Urinary markers aiding in the detection and risk stratification of prostate cancer

Nicholas Raja[1], Christopher M. Russell[2], Arvin K. George[2]

[1]Department of Urology, University of Michigan Medical School, Ann Arbor, MI, USA; [2]Department of Urology, Michigan Medicine, Ann Arbor, MI, USA

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Correspondence to: Arvin K. George, MD. 1500 East Medical Center Drive, TC 3875 SPC330, Ann Arbor, MI 49102, USA. Email: arvinkgeorge@gmail.com.

Abstract: The purpose of this review is to highlight the role of existing and promising urinary biomarkers for the detection and prognostication of prostate cancer (PCa). A number of novel urinary biomarkers have been introduced into the clinical space, which in combination with clinical variables, have demonstrated an increased ability to select patients for biopsy and identify men at risk of harboring clinically significant PCa. Though a number of assays require further validation, initial data is promising and forthcoming results will ultimately determine their clinical utility and commercial availability. For the past 30 years, first-line screening for PCa has relied on measurement of serum prostate-specific antigen (PSA) levels and the results from a digital rectal exam (DRE). A large body of evidence from the last 3 decades indicates that these screening methods are problematic, and often inadequate for detecting clinically significant PCa. Extensive efforts have recently been made to identify and commercialize novel PCa biomarkers for more effective detection of PCa, either alone or in combination with current screening methods. This review article highlights problems with current screening standards, and discusses 6 urinary biomarker assays in terms of their ability to detect and risk-stratify PCa: prostate cancer antigen 3 (PCA3), TMPRSS2-ERG, second chromosome locus associated with prostate-1 (SChLAP1), ExoDx, SelectMDx, and Michigan Prostate Score (MiPS).

Keywords: Prostate cancer (PCa); biomarker

Introduction

Prostate cancer (PCa) is the most common cancer in men after skin cancers and is the second leading cause of cancer related deaths in males in the United States, causing an estimated 26,730 deaths in 2017 alone (1). Definitive diagnosis of PCa is made based upon the results of a prostate biopsy. For almost 30 years, the decision to biopsy a patient has relied upon elevated prostate-specific antigen (PSA) levels (generally greater than 4 ng/mL, though age-specific PSA thresholds are commonly utilized) or abnormal

findings on a digital rectal exam (DRE). It has become clear that neither PSA nor DRE alone, or even together, are accurate enough to reliably detect clinically significant prostate cancer (csPCa), defined as any PCa with Gleason Score (GS) ≥7. Thus, there has been a movement to develop new biomarkers for more effective detection of PCa. Several are now commercially available, others await FDA approval, and a multitude more are still being investigated. Particularly, great efforts have been expended to identify urinary biomarkers, often to be used as an adjunct to PSA screening. Prostatic secretions merge with the urine where

the ejaculatory duct joins the urethra. In patients with PCa, tumor cells are similarly excreted through the ejaculatory ducts, and thus can be collected in the urine for analysis. Unlike other sources of biomarkers, such as tissue or blood, urine collection is inexpensive and easy to obtain in large volumes without invasive procedures. Additionally, isolation of biomarkers is easier in urine, whose composition is less complex than that of blood (2).

Current screening standards: PSA and the DRE

PSA [also called gamma-seminoprotein or kallikrein-3 (KLK3)] is an enzyme secreted exclusively by prostatic epithelial cells and aids in liquefaction of the seminal coagulum (3). PSA is present in small amounts in the blood of healthy men, but can be elevated in individuals with PCa. Evaluation of serum PSA levels has been the standard of practice for PCa screening since 1989. However, it is has become clear that PSA alone is not an adequate metric for assessing cancer risk. Elevations in PSA are not specific for the presence of PCa and can be elevated in a variety of other conditions, including benign prostatic hypertrophy (BPH), prostatitis, or urinary tract infections (UTIs). Additionally, PSA carries a low positive predictive value (PPV) for PCa of approximately 30%, meaning less than one in three men with elevated PSA will have positive pathology on subsequent biopsy (4-7). Additionally, men with low PSA levels can develop PCa. The Prostate Cancer Prevention Trial (PCPT) identified high-grade PCa in 12.5% of men with PSA <0.5 ng/mL (8). Even in PCa-positive men, PSA values can fluctuate throughout the clinical course, making it an ineffective tool for tumor staging and grading and subsequent risk-stratification (9). Although attempts have been made to overcome these inadequacies by developing other PSA-based tests, metrics such as percent free PSA, PSA velocity, PSA density, and age-specific PSA ranges similarly suffer from lack of general specificity as conventional PSA measurement (10).

