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Review

Blood and urine biomarkers in prostate cancer: Are we ready for reflex testing in men with an elevated prostate-specific antigen?

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Abstract *Objective:* There is no consensus on the role of biomarkers in determining the utility of prostate biopsy in men with elevated prostate-specific antigen (PSA). There are numerous biomarkers such as prostate health index, 4Kscore, prostate cancer antigen 3, ExoDX, SelectMDx, and Mi-Prostate Score that may be useful in this decision-making process. However, it is unclear whether any of these tests are accurate and cost-effective enough to warrant being a widespread reflex test following an elevated PSA. Our goal was to report on the clinical utility of these blood and urine biomarkers in prostate cancer screening.

Methods: We performed a systematic review of studies published between January 2000 and October 2020 to report the available parameters and cost-effectiveness of the aforementioned diagnostic tests. We focus on the negative predictive value, the area under the curve, and the decision curve analysis in comparing reflexive tests due to their relevance in evaluating diagnostic screening tests.

Results: Overall, the biomarkers are roughly equivalent in predictive accuracy. Each test has additional clinical utility to the current diagnostic standard of care, but the added benefit is not substantial to justify using the test reflexively after an elevated PSA.

Conclusions: Our findings suggest these biomarkers should not be used in binary fashion and should be understood in the context of pre-existing risk predictors, patient's ethnicity, cost of the test, patient life-expectancy, and patient goals. There are more recent diagnostic tools such as multi-parametric magnetic resonance imaging, polygenic single-nucleotide panels, IsoPSA, and miR Sentinel tests that are promising in the realm of prostate cancer

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screening and need to be investigated further to be considered a consensus reflexive test in the setting of prostate cancer screening.

1. Introduction

Prostate cancer (PCa) screening has been marred by controversy, with some clinicians and researchers suggesting that the harms—*i.e.*, overdetection, overtreatment and biopsy-related complications—outweigh the 20% mortality reduction associated with prostate-specific antigen (PSA) testing [1,2]. To reduce this risk of overdetection, researchers continue to evaluate a variety of imaging and molecular diagnostic tools to better discriminate men at risk of PCa. One of the primary goals of contemporary management strategies of PCa is to limit definitive treatments—which are associated with urinary, sexual, and physical functional morbidity—to cancers that are clinically significant (*i.e.*, Gleason score 7 or higher) [3]. Although there has been a substantial adoption of active surveillance for low-risk PCa, the question remains whether screening strategies can be implemented to reduce the detection of low-risk PCa that poses a low risk of cancer-related morbidity or mortality [4,5].

PCa screening involves both the use of PSA testing and prostate biopsy. In the United States (US), current screening guidelines from the US Preventive Task Force (USPSTF) and American Urological Association (AUA) advocate for shared-decision making for men aged 55—69 years old with regards to PSA screening [6,7]. In Asia, where PCa incidence is historically lower than in Western countries, there is significant variability in PSA screening utilization [8,9]. Nevertheless, cases of PCa are rising in more Asian countries partly because of an increase in PSA screening [10,11]. Regardless of region, there is even more ambiguity about the use of prostate biopsy in men with rising and/or elevated PSA. The decision to pursue prostate biopsy carries its own risk and benefit assessment given that prostate biopsy is not a benign procedure [12]. One strategy that can provide clarity in whom to biopsy for an "abnormal" PSA test, is based on using a reflexive biomarker test to identify men at risk of harboring clinically significant PCa. In this review, we evaluate the current state of the literature with regard to urine- and blood-based biomarkers for reflexive testing.

2. Interpreting diagnostics biomarkers

Diagnostic biomarkers help determine the probability of disease in an at-risk patient or population. Its utility is based on the pre-test probability of disease in those being tested and the diagnostic accuracy of the test. A diagnostic biomarker in the screening setting is especially helpful if it can accurately determine that a patient has a low likelihood of significant disease and can avoid further testing. In PCa, a biomarker with a high negative predictive value (NPV) gives patients and providers confidence that foregoing a prostate biopsy will not result in missing clinically significant cancer.

The area under the receiver operator characteristic (ROC) curve is another frequently used statistical metric to determine a test's utility because it incorporates the true positive rate and false positive rate [13]. Area under the ROC curve, also known as the area under the curve (AUC), ranges from 0.5 to 1.0, with a 0.5 meaning that the test is no better than a coin flip at delineating the outcome of interest. An AUC of 1.0 has perfect association with the outcome of interest (*e.g.*, a PCa biomarker with an AUC of 1.0 suggests a positive test would always correlate with clinically significant cancer at biopsy). Another useful statistical tool to estimate if a test has clinical utility is the decision curve analysis (DCA) [14]. DCA is a method to evaluate the benefits of a diagnostic test across a range of patient preferences (accepting risk of under treatment to overtreatment) to facilitate decisions about test selection and use. DCAs are usually graphed with the *x*-axis being the range of threshold probabilities and the *y*-axis being the test's net benefit. Both AUC and DCA are metrics commonly reported in studies evaluating the utility of PCa biomarkers and other medical tests aimed at improving the detecting of clinically significant disease such as magnetic resonance imaging fusion prostate biopsies [15—18].

