Biomarkers for detection of clinically significant prostate cancer: contemporary clinical data and future directions

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Abstract: Use of serum prostate-specific antigen (PSA) testing for early detection of prostate cancer appears to reduce cancer-specific mortality. Due to the limited specificity of PSA for clinically significant [Grade Group (GG) ≥2] cancer, however, screening carries substantial risks, including frequent unnecessary prostate biopsies and overdetection of non-aggressive cancers. To that end, serum and urine biomarkers with improved specificity for GG ≥2 cancer have been proposed for clinical use following PSA. In the current article, we present clinical validation data for five such biomarkers: PHI, 4Kscore, SelectMDx, ExoDx, and MPS. For all studies, we specify the study population (overall biopsy referral *vs.* pre-specified PSA ranges), previous biopsy status (biopsy-naïve *vs.* previous negative biopsy), and the proportion of subjects diagnosed with GG ≥2 cancer. Outcomes include test performance characteristics: sensitivity, specificity, negative predictive value (NPV), and positive predictive value (PPV). Published data were used to compute the number of unnecessary biopsies avoided and number of GG ≥2 cancers missed if the biomarker had been used clinically to select for prostate biopsy. The evidence review is preceded by a primer on these and other clinically-relevant summary statistics.

Keywords: Prostate-specific antigen (PSA); prostatic neoplasms; biopsy; neoplasm grading biomarkers; tumor

Introduction

Prostate cancer (PCa) remains one of the most common malignancies and a leading cause of death in men worldwide (1-3). In the United States alone, PCa accounts for more than 20% of all cancer diagnoses in men, with 190,000 cases and upwards of 33,000 PCa deaths projected in 2020 (4). Screening with prostate-specific antigen (PSA) has been shown to reduce mortality in men with clinically significant PCa [Grade Group (GG) ≥2] (5-8) and remains the mainstay of PCa detection, despite well-studied drawbacks. PSA is prostate gland-specific, but not cancer-specific (9,10). As such, widespread PSA screening results in frequent negative prostate biopsy and overdiagnosis of indolent disease (11,12), subjecting patients to undue harms in the course of diagnostic evaluation (13). Meanwhile, traditionally only about one-third of men with elevated PSA are found to have PCa on biopsy, with even fewer harboring GG ≥2 disease (14,15).

The limitations of PSA have led to development of novel biomarkers aimed at better informing the risk of GG ≥2 cancer (16). We herein provide a review of serum and urine biomarkers clinically-available to aid in diagnosis of

Table 1 PCa Biomarkers and their characteristics

Biomarker assay	Biomarker components	Clinical variables	Assay output	Serum or urine
4Kscore	PSA, fPSA, iPSA, hK2	Age, prior biopsy status, & optional DRE	Likelihood of detecting GG ≥2 (0–100%)	Serum
PHI	p2PSA, fPSA, PSA	None	Likelihood of detecting GG ≥2 (continuous score)	Serum
SelectMDx	HOXC6, DLX1	Age, PSA, prostate volume & DRE	Likelihood of detecting PCa and GG ≥2 (0–100%)	Post-DRE Urine
EPI	PCA3, ERG, SPDEF	None	Likelihood of detecting GG ≥2 (score of 0-100)	Urine
MPS	PCA3, TMPRSS2:ERG, PSA	None	Likelihood of detecting GG ≥2 (score of 0-100)	Post DRE-Urine

PHI, Prostate Health Index; EPI, ExoDx Prostate Intelliscore; MPS, MyProstateScore; PSA, prostate-specific antigen; fPSA, free prostate-specific antigen; iPSA, intact prostate-specific antigen; hK2, human kallikrein 2; DRE, digital rectal exam; p2PSA, [-2]pro-prostate-specific antigen; HOXC6, homeobox C6; DLX1, distal-less homeobox 1; PCA3, prostate cancer antigen 3; ERG, ETS transcription factor ERG; SPDEF, SAM pointed domain-containing ETS transcription factor; TMPRSS2, transmembrane protease, serine 2.

GG ≥2 PCa, including the Prostate Health Index (PHI), 4-Kallikrein score (4Kscore), SelectMDx, ExoDx Prostate Intelliscore (EPI), and MyProstateScore (MPS) (*Table 1*).

