Serial Fecal DNA testing vs FOBT for Colorectal Cancer Screening in an Average Risk Population: a blinded prospective cross-sectional study.

Ravi Sharaf

A. Study Purpose and Rationale

Colorectal cancer (CRC) is the second leading cause of death in the United States, claiming 56,730 lives in 2004. 146,940 new cases are diagnosed each year, (age standardized incidence 53/100,000) making it 3rd in American cancer incidence, with a lifetime risk estimate of -61/6. Risk factors for CRC include but are not limited to inherited CRC syndromes, inflammatory bowel disease, diabetes mellitus and insulin resistance, alcohol use, smoking, radiation exposure, and red meat consumption.

The mode of presentation of CRC follows one of three patterns. Sporadic disease (ie average risk) accounts for 70% of CRC cases. It occurs in individuals in whom there is no family history of CRC. Inherited syndromes account for <1 0% of CRC cases. These are divided as to whether colonic polyps are a major disease manifestation. Poyposis syndromes include Familial adenomatous polyposis. (FAP) and the hamartomatous polyposis syndromes (eg, Peutz-Jeghers, juvenile polyposis), while those without polyps include hereditary nonpolyposis colorectal cancer (HNPCC, Lynch syndrome 1), and the cancer family syndrome (Lynch syndrome 11). Familial CRC accounts for:5 25% of CRC. Affected patients have a family history of CRC, but the pattern is not consistent with one of the inherited syndromes.

Regardless of their etiology, most CRC arises from adenomatous polyps (adenoma to carcinoma sequence (Figure 1)). Polyps are mucosal colonic protrusions that can be classified as hamartomatous (juvenile polyp), hyperplastic, or adenomatous. Only adenomas are premalignant, though <1% ever become malignant. Risk for malignant transformation are polyp anatomy (sessile), size (>2.5cm), and histology (villous). Polyps usually require at least 5 years of growth before becoming clinically significant.

The progression from adenoma to carcinoma is driven by the accumulation of genetic mutations, as elucidated in 19W by Fearon and Vogelstein. Each mutation confers a selective growth advantage to the colonic epithelia[cell. Important genetic events include:

1   Allelic loss of the APC (adenomatous polyposis coli) gene (5q2l), as indicated by premature truncation of the APC protein. This is an event thought to occur early in tumorigenesis. Loss of functional APC leads to dysregulation of B-catenin, a protein that controls intestinal crypt cell proliferation and differentiation via activation of the transcription factor T-cell factor 4.
2   Point mutations in Ras, a protooncogene involved in G protein signal transduction. K ras is most often affected. Mutation events occur late in tumorigenesis.
3   Allefic loss at the p53 gene (17p). The p53 protein (tumor-suppressor) is a DNA-binding protein that acts as a transcriptional activator of growth inhibitory genes, and is a late event during tumorigenesis.
4   Other genetic changes include DNA hypomethylation (consequent gene inactivation), BAT-26 deletion (microstallite instability marker), the presence of "Long" DNA (marker of abnormal apoptosis), and the allelic loss of DCC (a tumor suppressor gene involved in cell-cell or cell-matrix adhesion).

The molecular genetics of colorectal cancer provide the basis for fecal DNA screening tests, soon to be discussed.

The current CRC screening paradigm is derived from AGA recommendations released in 2003 (Figure 2). Screening algorithms for CRC depend on the mode of disease presentation (sporadic,

inherited, familial). We will focus on the screening of average risk individuals. Screening for colorectal cancer lowers both the mortality and the incidence of the disease and is recommended for persons 50 years of age or older. No single test is of unequivocal superiority, and the tests vary in terms of effectiveness, risk, and cost. Of note, an excellent screening test is one that's reproducible, accurate, feasible, minimally invasive, safe, broadly accepted, and effects on clinical decision/outcomes.

Available screening tests for CRC include (but are not limited to) colonoscopy, which is the reference gold standard, with a sensitivity in the 90% range. Fecal occult blood testing (FOBT), the only CRC screening modality for which randomized clinical trials have shown decreased mortality (-33% after 13yrs). The AGA recommends FOBT that is guiac-based, home-performed, involving 2 samples from each of 3 consecutive stools, subsequently non-rehydrated w/dietary/medication restriction (red meat/NSAIDs) peri-test. Sensitivity is estimated at 25 to 50% for CRC and 10-36% for adenomas.

FOBT, however, is indicative of how a single somewhat sensitive test can increase in sensitivity when repeated. By report, via annual testing, the sensitivity for CRC increases to 92%. Further, a 2005 study in the Annals of Internal Medicine comparing the sensitivity/specificity of digital FOBT (2-sample) and routine 6-sample-home gulac for CRC screening in 3121 asymptomatic persons, aged 50-75, found sensitivity for CRC was 4.9% vs 23.9% for the 2 window and 6-window guiac respectively. (Specificities 97.5% (office DRE/FOBT)and 93.9 (home FOBT).

