Comparison of Immunochemical and Guaiac-Based Occult Fecal Tests with Colonoscopy Findings in Symptomatic Patients

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Abstract: *Purpose*: The fecal occult blood test is recommended for detecting colorectal cancer in asymptomatic patients. These tests are based on the fact that colonic cancer and large polyps spontaneously tend to bleed. Whether these tests are of any use in symptomatic patients remains debated. Our aim was to compare guaiac-based test and immunochemical test in symptomatic patients requiring total colonoscopy, to correlate these results to colonoscopy findings and to establish the performance of these tests for mucosal colonic lesion detection other than colorectal cancer.

Methods: Prospective study aimed at including 100 consecutive symptomatic patients whose condition required a total colonoscopy. All patients had 12 hours before endoscopy both tests performed on the same stool sample.

Results: 113 patients were included of which 100 had complete colonoscopy. Guaiac-based occult fecal test and the immunochemical test had similar performances whatever the mucosal injury and whatever the location of the injury were found at colonoscopy.

Conclusions: Despite numerous previous studies reporting higher performances of the immunochemical test over the guaiac-based test, the incremental increase performance of the immunochemical test remains non significant in the setting of the current study. Therefore generalization of this test should be taken with caution.

INTRODUCTION

Colorectal cancer is the third leading causes of death in Western countries and its incidence has constantly increased over the past recent years. Both men and women are at risk for colorectal cancer. It is more common in those who are 50 years of age or older. The US Preventative Services Task Force (USPSTF) recommends that initial screening be performed for all men and women who are 50 years of age and older and who are at average risk for colorectal cancer. Thus, the fecal occult blood (FOBT) test is recommended as a screening test for colorectal cancer in the general asymptomatic population [1-3]. This test has been shown to contribute to reduce the mortality attributable to this form of cancer [4]. The choice of screening method is based on available testing resources. The task force stated in 2002 that there are insufficient data to determine which screening strategy is best in terms of the balance of benefits and potential harms or cost-effectiveness [5]. Among the proposed tests, the Guaiac-based FOBT (gFOBT) has been criticized for its low sensitivity ranging from 50-60%, whereas its specificity is as high as 95%. In this condition, sensitivity refers to the ability of the test to produce a positive result in a patient with colorectal cancer. g FOBT also produce false-positive results when patients have ingested red meat. In 2002, immunochemical fecal occult blood tests (FIT) were introduced. These tests use antibodies that specifically detect the globin portion of human haemoglobin. In the study by Morikawa, FIT sensitivity for advanced neoplasia was 27.1% and 65.8% for cancer [1].

In this study, we performed two different FOBT including gFOBT and FIT the day before total colonoscopy in 100 consecutive symptomatic patients that were hospitalized and whose clinical conditions required a colonic examination. The main aim of our study was to analyze the ability of each test to detect blood in the stool specimen harvested few hours before bowel cleansing start for colonoscopy and to compare their results to colonoscopy findings. Thus the colonoscopy was our gold standard (presence of blood or not). We then determine the performance of the two tests to detect blood. In this particular setting, the colonoscopy was not aimed at screening for colorectal cancer but for all kind of other colonic mucosal alterations.

PATIENTS AND METHODS

The study was approved by the local Ethics Committee and all patients received a written information and gave their informed consent before inclusion as we usual do [6]. This prospective study was performed in hospitalized patients whose clinical problems further required an endoscopic examination of the colon. The study was performed in 1999 in our division and designed to get a total of 100 consecutive patients in whom total colonoscopy could be realized. Inclusion criteria were as follows: indication for colonoscopy including abdominal pain, diarrhea, constipation or recent change in the bowel habit, anemia of unknown origin after normal upper endoscopy. Exclusion criteria were as follows:

