The Use of Biomarkers in Prostate Cancer Screening and Treatment

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Prostate cancer screening and diagnosis has been guided by prostate-specific antigen levels for the past 25 years, but with the most recent US Preventive Services Task Force screening recommendations, as well as concerns regarding overdiagnosis and overtreatment, a new wave of prostate cancer biomarkers has recently emerged. These assays allow the testing of urine, serum, or prostate tissue for molecular signs of prostate cancer, and provide information regarding both diagnosis and prognosis. In this review, we discuss 12 commercially available biomarker assays approved for the diagnosis and treatment of prostate cancer. The results of clinical validation studies and clinical decision-making studies are presented. This information is designed to assist urologists in making clinical decisions with respect to ordering and interpreting these tests for different patients. There are numerous fluid and biopsy-based genomic tests available for prostate cancer patients that provide the physician and patient with different information about risk of future disease and treatment outcomes. It is important that providers be able to recommend the appropriate test for each individual patient; this decision is based on tissue availability and prognostic information desired. Future studies will continue to emphasize the important role of genomic biomarkers in making individualized treatment decisions for prostate cancer patients.

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KEY WORDS

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Prostate cancer (PCa) screening and diagnostic methods have been guided by prostate-specific antigen (PSA) levels for over 25 years, yet PSA screening has become controversial due to increasing rates of overdiagnosis and overtreatment. The lifetime risk of an American man developing PCa is 1 in 6, whereas the lifetime risk of a man dying from PCa is 1 in 35.[1] This discrepancy has led to questions regarding the value of diagnosing low-grade, indolent PCa and has called into question the utility of PSA as a screening tool.

In 1986, the US Food and Drug Administration (FDA) approved PSA as an adjunctive test to the digital rectal examination (DRE) for PCa diagnosis in men aged 50 years. Subsequently, between 1985 and 1995, the PCa incidence in the United States doubled from 55 cases per 100,000 men to 110.[2] In addition, the rate of radical prostatectomy (RP) increased sixfold from 1984 to 1990.[3] More men were diagnosed with lower-grade, clinically indolent cancer while they were asymptomatic, leading to criticisms of overdiagnosis in 1.7% to 67% of patients—a value that varies depending on the study design used and underlying population characteristics.[4]

It is unclear how much PSA screening has contributed to the decline in PCa mortality since the mid-1990s. The Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial (PLCO)[5] did not find a mortality benefit in patients who were screened with PSA.[5] The European Randomized Study of Screening for Prostate Cancer (ERSPC) found that, in order to prevent 1 death from PCa, 1410 men must be screened and 48 must be treated.[6,7] In 2012, the US Preventative Services Task Force gave PSA testing a Grade D recommendation, suggesting against the use of PSA screening for men except in high-risk populations. The task force cited only minimal reduction in mortality but significant possible harms of screening and treatment, including infection, bleeding, erectile dysfunction, and urinary incontinence.[8]

PSA testing has limited sensitivity and specificity in detecting high-grade PCa. The Prostate Cancer Prevention Trial[9] found that a PSA threshold of 1.1 ng/mL was required to achieve sensitivity of 83.4%, but with an accompanying specificity of only 39.9%.[9] This study was unable to find a PSA cutoff point with both a high sensitivity and specificity; the commonly used cutoff of 4 ng/mL is associated with a sensitivity of 20.5% and a specificity of 93.8% for detecting any grade of PCa.[9] For PSA levels of 4 to 10 ng/mL, specificity ranges from 25% to 40%,[10,11] and 65% to 75% of men who measure in this range have a negative biopsy result.[12] In addition, PSA levels are commonly increased in noncancerous clinical conditions, such as inflammation, infection, trauma, and benign prostatic hypertrophy (BPH). Due to these confounding conditions, the positive predictive value (PPV) for PSA is only 25% to 40%.[13] Several modifications of serum PSA, including free PSA (fPSA), PSA velocity, and various PSA isoforms, have been studied as potential biomarkers that may add utility to the PSA blood test. However, these surrogate biomarkers are not tissue specific or cancer specific, ultimately limiting their utility as a screening tool.[14]

In an effort to improve PCa screening methods, a new wave of PCa biomarkers has emerged that have higher PCa specificity than PSA and its isoforms. Biomarkers are molecules whose detection or evaluation provides information about a disease beyond standard clinical parameters.[15] They can be detected in a variety of settings, including tissue samples, blood, and urine. Biomarkers can provide diagnostic as well as prognostic information, assisting providers in making disease predictions designed to guide treatment decisions on an individualized basis.

Here we present a nonsystematic review of 12 commercially available markers and tests for PCa. This review is intended to guide clinicians in the utilization of these tests in the appropriate clinical space. These tests have either been approved by the FDA or are offered under a laboratory's Clinical Laboratory Improvement Amendments (CLIA) certificate.

Decision: To Proceed With Biopsy?

Presently, 1.3 million men undergo biopsies annually in the United States, more than 85% of whom will not have significant PCa. Prostate biopsy is an invasive procedure with the resultant possibility of complications including bleeding, urinary retention, infection, and sepsis. In addition to the anxiety associated with such a procedure, these risks present a significant burden to any man considering biopsy. The decision to proceed with biopsy must be weighed against the high likelihood that low-grade disease will be detected, as well as the possibility of undergrading due to biopsy sampling error. Patients who require repeat biopsy after initial

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negative biopsy can also use various genomic tests to help consider the likelihood of finding cancer in the setting of persistent symptoms or elevated PSA levels.

