A Comparative Study of Three Fecal Occult Blood Tests in Upper Gastrointestinal Bleeding

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The purpose of this study was to evaluate the performance characteristics of three fecal occult blood tests (FOBTs): the chemical *o*-toluidine test, the immunochemical OC-Hemodia test, and the immunochromatographic Quick Chaser Occult Blood (QCOB) test, which detect human hemoglobin and transferrin simultaneously in cases of upper gastrointestinal (GI) bleeding. Included were 48 FOBT specimens in 48 consecutive admission cases of upper GI bleeding (endoscopy confirmed). We excluded those fecal specimens with an obvious tarry and bloody appearance. The QCOB test revealed the highest positive rates of 33/48 (68.8%), and significantly higher positive rates than that of the OC-Hemodia test and *o*-toluidine test ($p < 0.025$ and < 0.01, respectively). In the patient group with upper GI bleeding due to gastric and duodenal ulcers, the QCOB test had higher positive rates (68.6%) than did the *o*-toluidine test (34.3%) ($p < 0.01$). There was no fecal specimen that was positive for the *o*-toluidine test or OC-Hemodia test and was negative for the QCOB test. Our results reveal that the QCOB test has significantly higher positive rates of fecal occult blood than either the OC-Hemodia test or *o*-toluidine test. The QCOB test is better than the other two tests for detecting occult blood in patients with upper GI bleeding.

Key Words: fecal occult blood test, immunochromatography, hemoglobin, transferrin, upper gastrointestinal bleeding

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An expanding array of fecal occult blood tests (FOBTs) has emerged, each with technical advantages and disadvantages. The traditional chemical method and immunologic determination of hemoglobin (Hb) are the most commonly used screening tools for colorectal cancer or premalignant adenomas in asymptomatic, low-risk populations. Fecal blood is an ambiguous screening tumor marker for colorectal cancer, because blood that appears does not arise from the neoplasm itself but from the circulation. In addition, many cancers, including the majority of adenomas, do not bleed sufficiently or frequently enough to be detected by available tests. Fecal occult blood should be an important indicator in the diagnostic evaluation of patients with suspected gastrointestinal (GI) bleeding of any etiology, not just as an indication of colorectal cancer.

The traditional chemical FOBTs are based on peroxidase activity of the hematin portion of Hb. Because these tests involve the liberation of oxygen from hydrogen dioxide oxidizing chromogen, false-positive results may be produced by dietary substances containing sufficient amounts of hematin. Various chemicals may also interfere with the analysis and cause false-negative results. To overcome such disadvantages in chemical tests, immunologic tests using human Hb antibodies, which require no dietary restrictions, have high diagnostic accuracy [1–3]. Because of variable fecal hydration [4], Hb degradation by proteases [5], absorption by mucin, and the loss of Hb

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antigenicity due to enterobacteria [6,7] and anti-inflammatory drugs, there is no FOBT that yields both satisfactory specificity and sensitivity for colorectal neoplasia [8]. The immunochemical assay of the FOBT is also inadequate as a screening test for stomach cancer [9]. Detection of fecal transferrin (Tf), which is more stable in stool than Hb [10], provides an alternative way of diagnosing the disease in the upper digestive tract. In a study of patients with colon cancer and polyps, Miyoshi et al demonstrated that simultaneous determination of Hb and Tf in FOBTs improves the accuracy of screening [11,12]. The efficacy of FOBTs for detecting Hb and Tf simultaneously in upper GI bleeding cases has not been reported in the literature.

The purpose of this study was to evaluate the performance characteristics of three FOBTs: the chemical *o*-toluidine test, the immunochemical OC-Hemodia test, and the immunochromatographic Quick Chaser Occult Blood (QCOB) test, which detect human Hb and Tf simultaneously in cases of upper GI bleeding.

