Perspective

Novel non-invasive urine-based gene expression assay discriminates between low- and high-risk prostate cancer before biopsy

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Comment on: McKiernan J, Donovan MJ, O'Neill V, *et al*. A Novel Urine Exosome Gene Expression Assay to Predict High-grade Prostate Cancer at Initial Biopsy. JAMA Oncol 2016;2:882-9.

Abstract: Prostate-specific antigen (PSA)-based screening programs are controversial, and influential guideline panels have recommended against PSA screening in all men. A main limitation of PSA-based blood tests is the lack of a valid threshold to distinguish between a malignant and a benign condition. In addition, PSA screening generally fails to differentiate between low- and high-grade prostate cancer and thus, is not able to prevent patients from unnecessary biopsies. Alternative, urine-based tests have recently been developed and provide promising predictive accuracy regarding the aggressiveness of the disease. Two markers—prostate cancer antigen 3 (PCA3) and an androgen-related fusion protein (TMPRSS2-ERG)—were combined into a urine test several years ago. This Mi-Prostate Score (MiPS) significantly outperformed both PCA3 + PSA and PSA alone for the prediction of high-grade prostate cancer before biopsy. To date, these tests need pre-collection digital rectal examination to improve the predictive ability. Against this backdrop, a recent study presented a novel urine-based assay, using an exosome-derived gene expression signature. In a validation cohort of 519 patients, the area under receiver operating characteristic curve (AUC) showed superior predictive ability in the discrimination of Gleason scores ≥7 and <7 before biopsy, when compared to standard of care alone (0.73 *vs*. 0.63; P<0.001). Despite not showing better predictive accuracy than already existing urine-based tests, this novel exosome-derived assay allows pre-biopsy assessment without the need for digital rectal examination. Thus, any health professional can perform the test, which (I) spares the patient another digital rectal examination and (II) may facilitate clinical workflow.

Keywords: Exosomes; prognosis; prostatic neoplasms; prostate-specific antigen (PSA); urinalysis

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Over the past five years, prostate-specific antigen (PSA)-based screening programs have come under scrutiny given concerns about the accuracy of PSA and its downstream effects on diagnosis and treatment.

Specifically, PSA is not cancer-specific and thus, several benign conditions are associated with elevated serum PSA levels. Even though several adjuncts to conventional PSA have been proposed (e.g., PSA density/velocity, PSA doubling time, percentage of free PSA, and several isoforms), there is no optimal threshold value to distinguish between prostate cancer and benign conditions (1). Furthermore, evidence is scarce showing that those PSA 'modifiers' provide additional accuracy relative to serum PSA alone (2).

Moreover, the extensive use of PSA screening has led to a significant increase of diagnostic prostate biopsies and higher detection rates of clinically insignificant tumors, which will likely remain indolent over time (3). Hence, influential public health guideline panels, such as the

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United States Preventive Services Task Force (USPSTF), have recommended against PSA-based screening in all men (4) to minimize the risks of overtreatment of low-grade tumors, as well as associated health care costs and psychological burden to the patient (5,6). Nevertheless, those recommendations have potentially significant consequences for patients harboring intermediate- to high-risk disease, as diagnoses might be delayed up to a certain point where potentially curative treatment is no longer possible. Given the established survival benefit of surgery or radiation therapy in patients with high-grade prostate cancer [Gleason scores (GS) ≥7 or locally advanced clinical stages] (7,8), the ideal prostate cancer early detection tool would be able to (I) identify patients with high-grade tumors to initiate diagnostic and treatment pathways and (II) avoid unnecessary biopsies and overtreatment in men with low-grade or without malignant cancer.

The perfect biomarker for general use in prostate cancer management needs to meet certain strict criteria. In addition to the required high sensitivity and specificity, the ability to differentiate benign from malignant, as well as indolent from aggressive tumors, the ideal marker has to be an inexpensive, easily accessible, and ideally non-invasive test. The concept of urinary prostate cancer biomarkers is not novel. To date, two urinary markers have been identified and adopted into clinical prediction tools to improve prostate cancer diagnosis and risk assessment.

Prostate cancer antigen 3 (PCA3) was initially described in 1999 as a prostate specific messenger ribonucleic acid (mRNA), which was overexpressed up to 66-fold in more than 95% of prostate cancers (9). In several follow-up studies, PCA3 demonstrated superior predictive abilities compared to serum PSA. In 2006, the PCA3 assay was translated into the commercially available Progensa™ PCA3 test (10). PCA3 was included into a predictive nomogram (11), which was externally validated in 2010 (12). Importantly, de la Taille *et al*. showed that PCA3 was superior in predicting initial biopsy outcome, compared to total PSA, PSA density, and %free PSA (13). Thus, in 2012, the US Food and Drug Administration (FDA) approved the test as a decision tool for the repeat biopsy setting, given that the likelihood of harboring prostate cancer increases, as the PCA3 score is higher (14,15). Specifically, in a cohort of 127 patients with a suspicious digital rectal exam (DRE), and/or persistently elevated PSA levels, and previous suspicious histology on the initial biopsy, Auprich *et al*. confirmed that PCA3 was the best predictor of prostate cancer at first repeat biopsy, compared to total PSA alone (16). Nevertheless, its role to distinguish indolent from aggressive tumors remains equivocal. In a retrospective study of 305 patients who underwent radical prostatectomy, the PCA3-score was not an independent predictor of extraprostatic extension, seminal vesicle invasion or high-grade disease (GS ≥7) (17).

