Review Article
Prostate diseases and male voiding dysfunctions

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Beyond Prostate Specific Antigen: New Prostate Cancer Screening Options

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Prostate specific antigen (PSA) is one of the best-known biomarkers for screening, diagnosis and follow-up of patients for prostate cancer. Owing to several inherent limitations with PSA, various newer blood and urinary based biomarkers have been evaluated in pursuit of better detection and risk stratification of prostate cancer cases. A combination of these different markers, in adjunct with clinical risk factors, and recent advances in imaging promises to offer better diagnostic performance with clearer risk stratification guiding therapeutics. We carried out an extensive literature search for the different biomarkers available for screening and diagnosis of prostate cancer, compared their performance with serum PSA to allow clinicians to draw meaningful conclusions to offer their patients a more personalized medical care.

Keywords: Biomarkers; Prostate cancer; Prostate specific antigen; Screening

INTRODUCTION

The discovery of prostate specific antigen (PSA) has undoubtedly been one of the most important turning points in history for the diagnosis and management of prostate cancer. The introduction of this novel biomarker in the late 1980s brought about a paradigm shift in the diagnosis of prostate cancers, with a sudden increase in incidence of the disease, accompanied by a noteworthy stage migration; this resulted in improved survival for patients with prostate cancer.

Not to undermine the importance of the most widely used biomarker for screening, diagnosis, and follow-up of any solid tumor, there are controversies regarding the use of PSA in prostate cancer screening. These stem from the fact that using PSA as a screening tool saw an increase in detection of several indolent tumors, and subsequent "over-treatment" with radical treatment for these cancers, which probably would have never surfaced in the lifetime of the patient without screening. Additionally, the lack of specificity for cancer and lack of a true prognostic ability has pushed several researchers to look beyond PSA for newer markers with better sensitivity and specificity, as well as prognostic calibration for screening and diagnosing patients harboring clinically significant prostate cancer. This review looks at various blood and urine based markers available for screening of prostate cancer and how they perform in comparison to PSA.

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PROSTATE SPECIFIC ANTIGEN FOR SCREENING OF PROSTATE CANCER: CONTROVERSIES AND RECOMMENDATIONS

Owing to the lack of sensitivity and specificity of prostatic acid phosphatases, the earliest available biomarker for prostate cancer, the U.S. Food and Drug Administration (FDA) in 1986 approved PSA to be used for monitoring disease progression in prostate cancer patients. However, 8 years later, the FDA approved PSA to be used also for screening, when combined with digital rectal examination in men older than 50 years, despite weak evidence existing in favor of its accuracy as a screening tool. The reported sensitivity and specificity for PSA when used as a screening tool, is around 20% to 40% and 70% to 90%, respectively, depending on the cut-off values used [1]. The receiver operating characteristic (ROC) curve revealed the area under the curve (AUC) to be between 0.55 and 0.70 for the ability of PSA to detect cancer [1]. The poor specificity of PSA as a marker can be attributed to the fact that PSA is organ specific and not disease specific. Indeed, various conditions like urinary tract infections, prostatitis or even benign enlargement of prostate can cause raised PSA levels [2]. Contrary to this, patients with highly undifferentiated prostate cancers, or those with neuroendocrine or small cell cancer may have very low PSA levels and may still harbor significant tumor burden [3].

Two major randomized controlled trials, one in the United States of America and the other in Europe, tried to explore the benefits of screening for prostate cancer using PSA. The Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial randomized 76,693 men aged between 55 and 74 to PSA screening *versus* usual care. This trial failed to show any benefit of screening through 13 years of follow-up and the prostate cancer mortality rate was 1.09 (95% confidence interval [CI], 0.87–1.36) [4]. In contrast to this, The European Randomized Study of Screening for Prostate Cancer, which randomized 162,243 men aged between 55 and 69 to screening *versus* usual care, showed a survival advantage of 21% at the end of 13 years [5].

The low specificity and positive predictive value of PSA, combined with doubtful evidence in favor of using PSA as a screening tool for prostate cancer clearly reflects an unmet need for newer biomarkers for screening and diagnosis of prostate cancer; and for biomarkers which would reveal the true malignant potential of the underlying cancer.

