The Clinical Applications of Serum and Urinary Biomarkers in Prostate Cancer

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Abstract

At every stage of the prostate cancer journey from screening and diagnosis to management of advanced disease, patients and clinicians face dilemmas and decisions that can impact long-term outcomes. Although traditional risk stratification in prostate cancer is based on serum prostate specific antigen, clinical stage and Gleason score, in recent years, biomarkers have been developed that may be useful in several clinical scenarios. Biomarkers that can accurately predict an individual patient's risk, prognosis, and response to specific treatments could lead to improvements in decision-making and clinical care. Although there is evidence to support the use of biomarkers to guide management decisions, the optimal scenario in which to use them, how to interpret the results, and how to incorporate those results into clinical decision-making can be confusing. Nevertheless, in the era of personalized and precision medicine, it is important for clinicians to be aware of what tests are available, what clinical questions they seek to answer, and what limitations they have. This review focuses on the serum and urine biomarkers for the management of prostate cancer that have been under intense investigation in recent years.

Introduction

Prostate cancer (PCa) is a heterogenous disease with a highly variable clinical course and behavior. Traditional risk stratification of PCa has been based on standard clinical parameters such as prostate specific antigen, clinical stage, and biopsy Gleason scores. Following the widespread use of PSA testing, the pendulum swung towards overdiagnosis of clinically insignificant PCa and the subsequent overtreatment with its associated morbidity [1]. Traditional models and nomograms, although allowing risk stratification of patients to a certain degree, do not allow accurate prediction of outcomes for an individual patient with PCa, leading to potential undertreatment of high-risk disease [2,3]. Because of these limitations, leading to discordant care, many areas of uro-oncology have recognized the need for the development of reliable biomarkers to aid decision-making in various challenging clinical contexts. Finding the "ideal" biomarker that can accurately predict a patient's individual risk and improve on existing, validated risk stratification tools has become an area of intense research interest in the last decade.

Biomarkers can be classified according to the source (eg, serum, urine, or tissue) (Table 1) or the clinical decision juncture at which they are used (Figure 1). In this 2-part summary, we review commercially available blood, urine and tissue-based biomarkers and summarize the currently available data on their use in a variety of clinical scenarios.

The first section will focus on the serum and urinary PCa biomarkers. Blood and urine sources have the advantage of being non-invasive, easily attainable, and convenient for patients and physicians. The subsequent article will focus on tissue-based biomarkers [4].

Key Words

Biomarkers, non-invasive, serum, urine, prediction

Competing Interests

None declared.

Article Information

Abbreviations

%fPSA	percent free PSA
BPH	benign prostatic hyperplasia
csPCa	clinically significant prostate cancer
DRE	digital rectal examination
EPI	ExoDx Prostate Intelliscore
HOXC6	homeobox C6
PAP	prostatic acid phosphatase
PCa	prostate cancer
PSAD	PSA density
PSADT	PSA doubling time
PSAV	PSA velocity
SOC	standard of care
tPSA	total PSA

Serum Biomarkers

Prostate Specific Antigen

Measurement of serum PSA is the most widely used test to aid in the detection of early prostate cancer, despite its well-known limitations, including high false-positive rates, poor specificity for prostate cancer, which lead to over diagnosis.

PSA is not tumor specific as it is secreted by both benign and malignant prostatic tissue. Therefore, apart from PCa, many benign processes such as inflammation, benign prostatic hyperplasia (BPH), and trauma may lead to increased PSA values. PSA gained FDA approval for PCa screening in 1994 after it was shown to have higher sensitivity than prostatic acid phosphatase (PAP) in the detection of prostate cancer [5].

Historically, a serum PSA level above 4 ng/mL was the accepted cutoff to predict the potential presence of prostate cancer [6]. However, it has since been recognized that 20% of patients diagnosed with prostate cancer have a PSA lower than 4 ng/mL [7,8]. Furthermore, it has been reported that when using a PSA threshold of 4 ng/mL, the specificity in detecting PCa is only 12.8%, leading to high false-positive rates and unnecessary biopsies [9]. To enable better discrimination between PCa and BPH, the measurement of the ratio of free to total PSA was proposed as a more accurate indicator, and many studies have demonstrated its usefulness [10-13]. In a large prospective multicenter clinical trial of 773 patients with PSA levels between 2 and 10 ng/mL, it was shown that a percent free PSA (%fPSA) cutoff of 25% detected 95% of cancers and avoided 20% of unnecessary biopsies, with minimal loss in sensitivity [14]. A multivariable analysis showed %fPSA to be an independent predictor of prostate cancer (OR 3.2, 95% CI 2.5 to 4.1; $P < 0.001$) and more significant than total PSA (tPSA). Generally, the lower the %fPSA, the higher the risk of cancer. However, prostate volume has been found to have an influence on %fPSA. Stephan et al. reported diagnostic value of %fPSA in patients with prostate volume < 40cm³, but advised caution in using %fPSA in those with larger glands [15].

