Oncology

# Evaluation of an Epigenetic Assay for Predicting Repeat Prostate Biopsy Outcome in African American Men

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**OBJECTIVE** To evaluate an epigenetic assay performed on tissue from negative prostate biopsies in a group of African American (AA) men undergoing repeat biopsy, and to compare accuracy for predicting repeat biopsy outcome to prior studies conducted in predominantly Caucasian populations.

**MATERIALS AND METHODS** The study population consisted of 211 AA men from 7 urology centers across the United States; all of whom were undergoing 12-core transrectal ultrasound-guided repeat biopsy within 30 months from a negative index biopsy. All biopsy cores from the negative index biopsy were profiled for the epigenetic biomarkers *GSTP1*, *APC*, and *RASSF1* using ConfirmMDx for Prostate Cancer (MDxHealth, Irvine, CA).

**RESULTS** Upon repeat biopsy, 130 of 211 subjects (62%) had no prostate cancer (PCa) detected and 81 of 211 (38%) were diagnosed with PCa. Of the subjects with PCa, 54 (67%) were diagnosed with Gleason score (GS) ≤6 PCa and 27 (33%) with GS ≥7 disease. For detection of PCa at repeat biopsy, ConfirmMDx sensitivity was 74.1% and specificity was 60.0%, equivalent to prior studies ($P$ = .235 and .697, respectively). For detection of GS ≥7 PCa, sensitivity was 78% and specificity was 53%. The negative predictive values for detection of all PCa and GS ≥7 PCa were 78.8% and 94.2%, respectively.

**CONCLUSION** In this group of AA men, we successfully validated an epigenetic assay to assess the need for repeat biopsy. Results were consistent with previous studies from predominantly Caucasian populations. Therefore, the ConfirmMDx assay is a useful tool for risk stratification of AA men who had an initial negative biopsy. UROLOGY 128: 62–65, 2019. © 2018 The Author(s). Published by Elsevier Inc.

Men with 1 or more negative prostate biopsies present a dilemma for clinicians and patients themselves. Because of limitations of the current standard of care, 60%-70% of initial prostate biopsies fail to detect cancer, and 20%-30% of men receive false negative biopsy results.[1,2] The fear that cancer was missed leads to repeat biopsies, which increases health-care costs and exposes men to potential morbidity, including post-biopsy infection and sepsis.[3-5] There is an unmet need for more accurate diagnostic tools to improve risk stratification and to help identify men who are most likely to benefit from a repeat biopsy.

Cancer-specific DNA methylation occurs early in the oncogenic process, and these epigenetic changes can be detected in prostate biopsy tissue at a distance from the actual tumor through a cancer-associated field effect.[6] ConfirmMDx for Prostate Cancer (MDxHealth, Irvine, CA) is a multiplex epigenetic assay that measures DNA methylation of *GSTP1*, *APC*, and *RASSF1*. The assay detects the presence of cancer in adjacent histologically negative prostate tissue.[6] When performed on tissue from cancer-negative prostate biopsies, ConfirmMDx can improve accuracy for predicting repeat biopsy outcome relative to the current standard-of-care risk factors.[7] In a large, blinded clinical validation study, the assay yielded a negative predictive value (NPV) of 90% for detection of

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cancer at biopsy, but only 42 of 350 study subjects were African American (AA).[8]

Prostate cancer (PCa) incidence and mortality rates are significantly higher in AA men compared to Caucasian American men.[9] AAs have a 1.7-fold increased incidence and a 2.3-fold higher PCa mortality rate compared to US non-Hispanic Caucasians (North American Association of Central Cancer Registries [NAACCR] 2016 and National Center for Health Statistics [NCHS] 2016). Although other factors may contribute to these differences, it is increasingly evident that this disparity has a molecular basis.[10] In fact, differences in genetic alterations have been reported in malignant prostate tissues from patients of diverse racial backgrounds.[10,11] These findings have potential implications for molecular tests used for cancer detection and management as the same racial diversity could apply to biomarkers.

All published ConfirmMDx studies to date have been performed in predominantly Caucasian subjects. In this study, we evaluated the performance of ConfirmMDx for Prostate Cancer in a cohort of AA men undergoing repeat prostate biopsy.

## MATERIALS AND METHODS

The study population consisted of 211 AA men from 7 urology centers across the United States, all of whom were undergoing standard 12-core transrectal ultrasound-guided repeat biopsy within 30 months from a cancer-negative index biopsy. The sites identified eligible subjects in a retrospective, consecutive manner starting from patients biopsied at the clinics at study initiation in 2011. All subjects had been screened for PCa and had received their index biopsy based on known risk factors (eg, elevated serum prostate-specific antigen level, abnormal digital rectal examination, and clinical symptoms). Men with atypical small acinar proliferation at index biopsy were excluded. Histopathology review was performed on all available tissue samples to verify the absence of adenocarcinoma or atypical small acinar proliferation. We requested 40$\mu$ of each archived, formalin-fixed, paraffin-embedded tissue core block from the initial biopsy. However, in the event of limited excess tissue, a minimum of 20$\mu$ from formalin-fixed, paraffin-embedded tissue cores were used. After completion of all analyses with the epigenetic assay, clinical and patient characteristics were verified and updated with data from subsequent biopsies as necessary.

