Review Article

Investig Clin Urol 2021;62:1-13.
https://doi.org/10.4111/icu.20200395
pISSN 2466-0493 • eISSN 2466-054X

INVESTIGATIVE AND CLINICAL UROLOGY
ICUROLOGY

Clinical utility of current biomarkers for prostate cancer detection

Jeong Hyun Kim[1], **Sung Kyu Hong**[2]

[1]*Department of Urology, Kangwon National University School of Medicine, Chuncheon,* [2]*Department of Urology, Seoul National University Bundang Hospital, Seoul National University College of Medicine, Seongnam, Korea*

Although prostate-specific antigen (PSA) remains the most used test to detect prostate cancer (PCa), the limited specificity and an elevated rate of overdiagnosis are the main problems associated with PSA testing. Over the last three decades, a large body of evidence has indicated that PSA screening methods for PCa are problematic, although PSA screening significantly reduces PCa-specific mortality. A number of novel biomarkers have been introduced to overcome these limitations of PSA in the clinical setting. These biomarkers have demonstrated an increased ability to select patients for biopsy and identify men at risk for clinically significant PCa. Although a number of assays require further validation, initial data are promising. Forthcoming results will ultimately determine the clinical utility and commercial availability of these assays. Extensive efforts have recently been made to identify and commercialize novel PCa biomarkers for more effective detection of PCa, either alone or in combination with currently available clinical tools. This review highlights the role of existing and promising serum and urinary biomarkers for the detection and prognostication of PCa before prostate biopsy.

Keywords: Biomarkers; Diagnosis; Prostatic neoplasms

INTRODUCTION

Prostate-specific antigen (PSA) is widely accepted as a tumor marker for screening, diagnosis, monitoring, and risk prediction of prostate cancer (PCa). However, PSA has limitations as a screening biomarker owing to its low specificity and lack of sensitivity. Its positive predictive value (PPV) was shown to be only approximately 25% in a pooled meta-analysis [1], which leads to a large number of false-positive results. As a result, up to 75% of patients undergo unnecessary prostate biopsies [2]. Up to 30% of PCa cases, and among these 10% of aggressive PCa cases, are identified in patients with a normal range of PSA [3]. Conventional prostate biopsy has inherent discomfort, cost, and problems with undersampling [4], although its morbidity is low. In particular, the rate of unnecessary prostate biopsy is increased in patients with PSA levels in the range of 2.5–10 ng/mL, the so-called gray zone [2,5].

Screening for PCa remains a controversial issue, although the European Randomized Study of Screening for Prostate Cancer (ERSPC) and recent analyses from the Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial have provided evidence that PSA-based screening can significantly decrease PCa-specific mortality

Received: 1 September, 2020 • **Revised:** 9 October, 2020 • **Accepted:** 22 October, 2020 • **Published online:** 21 December, 2020
Corresponding Author: Sung Kyu Hong https://orcid.org/0000-0002-8344-6774
Department of Urology, Seoul National University Bundang Hospital, 82 Gumi-ro 173beon-gil, Bundang-gu, Seongnam 13620, Korea
TEL: +82-31-787-7343, FAX: +82-31-787-4057, E-mail: hsk823@chol.com

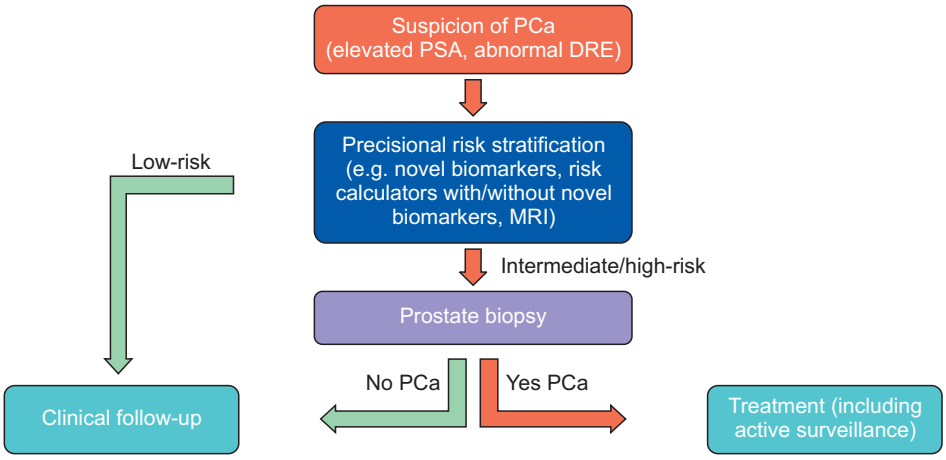

Fig. 1. Flowchart of men with elevated PSA and/or abnormal results on a DRE with a combination of risk stratification tools. PCa, prostate cancer; PSA, prostate-specific antigen; DRE, digital rectal examination; MRI, magnetic resonance imaging. Adapted from Osses DF, et al. Int J Mol Sci 2019;20:1637 [17].