Second, another important group of genes that are differentially expressed was identified in 2005: the ETS family (v-ets erythroblastosis virus E26 oncogene; ERG and ETS variant gene 1; *ETV1*). Tomlins *et al*. showed that these genes were overexpressed in approximately 57% of prostate cancer cases (18), and that this overexpression was most likely driven by an androgen-regulated fusion with the Transmembrane Protease, Serine 2 (TMPRSS2) (18). Following this, TMPRSS2-ERG fusion transcripts were shown to be detectable in urine samples (19). A meta-analysis of 61 studies evaluating men with fusion-positive prostate cancers did not find TMPRSS2-ERG to be a strong predictive marker of disease outcome after radical prostatectomy, as the fusion status was not associated with risk of GS ≥7 *vs*. GS ≤6 or GS =7 *vs*. GS ≤6 (20). These, along with results from other studies, suggest that TMPRSS2-ERG fusion may be able to predict tumor stage, however its association with GS or cancer-specific mortality remains unclear.

These two urine-based prostate cancer early detection biomarkers—PCA3 and TMPRSS2-ERG—along with serum PSA were subsequently combined into another urine test, the Mi-Prostate Score (MiPS) (21,22). MiPS + PSA outperformed both PCA3 + PSA and PSA alone for prediction of high-grade prostate cancer defined as GS ≥7 (22). Of note, both Progensa™ and MiPS require pre-collection DRE, which might be perceived as an invasive intervention and thus, do not meet the stringent definition of a 'perfect' detection tool.

Against this backdrop, a study by McKiernan *et al*. in *JAMA Oncology* found promising results for a novel urine-based gene expression assay to predict high-grade prostate cancer at initial biopsy (23). The authors used an exosome-derived gene expression signature, which included PCA3 and ERG RNA. While the underlying genes are not novel per se, McKiernan *et al*. were the first to isolate exosomal RNA without previous prostate examination, derive a molecular prostate cancer signature, and prospectively validate the predictive accuracy of this diagnostic tool. Exosomes are miniscule tissue-derived vesicles, which can be secreted by different cell types, including tumor cells, and carry proteins and RNAs that are representative of their tissue origin (24). In this study, 255 patients with serum PSA levels of 2–10 ng/mL were examined to assess the prognostic accuracy of the *ExoDx Prostate IntelliScore urine exosome assay*. The derived score was then validated in an intended-use population of 519 patients from 22 facilities in the United States. Patients were considered eligible if they had no history of prostate

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cancer or biopsy, were 50 years or older, and referred for initial prostate biopsy due to a suspicious DRE finding and/or serum PSA levels from 2–10 ng/mL. When estimating the area under receiver operating characteristic curve (AUC) for discrimination of GS ≥7 *vs.* GS <7 or benign disease, the novel urine exosome gene expression assay in combination with standard of care (PSA, age, race, and family history of prostate cancer) was superior to standard of care alone (AUC: 0.73 *vs.* 0.63; P<0.001) (23). Similar results were found when the target population was extended to include patients with a serum PSA level of 10–20 ng/mL.

This new tool relies on previously established genomic markers, but is solely first-catch urine-based. While the MiPS does incorporate previously established genes and serum PSA, the *ExoDx Prostate IntelliScore urine exosome assay* is different as it does not require a DRE (23).

Notably, the predictive accuracy of this novel exosome-derived test is no better than MiPS, which was previously introduced in 2015 (22) and relies on TMPRSS2-ERG, PCA3, as well as clinical variables included in the *Prostate Cancer Prevention Trial* risk calculator (PSA, family history, outcome of DRE, and prior biopsy) (25). While the AUC for MiPS was 0.779 for predicting high-grade cancer at biopsy in the validation cohort (22), McKiernan *et al.* reported an AUC of 0.73 in the external validation of 519 patients to discriminate between GS ≥7 *vs.* GS <7 or benign disease (23). However, it is convincing that the novel test can be conducted by any health professional without precise knowledge of the performance of an adequate DRE and not only by urologists or physicians. Also, patients could be spared another DRE, which may raise compliance and eventually facilitate the clinical workflow, indeed.

Despite these advantages, patients were considered eligible for the novel exosome-derived test if they presented with an elevated serum PSA ranging from 2–10 ng/mL (2–20 ng/mL in subanalyses) and/or a suspicious DRE. Whether a patient with suspicious DRE should undergo this test is a complex manner. Given that a substantial proportion of prostate cancers detected by DRE at PSA levels ≤4 ng/mL are associated with clinically highly aggressive tumors (26), it is debatable if a patient who presents with a suspicious DRE should undergo this test, as it is unlikely to change clinical decision-making.

Novel tumor targets are anxiously needed, and the combination of biomarker templates seems to be a promising approach to improve the prediction of prostate cancer and prostate cancer aggressiveness at biopsy. However, adequate internal and external validation of these markers are necessary. Specifically, prospective validation in randomly invited population-based cohorts is the gold standard to test the predictive accuracy of those novel markers. As such, the Stockholm 3 study group recently validated a new predefined model in a screening cohort of 113,082 men to identify high-risk prostate cancer (GS ≥7) with better accuracy than PSA alone (27). The model included a combination of several plasma protein biomarkers and performed significantly better than PSA alone (27). Nevertheless, regarding exosomes, further research is eagerly awaited. If researchers are able to gain higher yields of exosomes from urine samples, this may help in finding new bladder, prostate, or renal cancer-specific miRNA and mRNA biomarkers.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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