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Stool DNA Testing: Are We There Today?

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Screening for colorectal cancer is one of the most effective public health interventions. First-generation stool DNA tests demonstrated better sensitivity for colorectal cancer than fecal occult blood tests. Improvements to stool DNA tests have made them more sensitive and less complex. The newer marker panels can detect the vast majority of colorectal cancers and a large proportion of advanced adenomas, regardless of location in the colon. This review summarizes the development and advances to stool DNA testing for colorectal cancer.

INTRODUCTION

Screening for colorectal cancer (CRC) is highly effective in preventing CRC, which is why it has been endorsed by all major medical societies, receiving a Grade A recommendation from the United States Preventive Services Task Force (USPSTF).[1,2] Many screening tests have been recommended by guideline committees. In practice, colonoscopy and fecal occult blood tests, especially fecal immunochemical tests (FIT), are the predominant methods used, particularly colonoscopy in most settings.

An ideal cancer screening test should be non-invasive, easy to perform, convenient, safe, highly accurate, operator-independent, and inexpensive. Furthermore, since most cancer screening tests need to be repeated over time, patients should be willing to adhere to periodic testing. FIT testing has most of these advantages. It is non-invasive, performed at home on a single stool sample, safe, inexpensive, and operator-independent. However, FIT is known to miss up to 30% of cancers and 70-80% of the most precancerous polyps because bleeding rates may be low. For this reason, FIT is recommended as an annual test, something that most patients (and their physicians) find hard to do, or keep track of. Even when this is done, the nature of bleeding by colon cancers and polyps is such that those located in the distal colon are detected better than those in the proximal colon. Sessile serrated polyps, important new precursor lesions that do not bleed, are also unlikely to be detected by FIT. Moreover, false-positives occur if there is blood in the stool for a reason other than a cancer/polyp.

Given the limitations of FIT in terms of sensitivity, lower detection of proximal cancers and polyps, and poor adherence with annual testing, efforts have been made in the last two decades to develop a more sensitive stool-based test. A new multi-target stool DNA test (MT-sDNA; Cologuard®; Exact Sciences Corporation) goes beyond detecting occult blood in the stool because it also incorporates an analysis of abnormal DNA. This review will discuss the principle behind stool DNA testing, and summarize the recent findings of studies using the MT-sDNA test.

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Rationale for Stool DNA Testing

Until about 20 years ago, the notion that specific human gene mutations could be detected in stool among the enormous amount of bacterial DNA seemed an insurmountable hurdle. After all, human DNA represents only 0.01% of total stool DNA, the other 99.99% coming from non-human sources such as the microflora and diet.[3] Thanks to technological advances, analyzing DNA alterations in stool has become a reality.

There are several reasons why DNA is a promising analyte in stool. First, because so much is known about the molecular pathogenesis of colorectal neoplasia, highly discriminant markers can be chosen which represent the molecular pathways used by neoplastic, but not by normal, colonocytes. Second, unlike proteins for example, DNA is stable and can be amplified. Indeed, the use of sensitive PCR techniques with PCR inhibitors, and modern approaches for capturing DNA, has greatly facilitated the detection of even tiny amounts of mutated human DNA from stool. Third, DNA is exfoliated continuously from the surface of polyps and cancers, whereas bleeding from lesions is intermittent. Studies indicate that when normal colonocytes mature and eventually die, they undergo programmed cell death (anoikis) whereby their DNA is digested and phagocytosed by lamina propria macrophages and not much is shed into the lumen. However, since neoplastic colonocytes have impaired anoikis, proportionately more mutated DNA is shed into the lumen from these lesions. Fourth, the epithelial layer of an adenoma is much larger than the size of the polyp might suggest due to the extensive network of infolded, complex tubules. If one were to unravel the epithelial layer of a tubular adenoma, the surface area is approximately 250-fold larger than the polyp appears. As such, an adenoma measuring 2 cm in diameter has a surface area of approximately 800-1600 cm^2.[4]

Clinical Validation Studies

Numerous early studies used single and multiple DNA markers to demonstrate proof of concept that stool DNA testing was a viable approach (reviewed in 5). Some of these studies compared stool DNA tests to fecal occult blood tests and demonstrated superior sensitivity for CRC using the sDNA test.[6,7] However, these earlier molecular panels were cumbersome, expensive and still had suboptimal sensitivity for CRC and adenomas. They did, however, set the stage for the current version of the MT-sDNA test. The current test analyzes two highly discriminant methylated genes (*BMP3* and *NDRG4*), the seven most informative point mutations of the *k-ras* oncogene, a marker for total human DNA (*beta-actin*) and fecal hemoglobin.