[6,7]. A large proportion of PCa is latent, that is, it is never destined to progress or affect a patient's life. Approximately 50% to 60% of newly diagnosed PCa cases are at low risk for progression [8]. However, a substantial number of patients with low-risk PCa undergo some form of aggressive treatment regardless of risk. This has led to concerns regarding overdiagnosis and overtreatment. Active surveillance (AS) has emerged as an alternative treatment option for men with early-stage disease, in which any kind of definitive treatment is delayed and applied only if there is evidence of progression. Notwithstanding, detecting disease progression in a patient selected for AS remains a continuing challenge. Moreover, some patients with apparently low-risk disease actually harbor unfavorable disease owing to the inaccuracies of currently used repeat biopsy protocols [9]. The current problems inherent in the prostate biopsy procedure advocate for the development of noninvasive tools capable of predicting disease progression more accurately that are suitable for repeat measurements over time.

Refinements to the PCa diagnostic pathway focusing on the detection of only clinically significant PCa (csPCa) are needed to make the diagnostic pathway less burdensome to patients. In addition, the diagnostic pathway should be cost-effective and acceptable to the general population and health care providers [10]. Currently, many international guidelines recommend the use of risk stratification tools such as novel biomarkers, risk calculators (RCs), and magnetic resonance imaging (MRI) for the prediction of a positive prostate biopsy as reflex tests after an elevated PSA level [11-16]. This may support the process of shared, informed decision-making (Fig. 1) [17]; reduce the number of unnecessary biopsies by better identification of those men at risk for PCa; and better differentiate aggressive cancers from nonaggressive cancers.

Several biomarkers that can provide a higher degree of predictive accuracy for PCa and csPCa than currently available clinical tools are now actively investigated as novel tools to improve the detection of PCa before prostate biopsy and reduce unnecessary prostate biopsies. Table 1 summarizes the results of studies investigating the performance of serum and urinary biomarkers for the detection of PCa and reduction of unnecessary prostate biopsies. This review highlights the role of existing and promising serum and urinary biomarkers for the detection of PCa and csPCa before prostate biopsy.

PROSTATE HEALTH INDEX (PHI)

The phi is a simple blood test and a mathematical algorithm incorporating three different isoforms of PSA (total PSA [tPSA], free PSA [fPSA], and [-2]proPSA, i.e., p2PSA) combined in a mathematical formula: phi=(p2PSA/fPSA)×(tPSA)$^{1/2}$. fPSA has three subforms: benign prostatic hyperplasia-associated PSA (BPSA), inactive PSA (iPSA), and proPSA (Fig. 2) [18,19]. BPSA and iPSA are associated with benign tissues, whereas proPSA is associated with cancer [20]. It is possible to detect three truncated forms of proPSA (i.e., [-2], [-4], and [-5,-7]proPSA) in serum (Fig. 2) [19,21]. Of these three truncated forms of proPSA, p2PSA is the most stable form and is specific for PCa [20,22]. Development of the Access 2 immunoassay system (Beckman Coulter, Brea, CA, USA) by Beckman Coulter opened a new field of study for detecting PCa. Beckman Coulter developed the phi in partnership with the National Cancer Institute (NCI)'s Early Detection Research Network (EDRN), and the phi was approved by the U.S. Food and Drug Administration (FDA) in 2012.

Numerous studies [23-29] have reported that %p2PSA (i.e., the percentage of p2PSA to fPSA) and phi provide significantly better clinical performance for predicting PCa than

https://doi.org/10.4111/icu.20200395

Table 1. Studies investigating the performance of currently available serum and urinary biomarkers

