

Are We Ready for a Blood-Based Test to Detect Colon Cancer?

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Colorectal cancer (CRC)¹ remains one of the leading causes of death in the US despite improvements in early detection, changes in risk factors (e.g., decreased rates of smoking, decreased red meat consumption, and increased use of aspirin), and improved treatments (1). The US Preventive Services Task Force (USPSTF) and the National Comprehensive Cancer Network (NCCN) recommend that an individual age ≥ 50 years with no family history for CRC be screened (2, 3). The gold standard screening method for CRC is colonoscopy. Part of the barrier to widespread utilization of CRC screening is the “ick factor.” For colonoscopy, patients expressed the following reasons they did not undergo a colonoscopy: volume of bowel preparation, inadequate analgesia, no recommendation from primary physician, and embarrassment (4). Other CRC screening methods also have the ick factor, as they are stool-based [e.g., guaiac-based fecal occult blood test (FOBT), fecal immunochemical test (FIT), or stool DNA].

Circulating cell-free DNA (ccfDNA) testing is gaining momentum in clinical testing because ccfDNA can easily be isolated from the circulation (e.g., plasma) and other body fluids of patients. The largest uptake of ccfDNA testing has been in the arena of noninvasive prenatal testing as an alternative to maternal serum screening. ccfDNA is also a promising candidate for cancer testing since ccfDNA has genetic and epigenetic features similar to those of tumor DNA (5).

In this issue of *Clinical Chemistry*, Potter and colleagues describe a blood-based assay to detect ccfDNA methylated septin 9 (*mSEPT9*) and its ability to detect colon cancer (6). In the first part of the study, the authors describe some of the analytical performance characteristics of the *mSEPT9* assay, Epi proColon. Analytical validation requires proving that you can detect

what you say you can detect. The authors use DNA from a well-documented cancer cell line derived from cervical cancer as the positive reference material. It is interesting to note that the reference material is not derived from colon cancer cells, indicating that epigenetic changes (i.e., methylation) in *SEPT9* are not CRC specific. The other studies for analytical validation included limit of detection (i.e., sensitivity), cross-reactivity (e.g., specificity), and robustness.

The scoring of a positive vs a negative result with the Epi proColon assay for *mSEPT9* is determined by examining 3 independent reactions for presence or absence of amplification curves at 45 cycles. If any 1 of the 3 reactions is positive, then the result is called positive. Because the amplification control is a separate reaction, false negatives could occur if, for example, there was a pipetting error in the *mSEPT9* reactions. Such an occurrence may explain the 2 false negatives observed by Potter et al. Incorporation of an internal amplification control such as armored DNA could improve the assay. It is of additional interest to develop understanding of the 11 false-positive results observed in pooled serum from healthy donors. Was there low-level contamination present in these samples? Could any sort of correlation be determined between a strong or weak positive? Although the assay was scored as yes/no, semiquantitative information might be inferred from the results based on number of positive replicates and/or the cycle threshold value. Warren et al. (7) reported that *mSEPT9* ccfDNA can be detected in pregnant women, and perhaps some of the “normal” plasma may have been collected from a pregnant female.

The clinical validation described by Potter et al. is of more interest in determining whether *mSEPT9* could be used for screening colon cancer. The authors compared the diagnostic accuracy for CRC by colonoscopy with that of the Epi proColon assay in screened individuals with multiple ethnic backgrounds. Overall, clinical sensitivity was 68% (95% CI 53%–80%), clinical specificity 79% (95% CI 77%–81%), positive predictive value 8%, and negative predictive value 99%. These values are very similar to FOBT (clinical sensitivity 50%–79%) and FIT (clinical sensitivity 55%–100%) (8). All stage IV CRC was detected, although the numbers were small. Several false negatives were observed when *mSEPT9* was compared to colonoscopy.

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¹ Nonstandard abbreviations: CRC, colorectal cancer; USPSTF, US Preventive Services Task Force; NCCN, National Comprehensive Cancer Network; FOBT, fecal occult blood test; FIT, fecal immunochemical test; ccfDNA, circulating cell-free DNA.

The data in Supplemental Table 2 suggested that the rate of false negatives is influenced by stage of the cancer (6). It is possible that stage I cancer is associated with less ccfDNA in the blood than higher stages. Of note, the positivity rates for stage II and III were essentially the same. This may be a reflection of the small number of positive samples obtained.