BLOOD BASED MARKERS

1. Free prostate specific antigen

Cancerous cells release immature forms of PSA, which exist in blood complexed with several serum protease inhibitors, the most noteworthy of which is alpha-1 anti-chymotrypsin [6]. Normally, the unbound free PSA (fPSA) accounts for 5% to 40% of the total PSA (tPSA), which falls in patients with prostate cancer owing to the inability of immature forms of PSA to exit independently without complexing with protease inhibitors [7]. Christensson et al [7] compared the levels of non-complexed PSA (fPSA) in 144 patients of benign prostatic hyperplasia (BPH) with 121 patients with prostate cancer and found that the fraction of fPSA was significantly lower in patients of untreated prostate cancer than in those with BPH ($p<0.0001$). Two years later, Prestigiacomo and Stamey [8] studied the mean percentage levels of fPSA in 51 cancer patients and 48 patients of BPH, and found the levels to be 8.9% and 16.5% respectively, with the BPH patients having almost twice the levels seen in cancer patients. Luderer et al [9] compared the values of fPSA and tPSA in patients with prostate cancer, BPH, and healthy asymptomatic controls, and found that %fPSA performed better than tPSA in detecting cancer in the grey zone of tPSA from 4 to 10 ng/mL. Partin et al [10] showed a sensitivity of 95% and specificity of 20% when using a cut-off of 25% for fPSA for diagnosing men with prostate cancer whose PSA was from 4 to 10 ng/mL.

Apart from better diagnostic performance, fPSA has also been shown to reduce the proportion of unnecessary biopsies. In a prospective multicenter clinical trial evaluating 773 men, Catalona et al [11] showed that using a cutoff of 25% for %fPSA for men with PSA from 4 to 10 ng/mL significantly reduced the number of unnecessary biopsies, was an independent predictor of prostate cancer (odds ratio [OR], 3.2; 95% CI, 2.5–4.1; $p<0.01$), and performed better than age and tPSA in this cohort of patients.

With several studies proving the improved diagnostic capability of %fPSA as compared to tPSA for men with PSA from 4 to 10 ng/mL, the FDA approved the use of %fPSA for screening and diagnosis in this cohort of patients. The National Comprehensive Cancer Network

(NCCN) also suggest determining the %fPSA levels for men with PSA in this grey zone (4–10 ng/mL) [12]. Use of %fPSA beyond the level of 10 ng/mL of tPSA is not justified as the positive predictive value of tPSA reaches almost 80% beyond this level. Besides this, percentage fPSA also forms an integral component of other diagnostic tools such as the Prostate Health Index (PHI) and the 4K score, which will be discussed in the subsequent sections.

2. Prostate Health Index

In 2012, the FDA approved another proprietary test, the PHI, developed by Beckman Coulter Inc. (Brea, CA, USA), in collaboration with the National Cancer Institute (NCI) Early Detection Research Network. This test incorporates three individual PSA based biomarkers in a mathematical equation: (–p2PSA/fPSA)×√PSA. PHI was developed to better predict the possibility of presence of cancer and to direct biopsies in patients aged more than 50, with PSA from 4 to 10 ng/mL. In a prospective multicenter trial in the USA, which evaluated 892 men with PSA levels between 2 and 10 ng/mL and normal digital rectal examination, Catalona et al [13] evaluated the relationship between –p2PSA, fPSA, and tPSA and the biopsy findings. They found that the PHI outperformed both, the fPSA and tPSA, in terms of sensitivity and specificity; and the AUC for PHI exceeded that of free-to-tPSA ratio (0.724 *vs.* 0.670) for discriminating prostate cancer with Gleason 4 or greater from lower Gleason scores or negative biopsy [13]. These findings were confirmed in another multicenter prospective trial which showed that PHI outperformed all its individual components (–p2PSA, fPSA, tPSA) for detecting overall prostate cancer, or Gleason sum 7 or greater prostate cancer, and the authors concluded that PHI should be a part of the multivariable approach for aiming to reduce unnecessary biopsies and over-diagnosis [14]. Guazzoni et al [15] studied the performance of different isoforms of PSA and that of PHI in men with a PSA between 2 and 10 ng/mL and found that –p2PSA and PHI were the strongest predictors of prostate cancer and were significantly more accurate than tPSA, %fPSA, and PSA density in determining the presence of cancer at biopsy. Another multicenter study evaluating men with PSA in the range of 2 to 10 ng/mL found that 15.5% of the biopsies could be avoided, by using a PHI cut off of 27.6, while missing only 1.1% of aggressive cancers. The authors also demonstrated that addition of –p2PSA, %fPSA, and PHI to the base multivariable model significantly increased the accuracy by 6.4%, 5.6%, and 6.4%, respectively ($p<0.001$) [16]. Besides this, there are several validated nomograms which use PHI along with age, digital rectal examination (DRE) findings, and prostate volume, which have been shown to have a much higher predictive value than PSA alone, such as that developed by Lughezzani et al, and validated by the PRO-PSA Multicenter European Study Group (PROMETtheuS) [17,18]. Foley et al [19] developed two multivariable models, one based on PSA, and the other based on PHI, besides age, family history, abnormality on digital rectal examination, and previous biopsy results. The AUC for ROC curve for the PHI model was 0.77 while that of PSA was 0.71 for diagnosis of prostate cancer, and a decision-curve analysis showed a superior net benefit of the PHI model. The authors concluded that incorporation of these models using –p2PSA and PHI in diagnostic practice can help better stratify the risk of prostate cancer, and guide decision making regarding the need for a biopsy [19].