Despite above recommendations, screening rates remain low. In 1999 for U.S. residents age >50 years, only 20.6% had a home FOBT within I year, while 33.6% had undergone sigrnoidoscopy or colonoscopy within 5 years. Reasons for low screening rates include low levels of public awareness, public and professional attitudes about screening, and implementation barriers. The invasive nature of the screening tests is also a deterrent.

Noninvasive screening modalities are being formulated. Virtual colonoscopy is one. Fecal DNA testing is another. The fundamental idea behind the latter is that exfoliated tumor markers are released continuously into stool. Multiple studies on fecal DNA testing for CRC have been done (Ahlquist et aL, Dong et al., Rengucci et al., Tagore et al). Of varying quality, most studies were retrospective, done with variable blinding, using small numbers of archived frozen stools, primarily in pts with known CRC, with many lesions located distal to the splenic flexure. The DNA panels used vary. Permutations of the commercially available EXACT panel were used by AhIquist et al (CRC sensitivity of 91%, 82% for large adenomas w/ specificity of 93%) and Tagore (CRC sensitivity 63.5%, 57.1% for adenomas, Specificity 96.2%). Sensitivities from other studies have varied between 30 and 71 % for invasive CRC and 17 to 43% for adenomas. Of note, stool was tested only once for DNA. There is little published literature on the optimum frequency of DNA testing. Bradt et al found 93% concordance between one time vs three consecutive fecal DNA tests in pts w/known CRC. It is unclear whether EXACT has variations in sensitivity as the frequency of their assay varies.

The first large cross-sectional trial for Fecal DNA testing in an average risk population was published in 12/04 by Imperiale et al in the NEJM "Fecal DNA versus Fecal Occult Blood for Colorectal-Cancer Screening in an Average-Risk Population." Theu study was a prospective blinded cross-sectional trial in an average risk population comparing detection rates of Hemoccult 11 and the EXACT Fecal DNA test for CRC and CRC + adenoma w/high-grade dysplasia. The fecal DNA panel detected 16 of 31 invasive cancers, whereas Hemoccult 11 identified 4 of 31 (51.6 percent vs. 12.9 percent, P=0.003). The DNA panel detected 29 of 71 invasive cancers plus adenomas with high-grade dysplasia, whereas Hemoccult If identified 10 of 71 (40.8 percent vs. 14.1 percent, P<0.001). Among 418 subjects with advanced neoplasia (defined as a tubular adenoma at least 1 cm in diameter, a polyp with a villous, histologic appearance, a polyp with high-grade dysplasia, or cancer), the DNA panel was positive in 76 (18.2 percent), whereas Hemoccult 11 was positive in 45 (10.8 percent). Specificity in subjects with negative findings on colonoscopy was 94.4% for the fecal DNA test and 95.2% for Hemoccult 11.

The sensitivity for Fecal DNA testing in the Imperiale et al study is markedly lower than noted in prior studies, though it's the most rigorous of the fecal DNA literature. Fecal DNA testing has to potential to become a means of noninvasive screening that is equal in sensitivity to screening colonoscopy, and thus warrants further investigation. Not enough data is available right now to suggest the appropriateness

of an equivalence study. One method of increasing sensitivity, as indicated by FOBT, of a test may be through repetition. The Hypothesis for my Study Design is that serial testing with the EXACT fecal DNA panel on 3 consecutive stool will have better sensitivity than traditional Hemocult 11 home-FOBT testing. This time period was chosen given: a) the continuous nature of DNA shedding b) the fact that CRC mutagenesis occurs slowly (such that even if the interval between serial DNA tests in moths, new mutations are unlikely to be uncovered) c) pt acceptability with the procedure. Hemocullt If was chosen because it is the guiac-based test shown to have mortality benefits in RCTs proving FOBT's benefit. The EXACT panel was chosen, because as far as my research indicates, it is the most comprehensive available Fecal DNA screen and is currently considered the industry standard.

It is important to note that with time, the actual sensitivity of the EXACT Fecal DNA panel will improve with the addition to/refinement of the screening DNA panel, and that the cost of the test will decrease from it's current $400-800/test price range, making it more cost-effective than at present.

B. Study Design and Statistical Analysis

This study has a prospective, blinded, cross sectional design (tandem-testing) comparing detection rates between Hemocult 11 and the "EXACT" Fecal DNA test repeated on 3 consecutive stool samples or CRC and CRC/adenomas w/ high grade dysplasia in an average risk population. The predictor variables and outcome variables are dichotomous: the diagnostic tests themselves and absence/presence of CRC respectively. The null hypothesis is that there is no difference in sensitivity between serial DNA testing and FOBT. The alternative hypothesis is that serial fecal DNA testing has a sensitivity for detection of CRC of at least 75% while that of FOBT is 30% in an average risk population. With a Chi-Square Analysis, used to compare proportions of subjects in each of 2 groups that have a dichotomous outcome, and assuming a statistical power of 90 percent to detect a significant difference at a two-sided alpha level of 0.05, it is calculated that need 22pts w/CRC for statistical significance. The lifetime prevalence of CRC is estimated to be 6-7%, though individuals >50yr account for the majority of this prevalence. Difficult to find age-related prevalence, an estimation was made for total sample size based on prior studies, age-specific prevalence, and -20% non-completion rate, indicating that -3300 individuals would need to be enrolled. The actual number of patients screened for enrollment would obviously be higher. Results will be reported as sensitivies w/confidence intervals.