Other PSA metrics have also been employed to enhance the diagnostic and predictive capacity of PSA. These include PSA density (PSAD), PSA doubling time (PSADT), and PSA velocity (PSAV). PSA density is a quotient of serum PSA and prostate volume and may be a means of distinguishing between BPH and PCa [16]. A higher PSAD may not only indicate the presence of PCa but may also reflect the aggressiveness of the cancer. Studies have shown a correlation between higher PSAD and adverse PCa prognostic features [17]. A high PSAV and a shorter PSADT, especially in the setting of post-treatment biochemical recurrence (BCR), are associated with an increased risk of castration resistance, metastases, and cancer-specific and overall mortality [18].

Prostate Health Index (PHI)

Prostate Health Index (Beckman Coulter Inc., Brea, US) combines 3 quantitative kallikrein immunoassays—tPSA, %fPSA, and [-2]proPSA (p2PSA)—via a mathematical algorithm into a single score. The PHI received FDA approval in 2012 and is indicated as an adjunct to tPSA in men aged over 50 years with tPSA between 4 and 10 ng/mL and non-suspicious digital rectal examination (DRE) findings. Numerous validation studies have addressed the clinical utility of PHI. A prospective multi-institutional trial enrolled 892 men with no history of PCa, normal DRE, and tPSA of 2 to 19 ng/mL, and found that within this range of PSA, at a sensitivity of 80% to 95%, the specificity and area under the curve (AUC) of PHI exceeded those of PSA and %fPSA [19]. Similarly, Stephan et al. evaluated 1362 patients with tPSA of 1.6 to 8.0 ng/mL who underwent systematic biopsy with ≥ 10 cores, and showed that PHI (AUC = 0.74) outperformed %p2PSA (AUC = 0.72), p2PSA (AUC = 0.63), %fPSA (AUC = 0.61), and tPSA (AUC = 0.56) in predicting PCa [20]. Furthermore, these large series have demonstrated the advantage of PHI over %fPSA in distinguishing between high-grade disease (Gleason score ≥ 4 + 3) and low-grade disease or negative biopsies [19,20]. Catalona et al. showed that an increasing PHI was associated with a 4.7-fold increased risk of PCa and 1.6-fold increase in higher-grade disease. Other prospective studies have indicated the ability of PHI to detect aggressive (Gleason score ≥ 7) cancer with higher specificity than tPSA and %fPSA in biopsy naïve men, reducing the need for unnecessary biopsies [21].

The utility of PHI in detecting clinically significant prostate cancer (csPCa) was evaluated by Tosoian and colleagues, who reported that the median PHI density

TABLE 1.

Commercially available biomarkers that are FDA and Clinical Laboratory Improvements Amendments (CLIA) approved

| Biomarker	Molecular markers tested
Serum	
Prostate Specific Antigen (PSA)*	PSA
Prostate Health Index (PHI)* Beckman Coulter Inc, United States	tPSA, %fPSA, p2PSA
4K score OPKO Lab, United States	tPSA, fPSA, intact PSA, hK2
Urine	
Progensa Prostate Cancer Antigen 3 (PCA3)* Hologic, United States	PCA3
ExoDX Prostate (Intelliscore) Exosome Diagnostics Inc, United States	PCA3, ERG
Michigan Prostate Score (MiPS), United States	TMPRSS2-ERG mRNA, PCA3
Select MDX MDx Health, United States	HOXC6, DLX1
Tissue	
Confirm MDx MDxHealth, United States	DNA methylation (GSTP1, APC, RASSF1, ACTB)
Oncotype Dx Genomic Health, United States	RNA (17 gene expression)
Promark Metamark Genetics, United States	8 Proteins
Prolaris* Myriad Genetics, United States	RNA (46 gene expression)
Decipher Genome Dx Biosciences, United States	RNA (22 gene expression)

FDA-approved biomarkers

(based on prostate volume) was higher for those with csPCa (1.21) than for those with clinically insignificant PCa (0.70) and negative biopsies (0.53) with $P < 0.001$. Using a threshold of 0.43, PHI density was 97.9% sensitive and 38% specific for csPCa and 100% sensitive for Gleason ≥ 7 disease [22]. The authors concluded that discriminative ability of PHI density (AUC = 0.84) for csPCa was higher than PHI, PSA, PSAD, %fPSA, and prostate volume (AUC 0.52 to 0.79). Up to 38% of biopsies could be avoided while missing only 2% of

csPCa [22]. Another advantage of PHI over %fPSA is the lack of influence of patient age and prostate volume [19].