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1876-8202/09

blood in the stools, personal history of polyps. All eligible patients were included the day before colonoscopy. Participants were asked to prepare fecal samples from the same stool specimen by using the two collection kits provided by the nurse the day before colonoscopy and used as recommended by the commercial manufacturer. The collection of stool was performed before the start of bowel cleansing. Each time the sample was taken from two places of the faeces. The collection tubes were sent to the laboratory that performed the two different tests. gFOBT such as Fecatwin test detects the haem moiety of haemoglobin molecules by making use of the pseudoperoxidase activity of haem; haem releases oxygen from hydrogen peroxide, which then reacts with the colourless guaiac to form a blue dye. The detection limit for Fecatwin test (Fecatwin, Labsystems, Helsinky, Finland) is in the same range as for hemoccult and Hemofec and is about 0.7 to 2 ml of blood/100 g of stools [7]. FIT such as Obti test are said to be more sensitive because they use monoclonal or polyclonal antibodies raised against the globin moiety of human haemoglobin, detecting intact human haemoglobin or its very early degradation products. The detection limit for Obti test (Hexagon) is 0.88 mg of haemoglobin/g of stools.

No diet was administered before colonoscopy due to the short delay between admission and the endoscopy. The day before colonoscopy, patients received 3 L of a polyethylene glycol-based electrolyte solution for bowel preparation according to the instructions for use (Klean-prep, Norgine, Muttenz, Switzerland). Qualified gastroenterologists practicing in our division performed the colonoscopy. In all cases, the gastroenterologists were blinded to the results of the two tests. For the procedure, a standard colonoscope (PCF, Olympus, Zurich, Switzerland) was tentatively inserted into the caecum. Patients were excluded if the colonoscopic examination was incomplete because of problems with bowel preparations or failed colonoscopic insertion into the caecum. However, if the colonoscopy was incomplete because of the presence of an obstructing tumor, these results were included in the analysis. Sedatives such as midazolam were administered in each patient during the endoscopic procedure.

Macroscopic Findings During the Colonoscopy

During the colonoscopy, the location, the size and the nature of all lesions were recorded and imaged when appropriate. Endoscopists used biopsy forceps as a visual guide to better estimate the polyp size. All polypoid lesions were removed whenever possible or biopsied during the same colonoscopic session. Polyps < 5 mm in size were removed using hot biopsy forceps, whereas polyps > 5 mm in size were removed using diathermic snare. When we determined the performance of the two tests according to the location of the mucosal alteration, the distal colon included the descending colon, sigmoid colon and rectum, whereas the proximal colon included the transverse colon, ascending colon and caecum.

Pathologic Findings

Polyps as well as tissue specimens obtained after biopsy were fixed in formaldehyde for routine histologic examination and sent to the division of clinical pathology. Histologic characteristics of the polyps and the tissue samples included normal mucosa, hyperplastic polyp, adenoma, neoplasia, acute colitis and chronic colitis such as Crohn's disease, ulcerative colitis or ischemic colitis.

Statistical Analysis

Database management and statistical analysis were done using SPSS software (SPSS 10.0, SPSS Inc, Chicago, IL, USA). We used the χ^2 test and Anova test for comparison of proportion. A p value < 0.05 was considered significant.

RESULTS

Of the 113 consecutive patients enrolled in this study, a complete colonoscopy was performed in 100 patients (88.5%), whereas 13 were excluded because of an incomplete colonoscopy due to either insufficient bowel cleansing (8 cases) or inability to reach the caecum (5 cases) (Table **1**). The absence of diet before endoscopy may explain the number of incomplete colonoscopy. Indeed, many patients had food debris in the colon the day of the colonoscopy. There were no complications during colonoscopy. We were able to analyze data from each of the 100 patients who had a total colonoscopy. Among them, 56 were men and 44 were women aged between 21 and 90 years with a mean age of 67.8 ± 15.7 (Table **1**).

Findings performed throughout colonoscopy are described in Table **1**. Most of the colonoscopic findings are represented by polyps, colitis, diverticula and angiodysplasia. Polypectomy was performed in 19 cases during the same colonoscopic session, whereas 2 large and sessil polyps were removed ultimately during a second colonoscopy based on the histological analysis.

Among the 100 patients who undergone total colonoscopy, 42 had no colonic lesions that might explain a positive FOBT, whereas 58 had lesions (Table **1**) that either were bleeding (inflammatory colitis, ischemic colitis) or that might bleed or explain a positive FOBT. The sensitivity and the specificity of the gFOBT and of the FIT were 60% and 68% and 90 and 97%, respectively. The positive predictive value for the gFOBT and FIT was 89% and 97%, respectively (Table **2**).