Two large multicenter case-control studies were conducted with the MT-sDNA test before it was subjected to a pivotal validation study. One study used a non-optimized prototype of the MT-sDNA test and identified 87% of patients with CRC stages I-III, 54% of advanced adenomas, with 90% specificity.[8] Importantly, adenoma detection increased with larger adenoma size, and detection rates for CRC as well as adenomas was similar between the proximal and distal colon. The second case-control study used an optimized and automated MT-sDNA test.[9] Here, the sensitivity for CRC was 98% overall. Again, detection was not affected by tumor stage or location in the colon, and the specificity was 90%. The sensitivity for advanced precancerous lesions (which included advanced adenomas as well as sessile serrated polyps) was 57%, 73% and 83% for lesions ≥1 cm, >2 cm, and >3 cm, respectively.

Given the ability of the optimized, automated MT-sDNA test to detect the vast majority of cancers, a significant proportion of advanced precancerous lesions, a large, prospective pivotal validation study, called Deep-C, was performed.[10] This study enrolled over 10,000 asymptomatic, average-risk men and women across 90 North American sites. All subjects submitted a stool sample for MT-sDNA, as well as a commercial FIT test, and then underwent their screening colonoscopy. An independent clinical research organization oversaw the study, and the laboratory was blinded to the clinical findings. The age, gender, and ethnic distribution of the study population was nearly identical to that of the general U.S. population. The sensitivity of MT-sDNA versus commercial FIT was 92% vs. 74% for CRC overall, 93% vs. 73% for CRC stages I-III, and 94% vs. 70% for CRC stages I-II (all statistically significant). Advanced adenomas in this study was defined as adenomas or sessile serrated polyps >1 cm or any size adenoma containing villous features or HGD. Sensitivity of MT-sDNA for advanced adenomas was 42%, compared to 24% with FIT ($P<0.001$). Sensitivity for MT-sDNA was 66% for adenomas ≥2 cm, 42% for sessile serrated polyps ≥1 cm, and 69% for adenomas with HGD, compared to 43%, 5%, and 46%, respectively with FIT (all statistically significant). MT-sDNA detected advanced adenomas and sessile serrated

polyps in proportion to size, and was better than FIT for detecting lesions in the proximal colon. Specificity of FIT was higher than MT-sDNA. If patients with non-advanced findings at colonoscopy were included (for example, polyps < 1cm of any number), the specificity of MT-sDNA was 87% compared to 95% with FIT (P<0.001). However, if specificity was calculated using only patients with normal colonoscopies, the respective specificities were 90% and 96% with MT-sDNA and FIT (P<0.001).

Based on the Deep-C study (and the supporting data leading up to it), on August 11, 2014, the US Food and Drug Administration (FDA) approved the MT-sDNA test for use in general CRC screening. On the same date, the Center for Medicare and Medicaid Services (CMS) ruled that the test would be covered by Medicare at a frequency of every 3 years (based on mathematical modeling studies). Since the publication of the Deep-C study, very similar findings comparing MT-sDNA to FIT were observed in a screening trial of Alaska Natives.[11] In that study, the sensitivity of MT-sDNA vs. FIT for CRC (n=10) - 100% vs. 80% (P=0.48); for adenomas >2 cm - 62% vs. 29% (P=0.05); for sessile serrated polyps >1 cm - 67% vs. 11% (P=0.07), with specificity 93% vs. 96% (P<0.03).

Practical Clinical Questions

Based on the results of the Deep-C pivotal study, and the availability of Cologuard® in clinical practice, a variety of clinical questions arise which are on the minds of many healthcare providers, policy makers, payors and patients. I will offer my own position on these matters in this section.