The other common indication for prostate biopsy, an abnormal DRE, has also been shown to be ineffective for detection of PCa. During a DRE, the physician examines for changes in prostate size, shape, and texture. Suspicion for cancer is increased when the examination reveals a hardened prostate gland or nodularity. In the Prostate, Lung, Colorectal, and Ovarian (PLCO) Screening Trial, only 99 of 5,064 men (2%) who had an abnormal DRE in the setting of normal PSA were diagnosed with clinically significant PCa (11). Furthermore, though difficult to quantify, patient anxiety surrounding DRE can be very high, and has been reported as a significant barrier to PCa screening in the past (12,13).

A preliminary report from the United States Preventative Services Task Force (USPSTF) in April 2017 gave routine PSA screening in men ages 55–69 a grade C recommendation, an advisement to only screen on a case-by-case basis. In men age ≥70, PSA screening was given a grade D recommendation advising against routine screening. The American Urological Association's (AUA) recommendations differ slightly, and do recommend routine PSA screening in men ages 55–69 after informed discussion and shared-decision making. The downstream effects of these screening recommendations are as yet unknown, but indicate a need for better biomarkers for PCa detection. Additionally, current screening methods often lead to the diagnosis of early, localized, clinically insignificant cancers which may lead to intense anxiety on the part of the patient or overtreatment, with current treatment options carrying the risk of significant morbidity such as incontinence, erectile dysfunction, and hematuria (13-17).

Advances in genomics and proteomics have allowed us to understand the molecular biology of cancer to a level previously unattainable. Herein we discuss novel urinary biomarkers (*Table 1*) which promise to change future screening standards and improve the detection of clinically csPCa.

Methods

PubMed and MEDLINE database searches were conducted. Terms searched included "prostate cancer", "biomarkers", "urinary markers", "diagnosis", "prognostication", "risk-stratification", "urine assay", "gene panel", and "screening". All relevant studies were considered, regardless of year of publication. Biomarkers were selected for inclusion based on the amount of promise they show for use in clinical practice, based generally on commercial availability of the assay, the wealth of literature available regarding the marker, and the apparent effectiveness of the marker in regards to specificity, sensitivity, and area under the curve for diagnosis or prognostication of PCa.

Urinary biomarkers for PCa

Prostate cancer antigen 3 (PCA3)

One of the most widely studied urinary PCa biomarkers is

Table 1 Urinary Biomarkers for PCa

Marker(s)	Assay	Commercially available	Type of marker	Comments
PCA3	PRoGensa®	Yes	Diagnostic* (18-20)	Utility for prognostication remains controversial. PCA3 levels may be affected by androgen deprivation therapy (21-24)
TMPRSS2-ERG	–	No	Diagnostic* (10,25)	Similar to PCA3, this marker has unknown prognostic value and may be affected by androgen deprivation therapy (24,26,27)
SChLAP1	–	No	Diagnostic/prognostic (28,29)	SChLAP1 positivity has been shown to be associated with more aggressive disease (30)
PCA3, ERG, SPDEF	ExoDx	Yes	Diagnostic/prognostic (31)	SPDEF levels appear to correlate with tumor grade and metastatic potential (32). ExoDx is not currently approved by the FDA
HOXC6, DLX1	SelectMDx	Yes	Diagnostic (33)	In combination with conventional risk factors, a multimodal validation study demonstrated AUC of 0.90 for clinically significant PCa (34)
PCA3, TMPRSS2-ERG (+PSA)	MiPS	No	Diagnostic (35)	Cost-benefit analyses comparing MiPS with other non-urinary detection methods has yet to be performed (35)

PCa, prostate cancer; PCA3, prostate cancer antigen 3; MiPS, Michigan Prostate Score; TMPRSS2, androgen-related transmembrane protease, serine 2; ERG, ETS-related gene; SChLAP1, second chromosome locus associated with prostate-1; SPDEF, SAM pointed domain ETS transcription factor; HOXC6, homeobox C6; DLX1, distal-less homeobox 1.

