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Beyond PSA: Utilizing Novel Strategies to Screen Men for Prostate Cancer

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Abstract

Purpose of Review—The purpose of this article is to review blood and urine tests that are currently available and under investigation for a role in prostate cancer screening and detection.

Recent Findings—Compared to total PSA alone, its combination with percent free-to-total PSA contributes greater specificity for prostate cancer, and is a component of 2 newer blood tests called the 4kScore and Prostate Health Index. All three tests improve the prediction of high-grade disease and are commercially available options to aid in initial or repeat prostate biopsy decisions. PCA3 is a urinary marker that is currently available for repeat prostate biopsy decisions. Although PCA3 alone has inferior prediction of clinically significant disease and requires collection of urine after digital rectal examination, it may be combined with other clinical variables or other urine markers like TMPRSS2:ERG to improve performance. Little data is available to support a role for single nucleotide polymorphisms or other investigational markers in early detection.

Summary—Several commercially available blood and urine tests have to been shown to improve specificity of PSA for high-grade prostate cancer. Use of such tests would decrease unnecessary biopsy and overdiagnosis of indolent disease. Biopsy of men with moderately elevated PSA without use of such a reflex test should be discouraged.

Keywords

prostate cancer markers; PSA; 4Kscore; phi; PCA3

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Conflict of Interest: SL reports no conflict of interest related to this manuscript. HL holds patents for free PSA, human kallikrein 2, and intact PSA assays. AV and HL are named on a patent application for a statistical method to detect prostate cancer. The method has been commercialized by OPKO Health. AV and HL receive royalties from sales of the test and AV has stock options in OPKO Health.

Introduction

Although most prostate cancer (PCa) is detected through screening with prostate-specific antigen (PSA), the historical paradigm for screening and detection has significant limitations. Although highly sensitive for aggressive disease, PSA has only moderate specificity, resulting in unnecessary biopsies, with attendant risks of infection, and overdiagnosis of indolent cancers with potential for overtreatment. These issues have led to investigation into options beyond PSA that can be used in prostate cancer detection. Herein, we review the literature on other markers including commercially available blood (free PSA, the 4K score, the Prostate Health Index) and urine tests (PCA3, TMPRSS:2 ERG), and other new markers under investigation to aid in prostate biopsy decisions.

Free PSA

Based on the catalytic action of PSA, PSA in blood occurs predominantly covalently bound to alpha 1-antichymotrypsin ("complexed" PSA)[1], although some is unbound ("free" PSA). The ratio of free to total PSA (%fPSA) has been studied extensively as a marker for prostate cancer: a meta-analysis in 2006[2] included no fewer than 41 separate studies. Typical guidelines, such as those of the National Comprehensive Cancer Network (NCCN), recommend %fPSA as a reflex testing option for men with elevated PSA[3], with lower levels associated with a higher risk of high grade cancer. Free PSA is also an integral part of two multi-marker panels, the 4kScore and the Prostate Health Index. Research on these marker panels has provided additional evidence for the value of free PSA. For instance, in the US validation study of the 4kScore, removing free PSA from the marker panel reduced discrimination from 0.82 to 0.70[4].

4kScore

The 4kScore is a commercially-available assay run through the central laboratory of Opko Diagnostics, and is included in the 2016 NCCN guidelines as a secondary testing option prior to initial or repeat prostate biopsy.[3] The score presents to the clinician the patient's risk of biopsy-detectable high-grade prostate cancer (Gleason score 7 or greater) based on a prediction model including clinical variables (age, prior biopsy, DRE results) and measured levels of four kallikrein markers: total PSA, free PSA, intact PSA and hK2. To date, there have been 11 published studies on the four-kallikrein panel for biopsy prediction, including a total of 15,984 men. The model was originally developed using cohorts from the European Randomized trial of Screening for Prostate Cancer (ERSPC). The model was found to have much higher discrimination for high-grade cancer, in comparison with a "base" model that excluded the additional kallikrein markers, across a range of clinical settings, including men without prior screening[5,6], those previously screened[7,8], men undergoing repeat biopsy after initial negative biopsy[9], and men undergoing work-up prior to biopsy[10]. In a typical study[8], the AUC of the prespecified kallikrein model was 0.80 to predict high-grade cancer in previously screened men, compared to 0.71 for a model including age, PSA and DRE.