Study selection

The PubMed database was queried by biomarker name and resulting abstracts were reviewed in March of 2020. Our query included commercially-available serum- and urine-based biomarkers proposed for use following elevated PSA to improve the specificity of screening (17). Of note, preliminary MPS data was available at the time of initial review and formally cited at the manuscript revision stage. The primary outcome of interest was GG ≥2 PCa, and we included post-discovery (i.e., validation) studies that provided sensitivity and specificity for GG ≥2 PCa or provided sufficient raw data for calculation. We included studies of patients referred for prostate biopsy, in the vast majority of cases due to elevated PSA and/or abnormal digital rectal examination (DRE). Because these tests are proposed to aid in the diagnostic evaluation of at-risk men rather than primary screening, we included cohorts with a GG ≥2 prevalence >10%. To provide clinical context, data were stratified by study population [overall (i.e., all patients referred for prostate biopsy) *vs.* specific clinical criteria (i.e., specific PSA ranges)] and by biopsy status (initial *vs.* repeat biopsy). Each variable and summary statistic is defined in *Table 2*.

Measures of diagnostic performance

We reported the sensitivity, specificity, negative predictive value (NPV), and positive predictive value (PPV) of each biomarker at one or more threshold values [calculated values are labeled with an asterisk (*)]. The purpose of the following subsections is to briefly summarize relevant calculations and interpretation of common statistical parameters (18-20). Although beyond the scope of this review, it is important to consider that these measures are impacted by different study designs (i.e., matched) and potential confounders, requiring recalculation/adjustment for proper interpretation (21,22).

Sensitivity, specificity, biopsies avoided, and GG ≥2 PCa missed

Sensitivity and specificity are common measures of diagnostic accuracy that quantify the agreement of a test relative to a reference standard. Sensitivity and specificity depend on the threshold value used to identify positive (above the threshold) and negative (below the threshold) test results. Changing the threshold to increase sensitivity will necessarily decrease the specificity. In contrast to predictive values, sensitivity and specificity are inherent characteristics of a test and do not vary based on disease prevalence in the study population.

Sensitivity is the proportion of patients that have a positive test among all patients that truly do have the

Table 2 General Table components and statistical calculations

Study	Author, year
Population	Overall biopsy referral (i.e., all patients that underwent biopsy, regardless of indication) *vs.* specific clinical criteria
Bx Status	Initial *vs.* repeat biopsy
N	Sample size of study cohort
GG ≥2 (%)	Percentage of patients with GG ≥2 cancer in the study
Cutoff value	Assay threshold
Sn (%)	$\dfrac{TP}{TP+FN}$
Sp (%)	$\dfrac{TN}{TN+FP}$
NPV (%)	$\dfrac{TN}{TN+FN}$
PPV (%)	$\dfrac{TP}{TP+FP}$
Bx avoided (%)	Percent testing negative at the given cutoff value
Unnecessary Bx avoided (%)	Percent testing negative among those without the outcome (i.e., biopsies appropriately avoided based on testing, equivalent to specificity)
GG ≥2 missed (%)	100% - sensitivity
Model used	Assay + clinical variables included

Bx, biopsy; TP, true positive; TN, true negative; FP, false positive; FN, false negative; Sn, sensitivity; Sp, specificity; NPV, negative predictive value; PPV, positive predictive value. Statistical references: (18-20).

condition ("positivity in disease"); it is also called the true positive (TP) rate (20). In the current context, sensitivity represents the proportion of men that have a positive test among all that truly have GG ≥2 cancer. Specificity represents the proportion of patients with a negative test among all patients that truly do not have the condition ("negativity in health"). In this context, specificity is the proportion of men that have a negative test among all men that do not have GG ≥2 cancer. The false positive rate is equal to 1 – specificity.

As an example, we can apply the use of a biomarker test to determine the need for prostate biopsy: patients with a positive test do undergo biopsy, and patients with a negative test do not. For illustrative purposes, we will imagine that biopsy is 100% accurate for detecting GG ≥2 PCa if it exists. A test with 97% sensitivity would be positive for 97% of men with GG ≥2 cancer, appropriately leading to biopsy for those patients. The test would be negative in 3% of men with GG ≥2 cancer, leading to (inappropriately) not performing biopsy and therefore missing 3% of GG ≥2 cancers. As

such, the proportion of GG ≥2 cancers missed with a given testing approach can be calculated as 100%—sensitivity. This underscores the importance of a highly sensitive test when the main clinical aim is to avoid missing diagnoses (for example, when the condition is highly lethal but curable with treatment).

A test with 30% specificity will yield a negative test result in 30% of patients that do not have GG ≥2 cancer. As biopsy will be appropriately avoided in such men, specificity equals the proportion of unnecessary biopsies (i.e., biopsies that would have been negative/GG1) that were avoided through use of the test. This is in contrast to overall "biopsies avoided", which is simply the percentage of men that have a negative test result, without accounting for whether or not the negative test result accurately reflected the underlying disease state.

