Published in final edited form as:

Curr Opin Urol. 2015 January ; 25(1): 77–82. doi:10.1097/MOU.0000000000000133.

Urinary Biomarkers for Prostate Cancer in Current Opinion Urology (Dan Lin Editor)

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Abstract

Purpose of review—The field of urology has been beset by several major trends that have affected the early detection of prostate cancer. These stem primarily from a backlash against overdiagnosis due to PSA based screening efforts, and are epitomized by the US Preventative Services Task Force giving PSA-based prostate cancer screening a ‘D’ recommendation. Consequently, the active surveillance strategy for low risk prostate cancer has become commonplace, leading many to ask how best to follow these patients. More importantly, this public outcry has shifted the focus of early detection from an effort to diagnose any and all prostate cancers to an effort to diagnose only ‘high-risk’ cancer. Along with a trend for minimally invasive procedures, these forces have challenged the early detection field to more efficiently identify clinically significant prostate cancers at an early stage while limiting the number of biopsies.

Recent Findings—With FDA approval, PCA3 has emerged as the first bona-fide urinary biomarker for prostate cancer. Using the same platform, investigators have developed a second urinary test based on TMPRSS2:erg fusion. Recent literature supports use of these biomarkers as a combined panel that improves risk evaluation in the setting of prostate cancer detection. Early work for applying urinary biomarkers for active surveillance are underway. Other biomarkers in the pipeline will require further pre-validation and validation work.

Summary—Recent literature would support that urinary biomarkers have a clear role to supplement risk evaluation for men undergoing prostate biopsy and for prognostication.

Keywords

Prostate Cancer; Urinary Biomarkers; PCA3; TMPRSS2:erg

Introduction

Prostate specific antigen (PSA) is the most widely used noninvasive diagnostic test for prostate cancer and as such, it has led to the diagnosis and subsequent treatment for millions of American men. Since PSA’s introduction in 1989, the incidence of prostate cancer has risen which has benefited many but perhaps has also led to the overtreatment of low-risk prostate cancer in others. Randomized clinical trials examining the role of PSA based screening have either demonstrated a lack of, or only marginal efficacy when it is applied as

a screening test.^{1,2} This has led some, including the United States Preventative Services Task Force (USPSTF),^{3,4} to criticize its role as a prostate cancer screening test even as other professional societies recommends individualized discussion of PSA screening.⁵

This controversy arises from the fact that PSA is not prostate cancer specific; other common, non-malignant conditions such as benign prostatic hyperplasia (BPH), prostatitis, and even urinary tract infections may lead to its elevation.⁶ In practice, an elevated PSA will trigger a more specific, confirmatory test, the prostate biopsy, which is invasive and associated with bleeding and infectious complications.^{7,8} Attempts to address this has resulted in a number of PSA derivative tests such as percent free PSA^{9,10}, age-specific PSA ranges¹¹, PSA velocity¹² and -2proPSA^{13,14} but these are constrained by the same limitations as PSA itself - namely, confounding by benign prostatic conditions. At the heart of the PSA screening controversy is what to do if the PSA test is “abnormal” in light of the limited sensitivity and specificity.

Currently, an abnormal PSA test often leads to a prostate biopsy which when positive is often a low risk cancer. However, many men are reluctant to undergo a biopsy given fear of discomfort and complications. Indeed, the rise of resistant bacteria has increased the hospitalization rate after a prostate biopsy to nearly 3% largely due to ciprofloxacin resistance.⁸ Moreover, the anxiety incurred when a patient is told that he may have prostate cancer is considerable and difficult to quantify.

The USPSTF recommendation to not screen is based on using PSA as the sole test to drive the decision-making process.¹⁵ A possible alternative solution to not screening at all would be to improve the performance characteristic of the early detection process. One such approach is to supplement PSA with new biomarkers that provide supplementary information. The limitations of PSA as a stand-alone biomarker for early detection have led to an intensive search for other biomarkers.

Fortunately, a number of potential candidates have emerged thanks to high throughput genomic, proteomic and metabolomics efforts. Although PSA is a serum-based assay, biomarkers may be detected in all forms of body fluid, including urine. Indeed, the abundance of, and ease of, collecting voided urine holds the potential to quantitatively measure prostate cancer biomarkers that may be excreted or shed into the urine. This article will now focus on those biomarkers, which have been, or will likely be, translated into urinary assays.