A PCa diagnostic biomarker's clinical utility is not solely dependent on its statistical properties. First, a biomarker's performance must be seen in the context of already pre-existing multivariable clinical predictors such as Prostate Cancer Prevention Trial (PCPT), European Randomized Screening for Prostate Cancer (ERSPC), Chinese Prostate Cancer Consortium, and Korean Prostate Cancer Risk Calculators [19—22]. In order to be clinically useful, a biomarker must improve the diagnostic accuracy beyond the basic clinical factors that are included in these risk-calculators.

For the purpose of this review, we report available parameters of the diagnostic biomarkers that are currently available in clinical practice. We focus on NPV, AUC, and DCA in comparing reflexive tests due to their relevance in evaluating diagnostic screening tests. We also (1) engage in a critical assessment of the clinical utility of each biomarker, and (2) evaluate the benefit of testing relative to cost.

3. Evidence acquisition

Studies published after the year January 2000 and up to October 2020 were identified by electronic search of Medline (through PubMed) and Google Scholar. Keywords included "prostate cancer", "biomarker", "screening", and variations of known biomarkers including "PHI", "4Kscore",

"PCA3", "ExoDx", "SelectMDx", and "MiPS". References from the most recent relevant review literature that was not initially found in our search were utilized as well, in addition to references obtained from experts within the field. Studies from manufacturer websites were also evaluated to confirm the validity of the studies that were used.

4. Review of available biomarkers

A summary of the available serum and urine biomarkers is available in Table 1. The cost of each test was obtained from the 2021 Medicare Fee Schedule [23]. Due to the clinical and racial differences between patients in Western and Asian countries, whenever possible, we included studies from multiple regions and highlighted important differences [24].

5. Serum biomarkers

5.1. Prostate health index (PHI)

PHI is a serum test that uses total PSA (tPSA), free PSA (fPSA), and the [-2]proPSA isoform to calculate a risk score both clinically significant PCa and any PCa. The test is approved by US Food and Drug Administration (FDA) for men over the age of 50 years with no previous diagnosis of PCa, a benign digital rectal exam (DRE), and a total PSA of 4–10 ng/mL. PHI was been validated in multicenter prospective trials in North America and Europe [25–27]. The different PHI ranges in Western countries include 0–26.9, 27.0–35.9, 36.0–54.9 and 55+, which result in 9.8%, 16.8%, 33.3%, and 50.1% probabilities of detecting any PCa respectively [25,26,28].

Loeb et al. [29] found that in a cohort of men with prebiopsy PSA of 4.0–10.0 ng/mL, when using a PHI threshold of 27, the NPVs of finding any PCa and clinically significant PCa (Grade Group [GG] $\geq$2) were 89% and 97% respectively. In this study, the AUC of PHI alone was 0.708 for PCa overall and 0.707 for clinically significant PCa. The investigators found that the PHI score outperformed its individual components (total, fPSA and [-2]proPSA) in AUC for the detection of clinically significant PCa.

Two published studies have evaluated the impact of PHI on existing risk calculators. In comparing the PCPT risk calculator, PHI improved the AUC for detecting clinically significant PCa from 0.577 (PCPT only) to 0.697 ($p<0.001$) [29]. Foley and colleagues [30] similarly found that PHI incrementally improved the AUC of PCPT for predicting the presence of clinically significant PCa (AUC: 0.720 to 0.790, $p<0.01$). DCAs in both studies demonstrated the superior net benefit of including PHI to the risk calculators.

In a study of 569 Chinese men aged 55–75 years with PSA of 4–10 ng/mL with non-suspicious DRE, Chiu et al. [31] demonstrated that PHI had a higher AUC than PSA when both models were combined with prostate volume and age. This was true for any PCa or clinically significant PCa (0.78 *vs.* 0.71 and 0.83 *vs.* 0.70). In a multi-institutional study including both European and Asian sites, Chiu et al. [32] showed that at a PHI cut-off of <25 and the sensitivity for ruling clinically significant PCa, were 99% and 96% for Europeans and Asians respectively. Given the significantly lower rate of clinically significant PCa detection at a lower reference range in Asians compared to Europeans, the authors suggested that different PHI cut-offs should be used for Asians.

5.2. OPKO 4Kscore

The 4Kscore provides a risk score based on tPSA, fPSA, intact PSA (iPSA), and human kallikrein 2 (HK2). The test is currently under FDA review. The 4Kscore® panel provides a score of 0%–100%, which reflects the estimated probability that a patient will have clinically significant PCa on biopsy based upon the four serum markers and the patients clinical factors [33]. The 4Kscore itself is essentially

Table 1 The biomarkers as screening tools.