- **Is the sensitivity of MT-sDNA high enough to be an effective screening test?**

With a 93% sensitivity for stage I-III CRC, MT-sDNA is similar to that of colonoscopy, and significantly higher than the point sensitivity of 70% for FIT.[10] Thus, MT-sDNA misses about 7% of CRC, whereas FIT misses 30%. In the Deep-C study, of the 65 cancers, 13 were detected by MT-sDNA and missed by FIT, whereas only one was detected by FIT and missed by MT-sDNA. Because the compliance over time for CRC screening can be uncertain for an individual patient, in my opinion you want to make sure that the test will at least detect cancer at that single point in time, in case the patient does not return for regular surveillance. Also important for an effective screening test is its ability to detect those precursor lesions that are most likely to develop into cancer. In this regard, adenomas larger than 2 cm and those with HGD are among the most likely lesions to progress, and MT-sDNA outperformed FIT for detecting these lesions. Likewise, the sensitivity of MT-sDNA for detecting sessile serrated polyps ≥1 cm was 9-times greater than FIT, a finding that is readily understandable knowing that these subtle, often invisible lesions, do not bleed. It is worth considering that if an adenoma is missed by MT-sDNA, and the patient is compliant with follow-up testing, the programmatic sensitivity of the test will increase to a rather respectable level. Mathematical models suggest that assuming a screening frequency of every 3-years, and a polyp growth doubling time of 6 years, the point-sensitivity of 69% for HGD would yield programmatic sensitivities of 93% and 99% by the second and third screening rounds, respectively.[12]

- **Will patients accept this test?**

Because the MT-sDNA is a new test in clinical practice, there are limited data on patient acceptance. A prospective survey of 4,042 (84%) subjects participating in an earlier generation sDNA test revealed that sDNA testing received the same or higher mean ratings than guaiac based FOBT for most prep- and test-related features, and except for perceived accuracy, also received higher ratings than colonoscopy.[13] Post-marketing data may help answer this important question with respect to the new MT-sDNA test.

- **How often should MT-sDNA be performed?**

Again because the MT-sDNA test is new, there has not been enough time to do a study, which would offer guidance regarding screening interval. CMS has recommended a 3-year interval based on modeling studies. Other recent modeling studies support the 3-year interval and show that MT-sDNA every 3 years lies within the 98% of the efficiency frontier and provides greater than 90% of the Life Years Gained (LYG) with screening colonoscopy.[14]

- **With a higher false positive rate, won't this subject more patients to colonoscopy than FIT?**

It is true that more positive tests will generate more colonoscopies. However, at present, all individuals older than age 50 are recommended to have screening colonoscopy, so they would be referred for a test that is already recommended.

- **How do I interpret a positive MT-sDNA when the colonoscopy is normal?**

This does pose a clinical dilemma but several things

should be considered. First, one would want to make sure that the colonoscopy is of good enough quality to reasonably exclude any neoplasia. Second, for decades clinicians have been faced with the finding of a normal colonoscopy after a positive fecal occult blood test, and most tend to reassure the patient or repeat the stool test at some interval. Since the MT-sDNA test detects occult blood, it is possible that the false-positive result is from the hemoglobin component. Since the test results are reported as either positive or negative (the individual markers are not reported), there is no way to know if this is the case. Third, false-positive tests may be a function of age. Many genes become hypermethylated with age and we know from the Deep-C study that individuals over 65 years old had a higher false positive rate than those between ages 50-65 years. Fourth, there is concern that this situation might mean that a cancer higher up in the GI tract (or even the lungs) might be contributing abnormal DNA in the stool. However, as currently configured, the MT-sDNA has been optimized to detect colorectal, rather than upper GI neoplasia. Previous studies using an earlier generation sDNA test found no neoplastic pathology above the colon when upper endoscopy and CT scanning was performed on 60 consecutive patients with a positive sDNA and negative colonoscopy.[12]

- **Isn't this test too expensive?**

At present, the MT-sDNA list price is $649 (Medicare cost $509). However, this price includes a national 24 hour, 7 days a week, patient navigation and compliance service. Also, if the test is done every 3 years, the cost can be amortized to approximately $216 per year. Compared to not screening, the cost effectiveness ratio of $11,313 per quality-adjusted life year is within the acceptable range.[14]

- **Can I use this test in high-risk patients?**

No. The currently available MT-sDNA test was designed for, and tested only in, average risk, asymptomatic individuals. High-risk individuals with inflammatory bowel disease should not perform this test since they may have blood in the stool producing too many false positives. Studies are ongoing to develop an IBD-specific MT-sDNA test.[15] Also, the markers in this the panel have not been tested in patients with genetic susceptibility due to familial polyposis and Lynch Syndrome.