4Kscore

Kallikreins are a family of 15 related serine proteases that are known to alter cell growth regulation, increase extracellular matrix remodeling and degradation, and promote cell invasion and angiogenesis.[16] Human kallikrein-3 (PSA) and human kallikrein-2 (hK2) are the dominant forms and normally function to liquefy the contents of the vas deferens. They are formed as proproteins (pro-PSA and pro-hK2) and are cleaved to generate enzymatically active forms that can act on seminogelins. If they enter the circulation, they are rapidly bound by antichymotrypsin (ACT) or inactivated through proteolytic cleavage. The levels of both kallikreins increase in circulation as the tumor becomes more poorly differentiated, perhaps due to loss of tissue architecture.[17] Level of hK2 expression has been found to adequately discriminate between low-grade and high-grade disease, as well as organ-confined and non–organ-confined disease.[18–20] Analysis of the various forms of available PSA/hK2 (pro-PSA, active PSA, ACT-bound PSA and cleavage-inactivated PSA) in the circulation can suggest altered prostate biology.

The 4Kscore® Test (OPKO Health, Miami, FL) measures the plasma levels of the four different prostate-derived kallikrein proteins. Levels of these biomarkers are combined with certain clinical characteristics (age, DRE, prior biopsy status) to predict the risk of finding Gleason ≥7 disease on biopsy. It is designed for use in men with an elevated PSA level or abnormal DRE result who are considering an initial prostate biopsy, as well as in men with prior negative biopsy results and presently elevated PSA levels.

Multiple validation studies using the ERSPC cohort have shown significant discrimination of high-grade disease with incorporation of the 4Kscore as compared with a base model of age, total PSA (tPSA) level, and DRE result alone (area under the curve [AUC] 0.77, 0.81, 0.87 in the base model to 0.87, 0.84, 0.90 with 4Kscore).[21–23] In addition, the 4Kscore decreases the number of unnecessary biopsies by 49% to 57% among men being screened for the first time.[24] Among men who have had prior negative biopsy results, a similar increase in detection of high-grade disease and decrease in biopsy number was identified (AUC 0.71, 0.72 in the base model to 0.80, 0.83 with 4Kscore).[25,26] A validation trial in the United States by Parekh and colleagues[27] reported an AUC for predicting Gleason score of ≥7 of 0.82. The authors also showed that using a cutoff of 9% risk of high-grade disease on biopsy would result in a 43% reduction in the number of biopsies, while only conferring a 2.4% delay in diagnosis of high-grade cancer.

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The 4Kscore is not appropriate for men who have received a DRE in the previous 96 hours, men on a 5α-reductase inhibitor, or men who have undergone any therapy or procedure for symptomatic BPH, thereby limiting its utility in a large portion of the urologic population.

Prostate Health Index

The Prostate Health Index (PHI; Beckman Coulter, Brea, CA) is a blood test that analyzes levels of fPSA, tPSA, and [-2]proPSA (p2PSA) using the equation {(p2PSA/fPSA) × $tPSA^{1/2}$} to predict risk of Gleason ≥7 disease on biopsy. Here, p2PSA refers to a proprotein isoform of PSA with 2 amino acid proleader peptide sequence (normally pro-PSA has 7 amino acid leader amino acids), which was shown to have the most concentration in tumor tissues.[28] It reports risk of aggressive cancer as a four-tiered probability based on various score cutoffs. PHI is intended for use in men aged ≥50 years with serum PSA 4 to 10 ng/mL and negative DRE findings; it was approved by the FDA in 2012. PHI scores of 0 to 26.9, 27.0 to 35.9, 36.0 to 54.9, and ≥55.0 correlate with probabilities of Gleason ≥7 cancer on biopsy of 9.8%, 16.8%, 33.3%, and 50.1%, respectively.[29]

PHI is intended for use in men aged ≥50 years with serum PSA 4 to 10 ng/mL and negative DRE findings…

At PSA levels of 4.0 to 10.0 ng/mL, measuring the ratio of fPSA to tPSA significantly improves discrimination between PCa and benign conditions.[30] p2PSA has been identified as the most PCa-specific isoform of PSA,[31] and a higher percentage of p2PSA is associated with more aggressive PCa.[32,33] In 2011, Catalona and colleagues[29] published the results of a large, multicenter trial of 892 men with tPSA levels 2 to 10 ng/mL and normal DRE results. They found that the PHI assay as a whole has an AUC of 0.703.[29] They also report a fivefold increased risk of PCa in patients with a PHI value >55. A meta-analysis of 16 studies reported sensitivity of 0.85, specificity of 0.45, and an AUC of 0.70 in detecting PCa.[34] Stephan and associates[35]

reported increasing rates of PCa-positive biopsy results with increasing PHI scores, but do not offer a cutoff PHI score for detection of aggressive PCa. In addition, PHI has also been found to be a significant predictor of pT3 disease and Gleason score ≥7 at time of RP.[36]

Apifiny

Apifiny (Armune Bioscience, Kalamazoo, MI) is a blood test that measures the expression of eight PCa-specific autoantibodies. It is marketed for men with PSA >2.5 ng/mL who are considering initial biopsy, reporting a score on a scale of 1 to 100 that reflects risk of Gleason ≥7 on biopsy. A score of ≥59 reflects increased risk.

