Evaluation of diagnostic accuracy of screening by fecal occult blood testing (FOBT). Comparison of FOB Gold and OC Sensor assays in a consecutive prospective screening series

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ABSTRACT: We evaluated a new immunological fecal occult blood testing assay (FOB Gold, Sentinel = SENT) compared to the assay currently employed in the Florence screening program (OC-Hemodia, Eiken = OC). A total of 4,133 subjects were screened with both tests and underwent colonoscopy if positive (100 ng/mL Hb cutoff) to either test: 190 (4.59%) were positive (OC = 140 (3.4%); SENT = 131 (3.2%)). The relative sensitivity for 7 cancers was 100% with OC and 67.9% with SENT, and for 48 high-risk adenomas (HRAs) it was 77.0% with OC and 66.6% with SENT. The positive predictive value (PPV) for cancer+HRA was 31.4% for OC and 28.2% for SENT and the specificity was 97.7 for both. The differences were not statistically significant. Adding SENT to OC increased the positivity rate by 32% and the cancer+HRA detection rate by 25%, and decreased the PPV by 10%. Both tests were performed on the same tubes in 1,601 cases, and in 18 of 47 cases they differed on different tubes but not on the same tube, suggesting inhomogeneous Hb content or varying fecal matrix influence in different samples. SENT has practical advantages for screening (fully automated, high output, requires no dedicated instrument), a comparable specificity and a lower sensitivity, though the latter difference may be partially ascribed to differences in sampling and not to the assay itself. Because of the statistical insignificance of the differences, further studies are needed for confirmation.

Key words: Fecal occult blood testing (FOBT), Colorectal cancer, Screening

INTRODUCTION

Screening by fecal occult blood testing (FOBT) has been shown to be effective in reducing the colorectal cancer (CRC) mortality by several studies, including controlled clinical trials (1-8). Population-based screening is recommended as a preventive procedure by the European Community and is under implementation in several countries, including Italy (www.osservatorionazionale-screening.it) (9, 10).

Although randomized trials proving screening efficacy were based on guaiac FOBT, the favorable diagnostic performance of new immunological FOBT assays justifies their increasing use in screening practice (7, 8). In Florence a population-based FOBT screening program run by the Centro per lo Studio e la Prevenzione Oncologica (CSPO) has been ongoing since 1982, and evidence of the program’s efficacy has been provided by a case-control study (11). The performance of immunological FOBT was assessed by comparison studies with guaiac FOBT (8, 12, 13) and OC-Hemodia (Eiken Chemical Co, Tokyo, Japan, henceforth referred to as OC) has been in use since 2000 (14).

Implementing a nationwide screening program requires a very large number of FOBTs: for example, total national coverage in Italy would imply approximately 18 million FOBTs being offered every year. In such a scenario a growing interest of FOBT-producing companies is obvious, and an increasing number of new FOBT assays is being commercialized. The performance of new FOBT assays cannot be analyzed by new efficacy trials, and analysis is generally based on comparison of diagnostic accuracy with reference to FOBT assays of proven good performance.

The aim of the present study was to test the diagnostic efficacy of a recently commercialized immunological FOBT assay (FOB Gold, Sentinel, Milan, Italy, henceforth referred to as SENT). For this purpose a comparative prospective study was carried out within the Florence colorectal cancer screening program, using OC as the reference assay.

MATERIAL AND METHODS

The study was carried out within the Florence colorectal cancer screening program, inviting residents
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between 50 and 69 years of age to undergo biennial FOBT. Screening attenders from 3 municipalities of the Florence district were invited to participate in the study. Written information on the aims and modalities of the study was provided, further explanations were given by the staff delivering the FOBT kit, and signed informed consent was required. According to the screening protocol, subjects with a positive FOBT result were referred for total colonoscopy and double-contrast barium enema when colonoscopy was incomplete. Consenting subjects were provided with OC and SENT kits and asked to perform both samplings on the same bowel movement. No dietary restrictions were prescribed. Test tubes were delivered to the lab within 2 days of sampling and stored at 2-8°C before processing.