PCA3, also called "differential display code 3" (DD3). PCA3 is a long non-coding RNA (lncRNA) that is specific to the prostate and was first reported in 1999 by Bussemakers *et al.* (36). PCA3 is overexpressed in PCa relative to normal prostate tissue, and is present in up to 95% of PCa (36-39). Quantitative real-time polymerase chain reaction (qRT-PCR) for PCA3 detection was approved by the FDA in 2012 for clinical use in the USA, marketed as the PRoGensa® and later the uPM3™ assay. It is indicated for men age ≥50 with prior negative biopsies for whom standard of care includes repeat biopsy.

The "PCA3 score", calculated as an adjusted ratio of PCA3 to PSA mRNA (PCA3/PSA ×1,000), was found to be associated with the probability of diagnosing PCa in subsequent prostate Bx (40,41). There remains controversy as to the ideal cutoff score to use in order to rule out the need for repeat biopsy. PCA3 score values of 20, 35, and 50 have been examined in numerous studies, with sensitivities and specificities ranging from 52–82% and 79–89% depending on the selected cutoff point (10,25,42-47). A 2011 meta-analysis of 11 clinical studies suggested an optimal cutoff PCA3 score of 20, which imparted a sensitivity of 72% and a specificity of 53% for men undergoing repeat biopsy (40).

More recently, efforts have been made to integrate PCA3 score as a component of diagnostic nomograms.

One study by Hansen *et al.* in 2013 found that a nomogram incorporating PCA3 was far more accurate than one which did not include PCA3 score; concluding that 55% of men would be able to avoid a biopsy with ≤2% missed cases of csPCa (18). Additional studies have reported similar results, suggesting an important role for PCA3 in detection of PCa (19,20). Although PCA3 appears to be a reliable tool for the detection of PCa, studies disagree as to its prognostic value. Leyten *et al.* found that PCA3 score did not correlate with GS on biopsy or clinical tumor stage, while another group reported increasing tumor aggressiveness with higher PCA3 scores (21,22). Additionally, PCA3 levels in the urine may be significantly affected by androgen deprivation therapy, thus limiting the ability to monitor the clinical course of disease (23,24).

TMPRSS2-ERG

TMPRSS2-ERG is a chimeric protein composed of the androgen-related transmembrane protease serine 2 (TMPRSS2) and ETS-related gene (ERG). At the time of its discovery in 2005, TMPRSS2-ERG was the first known fusion gene implicated in PCa. This mutated protein results in aberrant expression of ERG, which contributes to oncogenesis by forcing progression through the cell cycle (48). Though TMPRSS2-ERG is the most common

gene recombination seen in PCa, it's prevalence appears to vary widely between ethnic groups—as high as 50% in western countries and as low as 11% in China (49-51).

Some studies have suggested that the expression of TMPRSS2-ERG in PCa carries a worse clinical prognosis. Hägglöf *et al.* showed that TMPRSS2-ERG was associated with increased cancer-specific mortality compared to patients whose tumors lacked ERG staining (52). However, other studies have failed to show clinical significance (53). Assays screening for TMPRSS2-ERG have been shown in multiple studies to improve detection of PCa. In one study, the combination of TMPRSS2-ERG with PSA allowed for a 50% reduction in repeat biopsies, with no reduction in either 10-year overall survival or 15-year PCa-specific survival (10). In particular, TMPRSS2-ERG is often combined with PCA3 due to the fact that PCA3 has high sensitivity for PCa and TMPRSS2-ERG has high specificity (25). The prognostic value of TMPRSS2-ERG is still under investigation, and results from multiple studies have been inconsistent. TMPRSS2-ERG was shown to improve predictive power in the European Randomised Study of Screening for Prostate Cancer risk calculator (ERSPCrc) in terms of predicting GS and stage (10). Another large multi-institutional trial by Tomlins *et al.* demonstrated that the addition of TMPRSS2-ERG and PCA3 to the PCPT nomogram significantly improved PCa risk prediction (AUC increased by 9–16%) (35). In contrast, studies by Cornu *et al.* and Leyten *et al.* showed that while TMPRSS2-ERG + PCA3 greatly increased PCa detection sensitivity, TMPRSS2-ERG was not correlated with GS in pathology specimens (26,27). Data also indicate that TMPRSS2-ERG may suffer from the same limitations as PCA3 when combined with androgen deprivation therapy (24).

