A Urine-Based Exosomal Gene Expression Test Stratifies Risk of High-Grade Prostate Cancer in Men with Prior Negative Prostate Biopsy Undergoing Repeat Biopsy

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Abstract

Background : Initial prostate biopsy often fails to identify prostate cancer resulting in patient anxiety, especially when clinical features such as prostate specific antigen (PSA) remain elevated, leading to the need for repeat biopsies. Prostate biomarker tests, such as the ExoDx™ Prostate (IntelliScore) , or EPI test, have been shown to provide individualized risk assessment of clinically significant prostate cancer at initial biopsy; however, the performance in the repeat biopsy setting is not well established.

Methods : As part of a previous prospective clinical validation study evaluating the performance of the EPI test, we collected first-catch, non-DRE urine samples across 22 sites from men with prior negative biopsy scheduled to undergo a repeat prostate biopsy to rule out prostate cancer. All men were 50 years or older with a PSA 2-10ng/mL. Exosomal mRNA was extracted and expression of three genomic markers, PCA3, ERG and SPDEF was measured. The resulting EPI score was correlated with biopsy results.

Results : 229 men with a prior negative biopsy underwent repeat biopsies. ExoDx Prostate demonstrated good performance ruling out high-grade (Grade group 2, GG2, or higher) prostate cancer (HGPCa) using the previously validated 15.6 cut point in the initial biopsy setting. The EPI test yielded an NPV of 92% independent of other clinical features and would have avoided 26% of unnecessary biopsies while missing only five patients with HGPCa (2.1%). Furthermore, the EPI test provided additional information at a cut-point of 20 and 29.6 with an NPV of 94%, potentially delaying 35% and 61% of unnecessary biopsies, respectively.

Conclusions : The EPI test provided good performance using the 15.6 cut-point for ruling out HGPCa / GG2 or higher in men undergoing a repeat prostate biopsy with a PSA of 2-10ng/ml. Furthermore, the test utilizes gene expression data independent of clinical features to predict the likelihood of HGPCa / GG2 on a subsequent needle biopsy.

Background

Prostate cancer (PCa) is a leading cause of cancer death among men in the United States, with more than 3.6 million men living with prostate cancer. It is estimated that 174,650 newly diagnosed cases occurred in 2019 [1]. Prostate needle biopsies are typically recommended for men with elevated serum PSA levels and/or a suspicious digital rectal exam (DRE) with added considerations based on family history, age, and race. The anxiety, pain, and potential complications associated with prostate biopsy are well documented [2–6]. Furthermore, a large percentage of newly diagnosed prostate cancers are indolent, clinically insignificant, and with low metastatic potential. These cancers typically do not require definitive treatment and may be managed most effectively with Active Surveillance (AS). The low sensitivity of PSA which contributes to the high frequency of newly-diagnosed low-risk PCa suggests that 60–70% of men may be able to avoid biopsy [7–10]. Compounding the challenge, the majority of initial biopsy tests do not find cancer [11]. For each negative initial biopsy, it is unknown whether elevated PSA levels and/or a

suspicious DRE facilitated the biopsy decision or if the biopsy simply missed a cancerous lesion due to sampling error, tumor heterogeneity and multifocality [12].

The European Randomized Study of Screening for Prostate Cancer (ERSPC) demonstrated that the overdiagnosis and overtreatment of PCa resulting from current practice requires more contemporary and improved methods to identify high-grade disease [13]. Novel diagnostics that provide additional clinical value to risk stratify high-grade PCa (Gleason Grade, or GG $\geq$ 2) have been developed with a primary objective to avoid biopsies for patients with an increased likelihood of having benign or non-aggressive (GG $\leq$ 1) disease [14,15].

The ExoDx Prostate assay relies on the isolation and analysis of urinary exosomes, which are small lipid bilayer membrane extra-cellular microvesicles (typically 30–200 nm in diameter) that are secreted from all living cells. Exosomes contain RNA, DNA, and protein, and have been identified in a variety of biofluids such as blood, cerebrospinal fluid, and urine. They are particularly promising for RNA expression profiling given their protected microanatomic environment [16–19]. A validated, non-DRE urine-based gene signature from normalized PCA3 (prostate cancer antigen 3) and ERG (V-ets erythroblastosis virus E26 oncogene homologs) and SPDEF has been validated in men undergoing initial prostate biopsy with an equivocal PSA. The ExoDx Prostate (EPI) test has been extensively validated in two prospective multi-center US studies for biopsy naive patients [14,15].

