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Towards the Detection of Prostate Cancer in Urine: A Critical Analysis

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Abstract

Introduction—Prostate specific antigen (PSA) and digital rectal exam (DRE) have low specificity for the detection of prostate cancer (PCa) and poorly predict the presence of aggressive disease. Urine is readily available, non-invasive, and represents a promising source of biomarkers for early detection and prediction of PCa prognosis. The goal of this review is to identify promising biomarkers for urine-based PCa, examine trends, and outline potential pitfalls.

Methods—Pubmed® and Web of Science® database searches of peer-reviewed literature on urine-based testing in PCa were performed. Original studies on this subject, as well as a small number of reviews, were analyzed including the strengths and weaknesses. We provide a comprehensive review of urine-based testing for PCa that covers the technical aspects including the methodology of urine collection, as well as recent developments in biomarkers spanning the fields of genomics, epigenetics, transcriptomics, proteomics, and metabolomics.

Results—The process of urine collection is subject to variability, which may result in conflicting clinical results. Detecting PCa in urine is technically feasible as demonstrated by numerous “proof-of principle” studies, but few markers have been validated in multiple large sample sets. Biomarker development using urine has been accelerating in recent years, with numerous studies identifying DNA, RNA, protein, and metabolite-based biomarkers in the urine. Advanced clinical studies have identified PCA3 and TMPRSS2:ERG fusion transcripts as promising RNA markers for cancer detection and possibly prognosis. DNA methylation analysis of multiple genes improves specificity, and represents a promising platform for the development of clinical-grade assays.

Conclusions—Urine-based testing is non-invasive and represents a rich source of novel biomarkers for PCa. Although urine demonstrates promise in detecting cancer, the ability to identify aggressive subsets of PCa needs further development.

Introduction

Prostate cancer (PCa) is the second leading cause of cancer death in men. The predominant tools of PCa detection are serum prostate specific antigen (PSA) and digital rectal exam (DRE). Despite their widespread use, PSA is a poor predictor of disease. As many as 65–70% of men presenting with an elevated PSA ranging between 4–10 ng/ml will have a negative biopsy result.¹ PSA isoforms add some additional specificity.² Elevated PSA levels

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necessitate the use of repeated biopsies which can be associated with significant morbidity, including sepsis, bleeding and hospitalization.³ Furthermore, up to 15% of PCa patients will present with a PSA level lower than the commonly used cut point of 4.0 ng/ml, leaving many cancers undetected.⁴ Another major challenge with current screening is the poor sensitivity in detecting clinically relevant cancers, especially high grade disease, which paradoxically express low PSA levels.⁵ Thus, development of more accurate screening tool for PCa, especially biologically aggressive disease, is critical.

Biological fluids with potential for PCa screening include prostate serum, semen, plasma, and urine. PCa and epithelial cells are shed into biologic fluids, particularly when the prostate is subjected to physical manipulation. This creates the potential for their noninvasive detection in either urine or expressed prostatic fluid. Urine is readily available, non-invasive and represents a promising source of biomarkers.

Methods

A Pubmed® and Web of Science® database search on peer-reviewed literature identified over 600 articles in the last 10 years using keywords including urine, biomarkers, PCa, screening and prognosis. Selection criteria included peer reviewed studies from English and non-English journals that: 1) evaluated urine specimen biomarker techniques, 2) validated biomarkers in multiple clinical samples especially large datasets, and/or 3) were novel. We reviewed 124 studies on the subject of urine-based testing for PCa, including several reviews. We expanded the discussion to include the methodology of urine collection, recent developments in biomarker research, and analysis of the strengths and weaknesses.

Results

Technical Aspects of Urine Collection

Urine-based screening relies on the presence of cancer products (DNA, RNA, proteins or metabolites) that are released either directly into the urine as cell-free markers or carried within prostatic cells that are shed into urine. Studies conducted on urinary biomarkers for PCa vary in their methods of urine collection. Müller *et al.* provided a detailed review on the various collection methods for DNA- and RNA-based tests.⁶ In many studies, prostatic massage or palpation is performed prior to urine collection with the idea of increasing sensitivity. However, the impact of prostatic massage has never been evaluated in a comparative study and its impact remains unclear. In an attempt to standardize urine collection, some studies recommend collection after an “attentive” prostate massage involving firm pressure (sufficient to depress the prostate by 1 cm) applied from the base to the apex and from the lateral to the median line for each lobe, with three strokes for each lobe.⁷ Other experimental variations include length of collection (overnight void versus 24-hour) and portion of the void to be used (first-stream versus mid-stream). Given the distance of the peripheral zone from the urinary outflow tract, urine-based tests may theoretically be less sensitive for peripheral cancers. However, studies on PCA3 did not find a difference in the levels of this marker in the urine of patients with peripheral versus transitional zone cancers.⁵

The stability of biological markers can vary with urine composition, pH, temperature, and processing time, which pose a challenge for standardizing the methods of urine storage prior to molecular detection. Studies whose primary outcome was recovery and analysis of human DNA in urine have concluded that storage of urine at room temperature with sodium azide over 30 days or frozen at –20°C with EDTA over 72 days are ideal.⁸ Since DNA is relatively stable in urine, DNA-based assays may require less effort for preservation. In contrast, the stability of RNA poses a challenge for RNA-based tests, since urinary cells

often contain fragmented RNA. Thus, the integrity and amount of RNA in urinary samples can be highly variable. Studies on RNA-based urine tests may require an adequate assessment of specimen stability and mRNA yield, or in some cases using PSA transcripts, as a surrogate for RNA quality.⁹