Biomarker	Provider	Source biomaterial	Certification	Outcome	Cut-off	NPV for CS PCa	AUC for CS PCa	NCCN	Cost (USD)
PSA	N/A	Blood	FDA	>0	None	85% at 4 ng/mL	0.577–0.767	-Multiple scenarios	$19
PHI	Beckman Coulter	Blood	FDA	0–55+	NR	97% at 27	0.707–0.790	-Consider	$499
4Kscore	OPKO	Blood	CLIA	0–100%	>7.5%	N/A	0.720–0.870	-Consider	$1185
PCA3	Progensa Hologic	Urine	FDA	0–100+	>25	98%–99% at 21	0.706–0.800	-Neg prior bx	$255
ExoDx	Exosome Diagnostics	Urine	CLIA	0–60+	>15.6	89%–98%	0.700–0.803	-Consider	$760
SelectMDx	MDxHealth	Urine	CLIA	0–100%	−2.8	94%–95%	0.672–0.850	-Investigational	$500
MiPS	Michigan Labs	Blood and urine	CLIA	0–100%	NR	90% for any PCa	0.779	-Investigational	$760

PSA, prostate-specific antigen; FDA, Food and Drug Administration; CLIA, Clinical Laboratory Improvement Amendments under Center of Medicare and Medicaid Services; NPV, negative predictive value; CS, clinically significant; PCa, prostate cancer; NCCN, National Comprehensive Cancer Network; USD, United States dollars; PCA3, prostate cancer antigen 3; PHI, prostate health index; MiPS, MiProstate Score; N/A, not applicable; NR, no recommended cut-off.

a personalized risk calculator as 100% minus the 4Kscore is the NPV [33].

Benchikh et al. [34] compared a "base" clinical predictive model routinely used in clinical practice (*i.e.*, age, PSA, and DRE) to a "full" model that included 4Kscore in a cohort of men from the ERSPC screening trial. The base model had an AUC of 0.767 (95% confidence interval [CI], 0.687—0.847) in predicting clinically significant PCa which improved to 0.870 (95% CI, 0.807—0.933) when the 4Kscore was incorporated. The authors found that they could reduce the number of biopsies in their cohort by 50% by applying a rule to only biopsy men with 20% or higher risk from their model. This strategy would miss 12 clinically significant cancers for every 1000 men with a clinical indication for biopsy [34].

A multi-institutional study that included 1012 men from 26 US centers compared the 4Kscore to a modified PCPT risk calculator [33]. The 4Kscore had an AUC of 0.820 compared to 0.740 for the modified PCPT risk calculator ($p<0.001$) in predicting clinically significant PCa. The investigators found that 30% of biopsies could have been avoided using a 4Kscore <6% to forego biopsy. This strategy would miss clinically significant cancer in 13 men (1.3% of studied men) [33]. In this study and a separate study using the Prostate Testing for Cancer Treatment (ProtecT) data, the 4Kscore had a greater than twofold net benefit when compared to the clinical risk calculator [33,35].

Darst et al. [36] studied the 4Kscore in a multiethnic population of African-Americans, Latinos, Japanese, Native Hawaiian, and Caucasian men and found that the 4Kscore alone was better at detecting aggressive cancer (defined as Gleason score 8 or higher, non-localized disease, or PCa death) and non-aggressive cancer than PSA alone. The benefit of the 4Kscore was most notable in detecting aggressive cancer in Japanese (AUC 0.805 [95% CI: 0.762—0.847] *vs.* 0.682 [95% CI: 0.630—0.734]), Latino (AUC 0.807 [95% CI: 0.750—0.864] *vs.* 0.692 [95% CI: 0.622—0.763]), and Native Hawaiian patients (AUC 0.929 [95% CI: 0.871—0.988] *vs.* 0.750 [95% CI: 0.634—0.866]). One limitation of this study, however, is that all of these patients, although different ethnicities, lived in the US, either Hawaii or Los Angeles.

6. Urinary markers

6.1. Prostate cancer antigen 3 (PCA3)

PCA3 is a gene that expresses a non-coding RNA that is significantly overexpressed by PCa cells [37]. The PCA3 assay measures voided mRNA copies of *PCA3* following a digital rectal examination, and reports a ratio of *PCA3*:*PSA* mRNA in the urine [38]. PCA3 was originally FDA-approved for men with a prior negative biopsy and no evidence of atypical small acinar proliferation (ASAP). The PCA3 assay provides a score between 0 and >100. Lower scores correlate with a decreased likelihood of cancer at biopsy. Based on prior studies, different thresholds such as 20, 25, and 35 have been recommended to decide whether a patient needs a biopsy but in actual clinical practice, PCA3 is best used as a continuum of risk [38—40]. Although PCA3 was FDA-approved for men with prior negative biopsy, it has been studied in biopsy-naïve men as well [41—43].

