see related editorial on page 2026

Comparison of Guaiac-Based and Quantitative Immunochemical Fecal Occult Blood Testing in a Population at Average Risk Undergoing Colorectal Cancer Screening

Dong Il Park, MD[1], Seungho Ryu, MD[2], Young-Ho Kim, MD[3], Suck-Ho Lee, MD[4], Chang Kyun Lee, MD[4], Chang Soo Eun, MD[5] and Dong Soo Han, MD[5]

OBJECTIVES: **Although some studies have shown that the quantitative, immunochemical fecal occult blood test (FOBT) (qFIT) has better performance characteristics than the standard guaiac-based FOBT (GT) for identifying advanced colorectal neoplasia (ACRN), there is limited information on test performance of these tests in average-risk populations.**

METHODS: **Seven hundred seventy consecutive average-risk patients from four centers who were undergoing screening colonoscopy also provided stool samples. Stool specimens from three consecutive bowel movements were applied to a hemoccult II test card (Beckman Coulter, Fullerton, CA) and OC-SENSA MICRO (Eiken Chemical, Tokyo, Japan) sampling probes at the same time. We measured the diagnostic value of the qFIT for detecting an ACRN by using three criteria: sensitivity, specificity, and likelihood ratios. A receiver operating characteristic curve for determining the qFIT cutoff values and the number of tests that best discriminated between ACRNs and other findings were determined.**

RESULTS: **Seventy-eight ACRNs were identified during colonoscopy. At all hemoglobin thresholds, the sensitivity of the qFIT was higher than that of the GT for cancer or ACRN. The sensitivity and specificity of the GT for detecting advanced adenomas, cancer, and ACRNs were 13.6%/92.4%, 30.8%/92.4%, and 16.7%/92.9%, respectively. Using the 100 ng/ml cut point and three-sample qFIT results, the sensitivity and specificity of the qFIT for detecting advanced adenomas, cancer, and ACRNs were 33.9%/90.6%, 84.6%/89.8%, and 43.7%/91.9%, respectively. The area under the curve for cancer indicated that using either 2 or 3 tests provided the best discrimination for cancer.**

CONCLUSIONS: **The qFIT provides a higher sensitivity for detecting ACRN and cancer than the GT, and has an acceptable specificity that significantly reduces the need for colonoscopic evaluation in the screened population.**

SUPPLEMENTARY MATERIAL is linked to the online version of the paper at http://www.nature.com/ajg

Am J Gastroenterol 2010; 105:2017–2025; doi:10.1038/ajg.2010.179; published online 25 May 2010

INTRODUCTION

Colorectal cancer (CRC) is one of the leading causes of cancer-related deaths in all industrialized countries (1). As a consequence of the characteristics of this cancer (a major effect on prognosis depending on the stage at diagnosis, and a long pre-clinical phase with frequent pre-cancerous lesions), substantial effort has been focused on devising an effective screening program. Colonoscopy is the most accurate test for detecting early cancers and for the detection and removal of advanced adenomas. However, because of its potential limitations, the availability of qualified endoscopists,

[1]Department of Internal Medicine, Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, Seoul, Korea; [2]Department of Occupational Medicine, Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, Seoul, Korea; [3]Department of Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea; [4]Department of Internal Medicine, Soon Chun Hyang University, Cheonan, Korea; [5]Department of Internal Medicine, Hanyang University Guri Hospital, Gyunggi-do, Korea. **Correspondence:** Dong Soo Han, MD, Department of Internal Medicine, Hanyang University Guri Hospital, Gyomun-dong 249-1, Guri-si, Gyunggi-do 471-701, Korea. E-mail: hands@hanyang.ac.kr
Received 9 October 2009; accepted 6 January 2010

as well as the higher cost, strategies, including the use of the fecal occult blood test (FOBT), have been proposed for large-scale population screening programs throughout the world.

Although annual or biennial guaiac-based FOBT (GT) screening reduces the incidence of CRC by 17–20% (2) and CRC mortality by 16–33% (3–5), the GT has been criticized due to poor sensitivity (6–8). For example, the sensitivity of the GT has been reported to be as low as 26% for cancers and 13% for large adenomas (6). A major disadvantage of the GT is the fact that it is not specific for human blood and can produce false-positive results when meats, fruits, or vegetables containing peroxidase are ingested (9). Also, bleeding in the upper gastrointestinal tract secondary to aspirin and non-steroidal anti-inflammatory drugs can produce false-positive tests. The false-positive tests, in turn, may lead to unnecessary colonoscopic evaluations, thus increasing the cost and risk of screening programs. False-negative tests may occur with ingestion of high doses of vitamin C, which will prevent detection of cancer and will provide a false sense of security for the patient. Efforts to develop a more sensitive GT have produced tests with poor specificity (10).

A laboratory-based, automated, quantitative immunochemical fecal occult blood test (qFIT) is human hemoglobin specific and eliminates the need for diet restriction, and, in addition, the hemoglobin quantification allows selection of a suitable threshold level for follow-up colonoscopy. Therefore, clinicians can adjust this threshold to take into account the patient's risk of advanced neoplasia and the availability of quality colonoscopy (11–14). A recent Israeli study showed a clinical laboratory-based qFIT that seems feasible for screening (15). They made full use of the quantitative nature of the test by measuring test characteristics at several fecal hemoglobin thresholds in high-risk patients who were scheduled for colonoscopy. Relative to an asymptomatic screening population, symptomatic patients were more likely to have both positive test results and the target disease. Therefore, it is difficult to apply their findings to an average-risk screening population because of the work-up bias. Also, they did not compare the qFIT with the standard GT.

In this prospective study, we compared the performance characteristics of the standard GT and the qFIT using different positivity cutoff values for the detection of advanced colorectal neoplasia (ACRN) or CRC in average-risk people undergoing screening colonoscopy, and determined the number of qFIT needed.