Prostate cancer antigen 3 (PCA3)

Prostate cancer antigen 3 (PCA3, also known as *Differential Display Code 3* or *DD3*) is the first urinary biomarker for prostate cancer to gain FDA approval. PCA3, a prostate-specific gene that is present in 95% of prostate cancers¹⁶, and significantly over-expressed in cancer tissue¹⁷. PCA3 is known to be a non-coding messenger ribonucleic acid (mRNA) with no resultant protein. Clinically, PCA3 mRNA is detectable in the urine and prostatic fluid of men with PCa. PCA3 mRNA levels are independent of prostate volume and serum PSA, but may be higher with larger, more aggressive tumors¹⁸. Although PCA3 has been approved in

Europe as the uPM3™ assay, PROGENSA®PCA3 Assay has only been approved by the FDA since 2012 with an indication for men with prior negative prostate biopsies.¹⁹⁻²²

With an initial cut-off value set at 50, this assay demonstrated a sensitivity of 69% and specificity of 79%²⁰. The nucleic acid sequence-based amplification urine uPM3™ assay has demonstrated similar results; with a cutoff of 0.5, sensitivity ranged from 66-82%, specificity from 76-89%, and negative predictive value from 84-87%^{19,21}. To date, studies²³⁻²⁵ have examined the performance of PCA3 and documented high sensitivity (52-58%) and specificity (72-87%)^{23,26} even in the setting of a prior negative prostate biopsy. In an affirmation of the FDA indication, a recent meta-analysis examining PCA3 for guiding repeat prostate biopsies examined 11 studies of moderate to high quality.²⁷ The synthesis of these data found that PCA3 cut off of 20 using the ProgenSA assay resulted in an optimized sensitivity for men undergoing a repeat prostate biopsy. Consistent with prior work, these investigators found that at this cutoff, there would be a significant reduction in unnecessary biopsies, by more than half. They also concluded that the cutoff of 20 is preferable to cut off of 35. These findings are further supported by the recent NCI Early Detection Research Network Validation Trial for PCA3.²⁸

Recent articles on PCA3

Since FDA approval, the literature has focused on applications of PCA3 to nomograms, and combinations with other diagnostic markers. In one study, investigators combined PCA3 information to clinical data among men undergoing an initial prostate biopsy.²⁹ In these cases, an extended prostate biopsy was performed and urinary PCA3 was collected prior to the biopsy. Diagnostic accuracy of PCA3 was compared using receiver operating characteristic curves. These models demonstrated that a PCA3-based nomogram significantly outperformed the clinical models without PCA3. Importantly, they found that only a few men with high grade prostate cancer would be missed, all the while, allowing up to 55% of men to avoid a prostate biopsy. Another group compared 3 nomograms that incorporated PCA3 and concluded that two provided good calibration and a high net benefit based on decision curve analyses. They also concluded that using these nomograms would have missed less than 6% of high grade prostate cancers while 48% of prostate biopsies could have been avoided.³⁰ Yet others have considered the head-to-head comparison of PCA3 the 4k (four kallikrein) score. In this analysis, PCA3 and the 4K score were added to the ERSPC risk calculator. In multivariable models, PCA3 and the 4k score appeared to be equivocal (AUC 0.73 vs 0.71, $p=0.18$), while both markers improved the base risk estimation.³¹

Another burgeoning area of research involving *pca3* has been to examine the role of PCA3 in selecting men for MRI directed biopsies. In a retrospective analysis of 163 men, investigators found that men with a multi-parametric 3 teslaMRI abnormality suggestive of prostate cancer had a significantly higher level of PCA3 compared to those who did not (52 versus 21, $p<0.001$).³² These findings are consistent with a prior report that concluded that sensitivity of PCA3 testing could be improved by addition of MRI in men who had previously negative biopsy.³³

TMPRSS2:erg Fusion

In 2005, Tomlins and Chinnaiyan reported on a unique gene fusion involving the rearrangement a member of the ETS oncogene family (erg) with the transmembrane protease serine 2, (TMPRSS2).³⁴ Prior to this report, fusions were not known to occur in prostate cancer. However, the investigators found that at least 50% of prostate cancer foci (involving 80% of prostate cancer cases) demonstrated this fusion. Importantly, this discovery holds potential to identify another common mechanism for the development of prostate cancer other than the oft-studied androgen pathway. In histologic studies, this fusion has been found to be the most specific prostate cancer biomarker yet with several studies consistently finding greater than 99% specificity.³⁵ Subsequently, a novel urinary assay was developed using the same technology platform as PCA3. Using this assay, Tomlins and colleagues examined how the quantification of TMPRSS2:erg fusion related to tumor volume.³⁶ In this study, erg+ cancer foci at the time of radical prostatectomy were assessed in terms of number of foci and also the summed linear tumor dimension (aggregate tumor size). With 41 radical prostatectomies, and a median of three tumor foci, they demonstrated a correlation with both the number of tumor foci and a total tumor dimension ($p < 0.001$). Given that they only looked at erg+ tumor foci, not surprisingly, PCA3 showed a weaker correlation.³⁶