The humoral immune response to cancer consists of the production of autoantibodies against a number of tumor antigens. In 2005, Wang and associates[37] identified a panel of 22 autoantibody biomarkers that, when present, were highly diagnostic of prostate malignancy, citing 88.2% specificity, 81.6% sensitivity, and an AUC of 0.93.[37] They used a T7-phage peptide display library to screen for biomarkers using 62 peptides against 96 biopsy tissues (48 positive and 48 negative for cancer). The Apifiny test incorporates detection of antibodies to 8 of these 22 identified biomarkers (*CSNK2A2*, centrosomal protein 164 kDa, *NK3 homeobox 1, aurora kinase interacting protein 1,* 5′-UTR *BMI1, ARF6,* chromosome 3′ UTR region *Ropporin/RhoEGF,* and *desmocollin 3*) that have roles in androgen response regulation, cellular structural integrity, and cell cycle regulation.[38] With this eight-autoantibody panel, Apifiny has reported to have an AUC of 0.69 for men with PSA >4 ng/mL, sensitivity of 0.603, specificity of 0.69, PPV of 0.299, and negative predictive value (NPV) of 0.888.[38]

Progensa PCA3 Assay

Progensa PCA3 assay (Hologic, Marlborough, MA), a long noncoding RNA also referred to as DD3, was identified as overexpressed in 53 out of the 56 tumor tissues analyzed and absent from the 18 control samples.[39] Follow-up studies showed it to have elevated expression in >90% of PCa.[40] Mechanistic studies identified its role in PCa cell survival, in part through its ability to regulate androgen receptor signaling.[41] The PCA3 assay was approved by the FDA in 2012 as a diagnostic test for PCa in the setting of a prior negative biopsy result. The assay is an in vitro nucleic acid amplification test measuring concentration of PCA3 and PSA messenger RNA (mRNA) molecules in a first catch post-DRE urine specimen. It uses the ratio of the levels of these two markers (eg, to negate the effect of increased PSA due to BPH and age) to calculate the PCA3 score, which is directly correlated with likelihood of positive biopsy result. It is designed for use in men aged ≥50 years who have had one or more negative biopsy results and who are considering a repeat biopsy.

Men with a score <25 are 4.56 times more likely to have a negative biopsy result than men with a score >25,[42] and lower PCA3 scores are associated with low-volume and low-grade disease.[43,44] Marks and associates[45] first evaluated the use of PCA3 in 226 patients undergoing repeat biopsy. At a score cutoff of 35, the group demonstrated a sensitivity and specificity of 0.58 and 0.72, respectively (AUC 0.68). Using a PCA3 score cutoff of 20, Wei and coworkers[46] reported an NPV of 88%, sensitivity of 0.76, and specificity of 0.52. Increasing PCA3 score also correlated with both higher probability of cancer and higher-grade cancer on biopsy. Using a PCA3 score cutoff of 25, Gittelman and coworkers[42] reported a sensitivity of 0.775, a specificity of 0.571, and an NPV and PPV of 90% and 33.6%, respectively.

PCA3 level is not elevated in acute inflammatory or infectious states, and is independent of prostate size. In addition, the PCA3 assay maintains its predictive power in men with BPH who are on long-term treatment with 5α-reductase inhibitors with nearly no loss of specificity over 4 years.[47] PCA3 expression level has been found in many studies to independently correlate with biopsy outcome[45,48–50] and tumor aggressiveness, as measured by tumor volume, tumor grade, and Gleason score.[43,48,51] However, other studies have found no significant correlation between PCA3 score and Gleason grade at biopsy.[45,52]

The current assay is approved for men undergoing repeat biopsy, although evidence is mounting that the PCA3 assay may have utility as a screening tool in men with elevated PSA values. However, the relation of PCA3 score with tumor aggressiveness and thus true prognostic value remains controversial. It is also important to note that the optimal PCA3 cutoff score is still subject to debate; Leyten and colleagues[53] showed an increase in sensitivity from 0.68 to 0.83 when lowering the cutoff from 35 to 25, and a corresponding decrease in specificity from 0.58 to 0.51.

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Michigan Prostate Score

The Michigan Prostate Score (MiPS) was released in 2013 and is an assay that incorporates serum

PSA level, urine PCA3 mRNA, and urine *TMPRSS2:ERG* mRNA. More than 50% of PCas harbor fusions between *TMPRSS2:ERG*, and multiple studies have shown that *TMPRSS2:ERG* fusions are more common in young men with early-stage PCa and in men presenting with low serum PSA values.[54,55] The MiPS test utilizes a urine sample, which must be taken no more than 1 hour after DRE. It is designed for use in men with an elevated PSA level who are considering initial biopsy, or in men with previous negative biopsy results who are considering a repeat biopsy. A score of 1 to 100 reflects the percent chance of finding any PCa on biopsy, and the score report also provides a risk estimate for detecting cancer of Gleason score ≥7.

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Tomlins and colleagues[56] found that the level of *TMPRSS2:ERG* transcript in the urine is associated with the presence of PCa, tumor volume, and Gleason score ≥7 both at biopsy and in prostatectomy specimens. Specificity of *TMPRSS2:ERG* is very high, at 93.2%.[53] Salami and colleagues[57] report an improved discriminatory ability for the combination of PSA, PCA3, and *TMPRSS2:ERG*, as opposed to each alone (AUC 0.88 for the combined test vs 0.72 for PSA, 0.65 for PCA3, and 0.77 for *TMPRSS2:ERG*). In a cohort study by Leyten and associates,[53] knowledge of MiPS score prior to biopsy would avoid 35% of biopsies. Tomlins and colleagues[58] also reported that using various percent cutoffs would avoid 35% to 47% of biopsies, while delaying diagnosis in only 1.0% to 2.3% of high-grade cancers.

