Accuracy of Detection of Colorectal Neoplasia Using an Immunochemical Occult Blood Test in Symptomatic Referred Patients: Comparison of Retrospective and Prospective Studies

Hirofumi Miyoshi, Masaki Oka, Kazunori Sugi, Osamu Saitoh, Ken-ichi Katsu and Kazuo Uchida

Abstract

Objective In this study the sensitivity and specificity of immunochemical tests for colorectal neoplasia were evaluated in retrospective and prospective studies.

Methods Four types of fecal blood tests – a chemical test (Hemoccult II) and three different immunochemical tests including a test which detects hemoglobin and transferrin – were performed in the retrospective study. In the prospective study the test for hemoglobin and transferrin was used for all patients that underwent total colonoscopy.

Patients One hundred seven patients with colorectal neoplasia, 57 with gastroduodenal bleeding, and 62 with normal digestive tracts were examined retrospectively. One thousand two hundred and ninety-eight nonspecifically symptomatic patients whose endoscopic examination was negative for hemorrhagic lesions in the upper digestive tract were examined prospectively.

Results In the retrospective study, sensitivities for the detection of colorectal cancers and adenomas with diameters ≥10 mm using the tests which detect hemoglobin and transferrin were 98% and 89%, respectively. These were the highest sensitivity among the four tests. The specificity of this test was 97%, which was higher than that of the Hemoccult II test. In the prospective study, the sensitivities of the tests for hemoglobin and transferrin for the detection of colorectal cancers and adenomas with diameters ≥10 mm were 79% and 33%, respectively. The specificity was 95%.

Conclusions The test for hemoglobin and transferrin showed the highest sensitivity and specificity for colorectal neoplasia in the retrospective study. The sensitivity and specificity of this test were not so high in the prospective study, but they may be clinically applicable in the evaluation of patients with various nonspecific symptoms.

Key words: hemoglobin, transferrin

Introduction

The effectiveness of fecal blood tests for the detection of colorectal cancer has been established (1). However, hemorrhage in the digestive tract is not a specific marker for colorectal cancer, so the diagnostic accuracy of such fecal blood tests is limited (2). Ahlquist et al (3) have prospectively studied the accuracy of the Hemoccult II and HemoQuant screening tests in high-risk groups, finding a sensitivity of 30% for colorectal cancer (3). In their previous retrospective study of referred patients, they have documented sensitivities of 67% and 97%, respectively, for the same tests (4). Assuming that the prospective study (which more closely resembles clinical test circumstances) more closely reflects test performance, the sensitivity of chemical tests in screening for colorectal cancer is low (5–9).

Recently, St. John et al (10) have reported results with an extremely sensitive and specific immunochemical test for detection of colorectal neoplasia in referred patients. However, these tests can be falsely negative due to the degradation of hemoglobin by intestinal bacteria (11–16), as the anti-hemoglobin antibodies used in these tests have a low sensitivity to degraded hemoglobin (17, 18). Therefore, screening for fecal occult blood using only immunochemical detection of hemoglobin still will miss occurrences of bleeding (19, 20). In the current study the sensitivity and specificity of the tests that detect both hemoglobin and transferrin (the Immunohemostick test), hemoglobin alone (the Immudia-HemSp test and OChemodia test), and chemical blood test (the Hemoccult II test) for various kinds of intestinal bleedings were compared in referred patients. From the results of the retrospective study, a prospective evaluation was conducted on what types of
Subjects