Tomlins and colleagues then reported on a series of over 1300 men at multiple centers undergoing prostate biopsy and radical prostatectomy.³⁷ In this sentinel paper, he correlated the urinary measure of TMPRSS2:erg fusion with prostate biopsy and post radical prostatectomy pathology. Using the PCPT nomogram as the standard for comparison, he separated the biopsy cohorts into three risk groups: lowest, intermediate and highest risk. By adding TMPRSS2:erg fusion and PCA3 to the PCPT nomogram, the adjusted risk prediction for cancer and for high grade cancer improved significantly ($p < 0.001$) with AUC increasing 9-16%. Moreover, this study also examined significant cancer at prostatectomy and demonstrated that higher TMPRSS2:erg fusion levels in the urine before surgery correlated to clinically significant findings at final pathology.³⁷ Following this publication, a number of other studies have reported on the use of TMPRSS2:erg fusion as a urinary assay. Typically, these have been in combination with PCA3 given that TMPRSS2:erg fusion has high specificity and sensitivity while pca3 has high sensitivity for prostate cancer.

In one such study, 45 men undergoing the biopsy were utilized to develop a simple clinical algorithm to predict prostate cancer on biopsy. In their approach, all men with a PSA greater than or equal to 10 would undergo a biopsy while men with a PSA less than 10 would only undergo biopsy if they had detectable levels of TMPRSS2:erg fusion or PCA3 in the urine.³⁸ Their analyses, while preliminary, would suggest that the combination of urinary biomarkers with PSA perform better than individual markers alone. Subsequently, another large multi-center series was published based in Europe.³⁹ In this trial from six centers, post DRE urine were collected prior to prostate biopsy prospectively in 497 cases. The authors compared base models using the European Randomized Study of Screening for Prostate Cancer (ERSPC) to the same models with TMPRSS2:erg fusion and PCA3 added. In their ROC analyses, they found that addition of these urinary biomarkers increased area under curve by at least 4%, with the improvements for predicting Gleason score, and clinical stage

largely coming from the addition of TMPRSS2:erg fusion. They concluded that incorporation of these new urinary biomarkers into clinical practice likely may lead to a considerable reduction in the need for prostate biopsy.³⁹ Other investigators have built upon the TMPRSS2:erg fusion body of work by considering other combinations of fusion markers. In one such study, other ETS transcription factor genes (ETV1,4,5) and common variants of the TMPRSS2:erg fusion were combined as a panel of gene fusion markers. In doing so, these investigators found that this panel was an independent predictor of high-grade cancer allowing clinical stratification of prostate cancer risk at the time of diagnosis.⁴⁰

These findings were then corroborated when Tallon and colleagues Incorporated TMPRSS2:erg fusion, PCA3 and the Prostate Health Index (PHI) into models predicting prostate cancer aggressiveness, defined as Gleason seven or higher cancer and tumor volume greater than or equal to 0.5 cc at radical prostatectomy.⁴¹ While they found that PHI was the best predictor of Gleason score, they also noted that TMPRSS2:erg fusion and PHI were independent predictors of extracapsular involvement by cancer. In contrast, only PCA3 appeared to be associated with cancer multifocality. They also demonstrated nicely in a series of multivariate analyses that adding these urinary biomarkers and PHI can significantly improve area under the curve by 10 to 13.8%, thus further validating the concept of multiplex biomarkers in clinical practice.⁴¹

Taken together, the series of published papers examining TMPRSS2:erg fusion and PCA3 demonstrate that they are consistently associated with prostate cancer diagnosis on biopsy, associated with various clinical pathological parameters at biopsy and radical prostatectomy, and add value to decision-making in the clinical setting. Not surprisingly, attempts to examine the urinary biomarkers and other prostate cancer settings are underway.

In one such study, investigators asked whether or not TMPRSS2:erg fusion along with PCA3 can stratify the risk of having aggressive cancer among men undergoing active surveillance for low risk prostate cancer. The setting of this study was the Canary Prostate Active Surveillance Study (PASS) where cases had urine collected at study entry.⁴² The levels of these urinary biomarkers were compared to the number of positive cancer cores, and cancer grade on their baseline biopsy, and seemed to stratify the risk of having aggressive cancer. However, in their receiver operating characteristic curve analyses, the addition of these two urinary biomarkers to PSA at baseline did not significantly improve the AUC of PSA model alone.⁴² Despite this, it remains an intriguing question whether or not these urinary biomarkers may be relevant in predicting subsequent biopsy and pathologic findings in men undergoing active surveillance.