TMPRSS2:ERG has not been found to correlate significantly with long-term risk of biochemical recurrence or PCa-specific mortality.[59] Although *TMPRSS2:ERG* gene fusions are reported to be associated with high-risk tumors, a more recent study reported no strong correlation between these fusions and long-term patient outcome.[60] In addition, the *ERG* rearrangements are less prevalent in men of African descent when compared with white men, 27% versus 54%, respectively. Finally, the report does not offer a low-risk/high-risk cutoff score.

Detection of the novel biomarkers HOXC6 and DLX1 in a post-DRE urine sample allows for individualized decision making according to probability of high-grade disease.

SelectMDx

SelectMDx (MDx Health, Irvine, CA) provides the likelihood of detecting any PCa on prostate biopsy, as well as the probability for high-grade versus low-grade disease. The test measures mRNA levels of distal-less homeobox 1 (*DLX1*) and homeobox C6 (*HOXC6*) in a post-DRE urine specimen and combines this with serum PSA, PSA density, DRE status, age, and family history of PCa.

HOXC6 regulates genes with both oncogenic and tumor suppressor activities, as well as several genes important for prostate morphogenesis and metastasis to the bone.[61] It is frequently overexpressed in patients with PCa, indicating an oncogenic role, and the degree of overexpression is directly associated with Gleason score.[62,63] *DLX1* is involved in neuroendocrine-epithelial differentiation, a characteristic associated with aggressive PCa.[64] Both the *HOXC6* and *DLX1* biomarkers have been shown to have independent value in predicting Gleason ≥7 PCa on biopsy, and are potentially involved in the onset of PCa.[65] In predicting the risk of high-grade PCa in men with PSA ≥4 ng/mL, Van Neste and colleagues[66] found that *HOXC6* and *DLX1* independently have an AUC of 0.73 and 0.65, sensitivity of 91% and 83%, and specificity of 33% and 16%, respectively. Together they have an AUC of 0.76, sensitivity of 91%, and specificity of 36%. When combined with the clinical parameters mentioned above, the AUC increases to between 0.86 and 0.90.[66]

Detection of the novel biomarkers *HOXC6* and *DLX1* in a post-DRE urine sample allows for individualized decision making according to probability of high-grade disease. It is designed to decrease the number of unnecessary biopsies; at a cutoff with a NPV of 98% for Gleason ≥7 PCa, total biopsies performed decreased by 42%, and unnecessary biopsies decreased by 53%.[66]

ConfirmMDx

ConfirmMDx (MDxHealth) is designed as a risk stratification tool for men with a negative prostate biopsy result and aims to reduce the number of repeat biopsies. It is a unique assay in that it analyzes epigenetic changes by detecting alterations in DNA methylation patterns of key tumor suppressor genes such as *GSTP1*, *RASSF1*, and *APC* in a prostate tissue sample. *GSTP1* is involved in DNA detoxification, *RASSF1* is involved in cell cycle regulation, and *APC* is involved in apoptosis, cell migration, and cell adhesion.[67] Hypermethylation of CpG islands in the promoter regions of these

genes is linked to the development of PCa.[67–69] The ConfirmMDx assay also takes advantage of the epigenetic field effect, in which normal cells that are adjacent to cancer foci may contain DNA methylation changes.[69]

The Methylation Analysis to Locate Occult Cancer (MATLOC) study conducted by Stewart and associates[70] demonstrated that the ConfirmMDx panel has a sensitivity of 68%, specificity of 64%, and NPV of 90%, decreasing the number of unnecessary repeat biopsies by up to 64%.[70] The Detection of Cancer Using Methylated Events in Negative Tissue (DOCUMENT) study by Partin and colleagues[71] further confirmed this test as the most significant independent predictor for PCa detection in a repeat biopsy when compared with age, race, PSA value, DRE result, and first biopsy histopathologic characteristics, and reports an NPV of 88%. Most recently, Van Neste and coworkers[72] reported that detection of low DNA methylation in histopathologically negative specimens had an NPV of 96% and an AUC of 0.742 for high-grade cancer, and that degree of methylation is directly correlated to grade of cancer. Zhou and investigators[73] also demonstrated higher GSTP1 methylation in higher Gleason grade tumors.

In terms of clinical decision making, Wonju[74] found a 4.4% repeat biopsy rate in men with a negative ConfirmMDx test result, as opposed to a 43% repeat biopsy rate reported in the PLCO trial. All of these biopsy results performed in the setting of a negative ConfirmMDx result were negative. When applying a probability threshold of 15%, Van Neste and colleagues[72] calculated that an additional 30 unnecessary repeat biopsies per 100 patients could be avoided.

Decision: Active Surveillance Versus Intervention?

Active surveillance (AS) has now become a standard of care in the treatment of very low-risk and low-risk PCa. However, AS tends to be underutilized, and most men with low-risk disease eventually receive surgical or radiation treatment.[75] The rate of progression necessitating intervention of men on AS protocols is approximately 40% over a 10-year period.[76] Epstein and colleagues[77] conducted a literature review of studies with >100 patients and found presence of Gleason score upgrading after RP from 6 to 7 in 35% of patients. Such data underscore the need to appropriately risk stratify patients after biopsy.

The rate of progression necessitating intervention of men on AS protocols is approximately 40% over a 10-year period.

The following biomarker tests are designed to guide management decisions and appropriately counsel postbiopsy patients regarding AS versus intervention.