All cancer-negative biopsy cores were tested in a random, blinded fashion using ConfirmMDx for Prostate Cancer, a multiplexed quantitative DNA methylation-specific polymerase chain reaction assay for the epigenetic biomarkers *GSTP1*, *APC*, and *RASSF1*.[6,12] The methylation ratio of all 3 genes was determined relative to that of the *ACTB* reference gene. Predetermined analytical cutoff values for determining methylation status of each gene were identical to those used in the independent MATLOC (Methylation Analysis to Locate Occult Cancers) and DOCUMENT (Detection of Cancer Using Methylation Events in Negative Tissue) studies.[7,8] Assay results were considered positive if any core yielded methylation signal above the pre-established threshold.

The clinical performance for detection of any cancer, or Gleason score (GS) ≥7 cancer, was assessed and compared to results from previous studies. The chi-square test was used to compare proportions, including sensitivity and specificity values across different studies, and Mann-Whitney test was used for comparing continuous variables.

## RESULTS

Clinical characteristics of the subject population are shown in Table 1. Upon repeat biopsy, 130 subjects (62%) had no PCa detected and 81 (38%) were diagnosed with PCa. Of the 81 subjects with PCa, 54 (67%) were diagnosed with GS ≤6 PCa and 27 (33%) with GS ≥7 disease. There was no difference in age, serum prostate-specific antigen level, or digital rectal examination result between PCa-positive and PCa-negative groups.

Table 2 shows ConfirmMDx clinical performance characteristics for detection of any cancer or high-grade PCa at repeat

**Table 1.** Subject demographics

| Parameter | | Cancer (N = 81) | Benign (N = 130) | *P* Value |
|---|---|---|---|---|
| Age (y) | Mean | 64 | 65 | .366 |
| | Median | 63 | 65 | |
| | Range | (46-86) | (43-83) | |
| Serum PSA (ng/mL) | Mean | 8.4 | 8.0 | .298 |
| | Median | 6.2 | 6.6 | |
| | Range | (0.6-61.6) | (0.8-30.8) | |
| DRE result (N, %) | Normal | 45 (55%) | 81 (62%) | .331 |
| | Suspicious | 15 (19%) | 9 (7%) | |
| | N/A | 21 (26%) | 40 (31%) | |
| Gleason sum(N, %) | 6 | 54 (66%) | | |
| | 7 (3 + 4) | 15 (19%) | | |
| | 7 (4 + 3) | 6 (7%) | | |
| | 8 | 4 (5%) | | |
| | 9 | 2 (3%) | | |
| Clinical stage (N, %) | T1c | 56 (69%) | | |
| | T2a | 7 (9%) | | |
| | T2c | 3 (4%) | | |
| | N/A | 15 (18%) | | |

DRE, digital rectal examination; N/A, not applicable; PSA, prostate-specific antigen.

**Table 2.** Clinical performance characteristics

| Detection of Any Cancer at Biopsy | | |
|---|---|---|
| Parameter | Value | 95% CI |
| Sensitivity | 74.1% | 63.1%-83.1% |
| Specificity | 60.0% | 51.1%-68.5% |
| Disease prevalence | 38.4% | N/A |
| PPV | 53.6% | 47.4%-59.6% |
| NPV | 78.8% | 71.5%-84.6% |
| **Detection of High-grade Cancer at Biopsy** | | |
| Sensitivity | 77.8% | 57.7%-91.4% |
| Specificity | 52.7% | 45.2%-60.1% |
| Disease prevalence | 12.8% | N/A |
| PPV | 19.4% | 15.8%-23.7% |
| NPV | 94.2% | 88.7%-97.1% |

CI, confidence interval; NPV, negative predictive value; PPV, positive predictive value.

biopsy. The 2 × 2 contingency tables used to generate these results may be found in Supplementary Table S1. For detection of PCa at biopsy, sensitivity was 74.1% and specificity was 60.0%. For high-grade cancer detection (GS6 or benign pathology considered "negative"), sensitivity was 77.8% and specificity was 52.7%. The NPVs for PCa and high-grade PCa were 78.8% and 94.2%, respectively.

We then compared ConfirmMDx clinical performance from this cohort to 2 published multicenter studies[7,8] conducted in primarily Caucasian populations (Table 3). For detection of cancer at repeat biopsy, there were no significant differences in sensitivity ($P = .235$) or specificity ($P = .697$). We also evaluated ConfirmMDx clinical performance by age range (Table 4). Subjects were divided into 3 groups: <55 years, 55-69 years, and ≥70 years old. No significant differences in sensitivity ($P = .418$) or specificity ($P = .213$) were observed between age groups, although the study was not specifically powered for this analysis.