METHODS

Patients

From December 2007 to November 2008, 1,020 consecutive, asymptomatic, average-risk people between 50 and 75 years of age who underwent screening colonoscopy from four tertiary medical centers (Kangbuk Samsung Hospital, Samsung Medical Center, Hanyang University Guri Hospital, and Soonchunhyang University Hospital) in South Korea were invited to participate in the study. The study protocol was approved by the institutional review boards of each participating hospital, which confirmed that the study was in accordance with the ethical guidelines of the Helsinki Declaration. All participants provided written informed consent. The participants were not enrolled into the study if any of the following criteria were present: a history of inflammatory bowel disease; symptoms of disease of the lower gastrointestinal tract, including visible rectal bleeding, a recent change in bowel habits, or lower abdominal pain that normally would require a medical evaluation; menstruation at the time of obtaining a stool specimen; hematuria; a positive FOBT in the past 12 months; a history of colon polyps or colon cancer; a colonoscopy or sigmoidoscopy within the past 5 years; or a family history of colon cancer with either a single affected first-degree relative ≥55 years of age or at least two affected first-degree relatives of any age. Other exclusion criteria included a medical condition that could increase the risk associated with colonoscopy (active cardiac or pulmonary disease, or any other serious disease) or would preclude any benefit from colonoscopic screening (cancer or any terminal illness), and inability to prepare the stool specimen. We did not exclude patients with long-term use of non-steroidal anti-inflammatory drugs or anti-coagulant therapy that was discontinued transiently for colonoscopy.

Stool samples

All participants received a verbal explanation of the tests and the study kits contained a short description of the study, written instructions on how to prepare the stool samples, three disposable paper floats, three qFIT collection tubes (OC-SENSA MICRO; Eiken Chemical, Tokyo, Japan), three GT test cards (hemoccult II test; Beckman Coulter, Fullerton, CA), sampling probes, and identification labels in a plastic zipper bag. No dietary or medication restrictions were advised, so as to achieve as high a compliance rate as possible. Stool specimens from three daily or consecutive bowel movements were collected and applied on hemoccult II test card windows and the qFIT sampling probes at the same time during the week before the colonoscopy was performed. After emptying the bladder and flushing the toilet, but before having a bowel movement, participants placed a disposable paper float in the toilet bowl to immobilize the stool for easy sampling. Before preparing the sample, the patient wrote his or her name and the date on the tube. Samples for the GT were spread directly onto the filter paper containing guaiac gum through oval spaces on the test kit card. Each card contained two guaiac-impregnated windows, and fecal material from each stool sample was applied to two sites on the card, for a total of six samples per patient. The patient inserted the qFIT sampling probe into several different areas of the stool and then re-inserted the sampling probe firmly into the qFIT collection tube before sealing. The probe tip with the stool sample was suspended in a standard volume of hemoglobin-stabilizing buffer. Samples for both tests were stored in the study kit container (a plastic zipper bag) at 4 °C until development within 2 weeks. Participants brought the samples of both tests to the hospital and submitted the samples to the research nurse on the day of the colonoscopy.

GTs were developed on the day of reception without re-hydration by trained staff and under strict quality control (double reading, control of frequency of positive tests, and reproducibility) at

each hospital. Readers of the GT were blinded to the patient's history and to the result of the qFIT. Samples for the qFIT were sent to the central analysis center within 2 days and processed immediately using an OC-MICRO instrument (Eiken Chemical), as described earlier. Test results for the GT and qFIT were sent to the statistician independently.

Colonoscopy and histologic examination

After a standard bowel preparation, all patients underwent colonoscopy with visualization of the entire colon, including the cecum. The endoscopist was not aware of the results of either FOBT. Patients with an incomplete examination or poor bowel preparation were excluded from the analysis. All polypoid lesions were removed during colonoscopy and sent to the on-site pathologists for histologic evaluation. Hyperplastic polyps were not included as neoplasia. Adenomas were classified by number, size, location, and histologic characteristics. The endoscopist estimated the adenoma size with calibrated open biopsy forceps, which were 7 mm in diameter. We prospectively classified adenomas by size as small (< 10 mm), or large (≥10 mm), and by histologic characteristics (tubular, serrated, tubulovillous, or villous). We classified dysplasia as low- or high-grade. If a patient had > 1 adenoma, the most advanced pathologic lesion or the largest lesion was included in the analysis. In addition, we regarded the pathologic findings as taking precedence over size. For example, if a patient had both a 10 mm-sized adenoma with low-grade dysplasia and a 9 mm-sized adenoma with high-grade dysplasia, the patient was regarded to have an adenoma with high-grade dysplasia. The term "advanced adenoma" refers to tubular adenomas with diameters of ≥10 mm, or to tubulovillous or villous adenomas, or those with high-grade dysplasia regardless of size. We re-examined all advanced adenomas < 10 mm in size to confirm the histologic diagnosis. Patients with intramucosal carcinoma or carcinoma *in situ* were designated as having high-grade dysplasia. Cancer was defined as the invasion of malignant cells beyond the muscularis mucosa. ACRNs were defined as advanced adenomas or invasive cancer.

Patients with diverticuli, hyperplastic polyps, or non-bleeding hemorrhoids were classified as having normal colonoscopic findings for the purpose of analysis. Cases diagnosed with colitis or other overt bleeding sources were excluded from the analysis.

Statistical analysis

We analyzed CRCs and advanced adenomas separately, and together as ACRNs. We reported polyp sizes and fecal hemoglobin measurements as the mean (s.d.s). As the distribution of fecal hemoglobin measurements was not normally distributed, we used non-parametric tests for non-transformed data. Fisher's exact test was used to evaluate the categorical variables and the means were compared by the Mann–Whitney test for two independent groups. We compared fecal hemoglobin measurements in independent diagnosis categories by the Kruskal–Wallis test. A Bonferroni correction for multiple comparisons was performed.

To classify a patient's fecal hemoglobin level as normal or abnormal, we used the following two thresholds: 100 ng/ml (buffer) and 75 ng/ml, respectively. We also repeated these analyses at different thresholds in increments of 25 ng/ml, ranging from 50 to 150 ng/ml.

We measured the diagnostic value of the qFIT for detecting ACRN by using the following three criteria: sensitivity, specificity, and likelihood ratios. In this study, a receiver operating characteristic curve (16) for determining qFIT cutoff values, and the number of tests that best discriminated between ACRN and other findings was derived by plotting the sensitivity vs. 1 minus the specificity for each qFIT value. The optimal cutoff point was defined as the closest point on the receiver operating characteristic curve to the point at a 1 minus specificity of zero and a sensitivity of 100%. The areas under the curves represent the probability that a subject chosen at random who had ACRN had a higher test value than a subject who did not have this complication.