Other urinary biomarkers under investigation

While TMPRSS2:erg fusion and PCA3 are both commercially available in the United States (PCA3 from HOLOGIC/ProgenSA and TMPRSS2:erg fusion may be ordered as part of the MI Prostate Score (MIPS) from the University of Michigan, ongoing work for a series of other biomarkers that may be quantified in the urine are on the way.

Going back to 2008, Tomlinson colleagues described SPINK1 expression as a possible molecular alteration among TMPRSS2:erg fusion – negative cancers.⁴³ Using a meta-

analyses, they not only identified this new biomarker, but suggested that it may be an independent predictor for biochemical recurrence following prostate cancer surgery. However, other urinary tissues that also express the marker may confound SPINK1 as a urinary test. The same group also described another novel biomarker related to PCA3. In this case, a long non-coding RNA⁴⁴ was found to be a promoter of aggressive prostate cancer via the SWI/SNF complex. This biomarker, SCHLAP1 (Second Chromosome Loss Associated with Prostate – 1) was observed to be overexpressed in a subset of aggressive prostate cancer cases including men with metastases and who have died from prostate cancer.⁴⁵ Further work demonstrated that this long non-coding RNA actually had cellular function (regulation of the chromatin modifying complex SWI/SNF) thereby antagonizing tumor suppressor.

Related to long non-coding RNAs is the idea of micro-RNA as a biomarker. These single-stranded RNA molecules are believed to function as a post-transcription regulators of gene expression. Profiling these expression patterns are intriguing and in one study of 29 men (either prostate cancer, BPH or healthy males), differential expression of two micro RNAs (1825, 484) were found to be associated with prostate cancer.⁴⁶ While far from conclusive, pursuing micro RNAs and other non-coding forms of RNA hold promise for development of new biomarkers and also a better understanding of prostate oncogenesis.

While this review focused on PCA3 and TMPRSS2:erg fusion, other potential urinary biomarkers include GSTP1, PSMA, and a variety of exosomes are under active investigation. These have typically been found to be promising but technical issues such as detection, and stability of the analyte in urine, have yet to be overcome.⁴⁷

Someday, urinary biomarker will help identify patients for which specific interventions are more likely to be successful. To date, only TMPRSS2:erg fusion has a clinical trial underway. In this trial, clinical scientists seek to determine whether or not TMPRSS2:erg fusion status are indicative of PARP (poly(ADP ribose) polymerase) inhibitor response among men with high risk cancer. PARP is necessary for cancer cells to repair damage and is a critical interacting partner of the TMPRSS2:erg fusion. Therefore, inhibition could improve response to androgen deprivation. Others have proposed use of small molecules such as pyrrole imidazole polyamide to break the fusion site as a means to repress prostate cancer tumor growth⁴⁸ and targeting SPINK1.⁴⁹

Limitations of Urinary Biomarkers

Despite the progress made on urinary biomarkers for prostate cancer, significant gaps in knowledge remain. First, is the uncertain role of these biomarkers in the cancer screening setting. We understand, that PSA as a standalone biomarker results in over detection but could the combination of PSA with these new urinary biomarkers improve performance such that screening can be limited to the detection of only those with high-grade cancer? Second, combinations of biomarkers have primarily focused on PCA3 and TMPRSS2:erg fusion. Given the burgeoning number of biomarkers, the potential combinations increase geometrically and deserve study. Indeed, it is likely that panel of biomarkers will perform better than simple pairing. Third, robust longitudinal studies of these biomarkers to examine

trends over time in men with and without prostate cancer have yet to be performed. Lastly, many of the pipeline urinary biomarkers still require further pre-validation and validation studies in order to move them into the clinical arena.

Conclusions

Recent literature would support that urinary biomarkers have a clear role to supplement risk evaluation for men undergoing prostate biopsy and for prognostication. Only two have been more thoroughly vetted: PCA3 and TMPRSS2:erg fusion and are commercially available. Future studies will likely focus on panels that combine urine, blood, and perhaps even tissue biomarkers, in addition to known clinical risk factors. On average, use of these urine biomarkers will allow the clinician to make better recommendations to their patients.

Acknowledgments

none

Conflicts of interest statement: research funding support from NCI, Exosome

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Key points

- PCA3 is the first urinary biomarker to be FDA approved in the US
- TMPRSS2:erg fusion, which is the most specific biomarker of prostate cancer to date, has a urinary assay using the same platform as PCA3
- It is recommended that TMPRSS2:erg be measured with PCA3 as a panel to assess the risk of prostate cancer
- Emerging applications of these and other biomarkers include active surveillance and risk stratification for treatment