A final statistical model for clinical use was built using data from the UK ProtecT trial (n=6129). The AUC for high-grade cancer was 0.82 for the four-kallikrein panel versus 0.74

for a model with PSA but without the other kallikreins[11]. The model developed in ProtecT was then externally validated in a prospective US cohort (n=1012) by an independent set of investigators[4]. The AUC of the pre-specified model was 0.82 vs. 0.74 for the widely used Prostate Cancer Prevention Trial (PCPT) risk calculator. The model was extremely well-calibrated and decision analysis demonstrated that use of the panel to determine indication for biopsy would decrease the number of unnecessary prostate biopsies, for instance, by about 50%, without delaying an undue number of high grade cancers (e.g. 24 per 1000 patients). Discrimination of the panel was superior in African Americans, although this difference was not statistically significant.

One key limitation of all studies that use biopsy as an endpoint is that Gleason score is only a surrogate. In a large population-based Swedish bio-bank study[12], the kallikrein model was very strongly associated with 15–20 year risk of distant metastasis or death from prostate cancer in men followed without screening. In one typical analysis, the Concordance Index for the kallikrein model was 0.875 compared to 0.805 for PSA alone. Critically, 10-year risk of metastasis was very low (e.g. ~0.2%) for men with a modestly elevated PSA (i.e. ≥2 or ≥3 ng/ml) but low risk from the kallikrein prediction model, even though these men were followed without DRE or repeat PSA. This suggests that using a low 4kScore to advise against biopsy in men with elevated PSA is a safe strategy. In an “impact study” conducted in routine US care, use of the 4kScore was found to reduce the incidence of biopsy by about 65%, demonstrating that urologists will use low scores from the model to make clinical decisions[13].

Prostate Health Index

The Prostate Health Index (phi) is a blood test combining total PSA, free PSA and −2proPSA using the following formula: ([−2]proPSA/fPSA) × √PSA. It was approved by the US Food and Drug Administration in 2012, and is included in the 2016 NCCN Guidelines as a secondary testing option for men making decisions about initial or repeat biopsy.[3] It is the least expensive of currently available commercial marker tests.

Large multicenter prospective studies in the US have demonstrated that at 95% sensitivity, phi has better specificity than total PSA and %fPSA.[14] Phi also has higher predictive accuracy for clinically-significant disease compared to its individual components of PSA, %fPSA and p2PSA.[15] Another multi-institutional study by de la Calle et al. reported that phi was a significant predictor of Gleason ≥7 disease on biopsies performed for elevated PSA and/or suspicious DRE, with AUCs of 0.82 and 0.78 in separate US study populations.[16] Using a phi threshold of 24 for biopsy would have avoided 36–41% of unnecessary biopsies and 17–24% of overdiagnosed indolent cancers.

Phi has also been validated internationally. Guazzoni et al. reported higher predictive accuracy using phi (AUC 0.76) compared to PSA density (61%), %fPSA (58%), and total PSA (53%).[17] A multicenter study by Lazzeri et al. of men undergoing biopsy for an elevated PSA +/− abnormal DRE showed that adding phi to a multivariable model with PSA and free PSA led to a significant improvement in predictive accuracy for high-grade PCa.[18] Using phi to determine the need for biopsy would avoid 16% of unnecessary biopsies while

missing only 1.1% of aggressive cancers. A comparative study from Sweden found that phi and the 4-kallikrein panel similarly improved the discrimination of high-grade disease on biopsy.[19]

Unlike the 4kScore, the reported phi value is based exclusively on PSA isoforms and does not already include other clinical variables like age, prior biopsy and DRE results. However, there are several validated nomograms using phi as a component of multivariable risk stratification. For example, Lughezzani et al. designed a nomogram combining age, prostate volume, DRE, prostate biopsy history and phi, which had an AUC of 0.80,[20] which was externally validated by the PRO-PSA Multicentric European Study Group (PROMETtheuS).[21] However, prostate volume may not be available at the time an initial biopsy decision is made. Another phi-based nomogram was reported by Foley et al. from Ireland including age, family history, DRE, previous negative biopsy and phi with an AUC of 0.79 for high-grade PCa overall, and an AUC 0.88 in men undergoing repeat biopsy.[22] In both cases, the nomogram using phi outperformed the same nomogram based on total PSA. Finally, the Rotterdam risk calculator smartphone app includes phi along with other variables for ease of use in the clinical setting.[23]

Finally, numerous studies have demonstrated a significant relationship between phi and adverse tumor features at radical prostatectomy, including pathologic stage, grade, tumor volume, and composite outcomes of clinically significant prostate cancer,[24–27] as well as biochemical recurrence.[28] Baseline and longitudinal values of phi are significant predictors of biopsy reclassification among men undergoing active surveillance.[2930,31]

Urinary PCA3, TMPRSS2:ERG, and MiPS

PCA3 is a noncoding mRNA that is overexpressed in prostate cancer tissue.[32] PCA3 can be measured in the urine following vigorous digital rectal examination (3 strokes/lobe) using commercially available assays.