Oncotype DX

The Oncotype DX assay (Genomic Health, Redwood City, CA) uses a fixed, paraffin-embedded prostate needle biopsy tissue sample to predict the aggressiveness of an individual patient's tumor, as reported by a Genomic Prostate Score (GPS) of 1 to 100. The test was approved for use in PCa in 2013, joining previous variations that were marketed for breast cancer and colon cancer. The quantitative reverse transcriptase-polymerase chain reaction assay measures the RNA expression levels of 5 reference genes (*ARF, ATP5E, CLTC, GPS1, PGK1*) and 12 genes representing 4 biologic pathways with known roles in PCa tumorigenesis: the androgen pathway (*AZGP1, KLK2, SRD5A2,* and *FAM13C*), cellular organization (*FLNC, GSN, TPM2,* and *GSTM2*), proliferation (*TPX2*), and stromal response (*BGN, COL1A1,* and *SFRP4*).[78] These are combined to generate a GPS ranging from 0 to 100; a higher GPS score is concordant with a higher chance of adverse pathology (primary Gleason pattern of 4-5 or disease that is no longer organ confined) after RP. It is designed to assist with risk stratification and further treatment decisions in clinically low- and low–intermediate-risk patients, specifically the decision to undergo AS or further treatment. The GPS is reported along with the patient's National Cancer Comprehensive Network (NCCN) clinical risk group to provide a percent likelihood of favorable pathology, freedom from high-grade disease, and freedom from non–organ-confined disease if the patient were to undergo RP. According to the NCCN guideline recommendations, Oncotype DX should be used for patients with very low- and low-risk disease at the time of diagnosis and a life expectancy of 10 to 20 years.

The Oncotype DX assay has previously been clinically validated in the settings of breast and colon cancer. Cullen and associates[79] further validated this assay's ability to predict adverse pathology and long-term outcomes in patients with PCa by obtaining GPS in a group of racially diverse, clinically very low-, low-, and intermediate-risk patients undergoing RP. They found that GPS is significantly associated with adverse pathology after adjusting for other clinical and pathologic features, and that high GPS is predictive of both high-grade and high-stage disease.

In analysis of adverse pathology, incorporation of the GPS improved the AUC of NCCN by 90%.[79] Due to the long-term follow-up of this study, the authors were also able to conclude that GPS is also a strong predictor of biochemical recurrence (BCR) and future metastases.

A study by Klein and colleagues[80] found that addition of the GPS score to existing clinical and pathologic factors expanded the low-risk population from 10% to 26%, and reclassified 35% of NCCN low-risk men to the very low-risk category, and 10% to the intermediate-risk category. With regard to clinical decision making, Badani and colleagues[81] found an overall 18% change in treatment recommendations after receiving GPS results in a cohort of 158 patients at 3 clinical institutions, a 19% to 21% decrease in RP recommendation, and a 33% decrease in RT recommendation. These changes in treatment decisions were found over all NCCN risk groups, but the NCCN low-risk group showed the greatest absolute recommendation change after a GPS of 37%. However, GPS score led to change in NCCN category in 39% of patients (lower in 35%, higher in 4%).

ProMark

The quantitative multiplex proteomic-based test, ProMark (Metamark, Waltham, MA) predicts potential cancer aggressiveness in patients with Gleason 3+3 or 3+4 disease on biopsy by measuring direct levels of eight proteins (DERL1, CUL2, SMAD4, PDSS2, HSPA9, FUS, pS6, and YBOX1) in a biopsy specimen through quantitative immunofluorescence. All of these proteins have roles in cell proliferation, stress response, and signaling pathway activities. ProMark reports individualized risk of Gleason ≥4+3 disease and/or non–organ-confined disease on a scale of 1 to 100, were an RP to be performed, and combines this with the patient's NCCN risk category.

In 2014, Shipitsin and colleagues[82] first reported a set of 12 protein biomarkers that were predictive of PCa aggressiveness and PCa-specific mortality despite sampling error. The AUC for the assay was reported at 0.72 for predicting lethal outcome, and 0.71 for predicting aggressive cancer. Further clinical validation was achieved by Blume-Jensen and associates,[83] who used 8 of the 12 candidate proteins and defined endpoints of "favorable" versus "nonfavorable," and "Gleason 6" versus "non-Gleason 6" in biopsy and prostatectomy specimens from 276 patients. The analysis for "favorable" pathology yielded an AUC of 0.68, and "Gleason 6" pathology yielded an AUC of 0.65. When combining the assay results with NCCN classification, AUC increased to 0.75. Similarly, when taking into account PSA, the percentage of positive cores and biopsy Gleason pattern, AUC for "favorable" pathology increased to 0.71. A risk score of 0.33 is recommended (on a scale of 0-1) as a cutoff for nonfavorable pathology, with sensitivity of 90%, PPV of 83.6%, and a false-negative rate of 10%.

PTEN/TMPRSS2:ERG

The *PTEN/TMPRSS2:ERG* (Metamark) molecular assay is for use in men with Gleason 3+3 or 3+4 disease on biopsy, as well as those with an atypical/high-grade prostatic intraepithelial neoplasia (HGPIN) diagnosis on biopsy. It helps to predict PCa aggressiveness by measuring the presence or absence of both the fusion gene *TMPRSS2:ERG* and the tumor suppressor gene *PTEN* in a biopsy; the presence of *TMPRSS2:ERG* and/or the absence of *PTEN* indicates a more aggressive cancer.