The NPV for PCA3 in ruling out clinically significant PCa on initial biopsy is reported to be as high as 98%—99% when using 20 as a cut-off [42,44]. The AUCs under the same criteria range from 0.78 to 0.83. Scattoni et al. [41] found that in biopsy naïve men, adding PCA3 to a model including PSA, fPSA, and prostate volume did not improve predicting accuracy (AUC 0.79 *vs.* 0.80, $p=0.690$) of detecting clinically significant PCa. Chevli et al. [45] found that in an analysis of 3073 men who underwent PCA3 prior to initial biopsy, PCA3 alone did not outperform PSA alone in prediction of clinically significant PCa (AUC 0.682 *vs.* 0.679, $p=0.702$), although it was better at detecting any PCa (AUC 0.697 *vs.* 0.599, $p<0.01$).

In a study of 500 Chinese men undergoing initial prostate biopsy, Wang et al. [46] showed that in men with PSA 4—10 ng/mL, the AUC of PCA3 was higher than PSA (0.750 *vs.* 0.614, *p*-value not provided) but not higher than PSA density (0.750 *vs.* 0.718, $p=0.590$). PCA3 did not perform better than PSA, %fPSA or PSA density in men with PSA >10 ng/mL. Ochiai et al. [47] showed similar findings in their study of 647 Japanese men. In men with PSA of 4—10 ng/mL, AUCs of PSA, fPSA/tPSA, PSA density, and PCA3 were 0.557, 0.647, 0.692, and 0.742 respectively. Although there was a significant difference between PCA3 and fPSA/tPSA ($p<0.05$), there was no difference between PCA3 and PSA density. Given that only 4% of men with PSA density <0.15 and PCA3 <20 had PCa, the authors concluded that using a combination of PCA3 with PSA density (not PCA3 alone) might be useful for selecting patients who could avoid an unnecessary biopsy.

Nevertheless, subsequent studies did show PCA3's predictive value. In a study of men undergoing initial biopsy ($n = 562$), adding PCA3 to the PCPT risk calculator improved prediction of clinically significant cancer compared to the PCPT risk calculator alone (AUC 0.780 *vs.* 0.740, $p<0.003$) [48]. In this study, the authors found that a PCA3 threshold of 20 would result in avoiding 41% of initial biopsies, while missing 31 men with clinically significant PCa (20.1% of clinically significant PCa, 5.5% of studied men). Hansen et al. [42] demonstrated that a PCA3 model using a cut-off of 21 added predictive value to their base model which included patient characteristics such as age, PSA, prostate volume, and DRE when detecting clinically significant PCa (AUC 0.829 *vs.* 0.775, $p<0.001$). In their DCA, when using a cut-off of 21, adding PCA3 to the model had a higher net benefit for all threshold probabilities >18%.

6.2. ExoDx prostate intelliscore (EPI)

EPI measures the exosomal RNA of *ERG* (ETS-related gene) and *PCA3* normalized to *SPDEF* (SAM pointed domain-containing Ets transcription factor) in voided urine without a prior DRE. Exosomes are small vesicles filled with cellular protein and RNA that are secreted from cells. They are useful in profiling RNA expression from tumor cells because they are very representative of their cell of origin [38,49]. There are no specific patient characteristic indications for EPI but it has mainly been studied in the biopsy naïve population [49—51]. It is not FDA-approved. Although this test is not a binary test, it does have a validated cut-off of 15.6 [49].

Its NPV for ruling out clinically significant PCa is reported to be 89%—98% [50,51]. Donovan et al. [51], in a retrospective analysis of 195 men with PSA 2—10 ng/mL and without prior biopsy, showed that using ExoDx improved predictive accuracy when added a baseline model which included PSA and DRE (AUC 0.803 [95% CI: 0.729—0.877] *vs.* 0.672 [95% CI: 0.577—0.768]). In a prospective study of 503 patients, McKiernian et al. [50] found that EPI alone, AUC 0.700, performed better than both the PCPT (AUC 0.63, $p=0.02$) and ERSPC (AUC 0.69, $p=0.001$) risk calculators in predicting clinically significant PCa. A cut-point of 15.6 would have avoided 26% of unnecessarily prostate biopsies, missing 7% of clinically significant PCa. The DCA demonstrated a net benefit of EPI as well compared to the risk calculators.

6.3. SelectMDx

SelectMDx is a urine biomarker measuring the expression of two mRNAs (homeobox C6 [*HOXC6*] and distal-less homeobox 1 [*DLX1*]) on urine sampled after a DRE and prostate massage in biopsy-naïve men [52]. SelectMDx is also currently not approved by the FDA. Its NPV in ruling out clinically significant PCa has been reported to be 94%—95% [53,54]. This two gene test was validated by Haese et al. [53] in 916 biopsy naïve men with a PSA less than 10 ng/mL. They demonstrated at the optimal risk score cut-off of −2.8. In this study, SelectMDx with the PCPT risk calculator had an AUC of 0.850 (95% CI: 0.830—0.880) compared to the AUC of 0.760 (95% CI: 0.720—0.800) for PCPT risk calculator alone in detecting clinically significant PCa. In men with PSA <10 ng/mL, a negative test would avoid 44% of biopsies, while missing 13% of patients with clinically significant disease (2.4% overall). DCA demonstrated a net benefit of adding SelectMDx. Van Neste et al. [54] showed that adding SelectMDx to the PCPT risk calculator had a significantly higher predictive accuracy than the risk calculator alone (AUC 0.900 *vs.* 0.770, $p<0.001$). Their DCA showed that adding SelectMDx to the PCPT compared to the risk calculator alone resulted in the largest net benefit in terms of accurately detecting men with clinically significant PCa.

