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Comparative evaluation of a new bedside faecal occult blood test in a prospective multicentre study

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SUMMARY

Background
Faecal occult blood testing is an established method of colorectal neoplasia screening. Guaiac-based tests are limited by poor patient compliance, low sensitivity, specificity and positive predictive value. Newer immunochemical-based tests, accurate but tedious, require a well-established laboratory set up. There is need for simpler immunochemical tests that can be performed at the out-patient clinic.

Aim
To compare the performance characteristics of a new bedside immunological test strip device with a sensitive Guaiac-based and established immunochemical test for detection of faecal occult blood in patients undergoing colonoscopy.

Methods
A total of 389 consecutive patients from four centres who were referred for colonoscopy also provided the stool samples for detection of occult blood without dietary restrictions. Stool tests performed were (i) Guaiac-based, (ii) immunochemical enzyme-linked immunosorbent assay and (iii) bedside immunochemical strip test.

Results
At the optimal threshold level, the sensitivity and specificity of the beside immunochemical strip test for detection of significant colorectal neoplasia (adenomas >1.0 cm and carcinomas) were 60% and 95%, respectively.

Conclusions
This bedside immunochemical strip test proved to be a simple, convenient, non-cumbersome and accurate tool with similar performance characteristics for detection of any bleeding lesion including colorectal neoplasia when compared with an established immunochemical faecal occult blood test.

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INTRODUCTION

Colorectal cancer (CRC) accounted for about 1 million new cases in 2002; its worldwide prevalence is only second to that of breast cancer. It is one of the leading causes of cancer death in western societies with 25-fold variation in its occurrence across the globe.[1] In Europe, there were estimated to be 376 400 new cases of CRC amounting to 203 700 deaths in 2004[2] while in the US there are expected to be 145 200 CRC registrations and 56 290 deaths this year.[3] It is estimated that about half of these deaths could have been prevented if patients would have undergone CRC screening.[4] It has been observed that most cases of sporadic CRC arise from pre-existing adenomatous polyps.

The metastatic CRC [stage IV tumour node metastasis (TNM)] has a dismal survival of <5% when compared with early CRC (stage I TNM).[5] The prevalence of the disease, with the proven ability of screening to reduce mortality makes population-wide screening of CRC a lucrative health goal. An ideal screening test must be non-invasive, acceptable to patients, simple, have high sensitivity and specificity, inexpensive, cost-effective, easy to implement across a large population and should be effective in reducing morbidity and mortality from CRC in the screened population.[6]

The most widely accepted non-invasive method for detecting CRC is faecal occult blood testing (FOBT). It is routinely used world over for CRC screening and surveillance and has been in use now for more than three decades. Screening in asymptomatic populations with FOBTs followed by colonoscopy if tested positive have reduced mortality rates by 15–33%.[7–10] However, sensitivity of FOBTs ranges from 26% to 69% for detecting CRC.[11–14]

There are two main types of commercially available FOBTs: 'Guaiac-based tests' in which Guaiac blue colour change occurs because of the pseudoperoxidase activity of haeme in the stool; 'immunochemical tests' which detect globin portion of human haemoglobin (Hb) in the stool using anti-Hb antibodies.[15] A major disadvantage of Guaiac tests is the fact that they are not specific for human blood and can produce false-positive results when meat, fruit, or vegetables containing peroxidase have been ingested. Also bleeding in the upper gastrointestinal (GI) tract secondary to aspirin and non-steroidal anti-inflammatory drugs (NSAIDs) can produce false-positive Guaiac-based tests.[16] This may lead to contraindicate colonoscopic examinations thus increasing the cost and risk of screening programmes. 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 to the patient. Because of these shortcomings Guaiac-based tests are being replaced by immunochemical faecal occult blood tests (IFOBTs), despite being more expensive.

The IFOBTs, which are widely used as screening tests in Japan, have been shown to be superior to the Guaiac-based tests in sensitivities, with similar specificity, as reported both in known CRC[17] and in asymptomatic populations.[13, 18] This is because IFOBTs do not detect blood from the upper GI tract, do not react with plant peroxidases and animal Hb. Thus, there is no need of dietary or medical restriction. One of the studies has shown 66% increase in patient participation with the elimination of dietary restrictions.[19] Furthermore, sensitivity of IFOBTs is high for adenomas,[17] intramucosal cancer[20] and Dukes A cancer.[21] On the basis of these reports, it is expected that screening with IFBOTs may provide larger effects on the incidence reduction of advanced CRC when compared with the Guaiac-based tests. However, most IFOBTs require a suitable well-equipped laboratory, i.e. an enzyme-linked immunosorbent assay (ELISA) reader, limiting their widespread use because of logistics and higher costs.