Following urine collection, either urinary sediment obtained after centrifugation or whole urine can be analyzed for biomarkers. The advantage of using urinary sediment over whole urine may be that data can be obtained from the majority of cells and cell fragments in the collected urine specimen increasing sensitivity. Drawbacks include the need for additional processing, the presence of crystals and other sediments that can inhibit assays, and the removal of the supernatant, which may have biological significance. In contrast, when commercial whole urine kits are used, an equal volume of urine is processed each time and any remaining specimen is discarded. Advantages of whole urine analysis include ease of collection and relative stability. To our review we found no studies that directly compared the efficacy of using urinary sediment versus whole urine. However, differences in the use of whole versus urinary sediment may explain some of the contradictory results regarding PCA3 and its correlation with clinical stage and Gleason score.¹⁰

Previous studies have verified the feasibility of using urine as a method of PCa cell detection. Prostate cells are directly released into the urethra through prostatic ducts. Attempts at detecting individual PCa cells in urine by using cytology are thwarted by unacceptably low sensitivities, although specificities are high.¹¹ Studies have estimated that only 10% of cells in the urine are of prostate origin and previous attempts at using a multiplex panel of markers in combination with cytology only reached a sensitivity of 15% for PCa detection.¹¹ Furthermore, when found cytologically, PCa cells in the urine occur almost exclusively in patients with high grade or advanced cancers.¹² Of interest, the presence of PCa cells may not be required for all urine-based assays, since DNA can also pass into urine via phagocytosis (i.e. macrophages engulf DNA from necrotic tumor cells, then appear in the urine).¹³

If the ultimate goal is to bring urine-based testing to the bedside, the technical challenges of urine assays must not be neglected. Thus far, the methods of urine collection have varied between studies, and markers in the urine can be subject to dilution depending on the amount of urine collected. To avoid the dilution of markers, groups preferentially choose the first void urine, which is most likely to contain prostatic fluid. Another potential problem is the presence of proteolytic activity in the urine by urokinase and other enzymes. Proteases found in stored urine have been found to degrade urinary albumin to a substantial degree.¹⁴ However, the extent to which proteases affect biomarkers in the urine is still unclear. The effect of prostate inflammation is another potential confounder. Serum PSA testing can be subject to false positives due to increased PSA leak in cases of prostatitis. Urinary biomarkers may be less influenced by the presence of prostate inflammation, but this has not been extensively analyzed. In one study, the PCA3 score was not falsely elevated in patients with chronic prostatitis or urethritis.¹⁵ Another challenge is the formation of precipitates and urinary crystals, which may trap biomarkers and underestimate the levels measured in the urine.¹⁶

Despite these pitfalls, urine presents advantages over other bodily fluids, such as plasma, serum, and prostatic fluids. It is easily obtained and non-invasive. Biomarkers generated by PCa are diluted into a smaller volume and immediately excreted upon urination. In contrast, biomarkers in blood and serum are diluted in the circulation (e.g. 4 liters) and may be altered by liver metabolism. Payne *et al.* analyzed four DNA methylation biomarkers *GSTPI*, *RASSF1*, *HIST1H4K*, and *TFAP2E* in urine and plasma and concluded that urine outperformed plasma in sensitivity for PCa.¹⁷

Biomarker Development

The composition of urine composition is complex and provides a rich source of biomarkers. Urinary biomarkers can be broadly classified into DNA, RNA, protein, and recently, metabolite-based markers (Figure 1). Many of these urine markers have been previously tested and validated in prostate tissues and prostatic secretions obtained from men with PCa.

DNA markers—Copy number changes occur in PCa and include either gene amplification or loss of heterozygosity (LOH).¹⁸ Regions frequently containing LOH in PCa include 7q, 8p, 10q, 12p, 13p, 16p, 17p, and 18q. Thuret *et al.* assessed the validity of a urinary LOH test by comparing it with the LOH in tumors from the same patients.¹⁸ They reported that LOH analysis detected PCa in urine with a sensitivity as high as 87% using a PCR-based assay. However, only 19 specimens were available for analysis. Interestingly, they also analyzed the genetic status of histologically “normal” prostatic cells located near the tumor and found that 53% of allelic deletions observed in tumor DNA were present in the associated histologically normal prostatic cells.¹⁸ This has interesting implications in that urine-based testing for LOH could take advantage of this “field defect” in epithelial cells and, therefore, may not always require the presence of cancer cells for diagnosis. Although aneuploidy (and LOH) is used routinely for bladder cancer detection in the form of the UrovysionTM test it has not been widely developed in PCa. Amplification of DNA has not yet been used as a method for detecting PCa in urine.

