Differentiating Molecular Risk Assessments for Prostate Cancer

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It is critically important to the evolving goals of prostate biopsy to find clinically significant cancer with lethal potential and avoid detection of indolent disease. Better tests and markers are required for improved detection of clinically significant prostate cancer and avoidance of biopsies in men with indolent disease. Currently, there are myriad alternative prostate cancer risk-assessment tests available derived from serum and urine that are designed to improve the specificity for detection of "significant" prostate cancer. Herein we discuss these tests and their clinical implications.

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KEY WORDS

Biomarkers • Prostate cancer • Prostate-specific antigen • PSA • Screening

The American Cancer Society estimates that in 2017 about 161,350 new cases of prostate cancer (PCa) will have been diagnosed in the United States, accounting for 19% of new cancer diagnoses in men; 26,730 will have died from the disease.[1] PCa mortality has substantially decreased over the past two decades, with the death rate estimated to have decreased 3% per year since 2009.[1] Early detection, largely achieved through screening with prostate-specific antigen (PSA), which is used almost ubiquitously among practicing urologists, has played an integral role in the declining death rate.[2-4] However, despite this, the United States Preventive Services Task Force (USPSTF) recommended against PSA screening for men over the age of 75 years in 2008, and then for all men in 2012. In 2012, the USPSTF gave annual PSA screening a D recommendation, meaning, "there is moderate or high certainty that the service has no net benefit or that the harms outweigh the benefits."[5] Since their 2012 recommendation, the USPSTF has revised their guidelines on PSA screening. They advise physicians to have individualized discussion of screening with men between the ages of 55 and 69 years. However, they still advise against PSA-based screening for men age 70 years and older.[6] PSA screening has largely transformed the management of this challenging disease; unfortunately, PSA is a prostate tissue–specific, not cancer-specific, marker. A PSA level >4 ng/mL leading to a prostate biopsy is common in clinical

practice; however, biopsies are subject to sampling error and false-negative results. In addition, overdiagnosis of indolent PCa have been estimated to be as high as 67%.[7] It is critically important in the evolving goals of prostate biopsy to find clinically significant cancer with lethal potential and avoid detection of indolent disease. As such, better tests and markers are required for improved detection of clinically significant PCa and avoidance of biopsies in men with indolent disease. Currently, there are myriad alternative PCa risk-assessment tests available derived from serum and urine that are designed to improve the specificity for detection of "significant" PCa. Herein we discuss these tests and their clinical implications.

Blood Biomarkers

Prostate Health Index

PSA is a serine protease secreted as a proenzyme. An isoform of pro-PSA with two amino acids, [-2]pPSA has been proven to be elevated in PCa tissue compared with non-neoplastic conditions (Figure 1).[8,9] Prostate Health Index (Beckman Coulter, Inc., Brea, CA; *phi*) uses a combination of this proenzyme, along with free PSA (fPSA) and total PSA (tPSA) to generate a *phi* score (Table 1). This score is designed to predict the likelihood of PCa in men presenting with an elevated serum PSA. The United States Food and Drug Administration (FDA) has approved the use of *phi* for men with PSA levels between 4 and 10 ng/mL. The National Comprehensive Cancer Network (NCCN) endorses that a *phi* score >35 could be useful in identifying PCa in patients who have never undergone prostate biopsy or have had a prior negative biopsy.[10]

In a multi-institutional trial of 892 men with PSA between 2 and 10 ng/mL with no prior prostate biopsy, Catalona and colleagues found that *phi* had a greater predictive value for PCa detection (AUC = 0.703) compared with %fPSA (0.648), [-2]pPSA (0.557), and tPSA (0.525) alone.[11] They also found that *phi* had significantly greater specificity at 95% sensitivity than %fPSA, [-2]pPSA, tPSA, and fPSA. At 90%, 85%, and 80% sensitivity thresholds, *phi* had a significantly greater specificity compared with %fPSA.[11] From the aforementioned trial, among 658 men with PSA between 4 and 10 ng/mL undergoing prostate biopsy, *phi* was determined to have the greatest predictive ability of clinically significant PCa (both Gleason ≥7 and Epstein significant cancer) when compared with %fPSA, [-2]pPSA, and tPSA.[12] *phi* was also validated in a multicenter European trial of 883 patients. The European study concluded that *phi* was the most accurate predictor of PCa (AUC = 0.68) compared with tPSA (0.51) and %PSA (0.64).[13] These data are concordant with additional studies that highlight the superiority of *phi* when compared with fPSA and tPSA.[14-17]