Ample evidence exists to suggest that PHI outperforms PSA as an initial screening tool, for risk stratification, deciding the need for biopsy, and for detection of clinically significant prostate cancer. Besides this, PHI has also been shown to correlate strongly with adverse pathological features following radical prostatectomies and with biochemical recurrence [20-23].

The European Association of Urology (EAU) strongly recommends the use of risk calculators such as PHI for avoiding unnecessary biopsies in patients with a normal digital rectal examination and PSA from 2 to 10 ng/mL [24].

3. 4K score

The 4K score, a commercially available assay offered by the central laboratory of Opko Diagnostics Inc. (Woburn, MA, USA), amalgamates together four prostate derived kallikrein proteins: tPSA, fPSA, intact PSA, and human kallikrein 2 (hK2), and was first introduced in March 2014. Besides the kallikrein markers, this model takes into account age, DRE findings, and previous biopsy results, and provides information regarding the probability of detecting high grade prostate cancer (Gleason sum 7 or greater). Most of the evidence on the performance of this four kallikrein model is derived from the European Randomized Study of Prostate Cancer Screening, and specifically

the Göteborg and the Rotterdam sections [25,26]. Vickers et al [25] evaluated the Göteborg cohort of 740 men undergoing biopsy and calculated the AUC for predicting cancer on biopsy for various models including the four kallikrein model and found that addition of fPSA, iPSA, and hK2 to tPSA improved the AUC from 0.72 to 0.84. A threshold cut-off of 20% risk of prostate cancer for biopsy would have reduced biopsies by 57% and would have missed only 3 out of 40 high grade cancers and 31 out of 152 low grade cancers in this cohort. The authors suggested that this model could be used to decide which patients should undergo a biopsy and which patients can continue with screening and defer biopsy until there was stronger evidence for malignancy [25]. Carlsson et al [26] evaluated 392 screened men participating in rounds 1 and 2 of the Rotterdam arm of the European Randomized Study of Prostate Cancer Screening. They too calculated AUC for different kallikrein based models to predict the aggressiveness of prostate cancer (pT3-4, extracapsular extension, Gleason grade >4, tumor volume >0.5 mL), and found the 4-kallikrein model to have the highest AUC (0.84, $p<0.0005$). They concluded that the use of this model would reduce the rates of immediate unnecessary active interventions [26]. The impact of the 4K score on the decision to biopsy the prostate was evaluated by Konety et al [27] in a clinical utility study in the USA. They evaluated the practice patterns of urologists over 35 centers in the USA which ordered the 4K score and found that the results of this test influenced the biopsy decisions in 88.7% of the patients with an abnormal PSA and/or DRE findings; a higher 4K score was associated with a higher likelihood of having a prostate biopsy ($p<0.001$) [27]. This is the largest study to date evaluating the practice patterns in the USA and supports use of the 4K score for risk stratification before tissue diagnosis for prostate cancer.

The 4K score has not yet been approved by the FDA for use, although the EAU recommends its use for avoiding unnecessary biopsies in patients with a normal digital rectal examination and PSA from 2 to 10 ng/mL [24].

URINE BASED MARKERS

1. Prostate cancer antigen 3

Prostate cancer antigen 3 (PCA3), also known as DD3 and first reported by Bussemakers et al [28] in 1999, is a non-coding RNA of unknown function, coded by a gene on chromosome 9q21-22. The interest of researchers in this non-coding RNA as a new biomarker for prostate cancer was based on the fact that it could be isolated from urine following a rectal massage of the prostate, was not produced by normal prostate tissue, lowly expressed byBPH, highly produced by malignant cells in the prostate, and the level of expression was not determined by prostatic volume [28].