Methylation of cytosine at CpG dinucleotides represents an epigenetic alteration in PCa that has been well characterized in tissues and is a promising area for urine biomarker development. In PCa pathogenesis, abnormal gene expression occurs resulting in altered levels of DNA damage repair genes, tumor-suppressors, cell cycle control genes, cell adhesion molecules, and signal transduction genes. Regulation of these genes can occur by DNA hypermethylation of their promoters resulting in a loss of gene expression. In urine as well as tissue specimens, aberrant DNA methylation can be detected by a number of methodologies including methylation-specific polymerase chain reaction (MSP), bisulfite sequencing, methylation-sensitive single-nucleotide primer extension (MS-SNuPE), and combined bisulfite restriction analysis (COBRA).¹⁹ Use of real-time quantitative MSP and the probe-based MethyLightTM assay in urine samples holds promise as a rapid, sensitive method of detection with the potential for high-throughput analysis of clinical samples.¹⁹

One well-known DNA methylation target is glutathione-S-transferase P (*GSTP1*), a gene involved in the detoxification of electrophiles and other damaging components. Numerous studies have confirmed hypermethylation of *GSTP1* in the urine samples of patients with PCa. In a paper summarizing these results,²⁰ the sensitivity of *GSTP1* in urine was poor, varying from 21.4% to 38.9%, but was improved by prostatic massage to 75%. The specificity of *GSTP1* methylation ranges from 93% to 100%.²⁰ These studies suggested that patients with an elevated PSA should undergo a complementary *GSTP1* methylation test to decide whether to perform subsequent biopsies.

To improve the lower sensitivity using single gene analysis, gene panels have been utilized with varying success (Table 1).^{17, 21–23} Overcoming low sensitivity, even with the use of multiple genes, is a major obstacle for DNA methylation-based tests. This is potentially due to the requirement of the presence of PCa cells. However, a major advantage is their high specificity, which can reach 100%, since targeted DNA aberrations are rarely present in controls.²⁴ If incorporated into the decision-making process on whether to pursue a subsequent biopsy, DNA markers would pose minimal risk of having false positives. Furthermore, the stability of DNA and ease of PCR-based methodologies makes DNA one of the most promising platforms for biomarker development. To date none are commercially available.

RNA markers—RNA urine markers have been developed to the greatest extent clinically to date. PCA3 (PCa Antigen 3) is a prostate-cancer specific gene that was discovered in 1999 by Bussemakers and Issacs and has been extensively reviewed.⁷ PCA3 is overexpressed in 95% of primary PCa specimens which demonstrate a 34-fold increase in expression.²⁵ Because no protein product has been detected for PCA3, assays using an RT-PCR platform were developed for urine. Initially, the PCA3 assay was known as the uPM3 diagnostic test, but the commercial platform is now called the ProgenSA® PCA3 assay (Gen-Probe) and uses Transcription Mediated Amplification (TMA) and Hybridization Protection (HP) for quantitation. The assay employs PSA transcripts as an internal control for RNA quality and to establish the presence of prostate specific nuclear material. The PCA3 to PSA ratio is used to generate a PCA3 Score. The use of an “attentive” prostate exam increases informative samples from 80 to 95%.⁹ In several studies using large cohorts of over 1000 men, PCA3 appears superior to PSA and percent free PSA with improved specificity (Table 2).^{26–29} The test does not appear to be greatly influenced by age, inflammation, trauma, the use of 5 alpha-reductase inhibitors or, importantly, prostate volume. Issues arise, however, with regard to the cutoff PCA3 score used to determine a positive test since the specificity decreases with a lower PCA3 Score. Thus, PCA3 represents a promising screening biomarker that will likely require utilization with other markers for screening given a low PCA3 score does not exclude cancer.

PCA3 now has FDA approval for its ability to predict cancer in patients with elevated PSAs and a negative biopsy. The clinical study submitted for FDA approval involved 495 eligible men at 14 clinical sites and had a negative predictive value of 90%. In this subpopulation, PCA3 was independently associated with PCa with an area under the curve (AUC) of 0.64–0.68 compared to PSA 0.52.³⁰ Multivariable models containing PCA3 have been developed to further improve diagnostic accuracy.^{26–29} PCA3 has also been reported to predict clinicopathological features including Gleason score, tumor volume, and stage;³¹ however, other studies have contradicted these results leading some to conclude that PCA3 has limited value in predicting aggressive cancers.³² Interest has arisen in determining if PCA3 can be used to identify patients with clinically insignificant PCa, but definitive studies are lacking.

Gene rearrangements, namely splice variants, have been described in hematologic malignancies and now appear to occur in PCa.³³ These fusions can lead to activation or inhibition of the fused transcripts. In 2005, Chinnayan found the first prostate gene fusions including one between transmembrane protease serine 2 gene (TMPRSS2) and E twenty-six (ETS) transcription factors, which include (*ERG*, *ET1*, *ETV4*, *ETV5*, and *ELK4*; *ERG* is predominant).³⁴ Hessels *et al.* analyzed urine in 78 men with PCa-positive biopsies and 30 men with PCa-negative biopsies, leading to the development of a clinical-grade assay for TMPRSS2:ERG that has a sensitivity of 37%.³⁵ Although the TMPRSS2:ERG fusion demonstrates usefulness as a screening tool, its prognostic utility is controversial. Rostad *et al.* found elevated TMPRSS2:ERG fusion transcripts correlate with high serum PSA, pathological stage, and Gleason score.³⁶ However, other contradictory studies found the converse.^{37, 38} Problems with TMPRSS2 include the lower frequency of the fusion in some populations leading to lower screening sensitivity³⁹, and the identification of a cutpoint that is applicable for all patient populations.