Retrospective study

During a 3-year period (January 1987 to December 1989) subjects with colorectal neoplasia were selected from groups of 93 patients with newly diagnosed colorectal cancer and 117 patients with newly diagnosed colorectal adenoma. These patients were endoscopically negative for bleeding upper digestive tract lesions and for gross hemorrhoidal bleeding. Patients were not receiving steroids or nonsteroidal anti-inflammatory drugs (NSAIDs). They were referred to our institute or others for surgery or polypectomy. Eligibility criteria included sufficient time for performance of the fecal blood tests before surgery or polypectomy without interfering with the timing of surgery and polypectomy. Patients had not obvious bleeding episodes and were not included if a mucosal biopsy specimen had been taken from the large bowel before testing. Patients meeting the above criteria included 21 symptomatic (diarrhea, constipation, abdominal fullness and pain) and 32 non-symptomatic patients with colorectal cancer (mean age: 65.0±8.5 years ±SD), 26 non-symptomatic patients with colorectal adenomas of ≥10 mm in diameter (mean age: 54.2±8.1 years), and 28 patients with colorectal adenoma with ranging 5 to 10 mm in diameter (mean age: 53.1±8.5 years). Subjects with gastroduodenal bleeding were selected from groups of 70 patients with newly diagnosed gastric cancer and 76 patients with peptic ulcer admitted over the 3-years period (January 1987 to December 1989). Fifty-seven patients with gastroduodenal bleeding (mean age: 52.1±8.8 years) in this study were consented to perform total colonoscopy and to collect three fecal samples. Actively bleeding lesions were excluded by total colonoscopy, and all of them were not receiving steroids or NSAIDs. Sixty-two controls (mean age: 48.7±8.2 years) consisted of volunteers in whom no lesions were detected by total colonoscopy and upper gastrointestinal endoscopy.

Prospective study

Subjects were selected from patients visiting our facilities from January 1990 to December 1995, who satisfied the following criteria: 1) between 45 and 80 years; 2) absence of bleeding lesions on upper gastrointestinal endoscopy. All patients had nonspecific abdominal symptoms. Exclusion criteria included the following: a) patients with melena, anemia, weight loss, emaciation, severe abdominal pain, or an apparent abdominal tumor; b) patients diagnosed with a colorectal neoplasm, inflammatory bowel disease, or other colonic disease; c) patients already diagnosed with a hematologic disease or malignancy involving other organs; d) patients with familial polyposis or other familial disorders with neoplasia; and e) patients who had received steroids or NSAIDs. All 1392 patients meeting these criteria consented to participate in this study. Forty-four patients failed to submit all these stool samples requested. Fifty-five patients did not arrive for their scheduled colonoscopy, including 5 of the patients not submitting all fecal blood test samples. After these 94 patients were disqualified, 1,298 subjects (703 men and 595 women; mean age 56.1±15.6 years) were included in data analyses. The fecal blood tests and colonoscopy were conducted in these patients at approximately the same time (maximum interval between fecal sample collection and colonoscopy, 30 days).

Collection of Fecal Samples

Retrospective study

All fecal samples were collected in a large bowl immediately following defecation and mixed thoroughly with a wooden spatula. Part of each fecal sample was then placed in a sealed, 3×4.5 cm vinyl chloride container and maintained at 4°C. Two to 8 hours later samples were transferred to a freezer and maintained at −20°C. The patients were given no dietary restrictions.

Prospective study

No patients were given dietary restrictions. When patients used a wash-out water closet with a flat pan (21) there were instructed to sit facing the tank, so that only a minimal amount of the stool would come into contact with the water. In the case of a syphonic water closet (21), in which the pan is filled with enough water, patients were instructed to empty the water while closing the original valve prior to defecation. In this prospective study, approximately eleven-twelfth of the patients used wash-out type, and one-twelfth used syphonic one. Nurses gave instructions for collection to the patients, who also received illustrated instructional brochures. A reagent stick (Fig. 1) was removed from a sheath and inserted into the specimen at several points. Three to 6 days of sample storage at room temperature did not affect the sensitivity of the test (16, 17).

Fecal Blood Tests

The following four fecal blood tests were performed as in the retrospective study: Hemoccult II; Immudia-HemSp, as a reversed passive hemagglutination test (9, 10, 22) (Fujirebio Inc., Tokyo); OC-hemodia Eiken, a latex slide test (23) (Eiken Chemical Co., Tokyo); and Immunohemostick (Kyoto Medical Lab Co., Kyoto; Fig. 1). The Immunohemostick test measures hemoglobin and transferrin simultaneously (17–20). Frozen samples were defrosted at room temperature for 1 to 2 hours, and then remixed in the vinyl chloride containers. All tests were performed according to the respective kit instructions. All technicians were blinded to clinical information and to the results of other tests. The Hemoccult II test was performed by experienced technicians who smeared aliquots from each sample onto both windows of the test cards and immediately added the catalyst solution without prehydration. A test card was reported as positive if either window developed a blue color within 30 seconds.