A recent study has evaluated the impact of PHI on clinical decision-making. In an observational study of 500 men, White et al. found that those who received a PHI test had a significantly lower biopsy rate compared to the control group (36.4% versus 60.3%; $P < 0.001$). The PHI test purportedly impacted physicians' management plans, including the decision to defer biopsies when

FIGURE 1.

Serum and urinary biomarkers according to their clinical applications
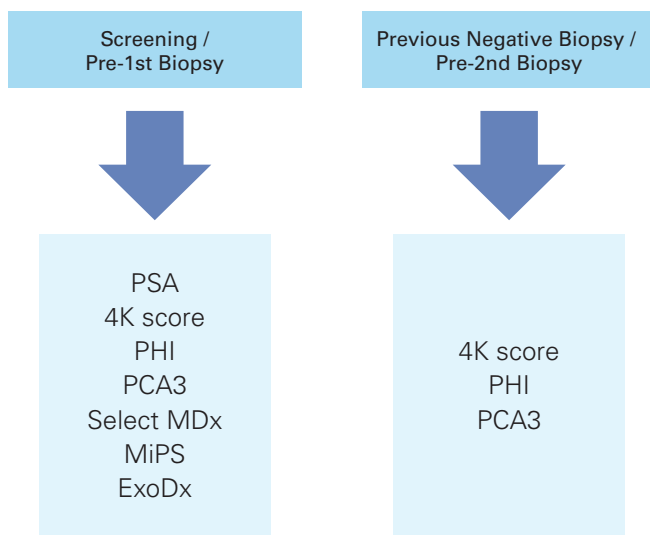
PHI score was low and to perform biopsies when the PHI score was intermediate or high [23]. Similarly, the prospective comparative analysis of 345 men by Tosoian et al. demonstrated that PHI testing reduced the rate of biopsy procedures whilst the detection of higher-grade cancers remained unchanged [24].

Another potential application of the PHI score is the integration into a multivariable model with multi-parametric magnetic resonance imaging (mpMRI). Gnanapragasam et al. found that adding PHI to mpMRI improved the prediction of overall and csPCa (AUC 0.71 and 0.75) compared with mpMRI or PSA alone (AUC 0.64 and 0.69) [24]. In determining whether to re-biopsy men with a negative mpMRI, PHI performed better than PSA and PSAD in identifying csPCa. With a PHI threshold of > 35, 42% of men avoided biopsy while missing only one of 21 significant cancers [25].

4K Score
The 4K score (OPKO Health, Miami, US) comprises a panel of 4 kallikrein (4K) markers including tPSA, %fPSA, intact PSA, and human kallikrein 2 (hK2) and combines clinical variables (age, DRE findings, and previous biopsy status) into a model to predict the likelihood of having csPCa (Gleason score ≥ 7) on prostate biopsy. It may have value in reducing unnecessary prostate biopsies [25]. The merit of the 4K score in the pre-biopsy setting has been extensively evaluated, particularly in Europe and the US [26–34].

Vickers et al. reported on 2914 men in the European Randomized Study of Screening for Prostate Cancer (ERSPC) who underwent prostate biopsy for PSA ≥ 3ng/mL, in which PCa was diagnosed in 28% of men [30]. Incorporating the 4K score with PSA and age significantly improved predictive accuracy with (AUC 0.78 versus 0.70) and without (AUC 0.76 versus 0.64) DRE results ($P < 0.001$). For every 1000 men, the addition of the 4K score would reduce the number of unnecessary biopsies by 513, albeit with the tradeoff that 12% of csPCa would be missed [30]. A smaller series of 262 men showed similar results, with the 4K score having greater diagnostic accuracy than "base" clinical models incorporating PSA, age, and DRE findings only, with AUC increases from 0.63 to 0.78 in PCa prediction and 0.77 to 0.87 for prediction of high-grade PCa [27]. Parekh et al. prospectively evaluated the diagnostic performance of the 4K score in 1012 men undergoing biopsy without restrictions on the indication for biopsy. Clinically significant PCa (Gleason ≥ 7) was found in 23% of patients in this cohort [31]. The 4K score outperformed the PCPT Risk Calculator 2.0 (AUC 0.82 versus 0.74; $P < 0.001$) in detecting csPCa and would have reduced the number of unnecessary biopsies by 58%. In a large representative cohort study of 40379 men from Sweden, Stattin et al. studied the 4K score for its ability to predict the risk of distant metastases [32]. Using a risk stratification approach to prostate biopsy using PSA and 4K score, they found that in patients with PSA ≥ 3 ng/mL, the risk of distant metastases at 5, 10, 15, and 20 years would be greater for the high-risk group with 4K ≥ 7.5% (2.4%, 5.6%, 9.9%, and 16.4%) than for the low-risk group with 4K < 7.5% (0%, 0.2%, 1%, and 1.8%) [32].