A modified version of the TMPRSS2:ERG assay in combination with the PCA3 test is now being evaluated. In a recent study involving 471 men, this combined assay increased the AUC from 0.66 to 0.75 when combined with the PCa Prevention Trial (PCPT) risk calculator in a community population.⁴⁰ The combined use of PCA3 and TMPRSS2:ERG may represent a promising urinary test for PCa.⁴¹

Other less extensively studied RNA markers for PCa include telomerase, α -methylacyl coenzyme A racemase (AMACR), and Golgi membrane protein 1 (GOLM1/GOLPH2). Telomerase activity has been identified in the majority of cancers and demonstrates activation in up to 93% of PCa cases.⁴² Botchkina *et al.* reported a 100% sensitivity and 88.6% specificity in urine for detection of PCa using a quantitative PCR telomeric repeat amplification protocol (TRAP) in 56 patients.⁴² Telomerase RNA can be detected in virtually all cancer types, including breast, lung, colon, kidney, prostate, bladder, uterus, ovary, pancreas, and liver but has been less extensively studied. A promising marker that is overexpressed specifically in PCa is AMACR. AMACR transcripts when used in combination with PCA3 in the urine of 92 patients was predictive of PCa with a sensitivity of 81% and specificity of 84%.⁴³ Future large cohort studies are needed to validate the use of AMACR as a screening tool.

As with DNA methylation markers, groups have assessed gene panels of RNA markers in an effort to maximize assay sensitivity. Recently published studies on RNA marker panels are presented in Table 1.^{35, 41, 44–47} Despite the effectiveness of multiplex RNA-based platforms, the high cost of using multiple RNA assays simultaneously may slow their widespread clinical application. Overall, RNA markers hold promise as initial screening tools, in conjunction with PSA testing, due to their high sensitivity relative to most DNA markers. The TMPRSS2:ERG/PCA3 urine test is promising and is currently being marketed to be used in conjunction with PSA testing.

Protein markers—In contrast to DNA and RNA, proteins are frequently secreted into bodily fluids and do not necessarily rely on the presence of cancer cells for detection. Furthermore, immunologic assays such as ELISA, the predominant tools for quantitative protein analysis, are relatively inexpensive, sensitive, and easily designed. A comprehensive list of candidate urine protein biomarkers examined in the literature is shown in Table 3. A multicenter study consisting of 165 patients examined the utility of the urine to serum PSA ratio and demonstrated an improved ROC curve when compared to serum PSA alone.⁴⁸ However, these results were contradicted in a smaller study⁴⁹ and are less likely to see widespread development given concerns over the use of widespread PSA screening.

While some of these markers hold promise, an ideal protein marker should be validated in independent studies and tested in large cohorts without having to rely on PSA. Matrix metalloproteinases (MMP) are a family of enzymes that function as extracellular proteases that are secreted in cancer as such represent a potential biomarker. Roy *et al.* identified a tumor-specific urinary MMP fingerprint by gelatin zymography in 148 patients and found that the presence of MMP-9 and MMP-9 dimer were independent predictors for identifying PCa with a specificity of 82% and sensitivity of 74%.⁵⁰ The presence of MMP-2 and 9 were confirmed in a smaller study that used zymographic tests to detect multiple forms of the same protein, but remain primarily qualitative.⁵¹ Larger studies have demonstrated the utility of these MMP isoforms in detecting ovarian and bladder cancer. Annexin A3, a calcium-binding protein, has an inverse relationship with cancer, which may be an advantage. Annexin A3 was analyzed in a blinded clinical study involving 591 patients at multiple centers and found in conjunction with PSA to have an ROC of 0.82.⁵² Endoglin (CD105) was found to be elevated in the urine of men with PCa, but required normalization to total urinary protein or urinary creatinine.⁵³ Recently, engrailed-2 (EN2) was found to detect PCa in urine with 66% sensitivity and 88% specificity using an ELISA-based assay.⁵⁴ A larger multicenter study is underway to evaluate the diagnostic potential of EN2.

The field of proteomics holds promise for biomarker discovery and has the advantage of analyzing thousands of peptides simultaneously. In a large proteomics study, 407 patient urine samples were analyzed using matrix assisted laser desorption-mass spectrometry time

of flight (MALDI-TOF) and found that two markers, uromodulin and semenogelin, could distinguish PCa versus BPH with 71.2% sensitivity and 67.4% specificity.⁵⁵ In 2008, Theodorescu *et al.* used CE-MS to identify and validate 12 novel urinary biomarkers for PCa. They found that mid-stream urine was uninformative, but first void urine was able to identify patients with PCa with 91% sensitivity and 69% specificity.⁵⁶ These studies require additional validation in larger cohorts.

Exosomes are secreted cell membrane vesicles that contain cytoplasmic RNAs and proteins and can be secreted into the urine. This new area is promising for the detection of PCa since it does not rely on the presence of PCa cells. Nilsson *et al.* found that exosomes were carriers for the *TMPRSS2:ERG* fusion and *PCA3* RNA.⁵⁷ In addition to RNA detection, proteomic profiling of exosomes in human urine is underway and may lead to new biomarker development for a variety of diseases, including PCa.⁵⁸ To date, no high-throughput techniques such as mass spectrometry and microarray have been used to evaluate the differences between exosomes of normal versus PCa patients.