All statistical analyses were performed using SPSS software (SPSS 13.0 for Windows; SPSS, Chicago, IL) and Stata 10.0 software. A two-tailed *P* value of < 0.05 was considered to be statistically significant.

RESULTS

Patients and colonoscopy results

Figure 1 shows a summary of the study. Of the 1,020 patients who were scheduled for screening colonoscopies, 891 agreed to participate. We subsequently excluded 121 patients, and 770 patients completed the study. The patients include 51.4% males, with a mean age of 59.3 years (s.d. 7.5). Seven patients had incomplete colonoscopic examinations because of inadequate bowel preparation or technical problems. We repeated colonoscopies in six patients and included these patients in the study sample. Only one patient who did not have a repeat colonoscopy was excluded from the study.

Adenomas were present in 278 (28.4%) patients (**Table 1**). These adenomas were classified as small (<10 mm) in 222 (28.8%) patients, and large (≥10 mm) in 56 (7.3%) patients. Advanced adenomas were present in 59 (7.7%) patients. Invasive cancer was detected in 13 (1.7%) patients. Of these patients with cancer, 10 patients were classified as at Dukes' stage A or B and three patients

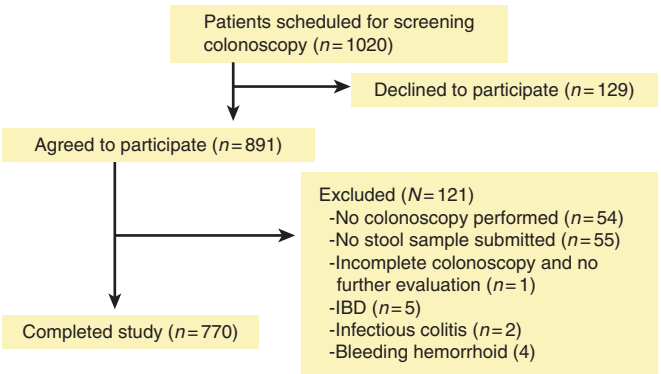


Figure 1. Study flow diagram. IBD, inflammatory bowel disease.

Table 1. Test results based on the colonoscopic findings

	Patients, *n* (%)	Mean lesion size (s.d.), mm	Positive GT, *n* (%)	Mean qFIT result (s.d.) (95% CI), ng/ml	Positive qFIT, *n* (%) 75 ng/ml	Positive qFIT, *n* (%) 100 ng/ml
Normal	479 (62.2)		35 (7.3)	50.7 (294.1), (4.8–100.2)	36 (7.5)	35 (7.3)
Adenoma	278 (36.1)	6.7 (5.6)	22 (8.0)	139.8 (516.9), (78.8–200.8)	46 (16.9)	40 (14.6)
Histology						
LGD	276 (99.3)	6.6 (5.5)	21 (7.6)	133.7 (472.8), (75.3–194.4)	44 (15.9)	39 (14.1)
HGD	2 (0.7)	20.0 (14.1)	1 (50.0)	985.5 (1287.6), (−10,583 to 12,554)	2 (100)	2 (100)
Size in diameter (mm)						
<10	222 (79.9)	5.6 (0.9)	15 (6.8)	58.3 (523.3), (8.62–224.4)	24 (10.8)	20 (9.0)
≥10	56 (20.1)	14.9 (7.7)	7 (12.5)	493.1 (921.3), (246.4–739.8)	22 (39.3)	20 (36.4)
Number						
<3	203 (73.0)	5.8 (5.1)	18 (9.0)	70.7 (342.7), (23.2–118.1)	25 (12.6)	20 (10.1)
≥3	75 (27.0)	9.2 (6.2)	4 (5.3)	326.8 (794.3), (144.1–509.6)	21 (28.4)	20 (26.7)
All non-advanced adenomas	219 (28.4)	4.8 (2.1)	14 (6.5)	68.7 (354.2), (21.5–115.9)	24 (11.2)	20 (9.3)
Advanced adenomas	59 (7.7)	13.7 (8.3)	8 (13.6)	403.5 (845.4), (183.2–623.8)	22 (37.3)	20 (34.5)
Proximal	33 (55.9)	14.4 (8.5)	7 (21.2)	641.7 (1021.1), (279.6–1,003.7)	19 (57.6)	18 (56.3)
Distal	26 (44.1)	12.8 (8.2)	1 (3.9)	101.3 (391.9), (57.0–259.6)	3 (11.5)	2 (7.7)
Cancer	13 (1.7)	33.8 (21.2)	4 (30.8)	845.5 (674.3), (447.0–1261.9)	12 (92.3)	11 (84.6)
Stages						
Dukes' A and B	10 (76.9)	30.5 (18.5)	3 (30.0)	689.3 (634.9), (235.1–1,143.5)	9 (90.0)	8 (80.0)
Dukes' C and D	3 (23.1)	45.0 (30.4)	1 (33.3)	1,405.0 (568.4), (6.97–2,817.0)	3 (100.0)	3 (100.0)
Location						
Proximal	11 (84.6)	36.4 (21.8)	3 (27.3)	836.5 (645.0), (403.1–1269.8)	10 (90.9)	10 (90.9)
Distal	2 (15.4)	20.0 (14.1)	1 (50.0)	953.5 (1,127.8), (91.7–1286.7)	2 (100.0)	1 (50.0)
ACRN	72 (9.4)	17.3 (13.9)	12 (16.7)	485.0 (831.3), (289.6–680.3)	34 (47.2)	31 (43.7)

ACRN, advanced colorectal neoplasia; CI, confidence interval; GT, guaiac-based fecal occult blood test; HGD, high-grade dysplasia; LGD, low-grade dysplasia; qFIT, quantitative immunochemical fecal occult blood test.

were classified as at Dukes' C or D. ACRN, including advanced adenoma and invasive cancer, occurred in 72 (9.4%) patients.

FOBT results

The GT was considered positive if any blue color appeared in the test slide window within 60 s after the addition of developer. We measured the hemoglobin content of each of the three consecutive fecal samples, and selected the highest value as a result. Overall, 61 (7.9%) patients were positive by GT, 94 (12.2%) patients were positive by qFIT at a threshold of 75 ng/ml, and 86 (11.2%) patients were positive by qFIT at a threshold of 100 ng/ml.