7. Combined serum and urine

7.1. Transmembrane protease serine 2 (*TMPRSS2*):*ERG* gene fusion via Mi-Prostate Score (MiPS)

The gene fusion of *TMPRSS2* and *ERG* creates a overexpression of the *ERG* oncogene driven by androgens [55]. This *TMPRSS2*:*ERG* gene fusion occurs frequently in PCa carcinogenesis [55]. The MiPS adds *TMPRSS2*:*ERG* mRNA (in post-DRE urine) to PCA3 and serum PSA to create a risk score. Its NPV for ruling out any PCa is 90% but its NPV in ruling out clinically significant PCa is unknown, although it is likely higher than 90% [56]. Tomlins et al. [57] showed that MiPS with the PCPT risk calculator had superior predictive accuracy compared to the PCPT risk calculator alone, demonstrating an AUC of 0.779 *vs.* 0.707 ($p<0.001$) for detecting clinically significant PCa. They found that a MiPS threshold of <15% would have avoided 36% of biopsies while missing 19 clinically significant PCa (8.5% of clinically significant PCa; 1.6% of studied men). On DCA, there was a clear net benefit of MiPS relative PCPTRC for detection of clinically significant PCa.

In Europe, Leyten et al. [58], in a study with 443 men undergoing biopsy for PSA >3 ng/mL, found that adding the MiPS score to the ERSPC risk calculator increased the predictive accuracy for any PCa. They did not calculate AUC for MiPS predicting clinically significant PCa but did show that *TMPRSS2—ERG* was a significant predictor for Gleason score, although PCA3 was not. The investigators also showed that combining PCA <25 and *TMPRSS2*:*ERG* <10 would have avoided 35% of biopsies while missing 11 cases of clinically significant PCa (9.6% of clinically significant PCa; 2.4% of studied men). Both of these studies suggested that MiPS has utility in reducing biopsies without missing many cases of clinically significant PCa.

It is important to note that the prevalence of *TMPRSS2*:*ERG* gene fusion varies significantly based on race. For example, Magi-Galluzzi et al. [59] showed that the gene fusion was present in 50% of Caucasians, 31.3% of African-Americans, and 15.9% of Japanese patients ($p=0.003$). Korean patients had the gene fusion present 21% of the time while the fusion was present in 46% of Northern Chinese and 78% of Southern Chinese patients. It is not understood how the variable rates of gene fusion prevalence will affect how MiPS is used in non-Western patients. Nevertheless, it may be important for providers to consider their patients' ethnicities prior to using MiPS as a diagnostic test.

8. Comparative effectiveness of tests

8.1. PHI *vs.* 4Kscore

A single study has performed a head-to-head comparison of PHI and the 4Kscore. Nordström et al. [60] performed this comparative analysis in a cohort of 531 men undergoing first-time biopsy for a PSA 3—15 ng/mL in Sweden from 2010 to 2012. The PHI and 4Kscore had AUCs of 0.71 (95% CI: 0.66—0.76) and 0.72 (95% CI: 0.67—0.78), respectively. Using a cut-off of 39 for PHI and 10% for 4Kscore, both tests would have spared 30% of biopsies, while missing 9.8%—10.5% of clinically significant cancers (2.6%—2.8% of studied men) [60]. On DCA, both had a mild net benefit relative to clinical models for detecting clinically significant PCa.

8.2. PHI *vs.* PCA3

In a head-to-head analysis, Seisen et al. [61] found that PHI was superior to PCA3 in predicting clinically significant PCa—defined as GG ≥ 2, positive biopsy cores >3, or >50% cancer involvement in any core in this study—among 138 biopsy-naïve men with an elevated PSA (4—20 ng/mL) and/or an abnormal DRE (AUC 0.550 *vs.* 0.800, $p=0.03$). PCA3 was, however, found to be more accurate for detecting any PCa when compared to PHI (AUC 0.710 *vs.* 0.650, $p=0.03$) [61]. Scattoni et al. [41] showed that PHI was more accurate than PCA3 in detecting PCa in patients without prior biopsies (AUC 0.69 *vs.* 0.57) although this difference was not statistically significant. They

demonstrated that adding PHI to a base multivariate model increased predictive accuracy by 5% (0.79 *vs.* 0.84) whereas adding PCA3 did not (0.79 *vs.* 0.80). In another study, Ferro et al. [43] did not find a difference between PHI and PCA3 in predictive accuracy for PCa (PHI AUC 0.77 [95% CI: 0.72—0.83] *vs.* PCA3 AUC 0.73 [95% CI: 0.68—0.79]).