Biomarker	Reference	Year	No. of patients	AUC	Result
Serum biomarkers					
PHI	Lazzeri et al. [27]	2012	222	0.67	At a phi cutoff of 28.8, 116 biopsies (52.25%) could be avoided, while 6 patients (8.4%) with PCa would have been missed. However, no patient with high-grade PCa would have been missed.
	Lazzeri et al. [28]	2013	158	0.73	At a phi cutoff of 25.5, 27 biopsies (17.2%) could be avoided, while 3 patients (4.2%) with PCa would have been missed and 2 patients (3.8%) with high-grade PCa would have been missed.
	de la Calle et al. [34]	2015	561	0.78	At a phi cutoff of 25, 40% of unnecessary biopsies could be avoided, and 25% of low-grade PCa would be reduced at the cost of missing 5% csPCa.
	Park et al. [30]	2018	246	0.76	At a phi cutoff of 22.9, 33 biopsies (21.3%) could be avoided, while 2 patients (1.3%) with PCa would have been missed. However, no patient with high-grade PCa would have been missed.
4K-score	Vickers et al. [39]	2008	740	0.83	Using a 20% risk of PCa as the threshold for biopsy, 424 (57%) biopsies could be avoided, while 31 (20.4%) of low-grade PCa and 3 (7.5%) of high-grade PCa would have been missed, respectively.
	Parekh et al. [43]	2015	1,012	0.82	Using a cutoff of 6% risk of csPCa, 30% of biopsies could be avoided, delaying diagnosis for 1.3% of patients with high-grade PCa.
	Braun et al. [44]	2016	749	0.78	Using a cutoff of 6% risk of csPCa, 17% of biopsies could be avoided, delaying diagnosis for 3.8% of patients with high-grade PCa.
Urine biomarkers					
PCA3	Marks et al. [51]	2007	226	0.68	Using a PCA3 cutoff of 35, the PCA3 assay had a sensitivity of 58%, a specificity of 72%, and an OR of 3.6. At a PCA3 cutoff of less than 20, the NPV was 0.88 in men undergoing repeat prostate biopsy.
	Seisen et al. [54]	2015	138		Phi outperformed PCA3 for detecting csPCa (AUC, 0.80 vs. 0.55; p=0.03) in a comparative study.
	Cantiello et al. [55]	2015	156		Both phi and PCA3 significantly improved the predictive accuracy for the endpoint of extracapsular tumor extension. However, only phi provided significant incremental predictive accuracy for the prediction of tumor volume >0.5 mL, pathologic GS ≥7, seminal vesicle invasion, and composite endpoint of csPCa.
TMPRSS2:ERG	Tomlins et al. [58]	2011	606		*TMPRSS2:ERG* score is associated with indicators of csPCa at biopsy and prostatectomy, including tumor volume, high pathologic GS, and Gleason upgrading.
	Salami et al. [61]	2013	45		The combination of PCA3 and *TMPRSS2:ERG* can improve their ability to predict results of prostate biopsy (AUC=0.88; specificity=90% at 80% sensitivity).
	Leyten et al. [62]	2014	443		The AUC of ERSPC-RC increased from 0.799 to 0.842 when PCA3 and *TMPRSS2:ERG* scores were added. In multivariable logistic regression analyses, only *TMPRSS2:ERG* added significant predictive value to ERSPC-RC for predicting biopsy GS (OR, 7.16; p<0.001) and clinical tumor stage (OR, 2.60; p=0.023), whereas PCA3 did not.
	Tomlins et al. [63]	2016	1,244		The AUC of PCPT-RC increased from 0.639 to 0.762 after adding both PCA3 and *TMPRSS2:ERG* scores for predicting PCa. The AUC was also increased to 0.779 for predicting high-risk PCa, when both markers were added.
EPI test	McKiernan et al. [67]	2016	519	0.73	At a predetermined cutoff of 15.6, EPI test yielded an NPV of 91% and a sensitivity of 92%, with 27% of patients having an EPI score below the cutoff. Applying a cutoff from the training cohort to serve as a threshold for biopsy in the validation cohort decreased unnecessary biopsies by 27% of patients, while missing only 8% of high-grade cancers.
	McKiernan et al. [68]	2018	503	0.70	A validated cutoff of 15.6 would avoid 26% of unnecessary prostate biopsies and 20% of total biopsies, with NPV of 89% and missing 7% of high-grade PCa. An alternative cutoff of 20 would avoid 40% of unnecessary biopsies and 31% of total biopsies, with NPV of 89% and missing 11% of high-grade PCa.
SelectMDx	Van Neste et al. [71]	2016	905	0.76	When this gene expression was combined with PSA, PSAD, DRE, previous negative prostate biopsies, age, and family history in a multimodal model, the overall AUC was 0.90 in the training set and 0.86 in the validation set. A total reduction of biopsies by 42% and a decrease of unnecessary biopsies by 53% were observed in this model, with an NPV of 98% for high-grade PCa.
	Hendriks et al. [72]	2017	172	0.83[a]	SelectMDx scores are significantly higher in patients with suspicious lesions in mpMRI (p<0.01), with an AUC of 0.83 for the prediction of mpMRI outcome.