The FDA, in 2012, approved an *in vitro* amplification based test called the Progensa PCA3 for patients suspicious of having prostatic malignancy but having a prior negative biopsy [29]. This was based on several studies demonstrating PCA3 to have a better predictive value than PSA. The Progensa PCA3 assay provides a score for PCA3 expression in the urine, which is a ratio of the PCA3 RNA to PSA RNA levels, and a score of <25 has been shown to be associated with a low likelihood of prostate cancer [30,31]. Marks et al [32] evaluated the PCA3 levels in urine samples of 233 men following a digital rectal massage, who had a PSA >2.5 ng/mL and a prior negative biopsy, and compared the abilities of PCA3 and PSA to predict the biopsy outcomes. The AUC for a ROC curve for PCA3 was 0.68 while that of PSA was 0.52. Using a cutoff of 35, PCA3 score had a sensitivity of 58% and specificity of 72%, with an OR of 3.6 [32]. In a prospective, multicenter study from Europe, Haese at al [33] compared the diagnostic accuracy of PCA3 score in 463 men and compared it to that of %fPSA. Using a cut off of 35 for the PCA3 score and 25% for %fPSA, PCA3 had a much higher diagnostic accuracy on repeat biopsy, and was independent of the number of previous biopsies, prostate volume, the PSA level, or the age of the patient. The score correlated positively with the Gleason grade, presence of high grade prostate intraepithelial neoplasia, the tumor volume and T stage. The authors concluded that PCA3 was a better predictor of outcomes of a repeat biopsy than other available biomarkers (%fPSA) and could avoid unnecessary biopsies, as well as better predicting the clinical stage and significance of prostate cancer [33].

Several authors have tried to establish the prognostic capabilities of this assay and have found a strong correlation between PCA3 scores and tumor aggressiveness. Nakanishi et al [34] correlated PCA3 levels with post prostatectomy tumor volume and Gleason score. PCA3 levels correlated significantly with total

tumor volume (r=0.26, p=0.008) and Gleason score (6 *vs.* 7 or higher, p=0.005). A multivariate analysis showed PCA3 to be the best predictor of tumor volume following prostatectomy (p=0.001) and the AUC from a ROC was 0.75 for predicting low volume tumor (less than 0.5 mL). The authors concluded that PCA3 was a strong reflection of tumor volume and aggressiveness and could be used for choosing between different treatment options (active surveillance *vs.* radical therapy) [34].

The FDA has approved the use of the Progensa PCA3 assay for patients with a prior negative biopsy and a high suspicion of harboring a malignancy, and its use is largely restricted to this population, rather than as an initial screening tool in place of PSA.

2. TMPRSS2:ERG fusion

Recurrent gene fusions involving the 5' untranslated region of *TMPRSS2* gene, one of the androgen regulated genes, and members of the ETS family of genes (*ERG*, *ETV1*, *ETV4*), have been demonstrated to be associated with prostate cancer with pathophysiologic, diagnostic and therapeutic implications [35,36]. In a quest to develop a non-invasive method to detect TMPRSS2:ETS gene fusions for detecting prostate cancer, a group of researchers from the University of Michigan explored the possibility of identifying this fusion in urine samples from patients diagnosed with prostate cancer using quantitative polymerase chain reaction [37]. They found a strong concordance between *ERG* overexpression and TMPRSS2:ERG overexpression with 42% of the patients exhibiting fusion transcripts in their urine. The researchers concluded that TMPRSS2:ERG gene fusions could be detected in the urine of prostate cancer patients and that it could be used as a diagnostic biomarker, although larger studies would be required to determine the sensitivity, specificity, and prognostic credibility of this biomarker [35].

Several isoforms of this gene fusion product are associated with pathogenesis of prostate cancer, the lack of tumor homogeneity, and the need for different assays to pick up each gene fusion separately, coupled with the lack of prognostic importance, has prevented this biomarker from gaining widespread acceptance. To add to this, the frequency of this gene fusion is variable across different population groups, with some groups of patients having very low expressions, which make it difficult to identify appropriate cut-offs for different populations [38].

To negate these shortcomings, this genetic fusion biomarker has been combined with the PCA3 marker to develop a much more specific and sensitive urine based marker than the two individual components themselves. This "combination" urinary biomarker has been shown to significantly improve the sensitivity of prostate cancer diagnosis [39,40]. Besides this, the combined PCA3 and TMPRSS2:ERG fusion scores have been shown to improve the diagnostic performance of PSA in detecting clinically relevant high grade prostate cancer on biopsy [38]. A commercially available assay combining these three biomarkers has been developed by the University of Michigan MLabs and is called the Mi-Prostate Score (MiPS). MiPS has been externally validated and has been shown to have better diagnostic performance in terms of risk stratification for detecting clinically significant cancers and avoiding unnecessary biopsies [41].