8.3. 4Kscore *vs.* SelectMDx

A prospective study of 128 patients with no prior biopsy and elevated PSA, compared 4Kscore with SelectMDx head-to-head [62]. It showed that the AUC for the 4KScore and SelectMDx to detect clinically significant PCa were 0.830 (95% CI: 0.710—0.949) and 0.672 (95% CI: 0.517—0.828; $p=0.036$), respectively. There was significant discordance (46%) between the two tests as well given a kappa coefficient of 0.184 using the 7.5% cut-off.

9. Economic evaluation

An important consideration in evaluating reflexive testing is the tradeoff between cost and efficacy of each biomarker. This assessment is traditionally performed using a cost-effectiveness analysis framework. Teoh et al. [63] conducted a simulation study to assess the cost-effectiveness of PHI in a cohort of Chinese men by comparing a PSA-based strategy (*i.e.*, offering biopsy for all patients with PSA 4—10 ng/mL) to a PHI-based strategy (*i.e.*, offering PHI to patients with PSA 4—10 ng/mL and offering a biopsy if PHI was >35.0). Using a Markov model of 25 screening cycles for men aged 50—75 years old with a negative DRE, the total cost per man using the PSA strategy was $27 439 *vs.* $22 877 (using 2019 US dollars) for the PHI strategy. The PHI strategy also had an expected gain of 0.35 quality-adjusted life years (QALYs).

Nichol et al. [64] showed similar findings in the US when comparing a PHI-based strategy to PSA alone. The model included men aged 50—75 years old and with a negative DRE. A Markov model of 25 screening cycles was used. A PHI was performed if PSA was either 2—10 ng/mL or 4—10 ng/mL. A patient was offered biopsy if the PHI score was >25. When the PSA range of 2—10 ng/mL was used, $1199 was saved and when the PSA range of 4—10 ng/mL was used, $443 was saved. There was an expected gain of 0.08 and 0.03 QALYs respectively.

Voigt et al. [65] compared a PSA screening strategy to 4Kscore screening strategy (cut-off 7.5%) in a simulated cohort of 100 000 men. Their cohort was modeled to be similar to Parekh et al.'s 4Kscore validation study [33] with 90% of patients aged 50—75 years old and 50%—60% of patients with PSA 4—10 ng/mL. They showed a cost savings of $169 million (2015 US dollars) if 4Kscore was used to help decide who should get prostate biopsy.

Govers et al. [66] studied the cost-effectiveness of SelectMDx in a population of American men with elevated PSA or abnormal DRE. In their Markov model, if SelectMDx was negative, no prostate biopsy was done and estimates of PCa specific mortality were based on the SPCG-4 study. They estimated that 311 879 men per year were to undergo prostate biopsy to detect localized PCa. They concluded that incorporating SelectMDx over an 18-year horizon would result in a cost-saving of $1694 (2015 US dollars) per patient with an average of 0.045 QALY gained [66].

Sathianathen et al. [67] did a cost-effectiveness analysis of PHI, 4Kscore, SelectMDx, and EPI in men with elevated PSA considering biopsy. Their model included men aged 50 years old with elevated PSA (3 ng/mL or greater). Biopsy was triggered when PHI was >24, 4Kscore risk was 7.5% or higher, EPI was 15.6 or higher or if SelectMDx was −2.8 or greater. The Markov model was run for 10 000 iterations. When compared to PSA, the EPI strategy (cost $3649) provided the highest QALY gain at 0.018 compared to 0.017 for 4Kscore (cost $4102), 0.014 for SelectMDx (cost $3442), and 0.009 for PHI (cost $3531). The total cost of the PSA strategy was $3863. The authors concluded that all of the studied biomarkers, except 4Kscore, were cost effective with EPI and SelectMDx being the most cost effective [67].

10. Discussion

In our analysis of the literature, we find that currently available reflex biomarkers largely provide some incremental value in predicting men who are at risk for any or clinically significant PCa. In general, the AUC ranges from 0.70 to 0.80 with NPV of 89%—99%. These tests are not perfect and have the potential to miss anywhere from 5% to 10% of clinically significant PCa. This is at the benefit of potentially avoiding biopsy in 20%—30% of men with elevated or rising PSA. These tests are more expensive than PSA but there is some evidence to suggest that they can lower health care expenditures on diagnosing PCa by reducing the number of biopsies.

Many of the studies evaluating these biomarkers were done in Western countries. This is a limitation that needs to be taken into account when using these biomarkers in Asian patients given that PCa incidence and mortality are different in Asian patients [10]. For example, Chiu et al. [32] demonstrated that higher PHI cut-off scores might be necessary rule out PCa in Chinese patients. Asian countries use different PCa risk calculators because Western calculators will overestimate PCa in Asian populations [24]. Reflexive biomarker-based risk tools may require adjustment for race/ethnicity or geography adjustment to account for differences in disease prevalence and aggressiveness.