Multiple studies showed greater diagnostic accuracy using urinary PCA3 versus total PSA or %fPSA PSA to identify PCa on repeat biopsy.[33,34] It was FDA approved in 2012 for men aged ≥50 with ≥1 previous negative biopsies for whom repeat biopsy is being considered, using a cutoff <25. PCA3 is also among the secondary testing options for repeat biopsy decisions in the 2016 NCCN guidelines, with a suggested cutoff of 35.[3]

Although PCA3 is not recommended in the NCCN guidelines for initial prostate biopsy decisions, several studies have examined its performance in this setting. A prospective, multi-institutional validation trial from the Early Disease Research Network included 562 men undergoing initial biopsy.[35] Prostate cancer was detected in 24%, 38%, 65% and 80% with PCA3 scores <20, 20–35, 35.1–60, and >60. Using a PCA3 cutoff of 60 in the initial biopsy setting was associated with 80% positive predictive value. However, high-grade PCa was found in 13% and 28% of initial biopsies performed at PCA3 levels <20 and 20–60, indicating that a much lower threshold would be required to avoid missing a significant number of high-grade cancers.

Overall, the data are conflicting with respect to the relationship of PCA3 with PCa aggressiveness. Multiple studies have failed to demonstrate a significant association between PCA3 with high-grade disease at biopsy, reclassification during active surveillance or adverse pathology at radical prostatectomy.[33,36–38] Moreover, the Prostate Health Index was shown to outperform PCA3 for the identification of clinically significant prostate cancer.[26,39] While there is clear evidence that the three PSA based markers - Prostate Health Index, 4kScore and free-to-total PSA ratio - have greater discrimination for high-grade compared to low-grade disease, it is not clear whether PCA3 preferentially detects clinically significant prostate cancer.

It is noteworthy that PCA3 can also be used together with other clinical variables as part of multivariable risk assessment tools. For example, Hansen et al. designed the first PCA3-based nomogram specifically to predict initial prostate biopsy results, including PCA3>21 along with age, PSA, DRE, and prostate volume.[40] Elshafei et al. created nomograms to predict overall and high-grade prostate cancer at initial biopsy using PCA3 along with age, race, family history, PSA, DRE and prostate volume.[41] At internal validation, the AUCs were 0.74 for any prostate cancer and 0.77 for high-grade disease, although it is noteworthy that prostate volume is not generally available at the time initial biopsy decisions are made. PCA3 has also been incorporated into existing risk prediction tools for men undergoing initial or repeat prostate biopsy, such as the PCPT risk calculator.[35]

Another way to use PCA3 and potentially improve its performance is through a combination urinary panel with other biomarkers such as TMPRSS2:ERG (T2:ERG), a gene fusion found in approximately 50% of prostate cancers. The new Mi-Prostate Score (MiPS) is a urinary marker test combining PCA3 and T2:ERG, which was recently shown to enhance the prediction of prostate biopsy outcome.[42] Among men undergoing initial biopsy, MiPS had an AUC of 0.79 for high-grade prostate cancer compared to 0.68 using PSA alone. Overall, the combination of PCA3 plus T2:ERG in MiPS had greater predictive accuracy than using either PCA3 or T2:ERG alone.

Single nucleotide polymorphisms

Single nucleotide polymorphisms (SNPs) in five chromosomal regions were reported to be significantly associated with PCa in a high profile publication in the *New England Journal of Medicine*[43]. Although each SNP was only modestly associated with PCa, the authors found a strong cumulative association with the disease. These findings have been widely reported and the authors announced plans to market a genetic test. However, despite very strong evidence of an association between the genotypes and cancer – that is, the p-value was very low - the genotypes have limited predictive ability, with an AUC of 0.57, little better than a coin flip[44]. Moreover, the authors did not evaluate whether the SNPs added predictive accuracy to PSA; subsequent research has found that when the SNPs were added to a predictive model that including PSA, the AUC was only marginally improved[45]. Subsequent GWAS studies have reported that about 30 different independent SNPs in multiple loci across many different chromosomes associate with risk of PCa diagnosis[46], some of which also are suggested to associate with disease outcome[47]. However, there is little if any evidence to suggest that the uncovering of any of these additional risk-associated SNPs have

direct clinical implications. For instance, in a study on the large prospective Malmö Diet and Cancer cohort, only 7 out of 50 previously identified PCa risk SNPs were associated with the risk of advanced or aggressive disease and their discrimination (0.57) was far less than PSA (0.79)[48]. An accompanying editorial, entitled “Time to Move On”, concluded “SNPs should probably not be further pursued in the context of detection … of prostate cancer”[49]. Thus, although ongoing investigation continues into genetic risk factors for PCa, there is no clinical role for SNPs in screening protocols at this time, although investigators continue to address whether there will be a clinical role for SNPs in biomarker-encoding prostate risk loci, which strongly influence biomarker levels in blood.[50]

The recent Stockholm 3 (STHLM3) study from Sweden examined the use of a model including genetic polymorphisms along with plasma biomarkers and other clinical variables to predict prostate biopsy outcome.[51] This particular panel is not yet commercially available and further study is necessary to determine the incremental cost-effectiveness of including genetic markers into predictive models.