Negative and positive predictive values

Unlike sensitivity and specificity, predictive values are

dependent on the prevalence of the outcome in the population. Among all patients with a negative test, the NPV is the proportion of patients that truly do not have GG ≥2 disease. For instance, a biomarker with 90% NPV for GG ≥2 PCa indicates that there is a 90% probability of not having GG ≥2 disease in patients who test negative. By contrast, among all men with a positive test the PPV is the proportion of men that do have GG ≥2 disease. If an assay has a PPV of 30% then a positive test indicates a 30% probability of having GG ≥2 PCa. It is additionally assumed for the purpose of this review that the patient/population presenting for biomarker testing is consistent with the population in which assay performance measures were derived. Thus, we have aimed to clearly summarize pertinent clinical data for all studies.

Discrimination

The area under the receiver operating characteristic curve quantifies the ability of a test to discriminate between those with and without the outcome. The AUC measures the area under the ROC curve, which plots the sensitivity versus the false positive rate for all potential threshold values. Importantly, the false-positive rate (1 – specificity) is the probability of a positive test result when the condition is absent. Meanwhile, the false-negative rate (1 – sensitivity) is the probability of a negative test result when the condition is present. For binary outcomes, the AUC is identical to the concordance statistic (c-statistic) (23). While the AUC provides a broad measure of performance across all potential thresholds, it values all potential cutoffs equally, making it limited for interpreting potential clinical utility of a test. For example, a test threshold that fails to detect 75% of high-grade cancers (25% sensitivity) would not be acceptable for clinical use, and therefore the false positive rates that determine the AUC are irrelevant at such low sensitivities. Yet the area under the curve for sensitivity 0–25% contributes the same proportion to the overall AUC as the clinically-meaningful sensitivity range of 75–100%. In the setting of GG ≥2 prostate cancer, useful tests generally have sensitivities of at least 75%, and, ideally, >90%. Therefore, while we present AUC as a broad measure of discrimination, we have focused on the accuracy of tests at specific thresholds presented in the literature. The performance of various testing approaches to support clinical decision-making is best summarized with decision-analytic measures such as decision curve analysis reviewed elsewhere (24).

Biomarkers

The Prostate Health Index (PHI)

The Beckman Coulter PHI is a blood-based assay that combines [-2] proPSA (p2PSA), free PSA (fPSA), and total PSA (tPSA) into a single score to predict likelihood of PCa on biopsy. Initial data revealed that use of PSA isoforms such as percent free PSA (%fPSA = fPSA/tPSA) could improve PCa detection relative to PSA (25), and additional evidence supporting the use of PSA isoforms led to the development of PHI. In a 2011 multicenter study of 892 patients, PHI demonstrated greater AUC (0.70) than its individual components [p2PSA (AUC 0.56), fPSA (AUC 0.62), and tPSA (AUC 0.53)] for PCa in men with PSA 2–10 ng/mL and normal DRE (26). Subsequent studies in the overall referral population (27-29) and in men with PSA 2–10 ng/mL (30-35) demonstrated improvements in AUC ranging from 0.06 to 0.25 relative to PSA-based models.

More relevant to contemporary practice, a number of studies have characterized the use of PHI for predicting GG³2 cancer (*Table 3*).

In 395 men referred for initial biopsy, regardless of PSA level, de la Calle *et al.* showed that a PHI cutoff of 24 demonstrated 92% sensitivity, 30% specificity, 89% NPV, and 37% PPV for GG ≥2 PCa. Using this cutoff could have avoided 21% of biopsies and 30% of unnecessary biopsies while delaying the diagnosis of 8.2% of GG ≥2 cancers (36).

Several studies have assessed PHI within specific PSA ranges in biopsy-naïve men. Among three such studies, PHI was shown to discriminate GG ≥2 PCa on biopsy with AUC 0.71 (N=503, PSA 2–10 ng/mL and normal DRE) (39), AUC 0.71 (N=531, PSA 3–15 ng/mL) (37), and AUC 0.80 (N=138, PSA 4–20 ng/mL) (38). Improvements in accuracy compared to PSA-based models ranged from 0.08 to 0.13 across these studies. Sensitivities and specificities using various PHI thresholds are listed in *Table 3*. Notably, Nordström *et al.* and Seisen *et al.* found that PHI outperforms base clinical models of PSA and age (AUC 0.71 *vs.* 0.55) (37) and PSA density (PSAD) (AUC 0.80 *vs.* 0.68) (38), respectively, thus helping to discriminate between GG1/benign and GG ≥2 tissue.