The Immudia-HemSp test used formalin-fixed chicken eryth-
Figure 1. The Immunohemostick test. The feces collector in this test consists of a stick (3 mm trick and 13 cm long) and a sheath (7.5 cm long) (A). The stick has a small hole 6 mm from the tip. Approximately 10 mg of stool is collected in the hole after several insertions into feces. Diluted anti-hemoglobin Ao monoclonal antibody and anti-transferrin polyclonal antibodies coat the surface of the stick up to 2.3 cm from the tip (B). The stick is designed so that when the collected stool sample contains hemoglobin or transferrin, it immediately reacts with the antibodies (18). The stick is stored in the sheath after fecal sampling. Rabbit antihemoglobin antibody and anti-transferrin polyclonal antibodies coat the surface of the stick. The sheath is designed so that when the collected stool sample contains hemoglobin or transferrin, it immediately reacts with the antibodies. Two prepunched disks with fecal smears were removed from each test card and placed in diluent in the wells of a microtiter plate. After elution for 10 minutes, serial dilutions of the fecal extract were prepared, and the chicken erythrocytes were added to wells that contained 1:4 and 1:8 dilutions. After incubation for 20 minutes at room temperature, hemagglutination was assessed visually and compared with a positive control. The test was interpreted as positive for occult blood if hemagglutination occurred at a 1:8 dilution. The OC-hemodias test used latex particles coated with anti-human hemoglobin antibody. Fecal samples were suspended in buffer solution in the specimen container. One drop of latex reagent was dropped within a circle on the test slide, and two drops of fecal suspension were added to the slide. Agglutination was assessed visually after the test slide was gently rotated for 3 minutes. For the Immunohemostick test the reagent stick (Fig. 1A) was inserted into a sample several times and then wiped with paper and inserted into a test tube after saline rinsing. Rabbit antihuman hemoglobin antibody and rabbit antihuman transferrin antibody, labelled with alkaline phosphatase, were used in the secondary reaction at 37°C for 1 hour. Then the alkaline phosphatase reaction was allowed to proceed for 30 minutes, at which point the color of each stick in the test tube was quantified using a colorimeter (Sanko Junyaku Company, Tokyo) at 510/630 nm. A sample was judged to be positive when the value was equal to or greater than 0.220 (19). For each of the above methods a patient was considered to have tested positive if one or more samples were positive when assessed without knowledge of results from the patient's other fecal blood tests or samples.

Only the Immunohemostick test, performed three times for each patient, was used in the prospective study. A specimen was considered to contain occult blood when at least one of the three samples was judged to be positive. The test results were masked at the time of colonoscopy so that these results did not influence diagnoses.

Diagnosis in the Prospective Study

We attempted to perform total colonoscopy after gut lavage with polyethylene glycol in all patients. The colonoscopy was terminated in 6% of the patients (78 cases) because the patient complained of severe pain. These patients underwent barium studies to examine the area in question. The size of colorectal adenomas was assessed by comparison with the biopsy forceps. Patients with colorectal neoplasia were classified on the basis of the size and histopathology of their lesion: colorectal cancers, adenomas ≥10 mm in diameter, or adenomas with diameters from 5 mm to 10 mm. Patients with adenomas of less than 5 mm in diameter were not considered a pathologic subgroup. Cases with other colorectal lesions such as hemorrhoids, angiodysplasia, rectal ulcer, and nonspecific colitis (colonoscopic evidence of local redness or mucosal opacity and pathologic evidence of inflammatory cell infiltration) were analyzed separately. Patients with colorectal cancer underwent surgical resection, and patients with adenoma underwent polypectomy. Patients with colorectal neoplasia underwent repeat colonoscopy within 1 year of the first examination. The patients having various symptoms continuously were treated conservatively and underwent some examinations including abdominal ultrasonography and barium study of small intestine. As to results obtained from the tests were referred in respective diagnoses.