The FOBT used as the reference method was OC-Hemodinia, an automated assay developed with the 10-tube rack OC Sensor (Eiken Chemical Co, Tokyo, Japan), a dedicated instrument that in our setting allowed an average processing of approximately 100 tests per hour. The assay is based on the flocculation reaction between human HbA and multiple monoclonal anti-HbA latex-adsorbed antibodies. The HbA concentration is measured by reading flocculation as an optical change (increased adsorbance at 660 nm) compared to a standard calibration curve. The inter-series coefficient of variation in our lab was 3.3% (156 ± 5.2) at a low and 3.8% at a high (601 ± 22.9) Hb level.

The FOBT assay tested in the present study was FOB Gold (Sentinel Ch, Milan, Italy), a fully automated assay developed with the Aeroset instrument (Abbott Diagnostics, Abbott Park, USA), allowing in our setting an average processing of approximately 500 tests per hour. The assay is based on the flocculation reaction between human HbA and polyclonal anti-HbA polystyrene-adsorbed antibodies. The HbA concentration is measured by reading flocculation as an optical change (increased adsorbance at 572 and 804 nm) compared to a standard calibration curve. The inter-series coefficient of variation in our lab was 3.3% (108.4 ± 7.5) at a low and 3.7 (359.4 ± 13.3) at a high Hb level.

Sampling tubes were blindly processed in 2 different labs (OC at CSPO, SENT at the Careggi Hospital). After verifying the alignment of the instruments, invariance of calibration, and control materials, a positivity cutoff of 100 ng/mL, currently used in the screening program, was chosen for the study. According to the screening protocol, subjects with positive findings at either OC or SENT were invited to undergo diagnostic assessment.

The reference standard to compare the diagnostic accuracy of OC and SENT was histological diagnosis of CRC or adenoma, obtained at diagnostic colonoscopy or at further histology (operative colonoscopy, surgery). For the purposes of the study, adenomas were assumed to be “high risk” (HRA) if a) larger than 9 mm, or b) with a villous or tubulo-villous histological pattern (>20%), c) with high-grade dysplasia, or d) 3 or more, of any size. All other adenomas were categorized as “low risk” (LRA).

Test performance was compared in terms of a) positivity rate, b) CRC + HRA detection rate per 1,000 screened subjects, c) sensitivity, specificity, and positive predictive value (PPV) for CRC + HRA. Subjects with a positive test result but not willing to undergo colonoscopy were assumed to have a negative outcome and were included in the denominator when accuracy was determined. Accuracy values were calculated also after adjustment for non-compliance to colonoscopy. Statistical analysis of observed differences was performed according to the chi-square (χ²) and McNemar tests, with statistical significance being set at p<0.05.

In order to ascertain whether discrepant results between OC and SENT could be attributable to differences in the sampled material or to the tests themselves, both tests were performed on the same tube in a subset of cases (15).

Between September 2003 and March 2005, 8,000 resident subjects in the 50-69-year age range were invited, and 4,187 (52.3%) complied, returning both OC and SENT tube to the labs. We excluded from evaluation 54 cases (0.76%) with insufficient material in one tube (OC = 21 (0.5%); SENT = 33 (0.79%). The final evaluation was thus performed on 4,133 subjects (2,117 women, 2,016 men; age range 50-70 years, average age 60 years).

RESULTS

Of 4,133 tested subjects 190 (4.59%) were positive at least at one test (OC = 140 (3.4%); SENT = 131 (3.2%)), and were invited to colonoscopy assessment. Concordant positive cases between OC and SENT were 42.6% (81/190, Cohen’s κ = 0.50). Fifteen of the 190 (7.8%) positive subjects refused colonoscopy assessment (OC+/SENT− = 6, OC−/SENT+ = 1, OC+/SENT+ = 8).