In 2015, Loeb *et al.* assessed prospectively collected, multicenter data on 658 men with PSA 4–10 ng/mL and normal DRE, of which 21% had a history of prior negative biopsy. Using a PHI threshold for biopsy of 28.6 led to 90% sensitivity and could've avoided 30% of unnecessary biopsies, thus demonstrating a potential role of PHI in further risk stratification

Table 3 Performance characteristics of PHI in predicting GG≥2 prostate cancer by population and biopsy status

PHI studies	Population	Bx status	N	GG≥2 (%)	Cutoff value	Sn (%)	Sp (%)	NPV (%)	PPV (%)	Bx avoided (%)	Unnecessary Bx avoided (%)	GG ≥2 missed (%)	Model Used
de la Calle et al., 2015 (36)	Overall BxRef	Bx-naive	395	31	24	92	30	89	37	21*	30*	8.2	PHI only
Nordström et al., 2015 (37)	PSA 3-15	Bx-naive	531	25	26	96*	12*	–	–	8.5	12	4.5	PHI only
					39	90*	40*	–	–	30	40	9.8	
					47	76*	61*	–	–	45	61	24	
Seisen et al., 2015 (38)	PSA 4-20 and/or abnormal DRE	Bx-naive	138	28	40	67	74	85	50	62*	74*	33*	PHI only
Chiu et al., 2019 (39)	PSA 2-10, normal DRE	Bx-naïve	503	23	25	99	10	–	–	9.7	10*	1.7	PHI only
					32	95	28	–	–	23	28*	5.2	
					35	94	37	–	–	33	37*	6.1	
					40	90	48			40	48*	10	
					45	78	59	–	–	51	59*	23	
					55	53	72	–	–	68	72*	52	
Loeb et al., 2015 (40)	PSA 4-10, normal DRE, age 50+	Mixed (21% RBx)	658	17*	28	95	27.4	–	–	–	27*	5*	PHI only
					29	90	29.7	–	–	–	30*	10*	
					32	85	38.9	–	–	–	39*	15*	
					34	80	46.4	–	–	–	46*	20*	

Bx, biopsy; Overall BxRef, Overall biopsy referral; RBx, repeat biopsy; Sn, sensitivity; Sp, specificity; NPV, negative predictive value; PPV, positive predictive value; PSA, ng/mL. Calculated values are labeled with an asterisk (*).

of patients meeting these clinical criteria (40). PHI was approved by the Food and Drug Administration (FDA) in 2012 for select men (at least 50 years of age, non-suspicious DRE, and PSA 4–10 ng/mL) (41), and the European Association of Urology (EAU) mention PHI may be offered in a subset of patients (PSA 2–10 ng/mL, non-suspicious DRE) to better define the risk of GG ≥2 cancer (42).

The 4-kallikrein score

The OPKO Health 4-kallikrein score (4Kscore) is a blood-based test consisting of a 4-kallikrein panel [PSA, fPSA, intact PSA (iPSA), and human kallikrein 2 (hK2)] plus age, prior biopsy status, and DRE findings (optional). The 4Kscore is reported as a percent likelihood of harboring GG ≥2 PCa (0–100%). Using data from the European Randomized Study of Screening for Prostate Cancer (ERSPC), four early

studies by Vickers and colleagues evaluated a 4Kscore (4K panel, age, and DRE status) threshold of 20% in biopsy-naïve referral populations. These data indicated that 36–60% of biopsies could have been avoided while missing 2.3–12% of GG ≥2 cancers, with AUCs ranging from 0.80 to 0.90 (43-46). Relative to the contemporary population in which biomarker testing is often applied (GG ≥2 PCa prevalence approximating 16–36%) (40,47-51), these cohorts were of lower risk (4.3–9.9% GG ≥2 PCa). This work set the foundation for future 4Kscore validation studies.

In 2015, Parekh and colleagues published a large prospective validation study of 1,012 men referred for biopsy (i.e., overall referral population) across 26 U.S. centers, of which 22% had a history of prior negative biopsy. The authors observed an AUC of 0.82 for predicting GG ≥2 disease, which significantly outperformed a modified Prostate Cancer Prevention Trial Risk Calculator (PCPT-RC) 2.0 clinical model (AUC 0.74). Based on this, use of

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Table 4 Performance characteristics of the 4Kscore in predicting GG ≥2 prostate cancer by population and biopsy status