These tests, even when combined with a pre-existing risk calculator like the PCPT risk calculator, can still miss anywhere from 5% to 10% of clinically significant PCa. Thus, these biomarkers may not provide enough confidence to convince a provider or patient to forgo a prostate biopsy in certain scenarios. Even after obtaining these biomarkers, certain higher risk patients or those who really wish to avoid biopsy may choose to obtain a multiparametric magnetic resonance imaging (mpMRI) prior to a definitive biopsy decision. The most recent NCCN guidelines for prostate cancer early detection [68] suggest that providers can consider these biomarkers and/or mMRI in men with PSA>3 ng/mL and/or suspicious DRE. This is because mpMRI-ultrasound fusion biopsy has been shown to more accurately find clinically significant PCa for men with no prior biopsies or prior negative biopsies [15,69—71]. mpMRI when combined with the ESPRC risk calculator had an AUC of 0.85 for detecting clinically significant cancer. Using mpMRI could avoid 36% of

unnecessary biopsies while missing only 4% of clinically significant cancers [72]. However, the role of mpMRI as a triage test to avoid prostate biopsy is unclear. Ahmed et al. [73] in the PROMIS trial studied the accuracy of 1.5 T mpMRI in ruling out clinically significant PCa in 576 men. Their subjects underwent an mpMRI and then both a transperineal and transrectal biopsy in the same setting. When defining clinically significant PCa as GG $\geq$2, mpMRI's NPV (using Prostate Imaging Reporting & Data System [PI-RADS] $\geq$3) for ruling out clinically significant PCa was 76%. Their study showed that using mpMRI to triage men could allow 27% of patients to avoid a primary prostate biopsy.

The most recent NCCN guidelines state that it is still not known how these novel biomarkers can be applied in optimal combination with MRI [68]. Nevertheless, incorporating the newer prostate biomarkers and mpMRI into predictive algorithms like the PCPT and EPSRC risk calculators is likely necessary when deciding whether a patient can forego prostate biopsy. There are already data that combining biomarkers with mpMRI increases diagnostic accuracy. For example, Hsieh et al. [74] in a prospective study combined PHI with 3 T mpMRI to detect clinically significant PCa in 102 men. The AUC of combining PHI and mpMRI was higher than PHI alone (0.873 *vs.* 0.735, $p=0.002$) and mpMRI alone (0.873 *vs.* 0.830, $p=0.035$). When using a threshold of PI-RADS $\geq$3 and PHI $\geq$30, the authors concluded that 50% of biopsies would have been avoided while missing only 4.2% of clinically significant PCa. Using PHI$\geq$30 alone would have avoided 35.3% of biopsies while missing 8.3% of clinically significant PCa. In a similar study, Druskin et al. [75] studied the combination of PHI density with mpMRI in 241 men. Subjects had an elevated PSA, negative DRE and no prior diagnosis of PCa. PHI density alone had an AUC of 0.78 while a model including PHI density with mpMRI showed an AUC of 0.90. They showed that 100% of men who had an MRI with PI-RADS $\geq$3 or PI-RADS $\leq$2 with a PHI density $\geq$44, had clinically significant PCa.

The main downside to using these novel biomarkers as screening tools is cost. PSA remains in use because it is familiar to most physicians and it is relatively inexpensive. These biomarkers generally cost at least four times as much as a PSA, which costs roughly $20 (Table 1). Despite the initial higher cost, there is some evidence that these diagnostic biomarkers may actually save patients and the healthcare system money in the long run in the diagnosis component of PCa care. If a patient is successfully able to avoid a prostate biopsy and all of the negative consequences that may result from treating clinically insignificant cancer, the upfront cost of a novel biomarker would be worth it. Patients may be willing to pay for a more expensive test initially if it will truly help prevent future unnecessary tests and treatment. However, the studies that look at the costs of these tests often do not take into the account the costliness of missing a clinically significant cancer. Even missing a small percentage of clinically significant cancer is incredibly costly to the patient and healthcare system. Nevertheless, it is also possible that as the cost of these tests approaches the cost of PSA, these new biomarkers could potentially supplant PSA as the initial PCa detect screening test. Similarly, mpMRI is not used for screening due to its cost as Kim et al. [76] estimated that widespread adoption of mpMRI prior to biopsy would cost $3 billion annually, roughly 15% of the entire cost of managing PCa. mpMRI also has known issues with interpreter variability, and therefore may not be as readily reproducible as biomarker laboratory results [77]. Until the cost of biomarkers and mpMRI decreases, the best way to use biomarkers and mpMRI is in a stepwise process, as suggested by the NCCN guidelines, based on risk stratification.