Seven CRCs were detected in the assessed subjects. They were all OC positive (crude detection rate 1.69‰, adjusted 1.88‰), whereas only 5 were SENT positive (crude detection rate 1.21‰, adjusted 1.28‰). Forty-eight subjects were detected as having HRA, 37 being OC positive (crude detection rate 8.9‰, adjusted 9.6‰), and 32 being SENT positive (crude detection rate 7.7‰, adjusted 8.3‰). The relative sensitivity for cancer was 100% for OC and 67.9% for SENT, whereas the relative sensitivity for HRA was 77.0% for OC and 66.6% for SENT. The adjusted PPV for cancer was 5.5% (crude 5.0%) for OC and 4.0% (crude 3.8%) for SENT. The adjusted PPV for CRC+HRA was 34.9% (crude 31.4%) for OC and 29.8% (crude 28.2%) for SENT. The specificity for CRC+HRA was 97.7% for both methods. The data are summarized in Table I. None of the differences in performance between OC and SENT reached statistical significance.
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A consecutive set of 1,601 cases was selected to perform both tests on both tubes. A positive result was observed at least in 1 of 4 determinations in 79 subjects (4.94%), 32 (40.5%) being positive at all 4 determinations. Discordance between OC and SENT (at least 1 negative result) was observed in 47 subjects; this was possibly attributable to inhomogeneous sampling (concordant result at both tests on the same tube) in 18 cases, and to differences in assay performance (discordance on the same tube) in 29. Data on discordant tests are summarized in Table II.

**DISCUSSION**

The use of fully automated immunological FOBT assays would be highly desirable in a national population-based screening program because of the large number of tests required, the large number of laboratories involved, the need for proper standardization and easy quality control procedures, and, last but not least, the substantial advantage in terms of costs for staff and instrumentation. The SENT assay has ideal characteristics from this point of view, as it allows a fully automated process, a short
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processing time, and a high output, using the standard instrumentation currently used in the clinical chemistry laboratory routine, as compared to OC, which involves a limited-output automated process and the use of a dedicated instrument.

For this reason we thought that a comparison of SENT with OC, as far as diagnostic accuracy is concerned, would be particularly interesting. Unfortunately, the results of the present experience show that the SENT assay is as specific as the OC assay but considerably less sensitive in the detection of CRC and HRA. The latter difference did not reach statistical significance, possibly due to the limited sample considered, but for the time being it represents a major limitation to the use of SENT in current practice.

Our data need confirmation from a larger study providing sufficient statistical power, and also a more detailed analysis of the observed differences from a stricter analytical point of view: our study design was essentially clinical, and did not allow detailed investigation of analytical aspects. Nevertheless, double testing on the same samples in a subset of cases suggests that part of the observed differences may be attributable to inhomogeneous Hb content or to a different influence of the fecal matrix in different samples from the same bowel movement, rather than to an intrinsically different performance of the 2 assays. Such an effect, however, is expected to occur in a random fashion in OC and SENT tubes, and does not modify the finding of a lower sensitivity of SENT as compared to OC.

Assay comparison was further influenced towards higher discrepancy of results by the use of a defined positivity cutoff, which is, however, the rule in screening practice to allow clinical decisions but implies that minor differences within the expected range of variability are equally assumed to be discrepancies (as far as accuracy is concerned) as major differences. As the optimal cutoff for quantitative immunological FOBT is still under discussion (14), the results of the present study, where a cutoff of 100 ng/mL Hb was used, cannot be generalized to scenarios using another cutoff for clinical purposes.

Although the study was not aimed at evaluating the advantages of multiple compared to single FOBT assays, adding SENT to OC on the same bowel movement increased the detection rate of CRC-HRA by 25% (absolute increase 2.6‰), while increasing the referral rate to colonoscopy by 32% (absolute increase 1.1%) and decreasing the PPV by 10% (absolute decrease –3.5%). These findings confirm that the reproducibility and reliability of a single FOBT sampling may be suboptimal due to inhomogeneous Hb content and/or variable interference of the fecal matrix, and stress the need for further investigation of the pros and cons of multiple testing on the same or on different bowel movements. A prospective study of single versus double FOBT is ongoing in our center and will be the subject of a separate report.

In conclusion, we found that the SENT assay is less sensitive than the OC assay for CRC and HRA, but since the observed difference did not reach statistical significance, further studies are needed for confirmation; also needed is a detailed study of the analytical aspects in order to investigate intrinsic accuracy differences between the 2 assays.

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