Materials and Methods

Samples

We collected 48 consecutive fecal specimens for FOBTs from 48 adult admission patients with upper GI bleeding in the Division of Gastroenterology, Department of Internal Medicine, Kaohsiung Medical University Hospital. Among those 48 upper GI bleeding cases, 47 cases were confirmed by GI endoscopy, which was performed by visiting gastroenterologists from the same hospital. In one case, endoscopy was not performed because the patient had open pulmonary tuberculosis. The clinical characteristics of these upper GI bleeding cases are described in Table 1. The mean age was 67.1 ± 14.8 years (range, 27–96 years). The most common causes of upper GI bleeding were gastric and duodenal ulcers (72.9%). All patients received detailed explanations of the study methods, including the process of collecting fecal samples. Fecal specimens with a bloody and tarry appearance due to active bleeding were excluded, and all suitable samples were processed immediately after collection without refrigeration.

Table 1. Clinical characteristics of 48 upper gastrointestinal bleeding cases

Characteristic	No. of cases	%
Male	27	56.3
Female	21	43.7
Etiology		
Gastric and duodenal ulceration	35	72.9
Esophageal varices	6	12.5
Polyp	4	8.33
Gastric cancer	3	6.25

Reagent kits

o-Toluidine test kits (Shih-Yung Medical Instruments, Taipei, Taiwan) were used for conventional chemical methods. The detection sensitivity of the *o*-toluidine test is 1:80,000. Latex agglutination OC-Hemodia reagent kits (Eiken Chemical, Tokyo, Japan) were used for the immunologic method and this test was processed automatically by an OC-Sensor machine. The measurement range of this latex agglutination method is 50–2,000 ng/mL, and the pathologic cutoff value was assigned as 100 ng/mL. The QCOB test (Mizuho Medy, Tosu, Japan) detected Hb and Tf simultaneously by the immunochromatographic method, and its one-step assay procedure can be conducted as easily as a commercial urine pregnancy test. When specimen suspension is dispensed onto the specimen area, any human Hb and Tf that are present migrate to the area between the specimen area and the result area, where they bind with colloidal gold conjugated to rabbit anti-human Hb and mouse monoclonal anti-human Tf to form antigen–antibody immunocomplexes.

According to the immunochromatographic principle, these antigen–antibody immunocomplexes migrate to the test result area, where they are caught by immobilized rabbit anti-human Hb and mouse monoclonal anti-human Tf to form sandwich complexes, made apparent by an easily readable purple–red color signal, which indicates the presence of human Hb and human Tf in the specimen. The measurement range of human Hb or Tf is 50–800 ng/mL. The detection sensitivities of both human Hb and Tf were 300 ng/mL. The detection sensitivities of these three tests were confirmed with the standard materials provided by their manufacturers.

Statistical methods

Statistical significance was evaluated using the χ^2 test. A p value of less than 0.05 was defined as statistically significant.

Results

The frequencies of the positive rates of three FOBTs in 48 upper GI bleeding specimens are shown in Table 2.

The QCOB test was positive in 68.8% (33/48), the highest among the three tests. The *o*-toluidine test was positive in 39.6% (19/48), and the OC-Hemodia test was positive in 45.8% (22/48). The QCOB test detected occult blood at significantly higher positive rates than did the OC-Hemodia test ($p < 0.025$). Of the 48 fecal specimens of upper GI bleeding tested by the QCOB test, 17 (35.4%) were positive for both substances, six (12.5%) were positive for Hb only, 10 (20.8%) were positive for Tf only, and 15 (31.3%) were negative for both substances.

The frequencies of positive rates for the various FOBTs in upper GI bleeding are shown in Table 3. The occult blood positive rate for the QCOB test was 68.6% (24/35), and 34.3% (12/35) for the *o*-toluidine test in the gastric and duodenal ulcer groups of patients with upper GI bleeding. There was a significant difference of positive rates between the QCOB test and the *o*-toluidine test ($p < 0.01$). All three gastric cancer cases tested positive in these three occult blood tests.