4Kscore studies	Population	Bx Status	N	GG ≥2 (%)	Cutoff value (%)	Sn (%)	Sp (%)	Bx avoided (%)	Unnecessary Bx avoided (%)	GG ≥2 missed (%)	Model Used
Parekh et al., 2015 (47)	Overall BxRef	Mixed (20% RBx)	1,012	23	6	94*	39*	30	39*	5.6*	Full 4K model
					9	90*	56*	43	56*	10*	
					12	86*	67*	51	67*	14*	
					15	79*	76*	58	76*	21*	
Zappala et al., 2017 (52)	Overall BxRef	Mixed (20% RBx)	1,012	23	7.5	93*	47*	36	47*	7.4*	Full 4K model
					7.5	93*	46*	36	46*	6.9*	4K model without DRE
Punnen et al., 2018 (49)	Overall BxRef	Mixed (20% RBx)	366	36	6	99*	27*	17	27*	1.5	Full 4K model
					7.5	97*	34*	22	34*	3	
					9	96*	39*	25	39*	4	
					12	92*	49*	31	49*	8	
					15	87*	57*	37	57*	13	
Bryant et al., 2015 (53)	Overall BxRef	Mixed	4,765	13.3	4	96*	31*	27	31*	4.5*	4K panel and age
					6	90*	49*	43	49*	11*	
					8	83*	64*	56	64*	17*	
					10	77*	74*	64	74*	23*	
Braun et al., 2016 (48)	Overall BxRef	Unknown	749	21	4	99*	11*	8.7	11*	1.4*	4K panel and age
					6	96*	21*	17	21*	3.8*	
					8	94*	30*	24	30*	6.3*	
					10	92*	39*	31	39*	7.7*	
					12	89*	48*	38	48*	12*	
Wysock et al., 2020 (51)	Overall BxRef	Unknown	50	44	7.5	96	32	–	32*	4.5*	Full 4K model
					12	90	54	–	54*	9.9*	
Nordström et al., 2015 (37)	PSA 3-15	Bx-naive	531	25	10	90*	40*	30	40*	11	4K panel and age
					15	78*	61*	45	61*	22	
					20	64*	77*	56	77*	37	

Bx, biopsy; Overall BxRef, Overall biopsy referral; RBx, repeat biopsy; 4K panel, PSA, fPSA, iPSA, and hK2; Full 4K model, 4K panel, age, prior biopsy status, and DRE; Sn, sensitivity; Sp, specificity; PSA, ng/mL. Calculated values are labeled with an asterisk (*).

4Kscore could have potentially avoided 30–58% of biopsies across different thresholds (6%, 9%, 12%, and 15%) while missing 1.3–4.7% of GG ≥2 cancers (47). These findings are corroborated by more recent studies using the 4Kscore in the overall referral population, which have yielded AUC ranges from 0.81 to 0.83 with improvements in AUC of 0.07 to 0.16 compared to a PSA-based clinical model and a urinary biomarker assay (SelectMDx), respectively (49,51).

4Kscore validation studies aimed at detection of GG ≥2 cancer are illustrated in *Table 4*.

SelectMDx

The SelectMDx (MDxHealth) urine-based assay incorporates biomarkers homeobox C6 (HOXC6) and distal-less homeobox 1 (DLX1) with clinical factors of age, PSA,

Table 5 Performance characteristics of SelectMDx in predicting GG ≥2 prostate cancer by population and biopsy status

SelectMDx Studies	Population	Bx status	N	GG ≥2 (%)	Cutoff value (%)	Sn (%)	Sp (%)	NPV (%)	PPV (%)	Bx avoided (%)	Unnecessary Bx avoided (%)	GG ≥2 missed (%)	Model used
Haese et al., 2019 (50)	Overall BxRef	Bx-naïve	916	28	12	93	47	95	43	–	47*	7*	Full SelectMDx
Wysock et al., 2020 (51)	Overall BxRef	Unknown	50	44	7.5	55	75	–	–	–	75*	46*	Full SelectMDx
					12	46	79	–	–	–	79*	55*	
Haese et al., 2019 (50)	PSA <10	Bx-naïve	715	21	12	89	53	95	34	44	53	11*	Full SelectMDx

Bx, biopsy; Overall BxRef, overall biopsy referral; RBx, repeat biopsy; Sn, sensitivity; Sp, specificity; NPV, negative predictive value; PPV, positive predictive value; PSA, ng/mL; Full SelectMDx, HOXC6, DLX1, age, PSAD, and DRE findings; Modified SelectMDx, HOXC6 TDRD1, DLX1, and PSA (no other clinical factors were incorporated). Calculated values are labeled with an asterisk (*).

prostate volume, and DRE findings to estimate percent likelihood of PCa and percent likelihood of GG ≥2 on biopsy. SelectMDx validation studies aimed at detection of GG ≥2 cancer are summarized in *Table 5*.