Other markers

Given the modest specificity of the PSA-test, it is not surprising that a large number of alternative markers have been proposed to aid PCa detection. For instance, in just two weeks of Medline updates we found papers on long non-coding RNAs[52], ALKBH3[53], two different approaches to MicroRNAs[54,55], a surface-enhanced Raman scattering (SERS)-based microdroplet sensor for PSA[56], urinary carbonic anhydrase IX splicing messenger RNA[57], prostate specific membrane antigen (PSMA)[58], and platelet factor-4 (PF-4)[58]. Here we review some of the most prominent recent markers.

Early prostate cancer antigen 2 (EPCA-2) was reported in a preliminary study comparing convenience samples to have very high specificity of 92%[59]. These results prompted considerable press coverage and claims[60,61] that EPCA-2 would “help eliminate tens of thousands of unnecessary biopsies at the same time that it detects many tumors that are now missed by [PSA]”. Subsequently, it has been alleged that the EPCA-2 assay is invalid[62] and a company that bought an interest in EPCA-2 has sued the developer of the test for scientific fraud.

Another marker that drew high-profile press attention was sarcosine, at least in part because it seems to validate the methodology of metabolomic profiling[63]. However, replication studies failed to find that sarcosine plus PSA predicts PCa any better than PSA alone[64].

The Engrailed-2 (EN2) protein is urine marker that, unlike PCA3, can be collected without prostate massage. This makes EN2 an attractive option for mass screening as, in principle, it could be obtained without the intervention of a health professional. However, the data on EN2 remain rather weak, with some preliminary studies on small cohorts suggesting a higher prevalence of EN2 positivity in urine in men with vs. without PCa[65], and reports of some statistically significant associations between EN2 before surgery and both tumor volume and stage[66]. As yet, there have been no large scale studies of EN2 providing data that could help elucidate its clinical role.

In a high profile paper from the *New England Journal of Medicine*, a panel of autoantibodies was claimed to discriminate almost perfectly between blood samples of men with and without PCa[67] with an AUC of 0.93. Ten years after publication, large validation studies have yet to be published.

Most recently, McKiernan et al. reported on the ExoDxProstate Intelliscore, which measures urinary exosomes from first-catch urine samples.[68] For men undergoing initial biopsy, the exosome panel had an AUC of 0.73 for high grade disease, compared to 0.61 for total PSA. Like many novel diagnostics, the exosome score has yet to undergo the sort of extensive validation studies that have been conducted for the markers recommended in guidelines, such as free-to-total ratio, Prostate Health Index, 4kScore and PCA3.

Conclusion

There are now several commercially available options beyond PSA that can aid in prostate biopsy decisions. PSA-based blood tests such as free PSA, the 4kScore and Prostate Health Index have been validated in dozens of studies involving many thousands of patients and offer greater specificity for clinically significant disease and reduce both unnecessary biopsies and overdetection of indolent disease. Urinary PCA3 is currently available as an additional option for men undergoing repeat prostate biopsy and has also been extensively validated. There is some early evidence that performance for identifying high-grade disease can be improved through combination with other markers such as TMPRSS2:ERG. Despite active investigation into single nucleotide polymorphisms and several other markers, a role for these tests in prostate cancer screening paradigms remains unproven. As more new markers continue to become available, head-to-head comparative data will be critical to determine the most cost-effective testing combination for use in different clinical scenarios.

Overall, PSA has inadequate specificity for high-grade prostate cancer and leads to unnecessary biopsy and overdiagnosis of indolent disease. Given the ready availability of reflex tests to improve the specificity of PSA for high-grade prostate cancer, biopsy of men with moderately elevated PSA without use of such a reflex test should be discouraged.

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Key Points

- Several markers are available as “reflex tests” to help aid prostate biopsy decisions
- Free PSA has been in wide clinical practice for many years and is a component of the newer 4kScore and Prostate Health Index tests.
- The 4kScore and Prostate Health Index are more specific than total PSA for clinically significant prostate cancer on initial or repeat biopsy.
- PCA3 is a urine test that can be used for repeat prostate biopsy decisions, but its relationship with aggressive disease is controversial.
- Reflex tests should be routinely conducted for men with moderately elevated PSA