PTEN is a tumor suppressor gene that is known to modulate a number of downstream targets with important roles in apoptosis and cell cycle progression. Specifically, it dephosphorylates the membrane lipid phosphatidylinositol-3,4,5 phosphate (PIP3) to phosphatidylinositol-4,5 phosphate (PIP2) thereby abrogating Akt membrane binding and its subsequent activation.[84] *PTEN* inactivation in cancer cells has been shown to be associated with high Gleason score and tumor progression.[85,86] Yoshimoto and colleagues[87] found that hemizygous PTEN deletion was found in 39% and homozygous deletion in 5% of tumor samples. Those samples with a homozygous deletion were more likely to have late biochemical recurrence ($P = .005$). Similarly, Schmitz[88] reported complete loss of PTEN expression in prostate cancer cells both in situ and metastatic to lymph nodes in 59% of cases.

The *PTEN/TMPRSS2:ERG* assay is reported in terms of binary results, and has not been shown to be significantly correlated with Gleason score at biopsy, pathologic stage, or other factors that indicate more aggressive disease at the time of surgery.[87] It is unique, however, in that it can be used in men with atypia or HGPIN on biopsy, and may lead to earlier diagnosis of potentially aggressive PCa.

Prolaris

The Prolaris (Myriad Genetics, Salt Lake City, UT) score is a quantitative measure of the average expression of 31 cell cycle progression (CCP) genes and 15 reference genes in either a biopsy specimen or an RP specimen to predict tumor aggressiveness and recurrence. The test was originally developed for breast cancer risk analysis. According to the NCCN guidelines, it is recommended for patients with very low- and

low-risk disease on biopsy and a life expectancy of 10 years.[89]

The Prolaris Biopsy score helps guide decision making toward AS versus surgery or radiation. Prolaris Biopsy scores range from 0 to 10, with each 1-unit increase reflecting a doubling of risk of disease progression. A score is also reported with its percentile distribution within a given AUA clinical risk group. The Prolaris Biopsy score is combined with the patient's age, PSA, clinical stage, percent positive cores, Gleason score, and AUA risk category to report a 10-year PCa-specific mortality risk. In a study of 585 men with clinically localized PCa on biopsy, Cuzick and colleagues[90] reported a relationship between CCP expression and PCa-specific mortality (hazard ratio [HR] of 2.08). After adjusting for Gleason score, PSA, extent of disease, and clinical stage, the CCP remained a highly significant independent predictor of PCa death (HR 1.76).

Prolaris after prostatectomy reports a score from 0 to 10, with each 1-unit increase reflecting a doubling of risk of BCR. The score is combined with the patient's preoperative PSA, Gleason score, and other clinicopathologic factors (surgical margins, extracapsular extension, seminal vesicle invasion, and lymph node invasion) to provide a 10-year risk of BCR. Cuzick and associates[91] found that increased expression of CCP genes in RP patients was predictive of BCR within 10 years (HR 1.74), and that CCP score, which reflects proliferative index, is predictive for death after disease progression ($P = .0007$). Similarly, Cooperberg and colleagues[92] reported that CCP score predicted BCR with an HR of 2.1 per unit increase in score. With regard to metastases, Bishoff and coauthors[94] found that in a combined cohort with 582 patients, CCP score was associated with both metastatic disease (HR 5.35) and BCR (HR 1.60).

The Prolaris score continues to show utility both in men considering definitive treatment for their PCa and in men who are considering adjuvant therapy. The level of CCP gene is directly correlated with tumor aggression and recurrence, and can also provide information on the risk of death due to PCa.

The Prolaris score continues to show utility both in men considering definitive treatment for their PCa and in men who are considering adjuvant therapy.

Decipher

The Decipher test (GenomeDx, San Diego, CA) is a genomic classifier that measures RNA expression of 22 different genes (*LASP1*, *IQGAP3*, *NFIB*, *S1PR4*, *THBS2*, *ANO7*, *PCDH7*, *MYBPC1*, *EPPK1*, *TSBP*, *PBX1*, *NUSAP1*, *ZWILCH*, *UBE2C*, *CAMK2N1*, *RABGAP1*, *PCAT-32*, *GLYATL1P4*, *PCAT-80*, and *TNFRSF19*), identified through extensive literature survey, in either a biopsy specimen or an RP specimen.[94] These 22 genes encompass several important biologic pathways such as cell proliferation, differentiation, cell motility and cell adhesion, cell cycle progression, immune modulation, and androgen receptor pathway. The Decipher score represents a continuous risk score called a genomic classifier and ranges from 0 to 1; a low risk score is 0 to 0.45, an average risk score is 0.46 to 0.6, and a high-risk score is 0.61 to 1.0.

According to NCCN guidelines, it is recommended for patients with adverse pathology after RP.[89] Decipher Biopsy reports 5-year risk of metastases, 10-year PCa-specific mortality, and risk of high-grade disease for men with any PCa on biopsy. Much of the data to support Decipher Biopsy come from studies done in prostatectomy specimens. Decipher score after prostatectomy conveys information to help with making decisions about adjuvant radiation therapy or observation after RP. It can also guide decisions regarding the use of hormone deprivation therapy in patients with biochemical recurrence. Its intended use is in men with high-risk pathology or high-risk clinical features after RP, reporting 5-year risk of metastases and 10-year PCa-specific mortality. Klein and colleagues[95] reported that in 337 Gleason 3+3 prostatectomy specimens, 20% had intermediate or high-risk Decipher scores, indicating a potentially more aggressive cancer in a low-risk biopsy. A 2016 report by Ross and colleagues[96] significantly and independently correlates Decipher score with incidence of BCR, metastasis, and PCa-specific mortality ($P < .01$). Five-year metastasis rate in a cohort of post-RP, clinically high-risk patients (PSA >20 ng/mL, Gleason 8, pT3b, or GPSM score 10) has been reported to be 2.4%, 6.0%, and 22.5% for patients with low Decipher scores (< 0.4), intermediate scores (0.4-0.6), and high scores (>0.6), respectively ($P < .001$).[97] In addition, Den and coworkers[98] found that Decipher scores can help guide timing of post-RP radiation therapy in men with high-risk pathology. Alshalalfa and coauthors[99] observed a distinct

… Decipher scores can help guide timing of post-RP radiation therapy in men with high-risk pathology.