In men with previously negative prostate biopsy but persistently elevated PSA, 4K score was found to have superior predictive capacity for high-grade cancers while minimizing unnecessary biopsies [32]. The 4K score showed higher discriminatory accuracy than PSA and DRE alone (AUC 0.68 versus 0.58, $P < 0.001$) in detecting PCa on repeat biopsy. For the prediction of high-grade (Gleason ≥ 7) PCa, the 4K score outperformed clinical factors alone (AUC 0.87 versus 0.76. $P = 0.003$). Using a 4K risk threshold of 15%, 712 repeat biopsies could potentially be avoided for every 1000 men, with only 3 of the 53 missed cancer diagnoses having a Gleason score ≥ 7 [32].

A comparison of 4K score and PHI in a populated-based cohort study of 531 men with PSA 3 to 15 ng/mL showed their predictive values to be similar in predicting any PCa (AUC 0.69 for 4K, AUC 0.70 for PHI), as well as high-grade PCa (AUC = 71.8 for 4K, AUC = 71.1 for PHI) [33]. Compared with a base model of age and PSA,

both 4K and PHI had higher AUC (*P* < 0.001). Using high-grade PCa risk thresholds of 10% for 4K and 39 for PHI, 29% of biopsies could potentially be avoided with the caveat that 10% of high-grade cancers could be missed [33].

Although there is little evidence to support their use in primary screening, both PHI and 4K scores are mentioned by the European Association of Urology (EAU), American Association of Urology (AUA), and National Comprehensive Cancer Network (NCCN) as potential markers that may be used to risk stratify patients in the early detection of PCa. Their benefits of predicting risk of high-grade PCa and reducing unnecessary biopsies in men with PSA from 2 to 10 ng/mL is recognized, as is their superiority over %fPSA [35].

Urinary Biomarkers

Progensa prostate cancer antigen 3 (PCA3)

PCA3 (Hologic, Marlborough, US) is a non-coding large chain RNA that is highly overexpressed in the majority of malignant prostate tissue compared with benign prostate tissue [36,37]. The Progensa PCA3 gene assay measures PCA3 mRNA concentrations in the first void urine collected after DRE [38]. In 2012, the FDA approved the use of PCA3 to facilitate the decision-making process to re-biopsy men with a previous negative biopsy. Multiple studies have evaluated the clinical utility of PCA3 in the early detection of PCa and as a prognostic marker in the active surveillance of patients with low-risk PCa [39–41].

A key European multicenter prospective study by Haese et al. correlated PCA3 scores of 463 men with previous negative prostate biopsies to the repeat biopsy outcomes, with a higher PCA3 score associated with a greater probability of a positive repeat biopsy [39]. Men who had a PCA3 score ≥ 35 had a 39% chance of having a positive biopsy compared with 22% in men with a PCA3 score < 35 (*P* < 0.001). The mean PCA3 score was significantly higher in men with a positive biopsy than in those with a negative biopsy (63.8 versus 35.5; *P* < 0.001). Furthermore, a PCA3 threshold of 35 provided greater diagnostic accuracy than a comparable %fPSA cutoff of 25%. PCA3 was found to be independent of tPSA, prostate volume, and patient age [39].

In a retrospective analysis of 13 men, Wu et al. reported that use of a PCA3 score threshold of 25 yielded sensitivity and specificity of 0.67 and 0.64, respectively [42]. Although PCA3 was found to be independently associated with PCa (AUC 0.64) in multivariable analyses, the highest diagnostic accuracy was derived from a model comprising PCA3, PSAD, PSA, DRE, and TRUS findings (AUC 0.82) [42].