C. Study Procedure

Subjects will be recruited from the academic-university setting of CPMC outpatient population. As per Webcis, -3000 -3700 screening colonscopies were performed from 2001-2005. (If these numbers are lower than expected, patient from another academic center in NY may be enrolled as well. The duration of the study is expected to be 3-5yrs). IRB submission/consents will be submitted to the respective institutions as necessary). Consecutive subjects will be enrolled, anticipating a wide variety of demographic groups. All subjects will provide 3 consecutive fecal samples for DNA testing, then complete three Hemoccult 11 cards before undergoing screening colonoscopy. Tests will be blinded. Stool samples will be analyzed for DNA abnormalities without knowledge of Hemoccult 11 or colonoscopy results and vice-versa. A centralized organization (independent of the sites/individuals collecting the data) will receive results from FOBT, fecal DNA testing, Golonoscopy and pathology testing and will conduct appropriate data analysis.

Subjects will be given instruction for fecal DNA stool collection as per published protocol (NEJM 12/04). The fecal DNA panel consists of 21 mutations: 3 in the K-ras gene, 10 in the APC gene, and 8 in the p53 gene; the microsatelliteinstability marker BAT-26; and a marker of long DNA. Data analysis will be automated. A positive result for any marker is considered a positive test.

Subjects will then be given 3 Hemoccult 11 cards to be completed in standard fasion (NEW 12/04).

Colonoscopy was performed as per the protocol of each site. Adequate colonoscopy requires travel to the cecum and Visualization of >90% mucosa. A finite group of colonoscopists will be responsible for all procedures. Biopsy specimens will be examined at CPMC, with centralized review. Subjects will be eligible for data analysis only if adequate specimens for FeCAL DNA Testing + FOBT are provided, and if colonoscopy is adequate. Subjects will be classified according to the most advanced lesion identified.

D. Study Drugs

There will be no study drugs used in this study.

E. Medical Device

There will be no medical devices used in this study.

F. Study Questionnaires

There will be no questionnaires used in this study.

G. Study Subjects

All participants will be at least 50 years old. Consider is being given as to to whether enrollment should weigh those individuals >65 yo, assuming there is no difference in the genetic epidemiology of sporadic disease.

Exclusion criteria include gastrointestinal bleeding within the preceding month, a change in bowel habits or a recent onset of abdominal pain, previous colorectal cancer or polyps, prior resection of any part of the colon, iron-deficiency anemia, or other coexisting visceral cancer. Persons who have undergone colonoscopy, sigmoidoscopy, or double-contrast barium enema within the preceding 10 years or who have had a positive fecal occult-blood test within the preceding 6 months will be excluded, as were those with inflammatory bowel disease, familial adenomatous polyposis or hereditary nonpolyposis colon cancer, more than one first-degree relative with colorectal cancer, or any first-degree relative with colorectal cancer before the age of 50 years. Persons unwilling or unable to undergo colonoscopy will also be excluded.

H. Subject Recruitment

Subjects will be recruited from consecutive patients from the physician practices involved. It is anticipated that this patient population should be sufficient

I. Confidentiality of Study Data

Information obtained about patients during this study will be kept strictly confidential. Each participant will be assigned a unique code number to keep track of their data and therefore no personal identifiers will be present on study data. Data collected will be accessible only to the investigators.

J. Potential Conflict of Interest

Some of the investigators in the CRC consortium have had paid-advisory roles for EXACT sciences. Documentation from EXACT will be obtained explicity noting these individuals, and that study results will be published regardless of potential negative publicity for the EXACT test.

K. Location of the Study

A multicenter trial that will obtain approval through each individual site's IRB before pt enrollment may start.

L. Potential Risks

Potential Risks Include Harm from the colonoscopy. Complications of colonoscopy include abdominal discomfort, bleeding, perforation, and infection. Risks from anesthesia (conscious sedation) if any is used are present Emotional harm from false + DNA testing.

M. Potential Benefits

Benefits include age-appropriate screening for CRC.

N. Alternative Therapies

Not applicable to this study

O. Compensation to Subjects

Subjects will not be compensated.

P. Costs to Subjects

The study will pay for FOBT testing and Fecal DNA testing. Colonoscopy should be covered by patient's individual insurance.

Q. Minors as Research Subjects

Not applicable to this study

R. Radiation or Radioactive Substances

Not applicable to this study