Metabolite markers—Metabolomics, seeks to distinguish the metabolite content of PCa cells from normal cells. Cancer metabolomics provide accurate read-outs of tumor cell physiology and biochemical activity making this an attractive option for biomarker development. In a landmark study, Sreekumar *et al.* used high-throughput liquid-and-gas-chromatography-based mass spectrometry to profile more than 1,126 metabolites in 262 clinical samples (including tissues, urine, and plasma) and found 176 common to all three.⁵⁹ Of these, 87 metabolites distinguished PCa from normal. A lead candidate sarcosine (N-methylglycine) was found to be associated with cancer progression to metastasis and had significant predictive value for cancer detection in urine samples when normalized to alanine or creatinine. However, an independent follow-up study of 106 patients was not able to reproduce the ability of urinary sarcosine (normalized to creatinine) to detect cancer although levels were higher than healthy controls.⁶⁰ These contradictory findings may be due to the cumulative effect of sample selection, urine handling, the normalization (alanine may be a better approach), or analysis methods. In a nested case control study examining methionine metabolites, urinary sarcosine and cysteine levels were significantly higher in 54 patients who had a cancer recurrence after treatment.⁶¹ The role of sarcosine as a urinary biomarker is still controversial and further validation studies are warranted. However, the finding of a link with more aggressive disease is encouraging with regard to a marker for significant PCa.

Conclusions

A multiplicity of studies has identified biomarkers for the non-invasive testing of PCa in urine. However, to date few biomarkers have reached the clinic and there are many reasons for this. The novel biomarker is required to be specific for PCa and not altered or expressed in other human tissues or diseases. The quality of the sample and analytical procedures utilized are critical components of these assays. Part of the variation in sarcosine results appears to result from the use of different assays.^{59, 60} Another challenge is developing a cutpoint for a positive test, an issue with the PCA3 test. The patient population clearly plays a role in the difficulty validating urine biomarkers and expanding testing to include the general population frequently leads to loss or decrease in sensitivity. Errors also result from the design and execution of many studies and the majority listed in this review should be considered exploratory. Finally, the cost of developing and validating a clinical grade assay is clearly beyond the majority of laboratory funding and requires the input of industry.

The ease of collection, low volume, direct access to the prostate, and excellent specificity are advantages of urine markers. *TMPRSS2:ERG/PCA3* has overcome many hurdles and

has already been marketed for clinical use. Recent FDA approval of PCA3 for patients with negative prostate biopsies provides validation of this approach. There are still challenges inherent in using urine including dilution, variability of collection methods, confounding effects of other urinary components, and biomarker degradation.

Despite numerous pitfalls, urine is clearly the next frontier for biomarker development for PCa. Several pressing issues remain however beyond developing more biomarkers that detect PCa. Selective markers that identify aggressive, clinically significant prostate cancer at an early timepoint are desperately needed. These cancers include high grade (Gleason 8–10) disease that has a elevated risk of disease progression. The failure of PSA to identify this important group has led to its criticism as a PCa marker and the downgrading of its use in screening.⁶² As scientists and clinicians think about future biomarker development, this aspect should be of paramount importance.

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References

1. Draisma G, Etzioni R, Tsodikov A, et al. Lead time and overdiagnosis in prostate-specific antigen screening: importance of methods and context. *J Natl Cancer Inst.* 2009; 101:374. [PubMed: 19276453]
2. Djavan B. Validity and legacy of prostate-specific antigen (PSA) and PSA-based parameters and isoforms in the new millennium. *Eur Urol.* 2010; 57:928. [PubMed: 20304552]
3. Gann PH, Fought A, Deaton R, et al. Risk factors for prostate cancer detection after a negative biopsy: a novel multivariable longitudinal approach. *Journal of clinical oncology: official journal of the American Society of Clinical Oncology.* 2010; 28:1714. [PubMed: 20177031]
4. Thompson IM, Pauler DK, Goodman PJ, et al. Prevalence of prostate cancer among men with a prostate-specific antigen level < or =4.0 ng per milliliter. *The New England journal of medicine.* 2004; 350:2239. [PubMed: 15163773]
5. Nakanishi H, Groskopf J, Fritsche HA, et al. PCA3 molecular urine assay correlates with prostate cancer tumor volume: implication in selecting candidates for active surveillance. *J Urol.* 2008; 179:1804. [PubMed: 18353398]
6. Müller H, Brenner H. Urine markers as possible tools for prostate cancer screening: review of performance characteristics and practicality. *Clin Chem.* 2006; 52:562. [PubMed: 16484332]
7. Vlaeminck-Guillem V, Ruffion A, André J, et al. Urinary prostate cancer 3 test: toward the age of reason? *Urology.* 2010; 75:447. [PubMed: 19586654]
8. Cannas A, Kalunga G, Green C, et al. Implications of storing urinary DNA from different populations for molecular analyses. *PLoS One.* 2009; 4:e6985. [PubMed: 19746164]
9. Groskopf J, Aubin SM, Deras IL, et al. APTIMA PCA3 molecular urine test: development of a method to aid in the diagnosis of prostate cancer. *Clin Chem.* 2006; 52:1089. [PubMed: 16627561]
10. Hessels D, van Gils MP, van Hooij O, et al. Predictive value of PCA3 in urinary sediments in determining clinico-pathological characteristics of prostate cancer. *Prostate.* 2010; 70:10. [PubMed: 19708043]
11. Fujita K, Pavlovich CP, Netto GJ, et al. Specific detection of prostate cancer cells in urine by multiplex immunofluorescence cytology. *Hum Pathol.* 2009; 40:924. [PubMed: 19368959]
12. Tyler KL, Selvaggi SM. Morphologic features of prostatic adenocarcinoma on ThinPrepR urinary cytology. *Diagn Cytopathol.* 2011; 39:101. [PubMed: 20146303]
13. Ahmed H. Promoter Methylation in Prostate Cancer and its Application for the Early Detection of Prostate Cancer Using Serum and Urine Samples. *Biomark Cancer.* 2010; 2010:17. [PubMed: 20657713]