AUC, area under the curve; PHI, Prostate Health Index; PCa, prostate cancer; csPCa, clinically significant prostate cancer; OR, odds ratio; NPV, negative predictive value; GS, Gleason score; ERSPC-RC, European Randomized Study of Screening for Prostate Cancer - risk calculator; PCPT-RC, Prostate Cancer Prevention Trial - risk calculator; EPI, ExoDx Prostate Intelliscore; PSA, prostate-specific antigen; PSAD, prostate-specific antigen density; DRE, digital rectal examination; mpMRI, multiparametric magnetic resonance imaging.
[a]:An AUC for the prediction of mpMRI outcome.

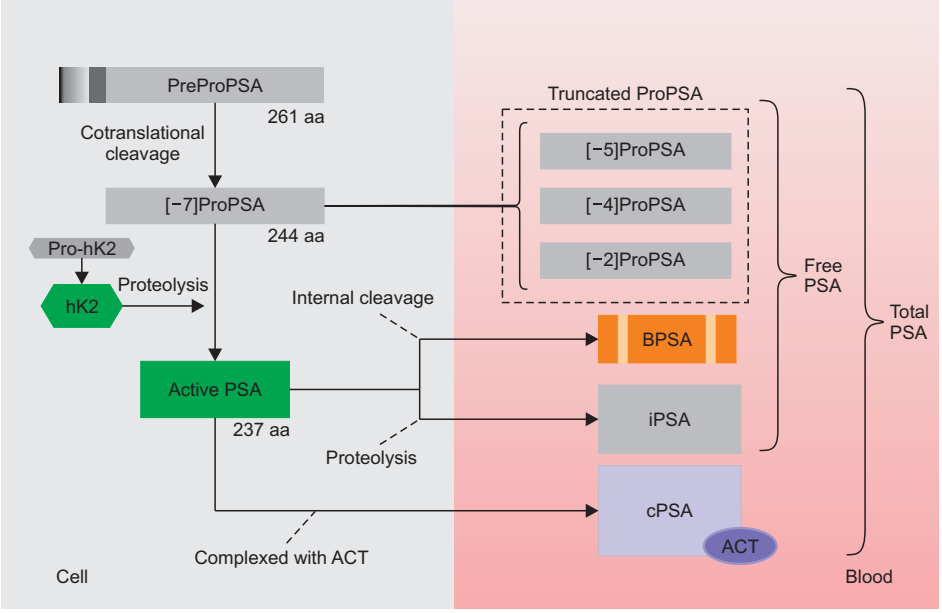

Fig. 2. Molecular forms of PSA. PSA, prostate specific antigen; BPSA, benign prostatic hyperplasia-associated PSA; iPSA, inactive PSA; cPSA, complexed PSA; ACT, alpha I-antichymotrypsin; hK2, kallikrein-related peptidase 2. Adapted from Filella X, et al. Pharmgenomics Pers Med 2018;11:83-94 [19].

Table 2. Summarized results of predictive accuracy for prostate cancer of tPSA, %fPSA, %p2PSA, and phi

Reference	AUC tPSA (95% CI)	AUC %fPSA (95% CI)	AUC %p2PSA (95% CI)	AUC PHI (95% CI)
Park et al., 2018 [30] (n=246)				
PSA≥3.5 (total)	0.683 (0.620–0.740)	0.68 (0.618–0.738)	0.761 (0.702–0.812)	0.797 (0.741–0.845)
3.5≤PSA<10 (subgroup)	0.556 (0.474–0.636)	0.685 (0.606–0.757)	0.74 (0.664–0.807)	0.763 (0.689–0.828)
Jansen et al., 2010 [24]				
Rotterdam (n=405)	0.585 (0.535–0.634)	0.675 (0.627–0.721)	0.716 (0.669–0.759)	0.750 (0.704–0.791)
Innsbruck (n=351)	0.543 (0.473–0.594)	0.576 (0.523–0.629)	0.695 (0.644–0.743)	0.709 (0.658–0.756)
Sokoll et al., 2010 [25] (n=556)	0.58 (0.53–0.64)	0.66 (0.61–0.71)	0.70 (0.65–0.75)	0.76 (0.72–0.81)
Guazzoni et al., 2011 [26] (n=268)	0.53 (0.47–0.59)	0.58 (0.52–0.64)	0.76 (0.71–0.81)	0.76 (0.70–0.81)
Lazzeri et al., 2012[a] [27] (n=222)	0.52 (0.45–0.59)	0.60 (0.53–0.67)	0.72 (0.66–0.78)	0.67 (0.61–0.73)
Lazzeri et al., 2013[b] [28] (n=158)	0.55 (0.47–0.63)	0.60 (0.52–0.68)	0.73 (0.66–0.80)	0.73 (0.66–0.80)
Stephan et al., 2013 [29] (n=1,362)	0.56 (0.53–0.59)	0.61 (0.59–0.64)	0.72 (0.70–0.75)	0.74 (0.71–0.76)