The positive rate of GT in patients with adenomas did not differ from the patients with normal colonoscopies (8.0% vs. 7.3%); however, the positive rate of qFIT at the 75 and 100 ng/ml thresholds was higher in patients with adenomas compared with that of patients with normal colonoscopies (16.9% vs. 7.5%, and 14.6% vs. 7.3% ($P<0.001$ and $P=0.002$), respectively (**Table 1**). Patients with adenomas ≥10 mm (493.1 ng/ml) in diameter, as well as patients with >3 adenomas (326.8 ng/ml), had significantly higher fecal hemoglobin levels compared with patients with normal examinations (50.7 ng/ml; $P<0.001$; **Table 1**).

Patients with villous or high-grade dysplasia did not have significantly higher positive rates of GTs (5/33 (15.2%) vs. 16/239 (6.7%), $P=0.09$), but had higher fecal hemoglobin levels (614.5 ng/ml vs. 74.2 ng/ml, $P=0.001$) than those with tubular adenomas with low-grade dysplasia (**Table 1**). Positive rates of GTs and the fecal hemoglobin measurements of patients with cancer were significantly higher compared with the patients with no neoplasia, regardless of cancer stage or site ($P<0.01$; **Table 1**). Positive rates of GTs and the mean fecal hemoglobin measurements of patients with ACRNs were significantly higher compared with patients with non-advanced adenomas or a normal colonoscopy ($P=0.005$ and $P<0.001$, respectively; **Table 1**).

We evaluated the intra-patient variation of the qFIT measurements. The correlation coefficients of the first and second qFIT samples, first and third samples, and second and third samples were 0.362, 0.603, and 0.584, respectively. These moderate correlations presumably reflect daily variations in blood loss.

Table 2. Performance comparison between GT and qFIT at the various hemoglobin thresholds

	Patients with true-positive result, *n*	Patients with false-negative result, *n*	Patients with true-negative result, *n*	Patients with false-positive result, *n*	Sensitivity (95% CI), %	Specificity (95% CI), %	Positive LR (95% CI), %	Negative LR (95% CI), %	Number of colonoscopies needed
AA									
Fecal Hb threshold									
≥50 ng/ml	26	33	628	83	44.1 (31.2–57.6)	88.3 (85.7–90.6)	3.8 (2.7–5.4)	0.6 (0.5–0.8)	4.2
≥75 ng/ml	22	37	638	73	37.3 (25.0–50.9)	89.7 (87.3–91.9)	3.6 (2.4–5.4)	0.7 (0.6–0.9)	4.3
≥100 ng/ml	20	39	644	67	33.9 (22.1–47.4)	90.6 (88.2–92.6)	3.6 (2.4–5.5)	0.7 (0.6–0.9)	4.4
≥125 ng/ml	17	42	651	60	28.8 (17.8–42.1)	91.6 (89.3–93.5)	3.4 (2.1–5.5)	0.8 (0.7–0.9)	4.5
≥150 ng/ml	16	43	655	56	27.1 (16.4–40.3)	92.1 (89.9–94.0)	3.4 (2.1–5.6)	0.8 (0.7–0.9)	4.5
GT	8	51	648	53	13.6 (6.0–25.0)	92.4 (90.2–94.3)	1.8 (0.9–3.6)	0.9 (0.8–1.0)	7.6
Cancer									
Fecal Hb threshold									
≥50 ng/ml	12	1	660	97	92.3 (64.0–99.8)	87.2 (84.6–89.5)	7.2 (5.7–9.2)	0.1 (0.0–0.6)	9.1
≥75 ng/ml	12	1	674	83	92.3 (64.0–99.8)	89.0 (86.6–91.2)	8.4 (6.5–10.9)	0.1 (0.0–0.6)	7.9
≥100 ng/ml	12	1	682	75	92.3 (64.0–99.8)	90.1 (87.7–82.1)	9.3 (7.1–12.2)	0.1 (0.0–0.6)	7.3
≥125 ng/ml	11	2	691	66	84.6 (54.6–98.1)	91.3 (89.0–93.2)	9.7 (7.0–13.5)	0.2 (0.1–0.6)	7.0
≥150 ng/ml	11	2	696	61	84.6 (54.6–98.1)	91.9 (89.8–93.8)	10.5 (7.5–14.7)	0.2 (0.1–0.6)	6.5
GT	4	9	690	57	30.8 (9.1–61.4)	92.4 (90.2–94.2)	4.0 (1.7–9.5)	0.8 (0.5–1.1)	15.2
ACRN									
Fecal Hb threshold									
≥50 ng/ml	38	34	627	71	52.8 (40.7–64.7)	89.8 (87.3–92.0)	5.2 (3.8–7.1)	0.5 (0.4–0.7)	2.9
≥75 ng/ml	34	38	637	61	47.2 (35.3–59.3)	91.3 (88.9–93.2)	5.4 (3.8–7.6)	0.6 (0.5–0.7)	2.8
≥100 ng/ml	32	40	643	55	44.4 (32.7–56.6)	82.1 (89.9–94.0)	5.6 (3.9–8.1)	0.6 (0.5–0.7)	2.7
≥125 ng/ml	28	44	649	49	38.9 (27.6–51.1)	93.0 (90.8–94.8)	5.5 (7.7–8.2)	0.7 (0.6–0.8)	2.8
≥150 ng/ml	27	45	653	45	37.5 (26.4–49.7)	93.6 (91.5–95.3)	5.8 (3.9–8.8)	0.7 (0.6–0.8)	2.7
GT	12	60	639	49	16.7 (8.9–17.3)	92.9 (90.7–94.7)	2.3 (1.3–4.2)	0.9 (0.8–1.0)	5.1

AA, advanced adenoma; ACRN, advanced colorectal neoplasia; CI, confidence interval; GT, guaiac-based fecal occult blood test; Hb, hemoglobin; LR, likelihood ratio; qFIT, quantitative immunochemical fecal occult blood test.