The false positives were also of interest in this study. The majority of false positives were observed in advanced adenomas (clinical sensitivity 18%–24%). This compares similarly to both FOBT (21%–35%) and FIT (15%–44%) (8). False positives also were observed in individuals with small polyps as well as those with a normal colonoscopy. The authors did not discuss the question of whether these positives were “strong” positives (e.g., had multiple positive reactions). It is estimated that colonoscopy misses about 4% of CRC, especially for right-sided colon cancer (9). Also, because *mSEPT9* is an epigenetic biomarker, this does not negate other potential cancer processes. The proof over time will be whether the cohort tested develops any cancers after appropriate follow-up. Many of the false positives for FOBT and FIT are understood to result from diets high in rare red meat and certain fruits and vegetables (e.g., horseradish, cantaloupe, raw turnips, broccoli, cauliflower, red radishes, and parsnips), medications (e.g., aspirin, vitamin C), hemorrhoids, and cleaning products in the toilet water (10). By comparison, the causes of false-positive ccfDNA *mSEPT9* assay results are less understood.

Are we ready for a blood-based test to detect colorectal cancer? The data for ccfDNA *mSEPT9* look promising. More studies will need to be done to understand false-positive and false-negative results. Testing for ccfDNA *mSEPT9* potentially is an alternative for patients that are noncompliant with colonoscopy, FOBT, or FIT. It is attractive since *mSEPT9* is a blood-based test and is easily collected during a routine medical appointment. There is no ick factor in a routine blood draw compared to stool-based testing or the preparation needed for a colonoscopy procedure. Ultimately whether guidelines such as USPSTF and NCCN will incorporate *mSEPT9* as a biomarker for CRC screening will be determined only after additional data have been generated. One thing is certain: early detection improves cancer survival rates. Because only approximately 65% of eligible people are getting the recommended screening for CRC (11), any test that improves screening compliance will save lives.

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References

1. Siegel R, Naishadham D, Jemal A. Cancer Statistics, 2013. *CA Cancer J Clin* 2013;63:11–30.
2. U.S. Preventive Services Task Force. Screening for Colorectal Cancer: U.S. Preventive Services Task Force Recommendation Statement. AHRQ Publication 08-05124-EF-3, October 2008. Agency for Healthcare Research and Quality, Rockville, MD.
3. NCCN guidelines on colon cancer, version 1.2014. http://www.nccn.org/professionals/physician_gls/f_guidelines.asp (Accessed June 10, 2014).
4. Harewood GC, Wiersema MJ, Melton LJ. A prospective, controlled assessment of factors influencing acceptance of screening colonoscopy. *Am J Gastroenterol* 2002;97:3186–94.
5. Utting M, Werner W, Dahse R, Schubert J, Junker K. Microsatellite analysis of free tumor DNA in urine, serum, and plasma of patients: a minimally invasive method for the detection of bladder cancer. *Clin Cancer Res* 2002;8:35–40.
6. Potter NT, Hurban P, White MN, Whitlock KD, Lofton-Day CE, Tetzner R, et al. Validation of a real-time PCR-based qualitative assay for the detection of methylated *SEPT9* DNA in human plasma. *Clin Chem* 2014;60.
7. Warren JD, Xiong W, Bunker AM, Vaughn CP, Furtado LV, Roberts WL, et al. Septin 9 methylated DNA is a sensitive and specific blood test for colorectal cancer. *BMC Med* 2011;9:133.
8. NCCN, American Cancer Society. Clinician's reference: fecal occult blood testing (FOBT) for colorectal cancer screening. nccn.org/wp-content/uploads/FOBTCliniciansReferenceFinal.pdf (Accessed June 10, 2014).
9. Bressler B, Paszat LF, Vinden C, Li C, He J, Rabeneck L. Colonoscopic miss rates for right-sided colon cancer: a population-based analysis. *Gastroenterol* 2004;127:452–6.
10. Deciphering diagnostics: what you need to know about FOBT. http://www.nursingcenter.com/nc/journalarticle?Article_ID=800657 (Accessed June 13, 2014).
11. CDC. Colorectal cancer screening rates remain low. <http://www.cdc.gov/media/releases/2013/p1105-colorectal-cancer-screening.html> (Accessed June 10, 2014).