Early work by Leyten *et al.* first identified three genes in urinary sediment for the detection of overall and GG ≥2 PCa: HOXC6, DLX1, and Tudor domain-containing protein 1 (TDRD1) (54). A follow-up study by Van Neste and colleagues focused on urinary mRNA levels of two of these genes (HOX6 and DLX1) (55). Unfortunately, the pertinence of these findings to contemporary practice are limited by study cohort characteristics, with mean PSA values greater than 10 ng/mL—well beyond the range in which additional testing is recommended by expert guidelines (17). Nonetheless, a model including HOXC6 and DLX1 was applied to a validation cohort of 386 men. Notably, the multigene model provided a lower AUC (0.86, 95% CI, 0.80–0.92) than a baseline model of clinical variables only (AUC 0.87, 95% CI, 0.81–0.93). When DRE findings were removed from the multigene model, the AUC improved to 0.90 (95% CI, 0.85–0.95). While these data were not particularly promising, they laid the groundwork for additional efforts.

A recent study of biopsy-naïve patients by Haese *et al.* evaluated SelectMDx in the overall referral population (N=916) and in a subgroup of men with PSA <10 (N=715). The full SelectMDx model consisted of urinary HOXC6 and DLX1 mRNA plus age, PSAD, and DRE result (without incorporating history of prior biopsy). In the overall biopsy-naïve population, SelectMDx demonstrated 93% sensitivity, 47% specificity, and 95% NPV. Similar findings were observed in men with PSA <10 ng/mL, with 89% sensitivity, 53% specificity, and 95% NPV. Notably, when assessed

without prostate volume (i.e., PSAD was replaced with PSA), SelectMDx had decreased sensitivity (87%), specificity (38%), and NPV (92%) (50). The inclusion of prostate volume in the SelectMDx model has implications for clinical use, particularly in biopsy-naïve men, given that measurement of prostate volume requires ultrasound or MRI.

In a more recent head to head comparison of SelectMDx and the serum 4Kscore, Wysock *et al.* showed SelectMDx to be inferior for detecting GG ≥2 PCa in the overall referral population. Although limited by a sample size of 50 men who underwent prostate biopsy, SelectMDx yielded an AUC of 0.67 (95% CI, 0.52–0.83) compared to the 4Kscore with an AUC of 0.83 (95% CI, 0.71–0.95) (51). The authors also report a discordance between the two biomarkers in guiding decision to biopsy, further illustrating the need for larger, prospective comparative studies to optimize clinical application.

ExoDx Prostate Intelliscore (EPI)

The Exosome Diagnostics EPI is a three-gene urinary assay that incorporates PCA3, ETS transcription factor ERG (ERG), and SAM pointed domain-containing ETS transcription factor (SPDEF) mRNA into a single numerical value (from 0 to 100) for detecting GG ≥2 disease.

In 2009, Nilsson *et al.* described use of urinary exosomes to detect PCa biomarkers in 11 men with PCa. The authors successfully detected PCA3 and TMPRSS2:ERG, two biomarkers with known specificity for PCa (56). Utilizing this principle, Donovan *et al.* developed a novel two-gene signature termed the EXO106 score (urinary PCA3 and ERG mRNA). This approach was novel in that urinary biomarker detection did not require pre-collection DRE. In

Table 6 Performance characteristics of ExoDx Prostate Intelliscore (EPI) in predicting GG ≥2 prostate cancer by population and biopsy status

ExoDx Prostate Intelliscore (EPI) Studies	Population	Bx status	N	GG ≥2 (%)	Cutoff value	Sn (%)	Sp (%)	NPV (%)	PPV (%)	Bx avoided (%)	Unnecessary Bx avoided (%)	GG ≥2 missed (%)	Model used
McKiernan et al., 2016 (58)	PSA 2-20	Bx-naïve	519	29	15.6	92	34	91	36	27	34*	8	Full EPI
McKiernan et al., 2018 (59)	PSA 2-20	Bx-naïve	503	31	15.6	93	26	89	37	20	26	7	Full EPI
					20	89	40	89	41	31	40	11	

Bx, biopsy; RBx, repeat biopsy; Sn, sensitivity; Sp, specificity; NPV, negative predictive value; PPV, positive predictive value; PSA, ng/mL; EXO106 model, PCA3 and ERG; Full EPI, PCA3, ERG, and SPDEF; PCA3, prostate cancer antigen 3; ERG, ETS transcription factor ERG; SPDEF, SAM pointed domain-containing ETS transcription factor. Calculated values are labeled with an asterisk (*).