The performances of both tests were not statistically different when analyzing only the data from the 40 patients who had fresh blood at colonoscopy due to either bleeding polyps, tumors or active colitis. Indeed, the sensitivity increased from 60 to 65% for the gFOBT and from 68 to 77% for the FIT whereas the specificity changed from 90% to 80% for gFOBT and from 97% to 85% for the FIT (Table **3**). Additionally, the performance of both tests were similar in the detection rate of invasive polyps (sensitivity 27% for gFOBT and 36% for FIT, p value non significant), and cancers (sensitivity 88% for both tests).

Finally, Table **4** summarized the results of both tests according to the location of the lesion that was bleeding during colonoscopy. Both test had better performances to detect occult blood when lesions of the colon were located on the left colon than on the right colon (Table **4**). gFOBT had the best negative predictive value when the lesion was located on the left colon.

Table 1. Characteristics of Study Population

Variable	Patients at Enrollment (%)	Eligible Patients	Failed Colonoscopy (%)
Number	113	100 (88.5)	13 (11)
Sex			
Male	61	56	5
Female	52	44	8
Age	68	67.8	70.1
Standard Deviation (SD)	15.4	15.7	13.2
Colonoscopy Findings			
Normal		42	
Abnormal		58	
Polyp < 5mm		11	
Polyp > 5mm		11	
Neoplasia		9	
Diverticulum		6	
Inflammatory colitis		12	
Angiodysplasia		5	
Rectal ulcer		3	
Ischemic colitis		3	
Vasculitis		1	
Rectal varice		1	
Stenosis		2	
Fresh blood at colonoscopy		40	

DISCUSSION

Early detection and removal of carcinomas and precancerous colonic adenomas can reduce mortality from colorectal cancer. Screening methods for colorectal cancer (FOBT) use the fact that colonic cancer and large polyps spontaneously tend to bleed [8]. These tests are aimed at detecting non-visible blood in the faeces, before there is any clinical evidence of bleeding. A variety of FOBTs are available including, guaiac, immunochemical and haem-porphyrin tests. Guaiac tests are generally best at detecting large, more distal lesions [9]. Because they depend upon peroxidase activity in the faeces, many variables may influence their results, including dietary factors, high doses of vitamin C, and aspirin that may cause gastrointestinal bleeding.

Table 2. Performances of gFOBT and FIT to Detect Blood

Test	gFOBT	FIT	P Value
Positive Test	39	41	
Negative Test	61	59	
Sensitivity (%)	60	68	ns
Specificity (%)	90	97	ns
Positive Predictive Value (%)	89	97	ns
Negative Predictive Value (%)	62	69	ns
False Positive Cases (%)	9	2	ns
False Negative Cases (%)	39	31	ns

Table 3. Performance of gFOBT and FIT to Detect Blood in Patients having Fresh Blood During Colonoscopy

Test	gFOBT	FIT	P Value
Positive Test	38	40	
Negative Test	62	60	
Sensitivity (%)	65	77	ns
Specificity (%)	80	85	ns
Positive Predictive Value (%)	68	77	ns
Negative Predictive Value (%)	77	85	ns
False Positive Cases (%)	2	15	ns
False Negative Cases (%)	35	22	ns

Immunochemical FOBTs have been reported to be more sensitive because they use monoclonal or polyclonal antibodies raised against the globin moiety of human haemoglobin, detecting intact human haemoglobin [1, 10]. They avoid interference from compounds which are known to affect the guaiac tests. The globin protein does not remain intact after passage through the upper gastrointestinal tract. Therefore, a positive FIT is specific for bleeding in the lower gastrointestinal tract. Recent studies report positive test results between 3% and 6% of screened populations. Van Rossum *et al.* have randomly compared gFOBT with FIT in a screening population [11] and found that differences in positive predictive value for cancer and advanced adenomas and cancer were, respectively, 2.1% (p = .4) and -3.6% (p = 0.5). However, the detection rate of the FIT may highly depend on the chosen cut-off values of haemoglobin/g of stool [12].