A prospective validation trial by Wei et al. included 859 PSA-screened patients undergoing initial biopsy and repeat biopsy after prior negative biopsy [41]. The authors demonstrated a positive predictive value (PPV) of 80% for PCA3 > 60 at initial biopsy and a negative predictive value (NPV) of 88% for PCA3 < 20 at repeat biopsy. Their findings supported the use of PCA3 in reducing unnecessary biopsies in men with a prior negative biopsy and concluded that for initial biopsy, a PCA3 > 60 significantly increases the probability of cancer detection [41].

In another European prospective multicenter study of 516 men, de la Taille et al. observed that the mean PCA3 score was higher in those men with a positive versus negative biopsy (69.6 versus 31.0; *P* < 0.001) and higher in men with csPCa. PCA3 scores > 35 had the highest diagnostic accuracy with sensitivity of 64% and specificity of 76%. PCA3 score was independent of age, total PSA and prostate volume and outperformed total PSA, PSAD, and %fPSA [43].

The ability of PCA3 to predict tumor volume and select low-risk patients for active surveillance was prospectively evaluated by Ploussard et al. who reported that PCA3 scores strongly correlated with tumor volume [44]. A PCA3 score > 25 was associated with a 3-fold increase in risk of csPCa. On multivariate analysis, a PCA3 score > 25 was a predictive factor for tumor volume > 0.5cm3 (OR 5.4; *P* = 0.01) and for significant cancer (OR 12.7, *P* = 0.003), suggesting its use as a tool in selecting better candidates for active surveillance [44]. Nakanishi et al. also found a significant association between PCA3 and tumor volume, with PCA3 being particularly useful in identifying low tumor volume < 0.5 mL (AUC 0.757) [45].

As PCA3 is the only urinary biomarker with FDA approval, the various guidelines do mention its use to risk stratify patients after a previous negative biopsy and to determine the need for a repeat biopsy. However, it is not recommended as a primary screening tool, and no threshold for the PCA3 score has been defined to guide decision-making.

ExoDx prostate intelliscore (EPI)

The ExoDx Prostate Intelliscore (Exosome diagnostics, Waltham, US) is a newer, novel urine exosome gene expression assay used to predict the risk of PCa on biopsy. Exosomes are 1 of 2 types of microvesicles found in prostate secretions [46]. Exosomes may be secreted by both normal and malignant tissues, but elevated exosome secretions have been found in malignant biofluids such as the urine of patients with PCa. Exosomes contain a portion of the parent cell cytoplasm containing proteins and RNA that closely resemble the

cell of origin [47]. Exosomes lack ribosomal RNA but are rich in mRNA, which acts as a unique genetic footprint of specific tumor cells and may give information about the specific tumor genotype that underlies the varying phenotypes that are seen in a heterogenous disease like PCa. Exosomes isolated from post DRE urine contain PCA3 and ERG (erythroblastosis virus E26 oncogene homologues) [47]. The EPI test has the advantage of not requiring a pre-collection DRE, making it non-invasive and more convenient. The test uses an algorithm independent of clinical variables to provide a risk score, with 15.6 being the threshold discriminating between high-grade and low-grade PCa [48].

McKiernan et al. compared the performance of EPI with biopsy outcomes in men with PSA ranging from 2 to 20 ng/mL, then went on to validate the prognostic score [49]. They found that incorporating EPI into the standard of care (SOC) improved the discrimination of high-grade disease from low-grade or benign disease. Validation in 519 patients showed the superior performance of EPI plus SOC (AUC 0.73) over SOC alone (AUC 0.63) in predicting high-grade disease ($P < 0.001$). Using an EPI cutoff score of 15.6, the authors concluded that 27% of biopsies could have been avoided, missing 5% of high-risk cancers [49].

A recent prospective randomized study by Tutrone et al. examined the clinical utility and the impact of EPI score on decision-making in men presenting for initial prostate biopsy with PSA 2 to 10 ng/mL [48]. A total of 1094 patients and 72 urologists were involved in the study. Sixty-eight percent of urologists were influenced by the EPI score in their decision to recommend or defer a biopsy, the main reason for noncompliance with EPI results being a rising PSA. Eighty-seven percent of patients with a positive EPI score were recommended to proceed to biopsy, leading to the detection of 30% more high-grade PCa than in the control arm. On the other hand, 63% of patients with a negative EPI score were recommended to defer prostate biopsy, and the authors estimated that 49% fewer high-grade cancers were missed because of biopsy deferral compared with SOC [48].