Pathologic Diagnosis

Biopsy specimens of colorectal neoplasia and specimens obtained from polypectomy or surgical resection were assessed by 3 pathologists, according to the World Health Organization diagnostic criteria (24, 25).

Statistical Analysis

Differences in sensitivity and specificity among the four fecal blood tests performed in the retrospective study were compared with 95% confidence intervals (CI). In the prospective study positive rates of diagnostic results were calculated and divided into 6 subgroups as colorectal cancer, adenomas with diameter ≥10 mm, adenomas with diameters <10 mm, other hemorrhagic lesions (non specific colitis, rectal ulcer, and angiodysplasia), hemorrhoids, and normal study.
Table 1. Comparison of Positivity Rates among Four Types of Fecal Occult Blood Tests in Referred Patients

<table>
<thead>
<tr>
<th>Diseases</th>
<th>Hemoccult II</th>
<th>Immudia-HemSp</th>
<th>OC-hemodia</th>
<th>Immunohemostick</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastroduodenal Bleeding*</td>
<td>38/57 (66.7%)</td>
<td>21/57 (36.8%)</td>
<td>12/57 (21.1%)</td>
<td>19/57 (33.3%)</td>
</tr>
<tr>
<td>Colorectal Cancer†</td>
<td>46/53 (86.8%)</td>
<td>49/53 (92.5%)</td>
<td>45/53 (84.9%)</td>
<td>52/53 (98.1%)</td>
</tr>
<tr>
<td>Adenoma ≥10 mm</td>
<td>20/26 (76.9%)</td>
<td>14/26 (53.8%)</td>
<td>14/26 (53.8%)</td>
<td>23/26 (88.5%)</td>
</tr>
<tr>
<td>Adenoma &lt;10 mm†</td>
<td>12/28 (42.9%)</td>
<td>7/28 (25.0%)</td>
<td>1/28 (3.6%)</td>
<td>12/28 (42.9%)</td>
</tr>
<tr>
<td>Normal</td>
<td>15/62 (24.2%)</td>
<td>4/62 (6.5%)</td>
<td>2/62 (3.2%)</td>
<td>2/62 (3.2%)</td>
</tr>
</tbody>
</table>

Immudia-HemSp: hemagglutination test (Fujirebio Inc., Tokyo). OC-hemodia: latex slide test (Eiken Chemical Co., Tokyo). Immunohemostick: enzyme-linked immunosorbent assay that detects hemoglobin and transferrin (Kyoto Medical laboratory, Kyoto). *These 57 patients include 28 with gastric cancers and 29 with active gastric or duodenal ulcers. †Twenty Dukes’A, 10 Dukes’B, and 23 Dukes’C. ‡Adenoma with diameters ranging from 5 to 10 mm. a, b, c, d, e, f, g: A significant difference was observed in the sensitivity or specificity between both tests.

Table 2. Immunohemostick Test Results in Prospective Study

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Positive test (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colorectal Cancer*</td>
<td>11/14 (78.6%)</td>
</tr>
<tr>
<td>Adenoma ≥10 mm</td>
<td>15/46 (32.6%)</td>
</tr>
<tr>
<td>Adenoma &lt;10 mm†</td>
<td>24/183 (13.1%)</td>
</tr>
<tr>
<td>Other Hemorrhagic Lesions‡</td>
<td>16/34 (47.0%)</td>
</tr>
<tr>
<td>Hemorrhoids‡</td>
<td>30/236 (12.7%)</td>
</tr>
<tr>
<td>Normal Study</td>
<td>41/785 (5.2%)</td>
</tr>
</tbody>
</table>

*There were eleven Dukes’A, two Dukes’B patients, and one Dukes’C patient in this prospective study. False-negative results occurred in three Dukes’A patients. †All of these patients had colorectal adenomas with diameters ranging from 5 to 10 mm. ‡There were 20 patients with nonspecific colitis, 9 with rectal ulcer, and 5 with angiodysplasia. The patients with hemorrhoids and other lesions, such as a cancer, an adenoma, or other hemorrhagic lesions, were included in “Other Lesions”.

