenberry CP Jr. and Weiss NS: A case-control study of screening sigmoidoscopy and mortality from colorectal cancer. N Engl J Med *326*: 653-657, 1992.

4 Schoenfeld P: Small and diminutive polyps: implications for colorectal cancer screening with computed tomography colonography. Clin Gastroenterol Hepatol *4*: 293- 295, 2006.

5 Hewitson P, Glasziou P, Irwig L, Towler B and Watson E: Screening for colorectal cancer using the faecal occult blood test, Hemoccult. Cochrane Database Syst Rev CD001216, 2007.

6 Whitlock EP, Lin J, Liles E, Beil T, Fu R, O'Connor E, Thompson RN and Cardenas T: Screening for colorectal cancer: An updated systematic review. Evidence Synthesis No. 65, Part 1. AHRQ Publication No. 08-05124-EF-1. Rockville, Maryland, Agency for Healthcare Research and Quality, October 2008.

7 Whitlock EP, Lin JS, Liles E, Beil TL and Fu R: Screening for colorectal cancer: An updated systematic review for the US Preventive Services Task Force. Ann Intern Med *149*: 638-658, 2008.

8 Klabunde CN, Frame PS, Meadow A, Jones E, Nadel M and Vernon SW: A national survey of primary care physicians' colorectal cancer screening recommendations and practices. Prev Med *36*: 352-362, 2003.

9 Allison JE, Tekawa IS, Ransom LJ and Adrain AL: Comparison of fecal occult-blood tests for colorectal-cancer screening. N Engl J Med *334*: 155-159, 1996.

10 Kronborg O, Jorgensen OD, Fenger C and Rasmussen M: Randomized study of biennial screening with a faecal occult blood test: results after nine screening rounds. Scand J Gastroenterol *39*: 846-851, 2004.

11 Sung J: Colorectal cancer screening: It's time for action in Asia. Editorial. Cancer Detect Prev *31*: 1-2, 2007.

12 Frommer DJ, Kapparis A and Brown MK: Improved screening for colorectal cancer by immunological detection of occult blood. Brit Med J *296*: 1092-1094, 1998.

13 Hoepffner N, Shastri YM, Hanisch E, Rösch W, Mössner J, Caspary WF and Stein J: Comparative evaluation of a new bedside faecal occult blood test in a prospective multicentre study. Aliment Pharmacol Ther *23*: 145-154, 2005.

14 Rozen P, Knaani J and Samuel Z: Comparative screening with a sensitive guaiac and specific immunochemical occult blood test in an endoscopic study. Cancer *89*: 46-52, 2000.

15 Sieg A, Thoms C, Lüthgens K, John MR and Schmidt-Gayk H: Detection of colorectal neoplasms by the highly sensitive hemoglobin-haptoglobin complex in feces. Int J Colorectal Dis *14*: 267-271, 1999.

16 van Rossum LG, van Rijn AF, Laheij RJ, van Oijen MG, Fockens P, van Krieken HH, Verbeek AL, Jansen JB and Dekker E: Random comparison of guaiac and immunochemical fecal occult blood tests for colorectal cancer in a screening population. Gastroenterol *135*: 82-90, 2008.

17 Wong BC, Wong WM, Cheung KL, Tong TS, Rozen P, Young GP, Chu KW, Ho J, Law WL, Tung HM, Lai KC, Hu WH, Chan CK and Lam SK: A sensitive guaiac faecal occult blood test is less useful than an immunochemical test for colorectal cancer screening in a Chinese population. Aliment Pharmacol Ther *18*: 941-946, 2003.

18 Seed PT and Tobias A: Summary statistics for diagnostic tests. Stata Techn Bull *59*: 9-12, 2001.

19 Ransohoff DF and Lang CA: Clinical guideline: Part I-suggested technique for fecal occult blood testing and interpretation in colorectal cancer screening. Ann Intern Med *126*: 808-810, 1997.

20 Ransohoff DF and Lang CA. Clinical Guideline: Part II-screening for colorectal cancer with the fecal occult blood test: A Background Paper. Ann Intern Med *126*: 811-822, 1997.

21 Lee JK, Liles EG, Bent S, Levin TR, and Corley DA: Accuracy of fecal immunochemical tests for colorectal cancer: Systematic review and meta-analysis. Ann Intern Med *160*: 171-181, 2014.

22 Sohn DK, Jeong SY, Choi HS, Lim SB, Huh JM and Kim DH: Single immunochemical fecal occult blood test for detection of colorectal neoplasia. Cancer Res Treat *37*: 20-23, 2005.