14. Kania K, Byrnes EA, Beilby JP, et al. Urinary proteases degrade albumin: implications for measurement of albuminuria in stored samples. *Ann Clin Biochem.* 2010; 47:151. [PubMed: 20150213]
15. Vlaeminck-Guillem V, Bandel M, Cottancin M, et al. Chronic prostatitis does not influence urinary PCA3 score. *Prostate.* 2011
16. Saetun P, Semangoen T, Thongboonkerd V. Characterizations of urinary sediments precipitated after freezing and their effects on urinary protein and chemical analyses. *Am J Physiol Renal Physiol.* 2009; 296:F1346. [PubMed: 19339629]
17. Payne SR, Serth J, Schostak M, et al. DNA methylation biomarkers of prostate cancer: confirmation of candidates and evidence urine is the most sensitive body fluid for non-invasive detection. *The Prostate.* 2009; 69:1257. [PubMed: 19459176]
18. Thuret R, Chantrel-Groussard K, Azzouzi AR, et al. Clinical relevance of genetic instability in prostatic cells obtained by prostatic massage in early prostate cancer. *Br J Cancer.* 2005; 92:236. [PubMed: 15655554]
19. Brena RM, Huang TH, Plass C. Quantitative assessment of DNA methylation: Potential applications for disease diagnosis, classification, and prognosis in clinical settings. *Journal of Molecular Medicine (Berlin, Germany).* 2006; 84:365.
20. Ploussard G, de la Taille A. Urine biomarkers in prostate cancer. *Nat Rev Urol.* 2010; 7:101. [PubMed: 20065953]
21. Hoque MO, Topaloglu O, Begum S, et al. Quantitative methylation-specific polymerase chain reaction gene patterns in urine sediment distinguish prostate cancer patients from control subjects. *J Clin Oncol.* 2005; 23:6569. [PubMed: 16170165]
22. Rouprêt M, Hupertan V, Yates DR, et al. Molecular detection of localized prostate cancer using quantitative methylation-specific PCR on urinary cells obtained following prostate massage. *Clin Cancer Res.* 2007; 13:1720. [PubMed: 17363525]
23. Costa VL, Henrique R, Danielsen SA, et al. TCF21 and PCDH17 methylation: An innovative panel of biomarkers for a simultaneous detection of urological cancers. *Epigenetics.* 2011; 6
24. Goessl C, Müller M, Straub B, et al. DNA alterations in body fluids as molecular tumor markers for urological malignancies. *Eur Urol.* 2002; 41:668. [PubMed: 12074786]
25. de Kok JB, Verhaegh GW, Roelofs RW, et al. DD3(PCA3), a very sensitive and specific marker to detect prostate tumors. *Cancer Res.* 2002; 62:2695. [PubMed: 11980670]
26. Deras IL, Aubin SM, Blase A, et al. PCA3: a molecular urine assay for predicting prostate biopsy outcome. *J Urol.* 2008; 179:1587. [PubMed: 18295257]
27. Chun FK, de la Taille A, van Poppel H, et al. Prostate cancer gene 3 (PCA3): development and internal validation of a novel biopsy nomogram. *Eur Urol.* 2009; 56:659. [PubMed: 19304372]
28. Roobol MJ, Schröder FH, van Leeuwen P, et al. Performance of the prostate cancer antigen 3 (PCA3) gene and prostate-specific antigen in prescreened men: exploring the value of PCA3 for a first-line diagnostic test. *Eur Urol.* 2010; 58:475. [PubMed: 20637539]
29. Tosoian JJ, Loeb S, Kettermann A, et al. Accuracy of PCA3 measurement in predicting short-term biopsy progression in an active surveillance program. *J Urol.* 2010; 183:534. [PubMed: 20006883]
30. Marks LS, Fradet Y, Deras IL, et al. PCA3 molecular urine assay for prostate cancer in men undergoing repeat biopsy. *Urology.* 2007; 69:532. [PubMed: 17382159]
31. Ploussard G, Haese A, Van Poppel H, et al. The prostate cancer gene 3 (PCA3) urine test in men with previous negative biopsies: does free-to-total prostate-specific antigen ratio influence the performance of the PCA3 score in predicting positive biopsies? *BJU Int.* 2010; 106:1143. [PubMed: 20230386]
32. Auprich M, Bjartell A, Chun FK, et al. Contemporary role of prostate cancer antigen 3 in the management of prostate cancer. *Eur Urol.* 2011; 60:1045. [PubMed: 21871709]
33. Pflueger D, Terry S, Sboner A, et al. Discovery of non-ETS gene fusions in human prostate cancer using next-generation RNA sequencing. *Genome Res.* 2011; 21:56. [PubMed: 21036922]
34. Tomlins SA, Rhodes DR, Perner S, et al. Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer. *Science.* 2005; 310:644. [PubMed: 16254181]