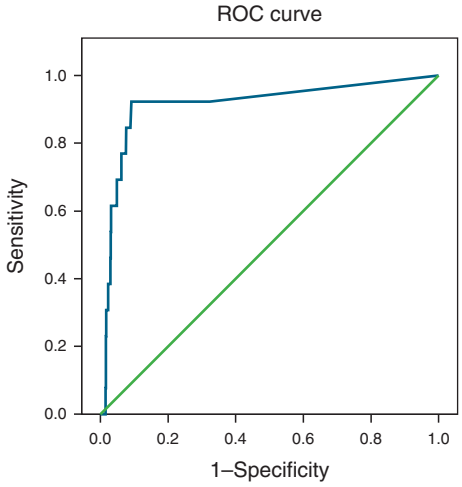
Performance comparison between GT and qFIT at the various hemoglobin thresholds

We measured the sensitivity, specificity, positive/negative likelihood ratios of the qFIT at different thresholds in increments of 25 ng/ml, ranging from 50 to 150 ng/ml and compared it with the GTs (**Table 2**). The lower the qFIT fecal hemoglobin threshold that was selected, the higher was the sensitivity for cancer or ACRN. Conversely, the higher the threshold that was selected, the

higher was the specificity for cancer or ACRN. At all hemoglobin thresholds, the sensitivity of the qFIT was higher than the sensitivity of the GT for cancer or ACRN. At thresholds of ≥125 and ≥150 ng/ml, the specificity of the qFIT was comparable to the GT for both cancer and ACRN.

The positive predictive values of the GT and the qFIT (using the 100 ng/ml cut point and three-sample qFIT results) for detecting advanced adenomas, ACRNs, and cancer were 13.1% (95% confidence interval (CI): 5.8–24.2)/23.3% (95% CI: 14.8–33.6), 19.7% (95% CI: 10.6–31.8)/36.0% (95% CI: 26.0–47.1), and 6.7% (95% CI: 1.8–15.9)/12.8% (95% CI: 6.6–21.7), respectively. The negative predictive values of the GT and the qFIT (using the 100 ng/ml cut point and three-sample qFIT results) for detecting advanced adenomas, ACRNs, and cancer were 92.7% (95% CI: 90.5–94.5)/94.2% (95% CI: 92.2–95.9), 91.4% (95% CI: 89.1–93.4)/93.9% (95% CI: 91.8–95.6), and 98.7% (95% CI: 97.6–99.4)/99.7% (95% CI: 98.9–100), respectively.

The fecal hemoglobin cutoff value that gives the best sensitivity and specificity for CRC was 118 ng/ml (**Figure 2**); however, this needs to be validated in a separate population.



Fecal Hb (ng/ml)	Sensitivity (%)	Specificity (%)
50	92.3	87.2
75	92.3	89.1
100	92.3	90.1
118	92.3	90.9
125	84.6	91.3
150	84.6	92.0
155.5	84.6	92.3

Figure 2. The best fecal hemoglobin cutoff value for detecting colorectal cancer with three quantitative immunochemical fecal occult blood tests. Hb, hemoglobin; ROC, receiver operating characteristic.

Table 3. Sensitivity and specificity for cancer and ACRN according to the number of samples

	≥75 ng/ml fecal Hb threshold		≥100 ng/ml fecal Hb threshold	
	Sensitivity (95% CI), %	Specificity (95% CI), %	Sensitivity (95% CI), %	Specificity (95% CI), %
AA				
qFIT 1	23.7 (13.6–36.6)	93.4 (91.3–95.1)	23.7 (13.6–36.6)	94.0 (91.9–95.6)
qFIT 1 or 2	27.1 (16.4–40.3)	91.4 (89.0–93.3)	27.6 (16.7–40.9)	92.4 (90.2–94.2)
qFIT 1, 2, or 3	37.3 (25.0–50.9)	89.5 (86.9–91.7)	34.5 (22.5–48.1)	90.4 (88.0–92.5)
GT (6 tests)	13.7 (6.0–25.0)	92.4 (90.2–94.3)		
Cancer				
qFIT 1	76.9 (46.2–95.0)	93.3 (91.2–94.9)	69.2 (38.6–90.9)	93.7 (91.7–95.3)
qFIT 1 or 2	92.3 (64.0–99.8)	91.4 (89.1–93.3)	84.6 (54.6–98.1)	92.2 (90.0–94.0)
qFIT 1, 2, or 3	92.3 (64.0–99.8)	88.8 (86.2–91.0)	84.6 (54.6–98.1)	89.8 (87.3–91.9)
GT (6 tests)	30.8 (9.1–61.4)	92.4 (90.2–94.2)		
ACRN				
qFIT 1	33.3 (22.7–45.4)	94.7 (92.8–96.2)	31.9 (21.4–44.0)	95.1 (93.3–96.6)
qFIT 1 or 2	38.9 (27.6–51.1)	92.9 (90.8–94.7)	38.0 (26.8–50.3)	93.8 (91.7–95.5)
qFIT 1, 2, or 3	47.2 (35.3–59.3)	91.1 (88.6–93.1)	43.7 (31.9–56.0)	91.9 (89.5–93.8)
GT (6 tests)	16.7 (8.9–27.3)	92.9 (90.7–94.7)		

AA, advanced adenoma; ACRN, advanced colorectal neoplasia; CI, confidence interval; GT, guaiac-based fecal occult blood test; Hb, hemoglobin; qFIT, quantitative immunochemical fecal occult blood test.
qFIT 1, the highest measurement of the first FIT; qFIT 1 or 2, the highest measurement of the initial two qFITs; qFIT 1, 2, or 3, the highest measurement of all three qFITs.

The number of qFITs needed to identify ACRN

The greater the number of tests performed per patient, the higher the sensitivity for cancer or ACRN, but conversely, the lower the specificity for cancer or ACRN at both the 75 and 100 ng/ml thresholds (**Table 3**). The area under the curve for cancer was the same when using the highest measurement of all three tests (qFIT1 vs. qFIT1, 2 or 3, 0.887 vs. 0.914, $P<0.05$) or the first two tests only (qFIT1 vs. qFIT1 or 2, 0.887 vs. 0.922, $P<0.05$), indicating that two tests give the best sensitivity and specificity for cancer (**Supplementary Figure 1** online). However, the area under the curve for ACRN was not significantly different when using the highest measurement of the first (0.723), the initial 2 (0.751), and all three qFITs (0.765) ($P>0.05$).