11. Future directions

As more than 200 PCa risk-associated single-nucleotide polymorphisms (SNPs) have been identified through large genome-wide association studies (GWASs), more SNP-based PCa tests are being developed [78,79]. Although individual SNPs by themselves do not have significant disease predictive value, when combined, polygenic SNPs panels can be a useful in risk stratifying individuals that are likely to have PCa. For example, Illumnia's OncoArray is a saliva-based test which contains about 80 000 PCa-specific markers [80]. In an analysis of more than 140 000 men of European ancestry, Schumacher et al. [78] identified 63 new PCa susceptibility loci. Using Oncoarray, they found the 1% of men who were at highest risk of developing PCa. These men were 5.7 times more likely than the general population to develop PCa. The top 10% of men more likely to develop cancer were 2.7 times more likely than the general population. Oncoarray is not commercially available yet but one important selling point for this test, per Schumacher, is that it will likely cost roughly $100. Ambryscore, another polygenic SNP panel from Ambrygen, can be used in male patients, 18—84 years old, and Northern European in ancestry with no personal or family history of a mutation in a PCa susceptibility gene. It uses 72 SNPs associated with PCa risk [81,82]. It is not offered as a stand-alone test but can be added onto any one of Ambrygen's multigene panels: ProstateNext, CancerNext, CancerNext Expanded, and CustomNext-Cancer [79,83].

Since these tests have been studied mainly in people of mainly European descent, more validation needs to be done with patients of other ethnicities. Studies investigating their clinical utility are limited. Few clinicians actually know how to use the information effectively. Nevertheless, polygenic SNP panels show promise in helping determine whether patients should undergo prostate biopsy or not. As they become more commercially available, they might be used in conjunction with or instead of pre-existing blood/urine biomarkers if they improve diagnostic accuracy. Although the pricing of these tests will likely fluctuate, a study demonstrated that the diagnostic benefit that SNP panels provide may prove cost-effective by decreasing PCa over-diagnosis [84].

IsoPSA is a newer serum test that determines risk for clinically significant PCa based on looking at specific isoforms of PSA that are linked to PCa in an aqueous 2-phase reagent system. It was first investigated by Klein et al. [85] who found that in a multicenter prospective study of 261 patients, IsoPSA alone had an AUC of 0.81 in detecting clinically significant cancer. The NPV at a cut-off of $\geq$17 was 96%. In a multicenter prospective validation study of 271 patients, Stovsky et al. [86] determined the AUC of IsoPSA alone for detecting clinically significant PCa to be 0.784 with the NPV being 93%. They estimate in a 1000-person cohort, 43% of

men would avoid unnecessary biopsy while missing only 2.2% of clinically significant cancer. Given this higher accuracy, IsoPSA is a promising test warrants further consideration as a more widely used biomarker.

The FDA recently approved new set of urine-based "liquid biopsy" tests called the miR Sentinel Tests. The Sentinel PCa tests finds patients with any PCa while the Sentinel CSTest differentiates patients from GG1 and GG2-5 PCa. Finally, Sentinel HG test differentiates patients with GG1-GG2 PCa from GG3-5 [87]. Wang et al. [87] used the assay, which extracts small noncoding RNAs (sncRNAs) from urinary exomes, to detect GG$\geq$2 with a sensitivity and specificity of 93% and 90% respectively with an NPV of 92%. The test is also able to differentiate between PCa *vs.* no cancer and GG1-2 *vs.* GG3-5 PCa with high accuracy. While still in their early stages of clinical testing, these non-invasive screening tests are very promising and warrant further investigation.

12. Conclusion

There are many PCa biomarker tests and they are roughly equivalent in predictive accuracy. The added benefit of each of these biomarkers is not incredibly substantial but still adds some clinical utility. Using the tests in a binary fashion should be avoided. Urologists will need to decide how to use the tests to determine probability as the current suggested cut-offs may not be sensitive enough. These biomarkers should also be understood in the context of the pre-existing risk predictors, the patient's ethnicity, life expectancy and quality-of-life goals, and cost. They may be cost effective and provide increased quality of life if they are truly able to avoid unnecessary biopsy and treatment.

mpMRI is a promising tool in prostate cancer diagnostics. The optimal combination of mpMRI with these biomarkers is unknown but may be complementary. Polygenic SNP panels are promising, emerging technology but do not have significant clinical utility yet. IsoPSA, a new serum test, and the miR Sentinel tests, a new set of FDA-approved urine liquid biopsies, are both promising. They show high sensitivity and specificity in detecting clinically significant PCa, warranting prospective investigation. IsoPSA and the miR Sentinel tests could be utilized more regularly for prostate cancer diagnosis if they are as accurate as the initial studies suggest but further studies are needed.

Author contributions

Study design: Edward K. Chang, Adam J. Gadzinski, Yaw A. Nyame.
Data acquisition: Edward K. Chang.
Data analysis: Edward Chang, Adam J. Gadzinski, Yaw A. Nyame.
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Critical revision of the manuscript: Edward K. Chang, Adam J. Gadzinski, Yaw A. Nyame.

Conflicts of interest

The authors declare no conflict of interest.

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