The latest NCCN guidelines do mention EPI as a potential investigative marker, but it is not currently incorporated into mainstream practice.

Mi prostate score (MiPS)

The MiPS assay was developed at the University of Michigan Rogel Cancer Centre. It is a urine multiplex analysis that combines PSA with 2 PCa specific biomarkers: PCA3 and an RNA marker that is found only when TMPRSS2 and ERG abnormally fuse. ERG is an ET transcription factor that is overexpressed in 57% of prostate cancers [50]. In more than 90% of cases that overexpressed ERG, there was found to be fusion with TMPRSS2. TMPRSS2-ERG (T2-ERG) fusion is thought be a strong indicator of PCa.

In a trial of 48 patients undergoing prostate biopsy, Salami et al. found an association between the presence of T2-ERG and PCa (OR 12.02, $P < 0.001$) [51]. Although PCA3 had higher sensitivity (93%), T2-ERG had the highest specificity (87%) in predicting PCa. T2-ERG also had better discriminative ability (AUC 0.77) compared with PCA3 (AUC 0.65) and serum PSA (AUC 0.72). Combining all 3 factors into a multivariable algorithm improved the AUC for cancer prediction to 0.88 with specificity of 90% and sensitivity of 80%, better than any individual marker alone [51]. Laxman et al. showed that compared with PCA3 alone (AUC 0.662), T2-ERG in combination with PCA3 and a multiplex panel of urinary transcripts (AUC 0.758) improved the early detection of PCa [52].

A validation trial in 1225 patients by Tomlins et al. assessed the ability of the multivariable MiPS model incorporating PSA, PCA3 and T2-ERG in predicting PCa and high-grade PCa on biopsy [53]. The authors showed that models incorporating T2-ERG had higher AUC than PSA in predicting any (0.693 versus 0.585) and high-grade (0.729 versus 0.651) PCa. The MiPS model incorporating T2-ERG outperformed other models incorporating only PCA3 and PSA in the detection of PCa ($P < 0.001$) [53].

The MiPS is currently an investigational tool within NCCN guidelines and currently not routinely used in mainstream practice.

Select MDx

Select MDx (MDx Health, Irvine, US) is a post DRE urine-based gene assay risk score that aims to predict a patient's risk of high-grade PCa. The algorithm measures the mRNA signatures of 2 genes implicated in prostate carcinogenesis—homeobox C6 (HOXC6) and distal-less homeobox 1 (DLX1)—and combines these with clinical factors such as age, family history, previous negative biopsies, and DRE. Leyten et al. initially described a 3-gene urinary panel including HOXC6 and DLX1 that was shown to have additional value over serum PSA and PCA3 in the detection of PCa and reducing the risk of overtreatment [54].

Van Neste et al. developed a model incorporating HOXC6 and DLX1 on a cohort of 519 patients and subsequently validated the risk score in an independent cohort of 386 patients in two prospective multicenter studies [55]. The authors identified the mRNA signature risk score in combination with PSAD and previous negative biopsies to be the most significant factors with an overall AUC approaching 0.90 (95% CI 0.85 to 0.95). Another model adding DRE as a risk factor

was also tested with an AUC of 0.86. When compared with the PCPTRC and PCA3, this model could reduce unnecessary biopsies by 53% with a NPV of 98% for Gleason ≥ 7 disease [55].

Correlating Select MDx with mpMRI results, a retrospective observational study of 172 patients reported a positive association between the risk score and the final PI-RADS grade [56]. Median Select MDx scores were higher in patients with a suspicious lesion on mpMRI than in those with a negative mpMRI ($P < 0.01$). Select MDx was also shown to have some value in predicting the mpMRI result with AUC of 0.83 compared with PSA (AUC 0.66) and PCA3 (AUC 0.65).

The cost effectiveness of Select MDx compared with SOC was evaluated by Dijkstra et al., who concluded that the judicious use of Select MDx to reduce the overdiagnosis and overtreatment of men with PCa and PSA > 3ng/mL could lead to reduction in costs and gains in quality adjusted life years [57].

Conclusions

A myriad of serum and urinary biomarkers have emerged with the goal of improving decision-making processes in the early diagnosis of localized PCa. Although a few have gained FDA approval, most are investigational and not used routinely in clinical practice. Biomarkers must add value beyond existing multivariable models to be cost-efficient and of overall benefit. Therefore, at this time, they must be used judiciously in the management of patients with suspected and known PCa.

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