Analyses by number of colonoscopy examinations needed

Using the positive predictive data for each type of FOBT, the number of colonoscopies needed was calculated so as to detect cancer or an ACRN in persons with a positive test (**Table 2**). The number of colonoscopies needed with the GT was 5.1 per ACRN and those with the qFIT ranged from 2.7 to 2.9 per ACRN, depending on the threshold chosen (**Table 2**).

Patients with false-negative and false-positive qFIT results

One case of cancer (proximal ascending colon, 15 mm, Dukes' stage A, 0 ng/ml of fecal hemoglobin level) and 39 advanced adenomas (30 cases <10 mm in size) had fecal hemoglobin measurements <100 ng/ml. Conversely, 55 patients without ACRN had a fecal hemoglobin measurement >100 ng/ml. These 55 patients have been evaluated and followed-up clinically for a mean of 9.1 months (s.d. 3.8).

DISCUSSION

This colonoscopy-controlled study in an average-risk population allowed for a detailed evaluation for quantitative, immunochemical determination of fecal occult blood. Many earlier studies have provided information on FIT sensitivity and specificity for the detection of CRC; however, colonoscopy was performed only in FOBT-positive cases (17,18). Assessment of a true-positive rate or sensitivity for FIT in an average-risk population is extremely difficult because FITs have not been performed on large populations of asymptomatic average-risk adults undergoing colonoscopy. The strengths of this study include the colonoscopy examination on all study subjects regardless of FOBT result, and administration of three consecutive standard GTs and qFITs in all subjects. No English-language publication has systematically compared the GT and fecal immunochemical hemoglobin content with total colonoscopy findings in an average-risk population.

Allison *et al.* (19) assessed the performance characteristics of a sensitive GT (Hemoccult Sensa; Beckman Coulter) and an FIT (FlexSure OBT (it is now called Hemoccult ICT in the United States); Beckman Coulter) for detecting left-sided ACRNs and cancer in an average-risk population with all negatives having flexible sigmoidoscopy. They reported the lower sensitivity and higher specificity of the FIT for detecting left-sided ACRNs (sensitivity, 33.1% vs. 44.4%; specificity, 97.5% vs. 92.1%, respectively) and cancer (sensitivity, 81.8% vs. 92.3%; specificity, 96.9% vs. 90.1%, respectively) compared with our study. In a large Japanese study (20) of nearly 22,000 asymptomatic, average-risk patients who had screening colonoscopy and were given an FIT over a 20-year period, the sensitivity/specificity of one-time FIT for detecting ACRN and invasive cancer was 27.1%/95.1% and 65.8%/94.6%, respectively. However, it was a retrospective study, performed only one-time FIT, and the proportion of study participants <40 years was nearly 20%, with most participants being 38 or 39 years of age at the time of testing. The sensitivity for detecting ACRN and cancer was similar to the 31.9% and 69.2%, respectively, that we obtained by using only the first fecal sample and a 100 ng/ml hemoglobin threshold.

Quantification of the FIT and evaluating the results of all three tests provided us with information on choosing the number of tests needed to be prepared and the test threshold for development, so as to obtain high sensitivity with the fewest number of tests and need for colonoscopy follow-up. In this study, when choosing a qFIT at the manufacturer-recommended threshold of 100 ng/ml, the number of colonoscopies needed to identify a patient with ACRN was only 2.7 compared with 5.1 with a GT. The mean fecal hemoglobin value increased in a clinically important and statistically significant way as the most advanced finding went from normal colon to non-advanced adenoma to advanced adenoma to cancer. At the manufacturer-recommended threshold of 100 ng/ml, the three-sample test sensitivity and specificity for detecting cancer were 84.6% and 89.8%, respectively, and sensitivity and specificity for detecting ACRN were 43.7% and 91.9%, respectively. Levi *et al.* (15) reported the test performance of a clinical laboratory-based qFIT by measuring the test characteristics at several fecal hemoglobin thresholds in high-risk patients who were scheduled for colonoscopy. At the fecal hemoglobin threshold of 100 ng/ml, the three-sample test sensitivity and specificity for detecting cancer were 88.2% and 89.7%, respectively, and the sensitivity and specificity for detecting ACRN were 61.5% and 93.4%, respectively. Patient spectrum can influence screening test performance by affecting disease prevalence. Thus, a qFIT may be more sensitive and specific in a relatively high-risk population, whereby some of all study participants manifest signs or symptoms that increase the likelihood of having both a positive test result and the target disease. That may be a reason for the better test performance for detecting ACRN compared with our study (sensitivity, 61.5% vs. 43.7%; specificity, 93.4% vs. 91.9%, respectively).

The qFIT was superior to the standard GT in test performance characteristics. As anticipated, the sensitivity of the qFIT was higher than that of the standard GT for both cancer and ACRN at all hemoglobin thresholds. Interestingly, the positive rate of GT in patients with adenomas did not differ from patients with normal colonoscopies (8.0% vs. 7.3%); however, the positive rate of qFITs at 75 and 100 ng/ml thresholds was higher in patients with adenomas compared with patients with normal colonoscopies (16.9% vs. 7.5%, and 14.6% vs. 7.3, respectively; $P<0.05$; **Table 1**). In the recent American Cancer Society-US Multi-Society Task Force CRC screening guidelines (21,22), a preference was stated for tests that

detect adenomas over tests that detect cancer. In fact, one of the advantages of the qFIT is that it is more sensitive for adenomas than is the standard GT, and qFITs showed more than a twofold higher sensitivity to adenomas in this study. Although the sensitivity of the GT for detecting neoplasia increased as the histologic stage progressed, even the sensitivity for detecting invasive cancer was only 30.8%. Advanced adenoma consists of lesions ranging from large tubular adenomas to early adenocarcinomas that vary widely in terms of the risks for progression to fatal cancer (20,23). Clark *et al.* (24) reported that only 2.5/1,000 polyps per year progress to cancer, and Ransohoff (25) reported a rate of ~1% per year. Therefore, although the sensitivity of the qFIT for detection of advanced adenomas was lower than that for the CRC in this study, programmatic testing and sensitivity should allow for good advanced adenoma detection.