SChLAP1

SChLAP1 (second chromosome locus associated with prostate-1) (also called LINC00913) was first characterized by Prensner *et al.* in 2013, and was shown to promote development of PCa by inhibiting SWI/SNF, a tumor-suppressing protein complex (30). SChLAP1 is expressed in a subset of PCa and is associated with more aggressive disease (30). Mehra *et al.* demonstrated that high levels of SChLAP1 expression were correlated with increasing GS and tumor stage (P<0.005) and increased time to development of lethal PCa (28). Additionally, quantifying SChLAP1 expression levels has been shown to be useful for predicting PCa metastasis within 10 years (OR 2.45; 95%

CI, 1.70–3.53, P<0.0001) (29). Assays utilizing SChLAP1 for PCa screening are not yet commercially available.

ExoDx prostate Intelliscore urine exosome assay

ExoDx is a urine exosome gene expression assay which measures a combination of ERG, PCA3, and SPDEF (SAM Pointed Domain ETS Transcription Factor). Higher level of SPDEF is thought to correlate with increased PCa aggressiveness and metastatic potential (32). One study by McKiernan *et al.* demonstrated that the ExoDx panel used in combination with PSA, age, race, and family history was better able to detect GS ≥7 PCa than these other variables alone (AUC 0.71), suggesting that 27% of biopsies could be avoided. In this study, the assay demonstrated a negative predictive value (NPV) of 91% and a sensitivity of 92%, missing only 12 of 148 GS ≥7 cancers (8%) (31). This gene panel became clinically available in September 2016; however, this test is only available from the manufacturer through a CLIA-approved (Clinical Laboratory Improvement Amendments) lab. It has not been approved by the FDA, and thus may not be covered by health insurance plans.

SelectMDx

SelectMDx is another commercially available combination assay utilizing HOXC6 and DLX1. HOXC6 (homeobox C6) on chromosome 12 and DLX1 (Distal-less homeobox 1) on chromosome 2 are two homeobox genes that have been found to be upregulated in PCa (54). HOXC6 plays a role in epithelial proliferation[41], but little is known about the oncogenic function of DLX1 (33). HOXC6 + DLX1 has been shown to be highly effective at predicting the presence of csPCa, with an overall AUC of 0.76 (33). When combined with other predictors including PSA density, DRE results, previous Bx, PSA, family history, and age, it reached an overall AUC of 0.90 (95% CI, 0.85–0.95) (34).

Though not currently included in the SelectMDx panel, there have been studies of HOXC6 + DLX1 combined with the protein TDRD1 (Tudor domain containing 1). TDRD1 was the first identified target of ERG, and upregulation of TDRD1 is highly correlated with ERG upregulation. One study found that this combination was more accurate in detecting clinically significant PCa than either PCA3 or PSA alone (AUC 0.77 *vs.* 0.62 PCA3 *vs.* 0.72 PSA) (24). Furthermore, the panel became even more effective when combined with PSA, with an AUC of 0.82 (24). NCCN 2017 practice guidelines state that SelectMDx is officially

still under investigation, and information will be reviewed as it becomes available.

Michigan Prostate Score (MiPS)

The MiPS is a combination of PSA + PCA3 + TMPRSS2-ERG developed in 2016 by Tomlins *et al.* MiPS uses a transcription-mediated amplification assay that generates scores for TMPRSS2-ERG and PCA3 by normalizing urinary mRNA with urinary PSA mRNA. A final multivariable regression generates the combined MiPS score. Tomlins *et al.* demonstrated significantly higher prediction rates for the presence of both PCa and csPCa (GS ≥7) when compared to PSA alone, PCA3 + PSA, and TMPRSS2-ERG + PSA (AUC 0.751, 0.585, 0.726, and 0.693 respectively) (35). Utilization of MiPS with the PCPT nomogram would have avoided 35–47% of biopsies, while only delaying the diagnosis of 1–2.3% of clinically significant PCa (35). MiPS is currently not commercially available, and the authors note that a cost-benefit analysis between MiPS and other non-urinary detection methods has not yet been performed (35).

Conclusions

In light of a mounting body of evidence highlighting the inadequacies of PSA and the DRE as screening tools for PCa, the development and utilization of novel biomarkers for better cancer detection is critical. Urine specimens in particular represent a low-cost, non-invasive, convenient source from which to measure the presence of biomarkers. Although utilization of the assays discussed in this review has not yet become standard of practice, they offer promising alternatives for both effective diagnosis and prognostication of PCa.

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Footnote

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