tPSA, total prostate-specific antigen; %fPSA, percentage of free PSA to tPSA; %p2PSA, percentage of p2PSA to free PSA; PHI, Prostate Health Index; AUC, area under the curve; CI, confidence interval.
[a]:An observational prospective study of a clinical cohort of men with previous negative prostate biopsies.
[b]:A nested case-control study from multicenter European cohort, the PROMEtheuS database.

tPSA or %fPSA (Table 2). Phi has demonstrated a higher accuracy than tPSA and %fPSA for predicting the presence of PCa at biopsy, showing area under the curves (AUCs) from 0.709 to 0.76. Furthermore, phi is associated with the aggressiveness of the tumor, showing higher levels in patients with aggressive PCa [27-29]. Similar results were shown by a recent prospective, multi-institutional study evaluating phi in Korean men [30], reporting AUCs of 0.76, 0.74, 0.69, and 0.56 for phi, %p2PSA, %fPSA, and tPSA, respectively, in patients with PSA in the gray zone (Table 2). There was also a significant association between phi and the Gleason score (GS) on biopsy (Spearman's rho=0.757; p<0.001). When phi was divided into quartiles, the proportion of high-grade PCa significantly and markedly increased as phi increased (Fig. 3). At the highest phi interval (phi>80), the chance of a positive biopsy result with high-grade PCa (GS≥7) was 100.0% (Fig. 3).

Phi is the least expensive ($80 in the USA) of the currently available commercial multiplex biomarkers and is suggested in the initial and repeat biopsy setting [31-33]. On average, using phi with a cutoff of ≥25 to biopsy could avoid 40% of biopsies and reduce 25% of GS 6 diagnoses at the cost of missing 5% of csPCa [34].

There has been a paradigm shift in PCa decision-making from a one-size-fits-all approach using tPSA toward multivariable risk assessment that takes the characteristics of individual patients into account. Given the substantial international evidence showing the superiority of phi over PSA, several tools have been created by combining phi with other

https://doi.org/10.4111/icu.20200395

clinical risk factors to aid in prostate biopsy decisions. For example, Lughezzani et al. [35] reported that the addition of phi to a multivariable model with age, prostate volume, digital rectal examination (DRE), and prostate biopsy history can lead to a statistically significant gain of 7% in predictive accuracy. They created a nomogram combining five variables that had an AUC of 0.80. Foley et al. [36] also created a multivariable phi-based nomogram including age, family history, DRE, previous negative biopsy, and either PSA or phi to aid in prostate biopsy decisions. The model using phi had an AUC of 0.77 for overall PCa and of 0.79 for high-grade disease. In a subset of men undergoing repeat prostate biopsy, the phi-based multivariable model had an AUC of 0.85 for any PCa and of 0.88 for high-grade disease. More recently, Loeb et al. [37] also reported that the addition of phi to the Prostate Cancer Prevention Trial risk calculator (PCPT-RC) and the ERSPC risk calculator (ERSPC-RC) can significantly improve the prediction of aggressive PCa (from AUC 0.58 to AUC 0.70 and from AUC 0.65 to AUC 0.71, respectively). These studies confirm that phi is a useful addition to multivariable nomograms for initial or repeat biopsy to improve the accuracy of risk stratification. ERSPC-RC including phi is easily accessible through the internet (www.prostatecancer-riskcalculator.com) or mobile applications (Fig. 4) [38] for easier use at the point of care.