Results

Retrospective study (Table 1)
A significant difference was observed in the sensitivity of the Hemoccult II and the Immunohemostick tests in their detection of gastroduodenal bleeding (difference: 33.3%, CI of difference: 17.7 to 49.0). The sensitivity of the Immunohemostick test for gastroduodenal bleeding was 33.3%. The Hemoccult II test was more sensitive than immunochemical tests for the detection of upper gastroduodenal lesions. There also was a significant difference in the sensitivity of the Immunohemostick and the Hemoccult II tests (difference: 11.3%, CI of difference: 1.3 to 21.3) and the Immunohemostick and the OC-hemodia tests (difference: 13.2%, CI of difference: 4.1 to 22.3) in their detection of colorectal cancer. The sensitivity of the Immunohemostick test for colorectal cancer detection was 98.1%. A significant difference in the sensitivity of colorectal adenomas with diameters ≥10 mm was observed between the Immunohemostick and the Immudia-HemSp tests (difference: 34.6%, CI of difference: 16.3 to 52.9) and the Immunohemostick and the OC-hemodia tests (difference: 34.6%, CI of difference: 16.3 to 52.9). The sensitivity of the Immunohemostick test for adenomas with diameters ≥10 mm was 88.5%. A significant difference was observed in the sensitivity of the Immunohemostick and the OC-hemodia tests in the detection of colorectal adenomas with diameters <10 mm (difference: 28.6%, CI of difference: 6.8 to 51.4%). The sensitivity of the Immunohemostick test in the detection of the adenomas with diameters <10 mm was 42.9%. A significant difference was observed between the Hemoccult II and the Immunohemostick tests (difference: 21.0%, CI of difference: 9.9 to 32.0) in their specificity. The specificity of the Immunohemostick test calculated from the data of positive rate for normal controls was 96.8% (Table 1).

Prospective study (Table 2)
The positive Immunohemostick tests were 151 (11.6%) in 1,298 patients. No lesions were found in small intestine by performing barium study for continuously symptomatic 52 patients. Sensitivities for colorectal cancer, adenomas with diameters ≥10 mm, and adenomas with diameters <10 mm were 78.6%, 32.6%, and 13.1%, respectively. The sensitivity for other hemorrhagic lesions and hemorrhoids were 47.0% and 12.7%, respectively. The prevalence rates of adenomas with diameters <10 mm (24/151, 15.9%) and hemorrhoids (30/151, 19.9%) in positive tests patients were nearly same as those in negative tests patients (159/1,147 and 205/1,147). The specificity of this test was 94.8%. The positive and negative predictive value of this test for colorectal cancer or adenomas with diameters ≥10 mm were 17.2% and 3.0%, respectively.

Discussion
In this retrospective study the sensitivity and specificity of four fecal blood tests were evaluated in patients with intestinal bleeding of various causes, none of whom were under dietary
restrictions for red meat or vegetables. The present results showed that the Immunohemostick test, which detects hemoglobin and transferrin simultaneously, had the highest sensitivity for the detection of colorectal cancer. While there were no statistically significant differences in the specificity of three types of immunochemical fecal blood tests (Table 1), the Immunohemostick test was more sensitive in the detection of adenomas which bleed less than cancers (18). This is because the anti-hemoglobin antibody, the sole antibody in the Immudia-HemSp and OC-hemodia tests, has a low sensitivity for hemoglobin degraded by bacteria and transferrin showed lower degradation (16, 18). In this retrospective study, the Immunohemostick and Immudia-HemSp tests may detect the bleeding from adenomas with diameters ranging from 5 mm to 10 mm, while our former study demonstrated that these tests fail to detect that from adenomas <5 mm (26).

In the current prospective study we used a fecal blood test with proven value in the retrospective study regarding sensitivity and specificity for detection of colorectal neoplasia. The protocol had to be simplified as much as possible to limit variation in the fecal collection process. The Immunohemostick test, an enzyme-linked immnosorbent assay was also adopted in the prospective study because it is a quantitative test. The Hemoccult II test, which has the same sensitivity as the Immunohemostick test for detection of adenomas with diameters <10 mm, has demonstrated reveals a high false positive rate in patients without bleeding lesions in the digestive tract if these patients are not subjected to dietary restrictions (27). The high yield of the Hemoccult II test for colorectal neoplasia probably also partly reflects incidental false-positive results.