Colonoscopy is considered the gold standard for CRC screening. But it is expensive, requires skilled endoscopist and may lead to life-threatening complications like perforation (one in 300 procedures).[22] However, complication rate varies in different studies and one of the recent studies in which more than 22 000 patients underwent screening colonoscopies (only diagnostic) by experienced endoscopists without any major complication or mortality.[23]

In a recent pilot study, we demonstrated a new immunological test strip device (bedside IFOBT) for the rapid, quantitative detection of FOB (Prevent ID CC, Preventis GmbH, Bensheim, Germany) and compared it with a widely used Guaiac-FOBT keeping ELISA-IFOBT as the gold standard. We showed that this bedside IFOBT is much more sensitive (76% vs. 30%) for detection of FOB than Guaiac-based test whereas specificity of both tests were comparable.[24] However, we did not perform colonoscopy in these patients.

In the present prospective study, we have evaluated the performance characteristics of this new bedside test for detection of FOB in patients undergoing colonoscopy. Experienced endoscopists carefully examined

the entire large bowel, recorded and reported the presence of any bleeding lesion that could cause positive FOBT. If any such lesion was found it was biopsied or snared if indicated. We also compared it with another established IFOBT (ELISA) and a sensitive Guaiac-based FOBT.

Our study is peculiar and different from previous studies in that the patient population was not only of CRC screening patients but also constituted the patients referred to or admitted with colonic symptoms and many of them had known clinical diagnosis. Thus, we evaluated performance characteristics of FOBT not only in detecting neoplasia but also in detecting presence of microscopic faecal blood whether it was because of neoplasia, inflammatory bowel disease (IBD) or dysentery.

MATERIAL AND METHODS

Ethics

The study protocol was approved by the ethics committee of Frankfurt University Hospital. Written informed consent was obtained from all participants before entering the study.

Patients and healthy controls

Patients from four different centres who were referred for colonoscopy were invited to participate in the study. There were 237 patients who either had known clinical diagnosis (e.g. IBD) or were symptomatic suggestive of colonic disease while as 150 healthy patients underwent CRC screening. Patients with active GI bleeding, menstruation and past history of total colectomy were not included in the study.

Patients given appointments for colonoscopy were asked to provide one stool sample for detecting faecal blood. No dietary restrictions were advised to the patients. Stool sample was collected before starting the bowel preparation in a sterile box for Guaiac and immunological tests whereas for the bedside IFOBT we provided patients with a simple stool-collecting device (see below). Stool samples of these patients with or without pathological findings were tested. In a standard questionnaire patient's clinical history was recorded.

After standard bowel wash colonoscopies were performed by the endoscopists, who were not aware of FOBT findings, till the caecum using the standard colonoscopes. Patients who were poorly prepared or in whom caecum could not be reached were not included in the study. Histology was obtained from the routine biopsies, polypectomies and/or from surgery. Adenomas were classified as large (>10 mm) or small (<10 mm) as judged during colonoscopies using biopsy forceps as measure to assess polyp size. All data were collected prospectively and recorded on standardized questionnaire forms. If a neoplastic obstructive lesion was found during colonoscopies, it was biopsied and the patient was included in the study despite colonoscopy not being complete.

Faecal occult blood tests

Stool samples were applied to the three windows of a Guaiac-FOBT Haemoccult (Beckman Coulter, Inc., Fullerton, CA, USA) and further processed without rehydration as indicated by the manufacturer. On an especially Guaiac resin impregnated filter paper stool sample was placed. Once it had dried alcohol-stabilized hydrogen peroxide was put on the paper. A positive test was indicated by colour change to blue after 60 s.

A human Hb ELISA (Immundiagnostik AG, Bensheim, Germany) was used as a highly sensitive IFOBT. This test is based on Hb antibodies-precoated microtitre plates. To 100 mg of faeces wash buffer was added, mixed and centrifuged. About 100 L of this solution was incubated on the microtitre plates precoated with anti-Hb antibodies. After washing anti-Hb a peroxidase-labelled antibody was added, and was incubated for 1 h. Then, the substrate was added and incubated for 20 min and finally the stop solution was added so the colour changed from blue to yellow. Absorption was determined with an ELISA reader at 450 nm against reference value. According to the manufacturer a cut-off level of ≤10 g Hb/mL of stool was used.