TABLE 1

Summary of Available Tests and Indications

Test	Specimen	Biomarkers	Clinical Endpoints	Target Patient Population	Management Guidelines	Study
Initial Biopsy						
PHI (Beckman Coulter, Brea, CA)	Blood	Levels of tPSA, fPSA, p2PSA (p2PSA/fPSA) × $tPSA^{1/2}$	Risk of HG cancer on biopsy (score 1-100)	Men ≥50 y with PSA 4-10 ng/mL and negative DRE result who are considering initial biopsy	Score 0-26.9: 9.8% risk of HG disease Score 27-35.9: 16.8% risk of HG disease Score 36-54.9: 33.3% risk of HG disease Score ≥55: 50.1% risk of HG disease	Catalona WJ et al[29]
Apifiny (Armune Bioscience, Kalamazoo, MI)	Blood	Circulating levels of 8 PCa-specific autoantibodies	Risk of HG cancer on biopsy (score 1-100)	Men with PSA ≥2.5 ng/mL who are considering initial biopsy	Score 1-58: low risk of HG disease Score ≥59: high risk of HG disease	Schipper M et al[38]
SelectMDx (MDx Health, Irvine, CA)	Urine	Expression of *DLX1* and *HOXC6*	Percent risk of Gleason ≥6 disease on biopsy Percent risk of HG cancer on biopsy	Men with elevated PSA value who are considering initial prostate biopsy	Low risk: routine follow-up and screening High risk: perform biopsy	Van Neste L et al[66]
Repeat Biopsy						
PCA3 (Hologic, Marlborough, MA)	Urine	Ratio of PCA3 and PSA expression	Risk of Gleason ≥6 disease on biopsy (score 1-100)	Men age ≥50 y who are considering repeat biopsy after initial negative biopsy	Score 1-25: low risk of cancer, safe to defer biopsy Score ≥26: high risk of cancer, perform repeat biopsy	Gittelman MC et al[42]
ConfirmMDx (MDxHealth)	Biopsy	Hypermethylation intensity of tumor suppressor genes *GSTP1*, *RASSF1*, and *APC*	Risk of PCa on repeat biopsy	Men who are considering a repeat biopsy after initial negative biopsy result	Negative: safe to defer biopsy Positive: repeat biopsy	Stewart GD et al[70]; Partin AW et al[71]; Van Neste L et al[72]
Initial or Repeat Biopsy						
4Kscore (OPKO Health, Miami, FL)	Blood	Levels of tPSA, fPSA, intact PSA, and human kallikrein-related peptidase 2	Percent risk of HG cancer on biopsy	Men with an elevated PSA or abnormal DRE result who are considering initial or repeat biopsy	Low risk (1%-7.5%): safe to defer biopsy with follow-up of PSA High risk (≥20%): perform biopsy	Vickers AJ et al[21,23,25,26]; Parekh DJ et al[27]

Test	Specimen	Biomarkers	Clinical Endpoints	Target Patient Population	Management Guidelines	Study
MiPS	Urine	Expression of *PCA3* and *TMPRSS2:ERG* Combined with serum PSA	Percent risk of Gleason ≥6 disease on biopsy Percent risk of HG cancer on biopsy	Men with elevated PSA value who are considering initial biopsy or repeat biopsy after initial negative result	Does not provide low- and high-risk cutoffs	Salami SS et al[57]; Leyten G et al[53]; Tomlins SA et al[58]
After Biopsy: Active Surveillance vs Intervention						
Oncotype DX (Genomic Health, Redwood City, CA)	Biopsy	Expression of 12 PCa-related genes	Percent likelihood of Gleason 3+3 or 3+4 disease on RP Percent likelihood of organ-confined disease on RP	Men with very low- and low-risk PCa based on NCCN risk group Men with Gleason 3+3 or 3+4 on biopsy	Does not provide low- and high-risk cutoffs Provides pathology risk information (GPS) relative to others in the same NCCN risk group	Cullen J et al[79]; Klein EA et al[80]
ProMark (Metamark, Waltham, MA)	Biopsy	Quantitative levels of 8 PCa-related proteins	Percent risk of developing aggressive disease (Gleason ≥4+3, non–organ-confined disease) based on ProMark score alone and when combined with NCCN category	Men with Gleason 3+3 or 3+4 on biopsy	Does not provide low- and high-risk cutoffs	Shipitsin M et al[82]; Blume-Jensen P et al[83]
PTEN/ TMPRSS2:ERG (Metamark)	Biopsy	Presence or absence of *PTEN* deletion Presence or absence of *TMRPRSS2:ERG* fusion	Cancer aggressiveness	Men with Gleason 3+3 or 3+4 on biopsy	Negative (intact *PTEN*, no *ERG* rearrangement): active surveillance Positive (*PTEN* deletion and/or *ERG* rearrangement): definitive treatment	Yoshimoto M et al[87]
Prolaris	Biopsy	Expression levels of 31 genes associated with cell cycle progression	Cancer aggressiveness (score 1-10) 10-y PCa-specific mortality risk	Men with PCa on biopsy	Does not provide low- and high-risk cutoffs Provides score relative to others in the same AUA risk category	Cuzick J et al[90]