A limitation in the use of *phi* is that it was designed to predict the probability of any PCa and not to stratify by risk, which makes the prediction of clinically significant disease difficult. Therefore, *phi* is ideally used in conjunction with other clinical parameters or nomograms. Lughezzani and colleagues developed a *phi*-based nomogram that also included patient age, prostate volume, digital rectal examination (DRE) results, and previous history of biopsy that had a predictive value of 75.1% for all PCa.[13] Foley and associates found that the addition of *phi* to the European Randomized Study of Screening for Prostate Cancer (ERSPC) risk calculator improved its discriminative

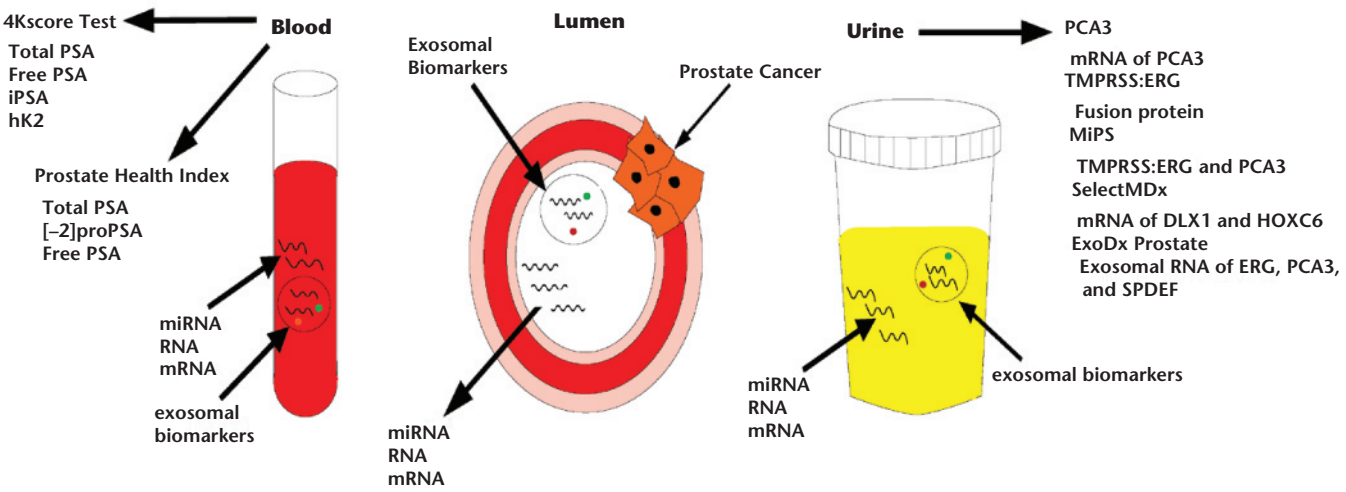

Figure 1. Blood and urine biomarkers in prostate cancer detection.

TABLE 1

Summary of Biomarkers in Detection of Prostate Cancer

Biomarker	Sample	Method	Regulation	Study Findings
Prostate Health Index	Serum	Isomer of precursor PSA found in higher concentrations in men with PCa	FDA approved for men with PSA 4-10 ng/mL CE-IVD	Improved predictive accuracy for overall PCa, clinically significant PCa vs %fPSA, [-2]pPSA, and tPSA
4KScore Test	Serum	Panel of kallikrein markers + clinical data	CAP accreditation	Improved Gleason ≥7 detection vs modified PCPTRC Addition of kallikrein panel improved high-grade cancer detection vs models based on clinical data
PCA3	Urine	Non-coding mRNA overexpressed in neoplastic prostatic tissue	FDA approved for men >50 years with at least 1 prior negative biopsy	Reduction in the burden of prostate biopsies among men undergoing repeat biopsy, but no consensus on cutoff
TMPRSS2:ERG	Urine	Fusion protein	CLIA accreditation	Improved predictive accuracy for PCa detection vs tPSA
MiPS	Urine	PCA3 + TMPRSS2:ERG + tPSA		Addition to models improved predictive ability for high-grade PCa detection
SelectMDx	Urine	mRNA levels of DLX1 and HOXC6 biomarkers	CAP accreditation CLIA accreditation	Greater prediction of high-grade PCa vs PCPTRC
ExoDx Prostate IntelliScore	Urine	Exosomal RNA or PCA3, TMPRSS2:ERG, SPDEF	CLIA accreditation	Improved ability to discriminate between low- and high-grade cancer vs clinical variables