The present analysis was designed to evaluate the performance of the ExoDx Prostate gene signature in a cohort of men with a history of prior negative biopsy and now presenting for a repeat biopsy.

Methods

STUDY POPULATION

First-catch, non-DRE urine samples were collected at 22 clinical sites (academic and community) in the USA from men with a prior negative prostate biopsy scheduled for a repeat biopsy between June 2014 and April 2015. The men were prostate cancer free, 50 years or older, and undergoing repeat biopsy for either a suspicious DRE and/or PSA level between 2-10ng/mL. Men with a history of invasive treatment for benign prostatic hyperplasia within 6 months or taking medications that affect serum PSA levels within 3-6 months were excluded. The prostate biopsies were reported by the local hospital/practice pathologist who was blinded to ExoDx Prostate results. The study protocol was approved by the Western Institutional Review Board, Olympia, WA and individual academic institutional review boards (Johns Hopkins Hospital, University of Michigan); all study participants provided written informed consent and were not compensated for participating in the study.

SAMPLE COLLECTION AND PROCESSING

First catch urine samples (25-50 mL) were stored at 4^{o}C for up to 14 days before shipment on ice to the central laboratory (Exosome Diagnostic Laboratory, Waltham, MA). At the Exosome Diagnostics CLIA Laboratory, samples were filtered (0.8um) and stored at -80^{o}C until further processing. For each sample, exosomal RNA was extracted, and the RNA copy numbers of ERG, PCA3, and SPDEF were determined. The methodology for urinary exosome isolation, primer generation, RNA extraction and normalization as well as reverse transcriptase polymerase chain reaction have been previously reported [14,15,20].

STATISTICAL ANALYSIS

The aim of the study was to assess the performance of the EPI assay for predicting clinically significant, HGPCa (Gleason 7, Grade group, GG 2 or higher) on repeat biopsy in men with a history of prior negative biopsy and a PSA level in the “gray zone” (2-10ng/mL). The original validated cut point of 15.6 from two prior prospective studies in men undergoing initial biopsy was utilized in the repeat biopsy setting and compared to EPI cut points of 20 and 29.6. The datasets during and/or analyzed during the current study may be available from the corresponding author on reasonable request.

Results

STUDY POPULATION

Between June 2014 and April 2015, non-DRE, first catch urine samples were collected from 1563 participants enrolled in a prospective validation study of the ExoDx Prostate test for men undergoing initial diagnostic prostate biopsy with a PSA between 2-10ng/mL. The enrollment included patients with a prior negative biopsy however they were excluded in the validation analysis by design. Here we show the outcome in the population that had at least one prior negative biopsy. Urine samples from subjects who had incomplete data and/or >49 ml were excluded. 229 patients met the criteria for the ‘Intended Use Population’: a prior negative biopsy and “gray zone” serum PSA levels (2-10 ng/mL). The median age was 65 years, and median pre-biopsy serum PSA was 6.1ng/mL (**Table 1**). Most subjects had no family history of PCa (75.1%) and 66.4% had a non-suspicious DRE with 71.6% of subjects of Caucasian descent and 14.4% African American.

BIOPSY OUTCOME

In 229 men undergoing repeat biopsy for a prior negative biopsy, the ExoDx Prostate test with the prior validated cut point of 15.6 (for the initial biopsy) demonstrated good performance in discriminating HGPCa, GG2 or higher from low-grade prostate cancer (Gleason 6, GG1) and benign disease on biopsy. The assay performance in the prior negative patients showed an NPV and sensitivity of 92% and 82%,

respectively. An EPI score less than or equal to 15.6 would have avoided biopsy in 26% of men (n=59) and delayed detection of ≥ GG2 disease in 5 men (2.1%). Importantly, only 3 men with GG3 or higher (1%) would have delayed detection in the repeat biopsy setting [11]. Using an EPI score of ≤20 or ≤29.6 would have avoided biopsy in 35% or 61% of men, respectively, thereby reducing anxiety and potential complications associated with an unnecessary biopsy **(Table 2).**

Discussion

Over 1 million transrectal ultrasonography guided (TRUS) prostate biopsies are performed annually in the US and Europe [22]. Although clinical information such as age, race, family history, or a suspicious DRE are triggers, most TRUS biopsies are driven by PSA screening. Biopsies are often associated with pain, bleeding, sepsis and, of significant concern, an increasing rate of antibiotic resistant infection [22,23]. A significant number (approximately 70% of men) are not found to have prostate cancer on initial biopsy, and this leads to patient anxiety because of the false negative rate as a result of prostatectomy under-sampling and tumor heterogeneity/multifocality. These concerns drive many men to undergo repeat biopsy. In fact, the Surveillance, Epidemiology, and End Results (SEER) data indicates that ~ 12% of men with a prior negative biopsy have a repeat biopsy within one year and 44% of men younger than 70 years old have a repeat biopsy [11,24].