One of the important advantages of the qFIT is that the test output is a continuous variable, which means that a clinician can choose an FIT positivity threshold to suit the patient's clinical characteristics. For example, consider the case of an older man who prefers to avoid colonoscopy because of the risk of a screening complication and has less potential benefit from detecting cancer because of his limited life expectancy. The threshold for screening an average-risk population might differ from that for a higher-risk population. The objective of the threshold for an average-risk population should be to increase specificity and reduce the proportion of false-positive results. Even small changes in test specificity result in large effects on the number of false positives in a screening study, which are responsible for unnecessary colonoscopic examinations.

Deciding how many fecal samples are needed and choosing the optimal fecal hemoglobin threshold for screening an average-risk population will also involve evaluating the cost benefit and accessing colonoscopy. Published experience on the number of qFITs to collect and the development of thresholds to use for optimal sensitivity and specificity has focused on identifying cancer, and not necessarily high-risk adenomas. Bleeding from a colonic neoplasm is often intermittent, so at present, on the basis of experience with GTs, we collect three fecal tests. This is in contrast to the annual 2-day FIT collection in the average-risk population as routinely used in Japan and Australia, the 1-day annual testing in Europe, and the 1-day biennial testing performed in Italy (26–31). In the Japanese experience, the threshold chosen is 150 ng fecal hemoglobin/ml of buffer vs. 100 ng/ml threshold used in Europe (11,30,31). The more tests analyzed per patient improve sensitivity, but decrease specificity. The number of samples and the fecal hemoglobin threshold has not been determined in Korea. In this study, we showed that two samples and a 118 ng/ml of fecal hemoglobin threshold provides the best discrimination for CRC in an average-risk Korean population.

Our study had several limitations. First, the positive rates of GT (7.9%) and qFIT (11.2% (100 ng/ml threshold)) were higher than earlier reports in an average-risk screening population. Although we only included asymptomatic, average-risk people for this study, the study sample from tertiary medical centers could be different from the general population, suggesting that the positive rate from a hospital-based study may be overestimated compared with population-based studies. Lack of dietary restriction could be a reason for high-positive rate for GT. Also, the number of qFIT tests (three tests) performed on each patient could be a reason for high-positive rate. Second, the GT (35/479 (7.3%)) and qFIT (35/479 (7.3%)) at 100 ng/ml thresholds showed the same false-positive rate (**Table 1**). Third, despite concern about varying fecal consistency and its effect on sampling, fecal hemoglobin content varied systematically by site, pathology, and lesion size. As the immunochemical test for blood requires antigenically intact globin, we expected the lower level of immunochemical fecal hemoglobin associated with cancer in the proximal colon and expected the same with advanced adenomas. We were surprised to find that the qFIT was less sensitive at detecting advanced adenoma located in the distal colon vs. the proximal colon. A larger mean lesion size in the proximal advanced adenoma compared with a distal location (14.4 mm vs. 12.8 mm) may be an explanation. Fourth, although we used colonoscopy as the gold standard, this type of examination can be imperfect, even when performed meticulously. Two studies in which tandem colonoscopies were performed calculated the miss rate of colonoscopy (32,33). These studies showed that miss rates between the first and second colonoscopy ranged from 12% to 13% for adenomatous polyps 6–9 mm in size and from 0% to 6% for those 1 cm or larger. It is important to note that these values probably are underestimated because they were calculated from the findings of the second colonoscopy. Studies using virtual colonoscopy reported miss rates of 12–12.5% for adenomas 1 cm or larger (34,35). Considering these results, we may have underestimated the performance of qFIT. We did not supplement colonoscopy by routine computed tomography colonography, which could have identified a greater number of adenomas. Fifth, 55 patients with false-positive qFITs (≥100 ng/ml of fecal hemoglobin without ACRN) did not have endoscopic re-evaluation and were followed clinically for a short period.

In conclusion, we have found that the qFIT provides a higher sensitivity for detecting ACRN and cancer than the GT with an acceptable specificity, and can significantly reduce the number of colonoscopies needed in an average-risk Korean population. In addition, the qFIT provides the ability to choose a level of sensitivity and specificity appropriate for the patient's clinical characteristics.

CONFLICT OF INTEREST

Guarantor of the article: Dong Soo Han, MD.

Specific author contributions: Participated in the design, analysis, interpretation, and reporting of the findings: Dong Il Park, Young-Ho Kim, Suck-Ho Lee, Chang Kyun Lee, Chang Soo Eun, and Dong Soo Han; participated in the analysis of data: Seungho Ryu. All authors approved the final version.

Financial support: The instruments, reagents, and research grant were provided by the Eiken Chemical (Tokyo, Japan) and Shinyang Diagnostics (Seoul, Korea). The funding sources had no role in the design, analysis, or interpretation of the study, or in the decision to

submit the manuscript for publication. The funding sources neither read nor commented on the manuscript or made suggestions for content.

Potential competing interests: None.

Study Highlights

WHAT IS CURRENT KNOWLEDGE

- ✓ The quantitative immunochemical fecal occult blood test (qFIT) has shown better performance characteristics than the standard guaiac-based fecal occult blood test (GT) for detecting advanced colorectal neoplasms (ACRNs) in high-risk population.
- ✓ Test performance in average-risk people undergoing screening colonoscopy needs to be evaluated.

WHAT IS NEW HERE

- ✓ We did a prospective study in a large population of average-risk people in which everyone was colonoscoped after having qFIT and GT.
- ✓ We confirmed with better evidence the observations of others that qFIT has a higher sensitivity for detecting ACRNs than GT, and has an acceptable specificity that significantly reduces the need for colonoscopic evaluation in the screened population.