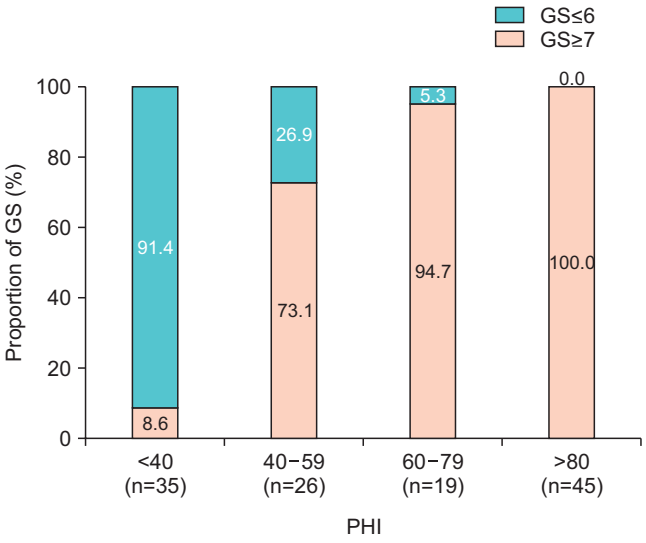

Fig. 3. Proportion of prostate cancer with Gleason score (GS)≥7 in relation to Prostate Health Index (phi) intervals.

4K SCORE

Several studies [39-42] have indicated that a panel of four-kallikrein markers (tPSA, fPSA, iPSA, and kallikrein-related peptidase 2 [hK2]) can be used to improve the predictive accuracy of biopsy outcome and reduce unnecessary biopsies. Using data from the Swedish section of the ERSPC (n=740), Vickers et al. [39] reported that a panel of four-kallikrein markers shows significantly better predictive accuracy of biopsy outcome in previously unscreened men with elevated PSA than does PSA alone (AUC from 0.68 to 0.83 for those without DRE and from 0.72 to 0.84 for those with DRE). They estimated that using a 20% risk of PCa as the threshold for biopsy could reduce the number of biopsies by 424 (57%) while missing only a small number of cancers (31 of 152 low-grade cancers and 3 of 40 high-grade cancers).

The four-kallikrein panel is conceptually similar to phi by using a combination of PSA-based markers (tPSA, fPSA, iPSA, and hK2). This panel has been commercialized by Opko Diagnostics with the name of the 4K score. The test combines the four-kallikrein panel together with patient age, DRE, and history of prior biopsy in an algorithm that calculates an individual patient's risk of csPCa on biopsy. The 4K score is currently not available in Europe. It costs

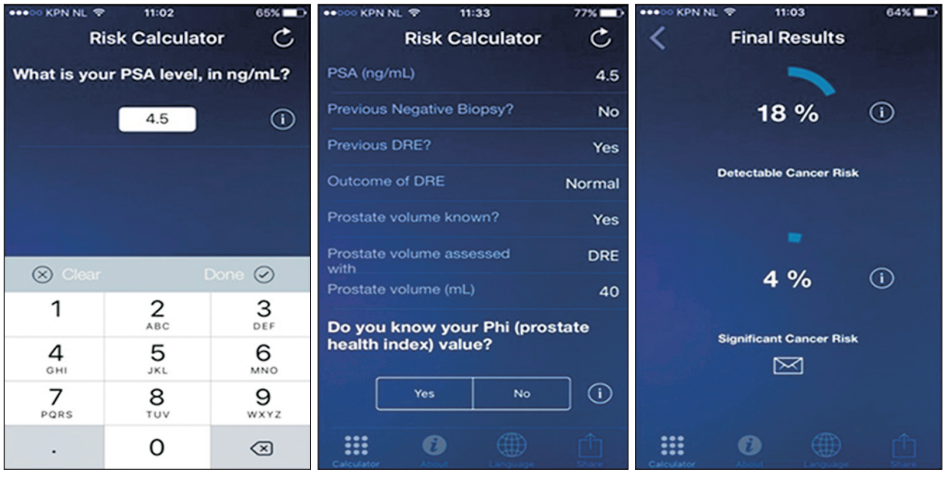

Fig. 4. Screenshots of a smartphone app of the European Randomized Study of Screening for prostate cancer risk calculator (ERSPC-RC), which includes the Prostate Health Index (phi). Adapted from Zhang K, et al. Asian J Urol 2017;4:86-95 [38].

around $500 in the USA.

A multi-institutional prospective trial in the USA [43] has shown that the 4K score could discriminate between patients with and without GS ≥7 with an AUC of 0.821 in 1,012 men undergoing a prostate biopsy. The authors of that study indicated that 30% of biopsies could be avoided using a cutoff value of 6%, delaying diagnosis for 1.3% of patients with high-grade PCa. Using a cohort of 749 men, Braun et al. [44] reported similar results, indicating that 17% of biopsies could be avoided using a threshold of 6% risk of high-grade PCa, whereas diagnosis would be delayed for 3.8% of patients with high-grade PCa. The AUC is 0.784 for a model including age, the four-kallikrein panel, and DRE [44].