CONCLUSION

Currently, in the U.S., approximately one-third of screen eligible individuals have not undergone screening. The reasons for this are multifactorial, and include barriers at the system, physician and patient level. There is currently a national campaign to increase CRC screening rates to 80% by 2018.[16] While efforts are ongoing to maximize the use of screening colonoscopy and FIT testing across the country, the performance characteristics and results from clinical studies using MT-sDNA offers a very reasonable and important contribution to our fight against one of the most preventable cancers. ■

References

1. Levin B, Lieberman DA, McFarland B, et al. Screening and surveillance for the early detection of colorectal cancer and adenomatous polyps, 2008: a joint guideline from the American Cancer Society, the US Multi-Society Task Force on Colorectal Cancer, and the American College of Radiology. *CA Cancer J Clin*. 2008;58:130-60.
2. Whitlock EP, Lin JS, Liles E, Beil TL, Fu R. Screening for colorectal cancer: a targeted, updated systematic review for the U.S. Preventive Services Task Force. *Ann Intern Med*. 2008;149:638-58.
3. Osborn NK, Ahlquist DA. Stool screening for colorectal cancer: molecular approaches. *Gastroenterology*. 2005;128:192-206.
4. Berger BM and Ahlquist DA. Stool DNA screening for colorectal neoplasia: biological and technical basis for high detection rates. *Pathology* 2012;44:80-8.
5. Potack J, Itzkowitz S. Practical advances in stool screening for colorectal cancer. *JNCCN* 2010;8:81-92.
6. Imperiale TF, Ransohoff DF, Itzkowitz SH, Turnbull BA, Ross ME; Colorectal Cancer Study Group. Fecal DNA versus fecal occult blood for colorectal-cancer screening in an average-risk population. *N Engl J Med*. 2004;351:2704-14.
7. Ahlquist DA, Sargent DJ, Loprinzi CL, et al. Stool DNA and occult blood testing for screen detection of colorectal neoplasia. *Ann Intern Med*. 2008;149:441-50.
8. Ahlquist DA, Zou H, Domanico M, et al. Next-generation stool DNA test accurately detects colorectal cancer and large adenomas. *Gastroenterology* 2012;142:248-56.
9. Lidgard GP, Domanico M, Bruinsma JJ, et al. Clinical performance of an automated stool DNA assay for detection of colorectal neoplasia. *Clin Gastroenterol Hepatol*. 2013;11:1313-18.
10. Imperiale TF, Ransohoff DF, Itzkowitz SH, et al. Multitarget stool DNA testing for colorectal-cancer screening. *N Engl J Med* 2014;370:1287-97.
11. Redwood DG, Asay ED, Blake ID, et al. Stool DNA testing for screening detection of colorectal neoplasia in Alaska Native people. *Mayo Clin Proc* 2016;91:61-70.
12. Ahlquist DA. Multi-target stool DNA test: a new high bar for noninvasive screening. *Dig Dis Sci* 2015;60:623-633.
13. Schroy PC 3rd, Heeren TC. Patient perceptions of stool-based DNA testing for colorectal cancer screening. Am J Prev Med. 2005;28:208-14.
14. Berger BM, Parton MA, Levin B. USPSTF Colorectal cancer screening guidelines: an extended look at multi-year interval testing. *Am J Manag Care*. 2016;22:e77-e81.
15. Kisiel JB, Yab T, Hussain FTH, et al. Stool DNA testing for the detection of colorectal neoplasia in patients with inflammatory bowel disease. *Aliment Pharmacol Ther*. 2013;37:546-554.
16. National Colorectal Cancer Roundtable 80% by 2018 fact sheet; 2014. http://nccrt.org/about/80-percent-by-2018/