## DISCUSSION

The results from this study provide external validation for the use of ConfirmMDx to help guide repeat biopsy decision-making for AA men. Furthermore, ConfirmMDx clinical sensitivity and specificity were equivalent to previously published studies conducted in primarily Caucasian men. This finding is especially important when considering that several PCa oncologic molecular pathways demonstrate substantial racial differences. Powell and Bollig-Fischer reported on multiple molecular PCa differences in AAs compared to Caucasian Americans including tumor suppressor genes, ERG, and single-nucleotide polymorphisms, in addition to various gene amplifications and deletions related to oncogenesis like androgen receptor signaling.[13] Some of these molecular differences may also affect molecular tests that aid in diagnosis of PCa.

Therefore, it is of special importance to investigate the scientific validity of specific molecular tools in AA men. It is also of note that molecular testing with this epigenetic assay has verifiable objective results that are reproducible and not subject to variability based on location, interpretation, or operator. Additionally, when molecular testing is used after validation in AA cohorts, it has the potential to positively impact health-care disparity related to PCa outcomes. Positively impacting health-care disparities in diseases related to specific populations is an important objective of the US Department of Health and Human Services.[14]

Similarly, in the recent 2017 US Preventative Services Task Force "Draft Proposal," research related to PCa diagnosis and treatment is specifically recommended: "*The USPSTF strongly encourages research on screening for and treatment of prostate cancer in African American men. It is important to consider both the potential additional benefits and harms to fully understand the value of screening. Studies are needed to confirm that African American men who undergo screening receive similar or greater reductions in prostate cancer mortality compared with men in the general population, as well as to explore the optimal screening frequency and whether beginning screening before age 55 years provides additional benefits in African American men. Studies are also needed to better understand strategies to mitigate harms and maximize benefits of screening, diagnostic follow up, and treatment*

**Table 3.** Comparison of clinical performance characteristics vs previous studies

| Parameter | MATLOC | DOCUMENT | This Study |
|---|---|---|---|
| Number of subjects | 483 | 320 | 211 |
| Sensitivity (95% CI) | 68% (57%-77%) | 62% (52%-72%) | 74% (63%-83%) |
| Specificity (95% CI) | 64% (59%-69%) | 64% (57%-70%) | 60% (51%-69%) |

DOCUMENT, Detection of Cancer Using Methylation Events in Negative Tissue; MATLOC, Methylation Analysis to Locate Occult Cancers.

**Table 4.** ConfirmMDx clinical performance by age range

| Parameter | <55 y | 55-69 y | ≥70 y |
|---|---|---|---|
| Total number of subjects | 28 | 113 | 69 |
| Cancer-positive (%) | 11 (39%) | 47 (42%) | 23 (43%) |
| Sensitivity (95% CI) | 64% (35%-85%) | 70% (56%-81%) | 83% (62%-94%) |
| Specificity (95% CI) | 77% (52%-91%) | 61% (49%-72%) | 52% (38%-66%) |

*(including active surveillance) in African American men. It is also important that research and quality improvement activities continue to work to eliminate disparities in access to high-quality care for men with prostate cancer".*

The objectives and findings of this study align with the US Preventative Services Task Force's recommendations for more research targeted in AA men at increased risk for PCa. In the context of increased concern about the risk of PCa in AA men, the decision to perform a repeat prostate biopsy after a negative initial biopsy needs to be considered carefully. The risk of missing a significant cancer in a population that has increased PCa mortality is a troubling concern. Therefore, to avoid the second biopsy, a tool that provides a high NPV has the potential for actionable utility. This epigenetic assay performed in that desired fashion by providing an NPV over 90%, and more specifically over 96% for high risk PCa. Although this study was multi-institutional and of broad geographic diversity, we encourage additional research studies that may contribute to this collective body of research in the AA population at risk for PCa.

One important limitation of this study is noted. All enrolled subjects received a repeat prostate biopsy. The results therefore apply to a group of men who, despite negative initial biopsy results, had clinical characteristics suspicious enough to recommend repeat biopsy. The true prevalence of PCa in patients who had an initial biopsy, but did not have a repeat biopsy, is unknown. Therefore, the results may not be generalizable to the population of AA men at large with a negative initial biopsy who do not have sufficient clinical suspicion to consider a repeat biopsy.

## CONCLUSION

The ConfirmMDx epigenetic assay improved the identification of AA men at risk for occult high-grade PCa upon repeat biopsy. Risk stratification was demonstrated for both the presence of PCa and high-grade PCa, with clinical sensitivity and specificity equivalent to previous studies performed on cohorts that were predominantly Caucasian. The assay's high NPV provides useful information to help identify men who could potentially avoid or delay an invasive repeat prostate biopsy procedure with its associated risks. Although the ConfirmMDx positive predictive value was lower than the NPV in this study, the assay did increase the rate of cancer detection and may help urologists better select men who would likely benefit from repeat biopsy to determine the presence of PCa.

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## APPENDIX

### *Supplementary Data*

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.urology.2018.04.001.