35. Hessels D, Smit FP, Verhaegh GW, et al. Detection of TMPRSS2-ERG fusion transcripts and prostate cancer antigen 3 in urinary sediments may improve diagnosis of prostate cancer. *Clin Cancer Res.* 2007; 13:5103. [PubMed: 17785564]
36. Rostad K, Hellwinkel OJ, Haukaas SA, et al. TMPRSS2:ERG fusion transcripts in urine from prostate cancer patients correlate with a less favorable prognosis. *APMIS.* 2009; 117:575. [PubMed: 19664128]
37. Petrovics G, Liu A, Shaheduzzaman S, et al. Frequent overexpression of ETS-related gene-1 (ERG1) in prostate cancer transcriptome. *Oncogene.* 2005; 24:3847. [PubMed: 15750627]
38. Winnes M, Lissbrant E, Damber JE, et al. Molecular genetic analyses of the TMPRSS2-ERG and TMPRSS2-ETV1 gene fusions in 50 cases of prostate cancer. *Oncol Rep.* 2007; 17:1033. [PubMed: 17390040]
39. Demichelis F, Fall K, Perner S, et al. TMPRSS2:ERG gene fusion associated with lethal prostate cancer in a watchful waiting cohort. *Oncogene.* 2007; 26:4596. [PubMed: 17237811]
40. Tomlins SA, Aubin SM, Siddiqui J, et al. Urine TMPRSS2:ERG fusion transcript stratifies prostate cancer risk in men with elevated serum PSA. *Sci Transl Med.* 2011; 3:94ra72.
41. Salami SS, Schmidt F, Laxman B, et al. Combining urinary detection of TMPRSS2:ERG and PCA3 with serum PSA to predict diagnosis of prostate cancer. *Urol Oncol.* 2011
42. Botchkina GI, Kim RH, Botchkina IL, et al. Noninvasive detection of prostate cancer by quantitative analysis of telomerase activity. *Clin Cancer Res.* 2005; 11:3243. [PubMed: 15867219]
43. Ouyang B, Leung YK, Wang V, et al. α -Methylacyl-CoA racemase spliced variants and their expression in normal and malignant prostate tissues. *Urology.* 2011; 77:249.e1. [PubMed: 21195844]
44. Laxman B, Morris DS, Yu J, et al. A first-generation multiplex biomarker analysis of urine for the early detection of prostate cancer. *Cancer Res.* 2008; 68:645. [PubMed: 18245462]
45. Ouyang B, Bracken B, Burke B, et al. A duplex quantitative polymerase chain reaction assay based on quantification of alpha-methylacyl-CoA racemase transcripts and prostate cancer antigen 3 in urine sediments improved diagnostic accuracy for prostate cancer. *J Urol.* 2009; 181:2508. [PubMed: 19371911]
46. Jamaspishvili T, Kral M, Khomeriki I, et al. Quadriplex model enhances urine-based detection of prostate cancer. *Prostate Cancer Prostatic Dis.* 2011; 14:354. [PubMed: 21788966]
47. Nguyen PN, Violette P, Chan S, et al. A panel of TMPRSS2:ERG fusion transcript markers for urine-based prostate cancer detection with high specificity and sensitivity. *Eur Urol.* 2011; 59:407. [PubMed: 21144642]
48. Irani J, Salomon L, Soulié M, et al. Urinary/serum prostate-specific antigen ratio: comparison with free/total serum prostate-specific antigen ratio in improving prostate cancer detection. *Urology.* 2005; 65:533. [PubMed: 15780371]
49. Pannek J, Rittenhouse HG, Evans CL, et al. Molecular forms of prostate-specific antigen and human kallikrein 2 (hK2) in urine are not clinically useful for early detection and staging of prostate cancer. *Urology.* 1997; 50:715. [PubMed: 9372881]
50. Roy R, Louis G, Loughlin KR, et al. Tumor-specific urinary matrix metalloproteinase fingerprinting: identification of high molecular weight urinary matrix metalloproteinase species. *Clin Cancer Res.* 2008; 14:6610. [PubMed: 18927302]
51. Di Carlo A, Mariano A, Terracciano D, et al. Matrix metalloproteinase-2 and -9 in the urine of prostate cancer patients. *Oncol Rep.* 2010; 24:3. [PubMed: 20514437]
52. Schostak M, Schwall GP, Poznanovi S, et al. Annexin A3 in urine: a highly specific noninvasive marker for prostate cancer early detection. *J Urol.* 2009; 181:343. [PubMed: 19012935]
53. Fujita K, Ewing CM, Chan DY, et al. Endoglin (CD105) as a urinary and serum marker of prostate cancer. *Int J Cancer.* 2009; 124:664. [PubMed: 19004009]
54. Morgan R, Boxall A, Bhatt A, et al. Engrailed-2 (EN2): a tumor specific urinary biomarker for the early diagnosis of prostate cancer. *Clin Cancer Res.* 2011; 17:1090. [PubMed: 21364037]
55. M'Koma AE, Blum DL, Norris JL, et al. Detection of pre-neoplastic and neoplastic prostate disease by MALDI profiling of urine. *Biochem Biophys Res Commun.* 2007; 353:829. [PubMed: 17194448]