REFERENCES

1. Parkin DM, Bray F, Ferlay J *et al.* Global cancer statistics, 2002. CA Cancer J Clin 2005;55:74–108.
2. Mandel JS, Church TR, Bond JH *et al.* The effect of fecal occult-blood screening on the incidence of colorectal cancer. N Engl J Med 2000;343:1603–7.
3. Hardcastle JD, Chamberlain JO, Robinson MH *et al.* Randomised controlled trial of faecal-occult-blood screening for colorectal cancer. Lancet 1996;348:1472–7.
4. Kronborg O, Fenger C, Olsen J *et al.* Randomised study of screening for colorectal cancer with faecal-occult-blood test. Lancet 1996;348:1467–71.
5. Mandel JS, Bond JH, Church TR *et al.* Reducing mortality from colorectal cancer by screening for fecal occult blood. Minnesota Colon Cancer Control Study. N Engl J Med 1993;328:1365–71.
6. Rozen P, Levi Z, Hazazi R *et al.* Quantitative colonoscopic evaluation of relative efficiencies of an immunochemical faecal occult blood test and a sensitive guaiac test for detecting significant colorectal neoplasms. Aliment Pharmacol Ther 2009;29:450–7.
7. Imperiale TF, Ransohoff DF, Itzkowitz SH, *et al.*, Colorectal Cancer Study Group. Fecal DNA versus fecal occult blood for colorectal-cancer screening in an average-risk population. N Engl J Med 2004;351:2704–14.
8. Allison JE. The role of fecal occult blood testing in screening for colorectal cancer. Pract Gastroenterol 2007;XXXI:20–32.
9. Macrae FA, St John DJ, Caligiore P *et al.* Optimal dietary conditions for hemoccult testing. Gastroenterology 1982;82:899–903.
10. Allison JE. Review article: faecal occult blood testing for colorectal cancer. Aliment Pharmacol Ther 1998;12:1–10.
11. Nakama H, Zhang B, Zhang X. Evaluation of the optimum cut-off point in immunochemical occult blood testing in screening for colorectal cancer. Eur J Cancer 2001;37:398–401.
12. Wong WM, Lam SK, Cheung KL *et al.* Evaluation of an automated immunochemical fecal occult blood test for colorectal neoplasia detection in a Chinese population. Cancer 2003;97:2420–4.
13. Launoy GD, Bertrand HJ, Berchi C *et al.* 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 2005;115:493–6.
14. Vilkin A, Rozen P, Levi Z *et al.* Performance characteristics and evaluation of an automated-developed and quantitative, immunochemical, fecal occult blood screening test. Am J Gastroenterol 2005;100:2519–25.
15. Levi Z, Rozen P, Hazazi R *et al.* A quantitative immunochemical fecal occult blood test for colorectal neoplasia. Ann Intern Med 2007;146:244–55.
16. Moses LE, Shapiro D, Littenberg B. Combining independent studies of a diagnostic test into a summary ROC curve: data-analytic approaches and some additional considerations. Stat Med 1993;12:1293–316.
17. Allison JE, Tekawa IS, Ransom LJ *et al.* A comparison of fecal occult-blood tests for colorectal-cancer screening. N Engl J Med 1996;334:155–9.
18. Castiglione G, Zappa M, Grazzini G *et al.* Immunochemical vs. guaiac faecal occult blood tests in a population-based screening programme for colorectal cancer. Br J Cancer 1996;74:141–4.
19. Allison JE, Sakoda LC, Levin TR *et al.* Screening for colorectal neoplasms with new fecal occult blood tests: update on performance characteristics. J Natl Cancer Inst 2007;99:1462–70.
20. Morikawa T, Kato J, Yamaji Y *et al.* A comparison of the immunochemical fecal occult blood test and total colonoscopy in the asymptomatic population. Gastroenterology 2005;129:422–8.
21. Levin B, Lieberman DA, McFarland B *et al.* Screening and surveillance for the early detection of colorectal cancer and adenomatous polyps, 2008: a joint guideline from the American Cancer Society, the US Multi-Society Task Force on Colorectal Cancer, and the American College of Radiology. Gastroenterology 2008;134:1570–95.
22. Levin B, Lieberman DA, McFarland B *et al.* Screening and surveillance for the early detection of colorectal cancer and adenomatous polyps, 2008: a joint guideline from the American Cancer Society, the US Multi-Society Task Force on Colorectal Cancer, and the American College of Radiology. CA Cancer J Clin 2008;58:130–60.
23. Allison JE, Selby J. Screening for colorectal cancer. N Engl J Med 2001;345:1850.
24. Clark JC, Collan Y, Eide TJ *et al.* Prevalence of polyps in an autopsy series from areas with varying incidence of large-bowel cancer. Int J Cancer 1985;36:179–86.
25. Ransohoff DF. Lessons from the UK sigmoidoscopy screening trial. Lancet 2002;359:1266–7.
26. Smith A, Young GP, Cole SR *et al.* Comparison of a brush-sampling fecal immunochemical test for hemoglobin with a sensitive guaiac-based fecal occult blood test in detection of colorectal neoplasia. Cancer 2006;107:2152–9.
27. van Rossum LG, van Rijn AF, Laheij RJ *et al.* Random comparison of guaiac and immunochemical fecal occult blood tests for colorectal cancer in a screening population. Gastroenterology 2008;135:82–90.
28. Bampton PA, Sandford JJ, Cole SR *et al.* Interval faecal occult blood testing in a colonoscopy based screening programme detects additional pathology. Gut 2005;54:803–6.
29. Morikawa T, Kato J, Yamaji Y *et al.* Sensitivity of immunochemical fecal occult blood test to small colorectal adenomas. Am J Gastroenterol 2007;102:2259–64.
30. Nakama H, Zhang B, Fattah AS. A cost-effective analysis of the optimum number of stool specimens collected for immunochemical occult blood screening for colorectal cancer. Eur J Cancer 2000;36:647–50.
31. Castiglione G, Grazzini G, Miccinesi G *et al.* Basic variables at different positivity thresholds of a quantitative immunochemical test for faecal occult blood. J Med Screen 2002;9:99–103.
32. Hixson LJ, Fennerty MB, Sampliner RE *et al.* Prospective study of the frequency and size distribution of polyps missed by colonoscopy. J Natl Cancer Inst 1990;82:1769–72.
33. Rex DK, Cutler CS, Lemmel GT *et al.* Colonoscopic miss rates of adenomas determined by back-to-back colonoscopies. Gastroenterology 1997;112:24–8.
34. Pickhardt PJ, Choi JR, Hwang I *et al.* Computed tomographic virtual colonoscopy to screen for colorectal neoplasia in asymptomatic adults. N Engl J Med 2003;349:2191–200.
35. Pickhardt PJ, Nugent PA, Mysliwiec PA *et al.* Location of adenomas missed by optical colonoscopy. Ann Intern Med 2004;141:352–9.