Lin et al. [45] recently evaluated the ability of the 4K score to predict the presence of high-grade PCa in 718 men enrolled for AS in nine centers. They showed that a clinical model including the 4K score significantly improved the accuracy of predicting reclassification compared with a clinical model including PSA at the first repeat biopsy, showing AUCs of 0.783 and 0.740, respectively (p=0.043). However, when the prediction of reclassification at subsequent biopsies was studied, there was no significant difference between the two models (AUC, 0.754 vs. 0.755).

The 4K score is currently recommended for patients undergoing initial and repeat biopsy. A systematic review to evaluate the performance of the 4K score in a prebiopsy setting showed a pooled AUC of above 0.80 for the discrimination of csPCa, which was highly consistent across 11 studies involving over 10,000 subjects [46]. On average, using the 4K score with a cutoff risk of 9% csPCa to indicate systematic biopsy could avoid 43% of biopsies at the cost of missing 2.4% of csPCa [39-44].

In a comparative study including 531 Swedish men undergoing first-time biopsy, the phi and 4K score showed similar discriminative abilities for the prediction of all PCa (AUC, 0.70 vs. 0.69) and high-grade PCa (AUC, 0.71 vs. 0.72) [47]. Both are serum-based simple blood tests that can reduce the number of unnecessary biopsies compared with screening with tPSA, representing important new options for reducing harm.

PCA3

Prostate cancer antigen 3 (PCA3) is a prostate-specific noncoding messenger RNA (mRNA) that is highly overexpressed in specific PCa cell lines and prostatic tumors [48,49]. PCA3 mRNA levels can be measured by using quantitative real-time polymerase chain reaction in a urine sample obtained after a prostate massage to obtain the maximum amount of prostatic cells. Measuring PSA mRNA allows for standardization of the number of PCA3 RNA copies by calculating the ratio of PCA3 to PSA (called the PCA3 score). The currently commercialized Progensa PCA3 test (Hologic Inc., Marlborough, MA, USA) is a robust urine test. It costs around $300 in the USA. According to the results of a meta-analysis [50], its overall sensitivity, specificity, and AUC values were 0.63, 0.88, and 0.82 in case-control studies and 0.65, 0.73, and 0.75 in prospective studies, respectively. That meta-analysis included 46 different studies, underlying that a cutoff of 35 was used in 26 institutions. However, the choice of the most appropriate cutoff for the PCA3 score remains controversial.

Marks et al. [51] demonstrated the superiority of the PCA3 over PSA by using a PCA3 assay in men (n=226) undergoing repeat prostate biopsy (AUC, 0.68 vs. 0.52; p=0.008). With the use of 35 as the most balanced PCA3 cutoff score, the PCA3 assay had a sensitivity of 58%, a specificity of 72%, and an odds ratio (OR) of 3.6. In particular, the authors reported that the negative predictive value (NPV) was 0.88 at a PCA3 cutoff of less than 20. Thus, the PCA3 score has remarkable utility in men who have one or more previous negative prostate biopsy results. The FDA approved this test as an aid for repeat prostate biopsy decisions in 2012.

Several studies [51-53] have investigated correlations of the PCA3 score with features of PCa aggressiveness such as tumor volume, GS, pT stage, and percentage of positive biopsy cores. However, data about the association of PCA3 score with csPCa are conflicting. PCA3 is not associated with locally advanced disease [53]. Thus, its utility is limited in the prediction of aggressive cancer.

Comparative studies have demonstrated that the phi outperforms PCA3 for the prediction of csPCa on biopsy [54,55]. Seisen et al. [54] reported that the phi outperforms PCA3 for detecting csPCa (AUC, 0.80 vs. 0.55; p=0.03). Similarly, Cantiello et al. [55] compared the performances of the phi and PCA3 to predict adverse pathologic features at radical prostatectomy. In a multivariable model, both the phi and PCA3 significantly improved the predictive accuracy for the endpoint of extracapsular tumor extension. However, only the phi provided significant incremental predictive accuracy for the prediction of tumor volume >0.5 mL, pathologic GS ≥7, seminal vesicle invasion, and the composite endpoint of csPCa [55]. Thus, many current guidelines recommend PCA3 with a cutoff of 35 in men with moderately elevated PSA for whom repeat biopsy is being considered.