195 biopsy-naïve men with PSA 2–10 ng/mL, an EXO106 cutoff of 10 provided high sensitivity (95%), specificity (50%), NPV (98%), and PPV (35%) for GG ≥2 PCa (AUC 0.76). The EXO106 model appeared to improve upon the standard of care (SOC) model of PSA, age, race, and family history of PCa (AUC 0.67), and combining EXO106 with SOC increased the AUC to 0.80 (57).

A key EPI validation study was conducted by McKiernan and colleagues in 2016 (58), and a 2018 utility study (59) included validation data relevant to this review (*Table 6*).

Both studies included independent cohorts of men with PSA levels ranging from 2–20 ng/mL presenting for initial biopsy (N=519 and N=503, respectively). In both cohorts, an EPI cutoff of 15.6 yielded similar sensitivity (92–93%), specificity (26–34%), NPV (89–91%), PPV (36–37%). Clinically, these data translated to avoidance of 20–27% of biopsies, while delaying the diagnosis of 7–8% of GG ≥2 cancers (58,59). These data support reproducibility and the potential use of EPI within this patient population.

MyProstateScore (MPS)

Previously named Mi-Prostate Score (MiPS), the clinically-available MyProstateScore (MPS) combines urinary PCA3 and T2:ERG with serum PSA in a validated model to predict GG ≥2 PCa (60). The resulting output is a continuous score from 0 (very unlikely to detect GG ≥2 PCa) to 100 (very likely to detect GG ≥2 PCa).

Individually, urinary PCA3 has been extensively studied and is FDA-approved for use in the repeat biopsy setting (55,61,62). Discovered in 2005, the T2:ERG gene fusion has been well-studied in tissue, where it has >99% specificity for cancer (63-65). Urinary detection of T2:ERG was achieved using a similar approach to PCA3, and urinary

T2:ERG has been associated with clinically-significant PCa in subsequent studies (66,67). Combining urinary PCA3 and T2:ERG in an 'either-or' approach, Sanda et al. reported 93% sensitivity, 33% specificity, 93% NPV, and 33% PPV for GG ≥2 disease. Application of the combined testing approach would have avoided 42% of unnecessary biopsies at the expense of missing 7% of GG ≥2 cancers (68).

In a large multicenter study, Tomlins and colleagues derived the multivariable MPS model to optimally combine PSA, PCA3, and T2:ERG for detecting GG ≥2 cancer. MPS was subsequently applied to an external validation cohort of 1,244 men with median PSA of 4.7 (IQR, 3.3–6.5), of which 20% had a history of a prior negative biopsy. On validation, MPS provided superior predictive accuracy (AUC 0.77) for GG ≥2 PCa relative to PSA (AUC 0.65) and the PCPT-RC (AUC 0.71). Across various threshold values, use of MPS would have resulted in substantial reduction in prostate biopsy, while missing only 1.0–2.3% of GG ≥2 cancers (60).

More recently, additional multi-institutional efforts have sought to establish a pragmatic approach to MPS testing. In the biopsy-naïve setting, the MPS threshold of 10 was applied to two external validation cohorts—one in the community setting and one in the academic setting. In the combined validation data (n=1,525), the MPS threshold of 10 provided 98% negative predictive value and 97% sensitivity for GG ≥2 cancer. These findings were confirmed in 1,242 patients meeting testing criteria consistent with the National Comprehensive Cancer Network (i.e., PSA 3–10 ng/mL or PSA <3 and suspicious DRE) and in clinically-pertinent subgroups (i.e., African-American men and men with suspicious DRE). Applied as a reflex clinical test, MPS would have prevented 33% of unnecessary biopsies while failing to detect only 3.0% of GG ≥2 cancers (69). These data are listed in *Table 7*.