CAP, College of American Pathologists; CLIA, Clinical Laboratory Improvement Amendments; fPSA, free prostate-specfic antigen; MiPS, Mi- Prostate Score; mRNA, messenger ribonucleic acid; PCA, prostate cancer; tPSA, total prostate-specific antigen; PCPTRC, prostate cancer prevention trial risk calculator.

ability to predict clinically significant cancer (AUC 0.78 vs 0.72; $P = 0.04$).[18] These results were validated by Loeb and colleagues when adding *phi* to the ERSPC and the Prostate Cancer Prevention Trial risk calculator (PCPTRC).[19] Their study also developed a new model including age, previous biopsy, prostate volume, PSA and *phi* with an AUC = 0.746.[19]

4Kscore® Test

The 4Kscore Test (OPKO Diagnostics, Woburn, MA) combines four prostate-specific serum biomarkers (tPSA, fPSA, intact PSA, human kallikrein 2) with clinical information to provide men with an accurate and personalized measure of their risk for aggressive PCa defined as any Gleason >6 disease (Figure 1). Clinical data including age, DRE, and prior prostate biopsy are combined in an algorithm with the biomarkers to predict the probability of aggressive PCa on biopsy (Table 1). The 4Kscore Test can be used prior to biopsy or after a negative biopsy and can predict the likelihood of metastatic disease in the next 20 years in otherwise healthy men who have a PSA ≥2 ng/mL.[20] Although it is not FDA approved,

the 4Kscore Test is certified by the Clinical Laboratory Improvement Amendments (CLIA) program of the Centers for Medicare and Medicaid Services. Similarly to *phi*, 4Kscore Test is considered to be potentially informative prior to initial biopsy or following a negative biopsy, as per NCCN guidelines.[10]

The 4Kscore Test was validated in a prospective study in the United States of 1012 patients across 26 centers. They found that for detection of Gleason ≥7, the 4Kscore Test demonstrated superior predictive ability when compared with a modified PCPTRC (AUC 0.82 vs 0.74). Using a cutoff of a 4Kscore ≥9%, the study showed avoidance of 43% of biopsies while only missing 2.4% of aggressive disease.[21] In another validation study, Vickers and colleagues found that the addition of the kallikrein panel improved detection of aggressive cancer compared with a model based on PSA, age, and DRE (AUC 0.78 vs 0.70) and a model based on PSA and age (AUC 0.76 vs 0.64). In this study, using a 4Kscore ≥20% cutoff, the number of biopsies would reduce by more than 50%, while missing 12% of aggressive disease.[22] The ability of the 4Kscore Test to predict detection of aggressive PCa on biopsy has been further established in a number of studies.[23-26] The 4Kscore Test has also been shown to be significantly associated with[27] and improved prediction of[28] higher pathologic grade in radical prostatectomy specimens. A population-based cohort study in Västerbotten, Sweden followed 12,542 men to determine their risk of distant PCa metastases. The group of men who had a 4Kscore Test result of 7.5% or lower were found to have a 1% and 1.8% chance of developing metastatic PCa by year 15 and 20, respectively.[20]

In May 2017, the price of the 4Kscore Test was reduced from $1900 to $595.[29] The cost might discourage the use of the 4Kscore Test in clinical practice, considering it has been shown to have similar predictive value in detecting all PCa compared with high-grade PCa.[25,30] However, many third-party payers will accept in-network payment, as does Medicare. Despite being an expensive test, the key clinical feature is its discerning ability to identify aggressive PCa (Gleason ≥7) that could potentially limit over-biopsy and over-treatment. One study has estimated that its use could save approximately $1 billion in healthcare costs in the United States.[31]