Test	Specimen	Biomarkers	Clinical Endpoints	Target Patient Population	Management Guidelines	Study
Decipher	Biopsy	Expression levels of 22 genes associated with high-risk PCa	5-y metastasis risk Likelihood of HG disease on RP 10-y PCa-specific mortality risk	Patients with localized disease on biopsy	Does not provide low- and high-risk cutoffs Low risk: active surveillance High risk: consider further treatment	Cooperberg MR et al[92]; Klein EA et al[95]; Ross AE et al[96]
After RP: Secondary Treatment vs Observation						
Prolaris (Myriad Genetics, Salt Lake City, UT)	Prostate	Expression levels of 31 genes associated with cell cycle progression	Risk of biochemical recurrence within 10 y (score 1-10)	Men who have undergone RP	Does not provide low- and high-risk cutoffs Provides score relative to others in the same AUA risk category	Cuzick J et al[91]; Cooperberg MR et al[92]
Decipher (GenomeDx, San Diego, CA)	Prostate	Expression levels of 22 genes associated with high-risk PCa	5-y metastasis risk 10-y PCa-specific mortality risk	Men with high-risk pathology or high-risk clinical features after RP	Low risk: observe with PSA monitoring, RT if PSA value rises High risk: adjuvant or early RT with further intensification of treatment as needed	Karnes RJ et al[97]; Den RB et al[98]

AUA, American Urological Association; DRE, digital rectal examination; GPS, Genomic Prostate Score; HG, high-grade (Gleason ≥7); fPSA, free prostate-specific antigen; NCCN, National Comprehensive Cancer Network; p2PSA, [-2]proPSA; PCa, prostate cancer; PHI, Prostate Health Index; PSA, prostate-specific antigen; RP, radical prostatectomy; tPSA, total prostate-specific antigen.

transcriptional profile for patients who developed metastases after RP in contrast to a similar profile for patients with no adverse outcomes and BCR, providing evidence for its utility to identify patients who would develop metastasis. In terms of clinical practice, Decipher score reclassified 60% of men to lower risk categories, and 39% of physicians changed treatment recommendations after reviewing Decipher results.[97,100,101]

Conclusions

The most recent update to the NCCN Guidelines for Prostate Cancer Early Detection includes the addition of various biomarkers to treatment algorithms.[89,102] As reflected in the guidelines, one biomarker cannot be recommended over another at this time. Additionally, these markers should not be used as first line in the diagnosis of PCa. Although many of these biomarkers offer valuable information on a case-by-case basis, they should be considered as one piece of the puzzle in counseling patients regarding their specific prostate malignancies.

Although we have come a long way in elucidating the underlying genetic changes in PCa and how they can guide management decisions, there is still much room for discovery and improvement. Genomic sequencing efforts over the past few years have shown the development of PCa to be driven additionally though copy number variations (CNVs).[103,104] CNVs have also been shown to be associated with aggressive disease and survival[103,105]; however, none of the genomic tests that are currently commercially available take CNVs into account when predicting aggressive disease or disease outcomes. In addition, a confounding factor in the general applicability and utility of these assays is the inherent intratumor heterogeneity and multifocality of PCa, which is not accounted for in any of the tests developed so far.

Currently available targeted therapy for PCa consists of mechanisms

of attack on the prostate cell membrane (prostate-specific membrane antigen [PMSA], prostatic acid phosphatase [PAP], and prostate stem cell antigen [PSCA]), tumor angiogenesis (bevacizumab, thalidomide, sunitinib), the androgen receptor pathway, and other cell proliferation pathways (mammalian target of rapamycin [mTOR] inhibitors).[106] As our knowledge of the general biology and immunology of PCa—as well as what genetically differentiates low-risk and high-risk cancers—increases, it is reasonable to predict that a greater number of targets will become available for increasingly specific cancer treatment, as well as improved prediction of tumor response to these targeted therapies.

Recent studies out of The Cancer Genome Atlas have led to the classification of PCa into different subtypes, yet the utility of this in the clinical setting awaits further evaluation.[104] The gamut of genetic and epigenetic changes that have been uncovered by next-generation sequencing studies have not been tapped, and in the future we envision drastic improvements and changes in the field of PCa diagnosis and prognosis. With the advent of cutting-edge tools, such as single-cell sequencing, and our ability to monitor cell-free DNA and RNA, novel tools for differentiating indolent from aggressive PCa will emerge, in addition to increasingly targeted therapy for specific PCa. ■

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MAIN POINTS

- Prostate cancer screening and diagnostic methods have been guided by prostate-specific antigen (PSA) levels for over 25 years, yet PSA screening has become controversial due to the potential for overdiagnosis and overtreatment.
- A new wave of prostate cancer biomarkers has recently emerged. These assays allow the testing of urine, serum, or prostate tissue for molecular markers of prostate cancer, and can provide information regarding both diagnosis and prognosis.
- There are numerous fluid and biopsy-based genomic tests available for prostate cancer patients that provide the physician and patient with different information about risk of future disease and treatment outcomes.
- One biomarker cannot be recommended over another at this time. Additionally, these markers should not be used as first line in the diagnosis of prostate cancer. Although these biomarkers can offer valuable information about an individual patient's disease, they should be considered as one piece of the puzzle in assisting physicians and patients in the decision-making process.

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