The current study was aimed to analyze the ability of gFOBT test and FIT to detect blood in the stool specimen harvested few hours before colonoscopy and to compare their results to the colonoscopy findings in patients who had abdominal complaints. In this study, the findings of the colonoscopy were used as the gold standard (presence of fresh blood or lesions that may bleed). Thus, the current study was absolutely not designed to screen our patients for

Table 4. Performances of g FOBT and FIT to Detect Blood According to the Location of the Lesion

Test Type Location	gFOBT		p Value	FIT		p Value
	Right Colon	Left Colon		Right Colon	Left Colon	
Positive Test	19	31		17	32	
Negative Test	54	56		56	55	
Sensitivity (%)	54	70	ns	61	85	ns
Specificity (%)	80	85	ns	85	85	ns
Positive Predictive Value (%)	37	68	ns	47	72	ns
Negative Predictive Value (%)	88	86	ns	91	94	ns
False Positive Cases (%)	20	15	ns	15	15	ns
False Negative Cases (%)	46	29	ns	38	14	ns

colo-rectal cancer but rather to test the performance of the two tests to detect blood in the stools of symptomatic patients for whom a total colonoscopy was required due to digestive complaints. Recent studies have provided information on the performance of FIT for the detection of colorectal cancer, in which the sensitivity ranged from 47% to 100%, and the specificity from 88 to 97% [13-15]. In these studies, however, colonoscopy was performed only in patient with positive test.

In our cohort, the rate of complete colonoscopy achievement was only 88.5% mainly because the bowel cleansing was not sufficient to allow a detailed investigation of the colon, a feature already reported in inpatients units. Colonoscopy was abnormal in 58 cases, polyps, cancer and colonic diverticula being the most frequent findings, whereas fresh blood was found in 40 patients. Surprisingly, the sensitivity and specificity of both screening tests were similar in our population, whereas the number of false positive cases were slightly higher in the gFOBT group than in the FIT group. To avoid this kind of problem, FIT for individuals with positive gFOBT have been suggested to decrease substantially the number of false positives in a screening programme for colorectal cancer [16]. When taking into consideration only patients with fresh blood detected during colonoscopy, FIT gained a non significant 12% increase over gFOBT (from 65 to 77%). Surprisingly, FIT was as good as gFOBT at detecting lesions located in the proximal colon versus in the distal colon, whereas Guitt *et al.* reported that among cancers the gain in sensitivity with FIT was confined to rectal cancer [17].

Our study has some limitations. First, it was not powered to definitely detect a significant difference between the two tests but our study was aimed to reflect the real life of an endoscopic unit. Moreover, the results are somewhat surprising because we expected a very significant difference between the two tests based on the current literature. The short delay between admission and colonoscopy made impossible the prescription of specific diet and this feature may have influenced the results of our study.

All these data taken together lead us to postulate that FIT can afford little or no advantage over gFOBT. Armitage *et al.* have already reported such results in population screening for colorectal cancer [18]. This is in contrast with the results of forensic studies performed at the crime scene where FIT has been demonstrated to be a powerful and robust tool as a confirmatory test for human blood [19]. Thomas *et al.* and St John *et al.* have reported that FIT were more sensitive only for symptomatic colorectal cancer than gFOBT [20, 21]. Robinson *et al.* found that the substantial positive predictive value for cancer warranted continuing evaluation in his study comparing a gFOBT with FIT in 1489 patients that completed both tests [22]. Another bias we may have in our study is the detection rate of the immunochemical test used by our laboratory. The detection rate was 0.88 mg hemoglobin/g of stool whereas Guitted *et al.* reported a detection rate of 0.1-0.2 mg hemoglobin/g of stool with more recent test provided by manufacturers [10]. This may be the pivotal difference between our study and more recent ones [10, 17]. Finally, using different positivity cut-off values for the immunochemical test is also of high importance and may explain various performances among studies reported by other groups [10, 15].

In conclusion, both gFOBT and FIT have, at least in our study, similar performances at detecting blood and colorectal cancer. Before generalization of the immunochemical test, we suggest that similar studies including large cohort of patients have to be undertaken.

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Received: February 26, 2009 Revised: April 27, 2009 Accepted: May 6, 2009