56. Theodorescu D, Schiffer E, Bauer HW, et al. Discovery and validation of urinary biomarkers for prostate cancer. *Proteomics Clin Appl*. 2008; 2:556. [PubMed: 19759844]
57. Nilsson J, Skog J, Nordstrand A, et al. Prostate cancer-derived urine exosomes: a novel approach to biomarkers for prostate cancer. *Br J Cancer*. 2009; 100:1603. [PubMed: 19401683]
58. Pisitkun T, Shen RF, Knepper MA. Identification and proteomic profiling of exosomes in human urine. *Proc Natl Acad Sci U S A*. 2004; 101:13368. [PubMed: 15326289]
59. Sreekumar A, Poisson LM, Rajendiran TM, et al. Metabolomic profiles delineate potential role for sarcosine in prostate cancer progression. *Nature*. 2009; 457:910. [PubMed: 19212411]
60. Jentzmik F, Stephan C, Miller K, et al. Sarcosine in urine after digital rectal examination fails as a marker in prostate cancer detection and identification of aggressive tumours. *Eur Urol*. 2010; 58:12. [PubMed: 20117878]
61. Stabler S, Koyama T, Zhao Z, et al. Serum methionine metabolites are risk factors for metastatic prostate cancer progression. *PLoS One*. 2011; 6:e22486. [PubMed: 21853037]
62. Chou R, Croswell JM, Dana T, et al. Screening for prostate cancer: a review of the evidence for the U.S. Preventive Services Task Force. *Ann Intern Med*. 2011; 155:762. [PubMed: 21984740]

Figure 1.
Urine biomarkers

Table 1

Marker panels

Group		No. of Patients	Sensitivity	Specificity	Limitations
DNA					
Hoque <i>et al.</i> ¹⁷	<i>p16, ARF, MGMT, and GSTP1</i>	73	87	100	Control population was 27% female
Payne <i>et al.</i> ²¹	<i>GSTP1, RASSF2, HIST1H4K, and TFAP2E</i>	192	94	27	No improvement in sensitivity or specificity of individual markers
Roupret <i>et al.</i> ²²	<i>GSTP1, APC, RASSF1, and RARB2</i>	133	87	89	Required bladder catheterization for urine collection
Costa <i>et al.</i> ²³	<i>PCDH17 and TCF21</i>	318	26	100	Low sensitivity
RNA					
Hessels <i>et al.</i> ³⁵	PCA3 and TMPRSS2:ERG	108	73	52	Low specificity
Laxman <i>et al.</i> ⁴¹	GOLPH2, SPINK2, PCA3, and TMPRSS:ERG	234	66	76	Cost and inconvenience of using 4 RNA markers in the clinical setting
Ouyang <i>et al.</i> ⁴⁴	AMACR and PCA3	92	81	84	AMCAR was not reported to be a strong predictor by Laxman <i>et al.</i>
Jamaspishvili <i>et al.</i> ⁴⁵	PCA3, AMACR, TRPM8, and MSMB	104	72	71	Use of markers that are unresponsive of prostate cancer when used alone
Nguyen <i>et al.</i> ⁴⁶	TMRPSS2:ERG subtypes	101	35	100	Low sensitivity
Salami <i>et al.</i> ⁴⁷	PCA3, TMPRSS2:ERG, and serum PSA	48	80	90	Small sample size, requires use of PSA

Table 2

Landmark PCA3 studies for prediction of PCa on biopsy

Group	Description	No. of Patients	Results
Deras <i>et. al.</i> ²⁶	Logistic regression models were generated using PCA3, serum PSA, DRE, patient age, and prostate volume, and subsequently cross-validated using a subset of the population	553	A model with an AUC of 0.75 was predictive regardless of serum PSA levels
Chun <i>et. al.</i> ²⁷	Large multicenter study demonstrating high predictive accuracy of PCA3 when incorporated into several multivariate models	809	A novel nomogram was developed with an AUC of 0.73
Roobol <i>et. al.</i> ²⁸	Repeat testing of PCA3 and biopsy in a randomized group of men with a PCA3 score 100 versus men with a PCA score <100	721	PCA3 has an AUC of 0.64 vs an AUC of 0.58 for PSA in a prescreened population
Tosoian <i>et. al.</i> ²⁹	Evaluated ability of PCA3 to predict short-term biopsy progression in a cohort of active surveillance patients	294	In men with low risk PCa, PCA3 alone could not be used to identify men with progression on biopsy

Table 3

Protein markers previously studied
5-alpha-reductase type 2
AGR2
Acidic fibroblast growth factor
α -methylacyl coenzyme A racemase
Annexin A3
BHUAE activity
Bladder tumor fibronectin
Bradeion
Calgranulin-B/MRP14
Endoglin (CD105)
Engrailed-2
Human kallikrein 2
IgG + IgA + IgM
MMP-2, MMP-9, and hMW MMP
Mitochondosome mainenance 5 protein (Mcm5)
Myeloid related protein-14
Nkx3.1
Nucleolin
Prostatic cancer antigen 1
Prostatic inhibin-like peptide
PSA
Ratio of scatter factor to creatinine ratio
Ratio of serum to urinary PSA
Ratio of transferrin to creatinine
Ratio of urinary PSA to serum PSA
Survivin
Telomerase
Thymosin beta-15
Tissue Factor
Transferrin