An immunological FOB test strip device (Prevent ID CC) was used. It is a one-step immunochromatographic assay for rapid quantitative detection of human faecal blood. One of its major advantages, which should make it more acceptable to the patients, is the simplicity and non-messy sample collection. Figure 1 demonstrates the convenience of this test. The stick of the sample collection device was sticked into the faeces (approximately 2 cm), it was then inserted into the collection device containing an extraction buffer solution and the assembly was shaken thoroughly. This procedure was repeated three times sampling the

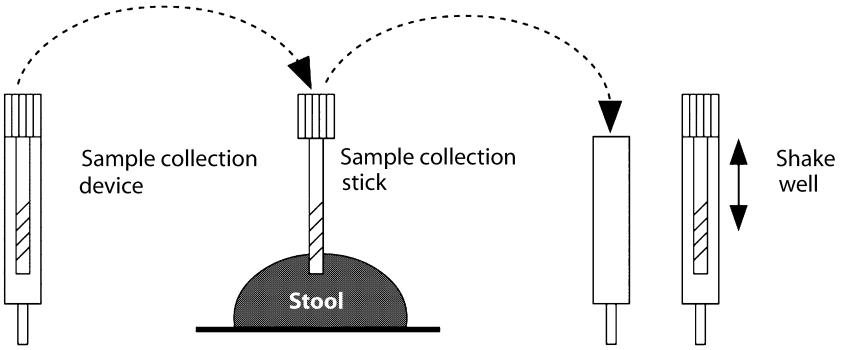

Figure 1. Sample collection for the bedside immunochemical faecal occult blood test (IFOBT; immunological test strip device; Prevent ID CC).

faeces from different sites. If the test was not performed within 1 day of sample collection it was stored at 2–8 C (as per the manufacturer's instructions). Two drops of this extracted sample were loaded into the sample opening of the test strip device, liquid faecal extract flows from the sample opening of the test device through the conjugate-release pad.

Principally it is a sandwich immunoassay in which gold-labelled anti-Hb antibodies bind to Hb (if present) in the sample. These complexes migrate over the test membrane until it passes the test zone, which consists of immobilized gold-labelled antibody–Hb complex and generates a violet band within 10 min (Figure 2a,b). To prevent false-negative an internal (control) line consisting of anti-IgG antibody has been added which indicates that the test has run properly. It has a very high sensitivity and can detect human Hb up to 10 g/mL.

The bedside FOBT was easy to use, and results did not depend on operator experience. All the three FOBTs were performed and reported by an experienced technician who was blinded to the patient's clinical profile.

Statistical analysis

For the statistical comparison of the non-parametrically distributed data, the Kruskall–Wallis ANOVA test was used (STATISTICA Version 5.0, StatSofts Inc., Tulsa, OK, USA). Sensitivity, specificity, positive predictive (PPV) and negative predictive (NPV) values of all the three FOBTs were determined.[25] Specificity was calculated for CR neoplasia as a whole group and not separately for adenoma and CRC. We used chi-square and Fisher's exact tests to compare differences in proportions amongst the tests. The *P*-values of <0.05 were considered as statistically significant (for sensitivity and specificity). Methods for proportions were used to calculate 95% confidence interval (CI).

RESULTS

A total of 387 consecutive patients underwent 407 tests, i.e. colonoscopies and FOBTs simultaneously. In 14 patients tests were repeated twice while in three patients they were repeated thrice. All the patients with repeat test were known cases of IBD. Their age ranged from 5 to 96 years (median age: 51 years). There was slight preponderance of females; 186 males and 201 females. The study duration was from January 2002 to December 2004. The major indications for colonoscopy were CRC screening, IBD, clinical suspicion of CR neoplasia or IBS, unexplained anaemia or diarrhoea, positive FOBT, etc. Comparative results of the test characteristics of asymptomatic screened patients and symptomatic patients are shown in Table 1. About 54 patients had evidence of CRC on colonoscopy (Table 2), the sensitivity of these tests to detect faecal Hb was 37.0%, 74.0% and 77.7% for the Guaiac-based test, the bedside IFOBT and the ELISA (cut-off <10 ng/mL) respectively (Table 3). Of 18 adenomas diagnosed, six were large (>10 mm) and 12 were small, i.e. <10 mm. The sensitivity of faecal Hb for any neoplasia (CR carcinoma plus large adenomas) was 29.1%, 59.7%, and 63.8% respectively (Table 3). Of 164 patients with normal colonic mucosa, 16 (9.7%) were positive with the Guaiac test, nine (5.5%) with the bedside IFOBT, whereas six (3.7%) were found positive with ELISA-IFOBT, i.e. false-positive.