3. Urinary mRNA (SelectMDx®)

A group of urologists from The Netherlands developed a multimodal model using urinary messenger RNA (mRNA) levels and traditional risk factors that could be used to identify patients with high grade PCa. They measured the urinary levels of HOXC6 and DLX1 and found that these were excellent predictors of high grade PCa with a AUC of 0.90 (95% CI, 0.85–0.95). They concluded that these urinary mRNA levels when combined with traditional risk factors could stratify better the risk for prostate cancer and aid in decision making [42]. SelectMDx® is a proprietary urine based, molecular diagnostic liquid biopsy from the MDxHealth (Irvine, CA, USA) which combines clinical factors with urinary levels of HOX6 and DLX1 and risk stratifies men at increased risk of harboring clinically significant prostate cancer who would benefit from an early intervention and diagnosis. This test has been shown to have a NPV of 95% for high risk cancers and reduces the need for any intervention, magnetic resonance imaging (MRI) or biopsy by 50% [43]. The EAU and the NCCN both recommend the use of SelectMDx for risk stratification of men with a normal DRE finding and a PSA between 2 to 10 ng/mL for directing future diagnostic procedures [12,24].

IMAGING AS A SCREENING TOOL: MULTIPARAMETRIC MAGNETIC RESONANCE IMAGING

The past few years have witnessed an unprecedented rise in the use of MRI for diagnosis of prostate cancers, not just for local staging but for detecting biopsy negative prostate cancers. Even among men who have never had a biopsy before, and who have raised serum PSA levels, multiparametric MRI (mpMRI) demonstrated promise in both the detection and exclusion of prostate cancer, using a template prostate mapping biopsy as reference [44]. Grenabo Bergdahl et al [45] studied 124 men from the 10th screening round of the Göteborg randomized screening trial and compared the performance of sequential screening (PSA+MRI) with conventional PSA screening and they concluded that a screening strategy with a lowered PSA cut-off followed by template biopsy in MRI positive men increased the detection of clinically significant cancers besides improving specificity. Nam et al [46] conducted a pilot study on 47 men to evaluate the role of MRI in primary screening of prostate cancer in an unselected population and found that the OR for having cancer was significantly higher for MRI (2.7; 95% CI, 1.4–5.4; p=0.004) than PSA (1.1; 95% CI, 0.9–1.4; p=0.21). The ReIMAGINE Prostate Cancer Screening Trial, sponsored by the University College London, Medical Research Council and Cancer Research (London, UK), is the largest trial underway to establish the role of MRI in screening for prostate cancer in the unselected population. Besides evaluating the performance of MRI as a screening tool, it also aims at determining the prevalence of MRI defined suspicious lesions and cancer in men across a spectrum of PSA levels. Patients with suspected malignancy on MRI proceed to have a targeted biopsy, while those with no lesions on MRI exit the study. This trial is presently recruiting and is expected to complete accrual by May, 2022. The results of this trial will bring to light the real performance of MRI as a screening tool as compared with PSA [47].

CONCLUSIONS

PSA, in spite of its inherent shortcomings, has been, and continues to be, the gold standard screening biomarker for prostate cancer. Advancements in the fields of genomic and proteomic technologies has enabled us to decipher the genetic basis and true biology of prostate cancers and make major advancements in the fields of diagnosis and therapeutics. Several newer biomarkers, be it the different isoforms of PSA or gene based markers, have come to the forefront with better diagnostic and prognostic performance than PSA. A combination of these biomarkers promises to offer the best in diagnostics, and comes closest to being an ideal biomarker. Several assays combining these individual biomarkers are already available, but large, multicenter studies are needed to establish the true performance of these assays and their acceptance as the screening method of choice over the standard PSA. mpMRI is also emerging as a promising tool to detect lesions early and screen patients for prostate cancer. A combination of imaging along with other blood and urine based biomarkers has the potential to have the highest sensitivity and specificity, and to detect the most clinically significant prostate cancer. This would not only improve the diagnostics but also guide the therapeutics of prostate cancer, enabling urologists to make a more informed decision regarding surveillance and radical treatment. Until such time, serum PSA continues to be the most widely accepted biomarker for prostate cancer screening.

Conflict of Interest

The authors have nothing to disclose.

Author Contribution

Conceptualization: PS. Supervision: PS. Writing – original draft: TN. Writing – review & editing: PS.

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