Urine Biomarkers

Prostate Cancer Antigen 3

Prostate cancer antigen 3 (PCA3) is noncoding mRNA that is overexpressed in prostatic tumors compared with non-neoplastic prostate tissue.[32,33] It is detectable in urine after vigorous DRE (Figure 1). The FDA has approved the use of PCA3 in men with at least one prior negative biopsy; a cutoff of less than 25 is associated with predicting a decreased risk of detecting PCa in men older than the age of 50 (Table 1). The manufacturer of the assay has changed the PCA3 cutoff value for a positive test from 35 to 25. The NCCN recommends a PCA3 cutoff of 35 in patients with PSA >3 ng/mL with previous negative biopsy when considering a repeat biopsy.[34] Published studies have shown superiority of PCA3 in predicting outcomes of prostate biopsy when compared with PSA and %fPSA.[35-37] Despite this, evidence points to PCA3 as a supplementary tool in the setting of at least one prior biopsy, rather than a sole predictor of PCa. In a multicenter trial of 859 men, Wei and colleagues demonstrated that a PCA3 cutoff of 20 would avoid a repeat biopsy in 46% of patients; however, this cutoff fails to diagnose PCa in 12% of patients and high-grade cancer in 3% of patients. When applying the same cutoff to the initial biopsy, a diagnosis of aggressive cancer is missed in 13% of patients.[38] Due to varying cutoff levels, its limited capability to differentiate between clinically significant cancers, and its increased cost compared with *phi*, the impact of PCA3 as a biomarker remains unclear and should only be considered in men with a negative prior biopsy.

TMPRSS2:ERG

The fusion protein TMPRSS2:ERG, which is another urine biomarker (Figure 1), is the result of the deletion and translocation of genetic material on chromosome 21, disrupting androgen signaling.[39,40] The most notable benefit of testing TMPRSS2:ERG is that this fusion protein is highly specific for PCa (Table 1).[41,42] At this time, TMPRSS2:ERG biomarker is not FDA approved. Tomlins and colleagues found that, on biopsy, TMPRSS2:ERG was significantly associated with an increase in clinically significant cancer by Epstein criteria and had a greater predictive value in diagnosing PCa when compared with tPSA. They also found that in men undergoing prostatectomy, urine TMPRSS2:ERG was significantly associated with increasing tumor size, Gleason score >6, and upgrade in Gleason score from biopsy.[43] Although the test is highly specific for PCa, TMPRSS2:ERG has limited sensitivity. Studies have shown improved diagnostic performance when combined with PCA3.[44,45] These findings were confirmed in a European prospective multicenter study.[46]

The Mi-Prostate Score (MiPS) combines these two urinary biomarkers (PCA3 + TMPRSS2:ERG)

with serum tPSA in order to predict the risk of any PCa and high-grade (Gleason >6) PCa on biopsy.[47] Tomlins and colleagues validated this diagnostic tool in a study of 1244 men undergoing prostate biopsy. They found that the predictive ability of MiPS to detect any PCa (AUC = 0.751) was significantly higher than that of PSA + PCA3 (0.726) PSA + TMPRSS2:ERG (0.693), and PSA (0.585). They also found the predictive ability of MiPS to detect Gleason >6 PCa (AUC = 0.772) was significantly higher than that of PSA + PCA3 (0.729), PSA + TMPRSS2:ERG (0.747), or PSA (0.651). The authors concluded that utilizing MiPS can reduce unnecessary biopsies.[48]

SelectMDx

SelectMDx (MDxHealth, Irvine, CA) is a urine-based molecular test that measures the mRNA levels of DLX1 and HOXC6 biomarkers (Figure 1). Leyten and colleagues found that a panel of DLX1, HOXC6, and a third biomarker, TDRD1, was found to have greater accuracy in predicting Gleason ≥7 PCa when compared with PCA3 and PSA (Table 1).[49] Van Neste and colleagues subsequently developed the SelectMDx tool in an initial cohort of 519 patients and its superiority in predicting high-grade (Gleason ≥7) PCa when compared with PCPTRC was validated in a cohort of 386 men.[50] Although not FDA approved, a recent British cost-effectiveness study determined that at a diagnostic sensitivity cutoff of 95.7% for high-grade (Gleason ≥7) PCa, SelectMDx demonstrated a savings of €128 ($143) and a gain of 0.025 quality-of-life years compared with using only PSA to select for prostate biopsy.[51] These data are encouraging and may portend future approval, which would facilitate more widespread use of this biomarker.