The main criterion for selection of patients in the prospective study was that the patients required further examination. It is important to determine what type of test should be performed in outpatients, all of whom have nonspecific symptoms (28). Many of these patients will be diagnosed with irritable bowel syndrome (29) and dyspepsia (30).

The fact that the patients selected in the prospective study may not have lesions that result in upper digestive tract bleeding is an important factor. The reason for this is indicated in the test results for referred patients in the retrospective study (Table 1). All of immunochemical fecal blood tests that were examined in this study may detect some of fecal blood with upper intestinal bleeding such as peptic ulcer and gastric cancer. Comparison of results obtained with other immunochemical tests indicate that detection of transferrin (which exists in only trace amounts in blood) did not ensure adequate sensitivity for the detection of upper gastrointestinal bleeding.

Fecal blood tests may not be expected to have high sensitivity for only colorectal neoplasia, because of detecting consequential lesions affecting test accuracy for colorectal neoplasia, such as trivial perianal lesions, angiodysplasia, and non-specific colitis (Table 2). Therefore, total colonoscopy was performed for all 1,298 patients in the prospective study, in order to detect these hemorrhagic lesions. While both gastroduodenal endoscopy and total colonoscopy were performed, all of bleeding lesions in digestive tract can not be detected. However, these patients may have no lesions in small intestine because we have performed barium studies of small intestine for continuously symptomatic patients.

The ability of the Immunohemostick test to detect adenomas with diameters ≥10 mm was also demonstrated in this prospective study. However, in contrast with the results of the retrospective study, it may be that the positive results yielded with this test for adenomas <10 mm (lesions from which bleeding can occur, Table 1) were serendipitous in the prospective study. Thus, differences were observed between the results of the retrospective study and prospective studies. This may be attributable to differences in the methods used to collect stool samples and selection bias of patients. While all feces excreted were thoroughly mixed in the retrospective study, stool samples in the prospective study were collected from a stick inserted into the excreted feces at several points. This suggests that blood from the large intestine was not homogeneously distributed throughout the feces.

In this prospective study adenomas were detected in approximately 18% of the selected patients. While patients with non-specific abdominal symptoms are thought to be at high risk for colorectal neoplasia, a high percentage of patients should be performed further examinations. Therefore, use of the fecal blood test may facilitate the determination of which patients must undergo invasive examinations (colonoscopy and barium enema study), thus allowing us to reduce the number of invasive tests performed.

In terms of cost-effectiveness it may not be recommended that physicians order the Immunohemostick test (about 3.5$ per test) in place of the Hemoccult II test for average-risk patients. The higher positive rate of the Immunohemostick test (12%) obtained in the current study was mostly attributed to the subject selection methods that symptomatic patients were examined. The cost-effectiveness of this test will be addressed by investigating the cause of the low specificity obtained in the prospective study.

With respect to the cancer-screening guidelines recommended by the American Cancer Society (31) or by the National Cancer Institute (32) (e.g. flexible sigmoidoscopy for persons older than 50 years), the protocol followed ultimately depends on the specific community of physicians (33). While a screening method using sigmoidoscopy can prevent deaths by colorectal cancer (34–36), up to 40% of colorectal neoplasms may go undetected (37). Further consensus among physicians may be obtained if rather than sigmoidoscopy, we adopted another strategy in which patients who have tested positive on the Immunohemostick test are then subjected to a total colonoscopy.

In summary, the relatively high sensitivity of the fecal occult blood test in detecting colorectal neoplasia was verified in the retrospective and in a strict prospective study. Because of the highly selected nature of subjects studied, caution should be taken so as not to overstate the detection sensitivity of the Immunohemostick test, these results cannot be extrapolated onto the screening setting. The application of this test would change the management of patients with non-specific symp-
tions that need total colonoscopy.

Acknowledgements: This study was supported by the Fund of Cancer Research from Hyogo Total Health Association in Japan and Kyoto Medical Science Laboratory, Hazukashi, Fushimi-ku, Kyoto 612 Japan.

References