About 141 (34.6%) patients revealed a lesion (bleeding) on colonoscopy which is fairly high when compared with screening population. This is because, ours was a hospital-based study, and mostly included symptomatic patients unlike the screening studies that usually includes asymptomatic healthy individuals. Active bleeding colonic lesions were found in 67 (43.2%) of total 155 IBD patients examined, 29 with

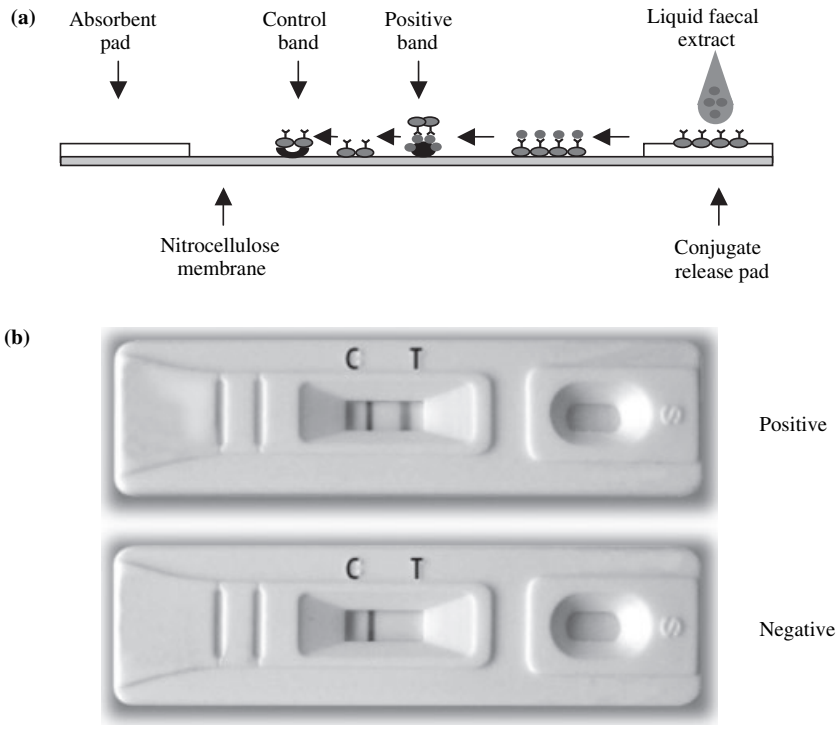

Figure 2. (a) Principle of the bedside immunochemical faecal occult blood test (IFOBT; immunological test strip device). After binding of faecal haemoglobin (blue dots) by antihaemoglobin antibodies, immuncomplexes are detected by a second nitrocellulose membrane-coupled antibody, resulting in the appearance of a positive band. The formation of a control band indicates the correct function of the test and unimpeded flow of faecal extracts through the device. (b) Photograph of Prevent ID CC test strips from a faecal specimen negative or positive for faecal occult blood.

Table 1. Test characteristics in screening and symptomatic patients

Diagnosis	*N*	Positive Guaiac-based test, *N* (%)	Positive bedside IFOBT, *N* (%)	Positive ELISA-IFOBT, *N* (%)
Screening patients				
Colorectal neoplasia*	6	2 (33.3)	4 (66.7)	4 (66.7)
Normal	150	17 (11.4)	8 (5.3)	5 (3.3)
Total	156	19 (12.2)	12 (7.7)	9 (5.8)
Symptomatic patients/patients with known disease				
Colorectal neoplasia*	66	19 (28.8)	39 (59)	42 (63.6)
IBD	155	39 (25.2)	68 (43.9)	68 (43.9)
Others	30	3 (10)	3 (10)	3 (10)
Total	251	61 (24.3)	110 (43.8)	113 (45.0)

* CRC + large adenomas.
CRC, colorectal cancer; IFOBT, immunochemical faecal occult blood test; IBD, inflammatory bowel disease; ELISA, enzyme-linked immunosorbent assay.

ulcerative colitis (UC) and 38 with Crohn's disease (CD). About 88 patients had inactive IBD (i.e. no evidence of bleeding lesion during colonoscopy), 22 UC and 66 CD. Two patients with pseudomembranous colitis had all the three FOBTs-positive in them. Sensitivity and specificity of rapid bedside test was found to be statistically significant in detecting any bleeding lesion (except adenomas) when compared with Guaiac-based tests.

DISCUSSION

The CRC and adenomas bleed intermittently[26] and positivity of FOBTs depend on degree of blood loss. Generally 2 mL of blood is necessary in stool to produce positive Guaiac-based tests.[5] When compared with CRC, adenomas bleed less frequently and also the bleeding is small in amount,[27] i.e. why sensitivity to detect them is lower. Also in the present study, the

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