23 Levi Z, Birkenfeld S, Vilkin A, Bar-Chana M, Lifshitz I and Chared M: A higher detection rate for colorectal cancer and advanced adenomatous polyp for screening with immunochemical fecal occult blood test than guaiac fecal occult blood test, despite lower compliance rate. A prospective, controlled, feasibility study. Int J Cancer *128*: 2415-2424, 2011.
24 Allison JE, Sakoda LC, Levin TR, Tucker JP, Tekawa IS, Cuff T, Pauly MP, Shlager L, Palitz AM, Zhao WK, Schwartz JS, Ransohoff DF and Selby JV: Screening for colorectal neoplasms with new fecal occult blood tests: update on performance characteristics. J Natl Cancer Inst *99*: 1462-1470, 2007.
25 Levi Z, Rozen P, Hazazi R, Vilkin A, Waked A, Maoz E, Birkenfeld S, Leshno M and Niv Y: A quantitative immunochemical fecal occult blood test for colorectal neoplasia. Ann Intern Med *146*: 244-255, 2007.
26 Cheng TI, Wong JM, Hong CF, Cheng SH, Cheng TJ, Shieh MJ, Lin YM, Tso CY and Huang AT: Colorectal cancer screening in asymptomaic adults: comparison of colonoscopy, sigmoidoscopy and fecal occult blood tests. J Formos Med Assoc *101*: 685-690, 2002.
27 Morikawa T, Kato J, Yamaji Y, Wada R, Mitsushima T and Shiratori Y: A comparison of the immunochemical fecal occult blood test and total colonoscopy in the asymptomatic population. Gastroenterol *129*: 422-428, 2005.
28 Nakama H, Yamamoto M, Kamijo N, Li T, Wei N, Fattah AS and Zhang B: Colonoscopic evaluation of immunochemical fecal occult blood test for detection of colorectal neoplasia. Hepatol Gastroenterol *46*: 228-231, 1999.
29 Nakama H, Kamijo N, Abdul Fattah AS and Zhang B: Validity of immunological faecal occult blood screening for colorectal cancer: a follow up study. J Med Screen *3*: 63-65, 1996.
30 Launoy GD, Bertrand HJ, Berchi C, Talbourdet VY, Guizard AV, Bouvier VM and Caces ER: Evaluation of an immunochemical fecal occult blood test with automated reading in screening for colorectal cancer in a general average-risk population. Int J Cancer *115*: 493-496, 2005.
31 Itoh M, Takahashi K, Nishida H, Sakagami K and Okubo T: Estimation of the optimal cut off point in a new immunological faecal occult blood test in a corporate colorectal cancer screening programme. J Med Screen *3*: 66-71, 1996.
32 Nakazato M, Yamano HO, Matsushita HO, Sato K, Fujita K and Yamanaka Y: Immunologic fecal occult blood test for colorectal cancer screening. Japan Med Assoc J *49*: 203-207, 2006.
33 Park DI, Ryu S, Kim YH, Lee SH, Lee CK and Eun CS: Comparison of guaiac-based and quantitative immunochemical fecal occult blood testing in a population at average risk undergoing colorectal cancer screening. Am J Gastroenterol *105*: 2017-2025, 2010.
34 de Wijkerslooth TR, Stoop EM, Bossuyt PM, Meijer GA, van Ballegooijen M, van Roon AH, Stegeman I, Kraaijenhagen RA, Fockens P, van Leerdam ME, Dekker E and Kuipers EJ: Immunochemical fecal occult blood testing is equally sensitive for proximal and distal advanced neoplasia. Am J Gastroenterol *107*: 1570-1578, 2012.
35 Parra-Blanco A, Gimeno-García AZ, Quintero E, Nicolás D, Moreno SG and Jiménez A: Diagnostic accuracy of immunochemical versus guaiac faecal occult blood tests for colorectal cancer screening. J Gastroenterol *45*: 703-712, 2010.
36 Chiu HM, Lee YC, Tu CH, Chen CC, Tseng PH, Liang JT, Shun CT, Lin JT and Wu MS: Association between early stage colon neoplasms and false-negative results from the fecal immunochemical test. Clin Gastroenterol Hepatol *11*: 832-8.e1-2, 2013.
37 Chiang TH, Lee YC, Tu CH, Chiu HM and Wu MS: Performance of the immunochemical fecal occult blood test in predicting lesions in the lower gastrointestinal tract. CMAJ *183*: 1474-1481, 2011.
38 Brenner H and Tao S: Superior diagnostic performance of faecal immunochemical tests for haemoglobin in a head-to-head comparison with guaiac based faecal occult blood test among 2235 participants of screening colonoscopy. Eur J Cancer *49*: 3049-3054, 2013.
39 Lüthgens K, Maier A, Kampert I, Sieg A and Schmidt-Gayk H: Hemoglobin-Haptoglobin complex: A highly sensitive assay for the detection of fecal occult blood. Clin Lab *44*: 543-551, 1998.

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