Table 7 Performance characteristics of the MyProstateScore (MPS) in predicting GG ≥2 prostate cancer by population and biopsy status

MPS studies	Population	Bx Status	N	GG ≥2 (%)	Cutoff value	Sn (%)	Sp (%)	NPV (%)	PPV (%)	Bx avoided (%)	Unnecessary Bx avoided (%)	GG ≥2 missed (%)	Model used
Sanda *et al.*, 2017 (68)	Overall BxRef	Bx-naïve	561	26	10 (PSA) 20 (PCA3) 8 (T2:ERG)	93	33	93	33	33	42	7	PSA or PCA3 or T2:ERG
Tosoian *et al.*, 2021 (69)	Overall BxRef	Bx-naïve	1,525	22	10	97	33	98	29	26	33	3	MPS
Tosoian *et al.*, 2021 (69)	Guideline-directed	Bx-naïve	1,242	22	10	96	32	97	28	26	33	3.7	MPS

Bx, biopsy; Overall BxRef, overall biopsy referral; RBx, repeat biopsy; Sn, sensitivity; Sp, specificity; NPV, negative predictive value; PPV, positive predictive value; PSA, ng/mL; Guideline-directed, PSA 3-10 or PSA <3 with abnormal DRE. The MPS model includes serum PSA and urinary PCA3 and T2:ERG scores; clinical variables are not required. Calculated values are labeled with an asterisk (*).

Commentary

Several serum and urine biomarkers have been proposed to improve detection of clinically-significant PCa and better inform clinical decision-making. These commercially-available tests have been validated to varying degrees in pertinent testing populations, and each appears to add diagnostic information beyond baseline clinical data.

Although promising, optimal application of these biomarkers remains to be determined. Continued work to refine the role of molecular biomarkers in prostate cancer early detection is ongoing, including efforts combining these markers with pre-biopsy prostate MRI. MRI has been used for risk stratification in the biopsy referral population and has been associated with improved detection of clinically significant PCa and reduced overdiagnosis of GG1 disease (70,71). However, the tradeoff in terms of significant cancers missed is concerning. Available data from largely expert centers cite a pooled NPV of 91% for clinically significant cancer in biopsy-naïve men but acknowledges significant heterogeneity across centers, with NPV as low as 63% (72). Variability in MRI accuracy exists within and across institutions. There is evidence supporting a wide variation in PIRADS score and cancer yield for individual readers, with clinically significant cancer detection rates ranging from 40% to 80% for lesions read as PIRADS 5 across radiologists at a single academic center (73). Furthermore, wide ranges in prostate MRI accuracy have been reported across institutions. For example, Westphalen *et al.* reported a PPV ranging from 35% to 49% in data collected from centers participating in the Society of Abdominal Radiology Prostate Cancer disease-focused panel, which comprises experts dedicated to prostate cancer

imaging (74). These data raise concerns in using MRI as an initial secondary test for PCa evaluation, and support consideration of objective testing platforms not dependent on reader expertise.

Practically, objective tests obtained in the course of standard urologic care provide notable advantages. We have reviewed several candidate markers for the detection of clinically significant prostate cancer. Acknowledging the limitations of cross-study comparisons, these markers appear to provide diagnostic accuracy on par with that of MRI obtained at expert centers. On the contrary, MRI provides a unique ability to target high-risk lesions and is currently supported by a more robust body of prospective data than most of the biomarkers described herein (70,71,75,76). These assays stand to benefit from additional prospective studies to identify clear approaches for clinical use in better-defined testing populations (i.e., biopsy-naïve men, those with a history of negative biopsy, and African American men). Certainly, as a combined approach, initial use of biomarkers to rule out one-quarter to one-third of unnecessary biopsies, followed by MRI to improve the diagnostic yield of invasive biopsy, is highly appealing. A number of clinical trials assessing the value of MRI and biomarkers are currently underway (77,78).

While the use of imaging and biomarker-based tools appear to provide clinical benefit relative to PSA alone (71,79), their impact on the cost of care is not fully characterized. In a 2018 cost-effectiveness analysis, Sathianathen *et al.* found that the use of biomarkers following elevated PSA reduced unnecessary biopsies by 24% to 34% and improved quality-adjusted survival relative to the standard of care (80). Currently, the cost

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and availability of these tools vary across practices and reimbursement policies. As practice patterns and policies are better established, further analyses will better define the cost-effectiveness of these diagnostic modalities in addition to their clinical accuracy. Ultimately, the ability to use these tests will likely depend on practical considerations such as reimbursement, which is similarly tied to clinical evidence. Thus, the ability to optimally apply available tools will depend on the production of quality data to demonstrate a measurable impact on meaningful clinical outcomes.

Conclusions

The goal of secondary testing is to reduce the harms associated with PSA-based screening while preserving its potential life-prolonging benefit. Multiple serum and urinary biomarkers have been validated for use in avoiding unnecessary biopsies in a proportion of men, while failing to detect a limited number of GG ≥2 cancers. Emerging data focused on clear applications of these markers in specific clinical settings and populations will better define their use and support more widespread adoption. As our shared goal remains to minimize harm due to prostate cancer and improve the patient experience, thoughtfully-designed and well-executed clinical research is essential to reaching this goal.

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