Fifteen fecal specimens tested negative in all three types of occult blood tests. No fecal specimen was positive with the *o*-toluidine test or the OC-Hemodia test that was negative with the QCOB test.

Table 2. Frequency of positive rates (%) of various fecal occult blood tests in upper gastrointestinal bleeding

o-Toluidine	OC-Hemodia	QCOB
19/48* (39.6)	22/48† (45.8)	33/48‡ (68.8)

QCOB = Quick Chaser Occult Blood.
*$p < 0.01$ by χ^2 test, compared with the Quick Chaser Occult Blood test.
†$p < 0.025$ by χ^2 test, compared with the Quick Chaser Occult Blood test.
‡The positive rates for hemoglobin and transferrin are 56.2% and 47.9%, respectively.

Discussion

The accuracy of screening tests for cancer can be evaluated, by a retrospective approach, to study both affected cancer cases and healthy subjects, although the provision of follow-up for all subjects who undergo an examination for false-positive cases presents certain operational limitations. Nevertheless, the immunochemical FOBT is widely used as a screening method for colorectal cancer [13,14], and several studies have suggested that immunochemical FOBTs are more efficient [15–17]. In our study, we used the OC-Hemodia test for immunochemical occult blood testing.

Among the three FOBTs used in this study, the OC-Hemodia test had the lowest pathologic cutoff value for fecal Hb (100 ng/mL assigned by the manufacturer). The cutoff value for fecal Hb with the QCOB test was 300 ng/mL. These values differed from those in a previous study by Nakama et al, as a result of different immunochemical and immunochromatographic methods [18].

When we evaluated the possibility of using these kits for detecting upper GI bleeding, if we only compared the results for Hb or Tf from the QCOB test, there was no significant difference in fecal occult blood positive rates with the OC-Hemodia test. Furthermore, when comparing combined results for Hb and Tf, the QCOB test revealed significantly higher positive rates of occult blood (68.8%) than did the OC-Hemodia test (45.8%) ($p < 0.025$). There have been several studies examining the diagnostic accuracy of FOBTs in the detection of stomach cancer and upper digestive tract disease [9,19,20]. The results were similar to our observations that chemical or immunochemical methods of detecting solely Hb have low rates of success. In the hospital-based case-control study of Nakama et al, the immunochemical test was positive in six (14.3%) subjects with stomach cancer, in 32 (76.2%) subjects with colorectal cancer, and in 10 (7.9%) healthy subjects, respectively, showing a significant difference in detection rates between

Table 3. Frequency of positive rates (%) of three occult blood tests in upper gastrointestinal bleeding specimens

Diagnosis	*o*-Toluidine	OC-Hemodia	QCOB
Gastric and duodenal ulcer ($n = 35$)	12/35 (34.3%)	15/35 (42.9%)	24/35* (68.6%)
Esophageal varices ($n = 6$)	1/6 (16.7%)	2/6 (33.3%)	3/6 (50%)
Polyps ($n = 4$)	1/4 (25%)	1/4 (25%)	2/4 (50%)
Gastric cancer ($n = 3$)	3/3 (100%)	3/3 (100%)	3/3 (100%)

QCOB = Quick Chaser Occult Blood.
*Compared with the *o*-toluidine test, $\chi^2 = 6.92$, $p < 0.01$.

subjects with stomach cancer and colorectal cancer ($p < 0.01$) [9]. Thus, the immunochemical FOBT was determined as an inadequate screening test for stomach cancer. The authors suggested that examination of the upper digestive tract is unnecessary in cases where the results of FOBTs are positive with no sign of colorectal disease. The false-negative results of immunochemical tests occur because Hb derived from the upper digestive tract is broken down in the intestinal tract. Therefore, the antigenicity of Hb is lost and cannot be detected by the immunochemical occult blood test [13,21]. In this study, 15 cases were negative in all three FOBTs. In addition to the lost antigenicity in the intestinal tract as mentioned above, the false-negative result of FOBTs was probably due to our exclusion of stool specimens with an obvious bloody and tarry appearance.