ExoDx™ Prostate IntelliScore

ExoDx™ Prostate IntelliScore (Exosome Diagnostics, Inc., Waltham, MA) analyzes exosomal RNA for three biomarkers (PCA3, TMPRSS2:ERG, and SAM pointed domain containing ETS transcription factor [SPDEF]) known to be expressed in men with Gleason ≥7 cancer (Table 1). The test is run on a first-catch, non-DRE urine specimen (Figure 1). It is not currently FDA-cleared. A validation study by McKiernan and colleagues demonstrated that the addition of this urine exosomal assay to standard of care variables (PSA level, age, race, and family history of PCa) was associated with improved discriminative ability between low (Gleason 6) and high-grade (Gleason ≥7) PCa.[52] With a cut-off with a negative predictive value of 91% and sensitivity of 92%, only 8% of high-grade PCa were missed while 27% of biopsies were avoided.[52]

Clinical Use

Both serum and urine markers are potentially beneficial in predicting the chances of finding cancer in patients who are found to have elevated PSA on routine screening or who have a rising PSA following a negative biopsy. With such a variety of biomarkers available, clinical utility is dependent upon understanding which tests to use and at which stage of care, as well as the characteristics of the patients in validating studies. PCA3 does not differentiate well between low-risk and clinically significant disease, which may limit its clinical use. Other biomarkers can such as *phi*, 4Kscore, ExoDx Prostate IntelliScore, SelectMDx, and TMPRSS2:ERG are purportedly more discriminatory in predicting the risk between low- and high-risk PCa. Additionally, these tests can be used in combination and along with other clinical data to predict clinically significant disease as in the case of MiPS and ExoDx. However, the definition of clinically significant PCa varies throughout the biomarker validation literature. Notably, Lamy and colleagues recently evaluated the clinical validity and utility of these biomarkers in a systematic review. Based on available data they noted that only *phi* and 4Kscore can accurately discriminate between aggressive and indolent PCa with a level of evidence equal to 1, with the other biomarkers falling short for routine clinical use.[53] These data, along with cost to the patient and availability, can provide additional information to the clinician to avoid the risk of unnecessary biopsies, and potential over-diagnosis and subsequent overtreatment.

Conclusions

There are multitudes of commercially available novel biomarkers that allow for improved prediction of PCa in men with an elevated PSA. The challenge to the practicing urologist is integrating these biomarkers into the management of men who are at risk for PCa based on PSA and other risk factors. The meticulous testing of these biomarkers by incorporation into clinical trials will aid in their widespread use and ability to guide PCa management. Although biomarkers should not replace standard clinical information and physician judgment, their use, along with the emerging use of imaging, can be a useful supplemental tool in the evaluation of men for PCa. In accordance with American Urological Association guidelines and best practice, clinicians should have informed discussions with their patients regarding the use of these PCa biomarkers. ■

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MAIN POINTS

- There are multitudes of commercially available novel biomarkers that allow for improved prediction of prostate cancer (PCa) in men with an elevated prostate-specific antigen (PSA). The challenge to the practicing urologist is integrating these biomarkers into the management of men who are at risk for PCa based on PSA and other risk factors.
- The meticulous testing of these biomarkers by incorporation into clinical trials will aid in their widespread use and ability to guide PCa management.
- Although biomarkers should not replace standard clinical information and physician judgment, their use, along with the emerging use of imaging, can be a useful supplemental tool in the evaluation of men for PCa.
- In accordance with American Urological Association guidelines and best practice, clinicians should have informed discussions with their patients regarding the use of these PCa biomarkers.

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