PSA screening for prostate cancer is challenged by the low sensitivity and poor specificity for prostate cancer detection. Risk assessment tools such as Prostate Cancer Prevention Trial risk calculator (PCPTRC) were originally developed to improve clinical risk assessment by combining multiple clinical features with PSA. In 2012, the USPSTF recommendation against PSA testing was due to the detection and aggressive overtreatment (surgery and radiation therapy) of low-grade PCa. Although the USPSTF revised its initial recommendation in 2018 based on study re-evaluation [27,28], the pendulum has swung away from PSA screening, and there is a risk the success in reducing PCa mortality will be lost. Since the 2012 USPSTF recommendation, an increase in metastatic prostate cancer has been observed, although it is actively debated [29]. The 2018 USPSTF update recommends PSA screening and shared decision making in men ages 55–69.

The ExoDx Prostate test – a genomic prostate biomarker of HGPCa, independent of clinical features or standard of care (SOC)- was developed and validated as a urine-based, exosomal gene signature from genes known to be involved in PCa initiation and progression: ERG, PCA3 and SPDEF [30–36].

The ExoDx Prostate test algorithm was developed and validated on the intended use cohort, i.e. men presenting for their initial biopsy with a PSA 2–10 ng/mL where it achieved an NPV of 91%, a sensitivity of 91% and 34% specificity [14,15]. It is well established in the literature that prevalence of prostate cancer is significantly lower in a prior negative biopsy population and that this clinical feature alone will favorably impact test performance when included in risk assessment models (and some commercial assays) [37–38]. To address this issue, we evaluated the accuracy of EPI in the prior negative biopsy

population as the test does not include any clinical variables such as prior negative biopsy, PSA, DRE outcome, family history in generation of the final risk score.

An ExoDx Prostate score less than 15.6 would have potentially avoided biopsy in 26% of men, and a score of less than 20 would have avoided a biopsy in 35% of men without missing additional HGPCa. As 2019 NCCN guidelines provide management options between GG2 compared to GG3 categories at diagnosis, it is important to note only 3 patients with GG3 or higher-grade disease were missed at the 15.6 or the 20 cut-point when examining repeat biopsies [39]. The ExoDx Prostate assay demonstrated a comparable NPV in prior negative biopsy men compared to men undergoing initial biopsy.

Study limitations include the sample size and lack of a central pathology review, which may have introduced some variability, specifically when reporting small volume cancers and the fact that there were no men in the study who underwent multi-parametric MRI imaging pre-biopsy. During the study period (2014 and 2015), MRI imaging was not standard of care in the USA. Nevertheless, all the study participating centers represent large urology group practices and academic centers with highly experienced uropathologists. A second prospective study in prior negative biopsy men is underway in the US to address the increasing use of mpMRI imaging and fusion biopsy in this population.

Conclusion

The ExoDx Prostate *(IntelliScore)* test is a validated, non-invasive urine exosome gene expression assay that performs equally well for men who have had a prior negative biopsy. The gene expression assay is more accurate than existing risk assessment methods, is not dependent on clinical features, and helps inform decision-making at both initial and or repeat biopsy time-points.

Declarations

Ethics approval and consent to participate:

The study protocol was approved by the Western Institutional Review Board, Olympia, WA and individual academic institutional review boards (Johns Hopkins Hospital, University of Michigan); all study participants provided written informed consent and were not compensated for participating in the study.

Consent for publication:

Not Applicable

Availability of data and materials:

The datasets during and/or analyzed during the current study may be available from the corresponding author on reasonable request

Author’s Contributions

JM, MD, VT, PT, SK had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Drs JM, MD contributed equally to this research and article.

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Abbreviations

PCA3, prostate cancer antigen 3; ERG, ETS (erythroblast transformation-specific) -related gene; SPDEF, SAM pointed domain-containing ETS transcription factor.

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Tables

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