In this study, 10 cases (20.8%) of upper GI bleeding were occult blood positive for Tf only. Schade et al [22] and Ward et al [23] observed that Tf is a glycoprotein that is highly resistant to bacterial degradation as well as to breakdown by digestive enzymes, and Tf was found to be the most stable, suggesting that it could perhaps be used as a marker of GI bleeding. In Miyoshi's studies, 18% of fecal specimens were positive for Tf alone, suggesting that Tf is stable in feces [11].

Our results revealed that QCOB tests have significantly higher positive rates than that of the QC-Hemodia method in upper GI bleeding cases. These false-negative findings via the immunochemical method were also seen in studies by Burton et al [24] and Tada et al [25]. Their observations demonstrated that bleeding from the GI tract is rarely detectable by immunologic tests as a result of the effects of intestinal bacteria and digestive juices.

In the studies by Rockey et al, the possibility is raised that a combination of a highly sensitive guaiac-based FOBT plus an immunochemical test could help to differentiate occult upper from lower GI bleeding [26]. They found that a positive SENSA test (guaiac test), as well as a positive immunochemical test, would raise the likelihood that bleeding is from the lower GI tract. In contrast, a positive SENSA test in the setting of a negative immunochemical test could be indicative of upper GI bleeding. Harewood et al compared three FOBTs in upper GI bleeding by ingested autologous blood and observed that the HQT (heme-porphyrin) test detected occult upper GI blood loss significantly more frequently than the HO (guaiac) test and HS (immunochemical) test [27].

The QCOB test by the immunochromatographic method is ready to use, without prior preparation of reagents and is machine independent. The one-step assay procedure can be handled easily by medical technologists, even for house-call patients. Therefore, this test can be a better choice for FOBTs in the countryside. However, the QCOB test is not cost effective compared with other methods.

Based on our observations from this study, the QCOB test revealed significantly higher positive rates of occult blood (68.8%) than did the OC-Hemodia test (45.8%) and the *o*-toluidine test (39.6%). Thus, the QCOB test (combination assay of human Hb and Tf) is superior to the other two tests for the detection of fecal occult blood in patients with upper GI bleeding.

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三種糞便潛血反應試驗於上腸胃道出血病例之比較

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本研究之目的在於：評估三種糞便潛血反應試驗：化學法 *o*-toluidine，免疫化學法 QC-Hemodia 以及能同時檢測人類血紅素及 transferrin 之免疫色層分析法 Quick Chaser Occult Blood (QCOB) 在上腸胃道出血病例之表現。吾人收集住院於高雄醫學大學附設醫院腸胃內科連續 48 個經胃內視鏡診斷為上腸胃道出血病例之 48 個糞便檢體，經排除掉明顯黑色及鮮血糞便檢體者。結果顯示：QCOB 試驗有最高之糞便潛血陽性率 68.8% (33/48)。QCOB 試驗此 68.8% 陽性率均較 QC-Hemodia 試驗及 o-toluidine 試驗者為高 ($p < 0.025$，$p < 0.01$)。上腸胃道出血病例中之胃及十二指腸潰瘍群 QCOB 試驗陽性率 68.6% 較 *o*-toluidine 試驗者 34.3% 為高 ($p < 0.01$)。吾人亦發現無任何糞便檢體 *o*-toluidine 及 QC-Hemodia 試驗陽性而 QCOB 試驗陰性者。本研究結果顯示 QCOB 試驗比 QC-Hemodia 試驗有較高之潛血反應陽性率。對上消化道出血病例，QCOB 試驗比起其他兩種檢驗在糞便潛血反應試驗上有較好的表現。

關鍵詞：糞便潛血反應試驗，免疫色層分析法，血紅素，上消化道出血

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