https://doi.org/10.4111/icu.20200395

TMPRSS2:ERG GENE FUSION

The *TMPRSS2:ERG* gene fusion is a genetic rearrangement of the *TMPRSS2* gene (an androgen-regulated transcriptional promoter) and the *ERG* oncogene, which commonly occurs in PCa [56]. Similar to PCA3, a *TMPRSS2:ERG* rearrangement can be detected in urine after DRE [57]. It can also be normalized to the amount of PSA mRNA to generate a *TMPRSS2:ERG* score. Hessels et al. [57] reported that the *TMPRSS2:ERG* fusion gene has greater diagnostic accuracy than tPSA, with a high specificity of 93% and a PPV of 94% for detection of PCa. Unlike PCA3, a *TMPRSS2:ERG* score is associated with csPCa [58,59]. Tomlins et al. [58] reported that the *TMPRSS2:ERG* score was associated with indicators of clinically significant cancer at biopsy and prostatectomy, including tumor volume, high pathologic GS, and Gleason upgrading in a large-scale multicenter study. Another population-based study found that *TMPRSS2:ERG* gene fusion was associated with an increased cumulative incidence ratio of 2.7 for developing metastases and PCa-specific mortality [59]. However, the low sensitivity of this biomarker reduces its value as a standalone test. Combining PCA3 with *TMPRSS2:ERG* can improve the prediction of PCa [57,58,60-63]. Robert et al. [60] have shown that the combination of these two markers can lead to a higher sensitivity than *TMPRSS2:ERG* alone (sensitivity: 93.6% vs. 45.8%) while preserving its high specificity (98.8% vs. 97.5%) at the prostatic tissue level. Salami et al. [61] also confirmed that the combination of these two markers can improve their ability to predict the results of prostatic biopsies (AUC=0.88).

In a prospective multicenter study (n=443) [62], both PCA3 and *TMPRSS2:ERG* had independent additional predictive values over ERSPC-RC parameters for predicting PCa in multivariate analyses (OR, 3.64; p<0.001 and OR, 3.28; p=0.002, respectively). The AUC of ERSPC-RC increased from 0.799 to 0.842 when PCA3 and *TMPRSS2:ERG* scores were added. Interestingly, in multivariate logistic regression analyses, only *TMPRSS2:ERG* added significant predictive value to ERSPC-RC for predicting biopsy GS (OR, 7.16; p<0.001) and clinical tumor stage (OR, 2.60; p=0.023), whereas PCA3 did not. A prospective study by Tomlins et al. [63] has also shown the value of PCA3 and *TMPRSS2:ERG* scores when they are added to PCPT-RC. AUC was increased from 0.639 for PCPT-RC to 0.762 after adding both PCA3 and *TMPRSS2:ERG* scores. PCA3 and *TMPRSS2:ERG* scores were also evaluated to predict high-risk PCa, with an AUC of 0.779 when both markers were added.

As a commercial test, the MiProstate Score (MiPS; University of Michigan, Ann Arbor, MI, USA) incorporates PSA, PCA3, and *TMPRSS2:ERG* to predict the risk of PCa and csPCa. MiPS costs around $700 in the USA. It is a promising test following PSA screening. However, it has not yet been validated in prospective studies or directly compared with other biomarkers.

ExoDx PROSTATE INTELLISCORE (EPI)

The EPI test (Exosome Diagnostics, Boston, MA, USA) evaluates urine-based exosomal RNA expression levels of three genes, utilizing *PCA3* and *ERG* (V-ets erythroblastosis virus E26 oncogene homologs) RNAs from urine normalized to *SPDEF* (SAM pointed domain-containing Ets transcription factor). Exosomes are small vesicles with double-lipid membranes that are secreted from cells. Exosomes encapsulate a portion of the parent cell cytoplasm. They are shed into various biofluids, including blood and urine (Fig. 5) [64]. They are rich sources of cellular proteins and RNAs. They are promising for profiling RNA expression in tumor cells because they are highly representative of their cells of origin and provide protection for mRNA during sample processing [65].

The EPI test is a simple urine test and somewhat unique since it does not require pre-catch DRE or special post-catch handling [66]. This test assigns an individual risk score for patients ranging from zero to 100 to help physicians evaluate a patient's risk for high-grade, potentially more aggressive PCa. Scores above a pre-defined cutoff are associated with an increased likelihood of high-grade PCa on a subsequent biopsy [67]. Thus, this test proposes to discriminate PCa with a GS of ≥7 from PCa with a GS of 6 and benign disease at initial biopsy.

McKiernan et al. [67] evaluated the diagnostic performance of the exosomal gene signature and demonstrated that the ExoDx panel used in combination with PSA, age, race, and family history was able to detect GS ≥7 PCa better than a standard-of-care variable alone (AUC, 0.73 vs. 0.63; p<0.001). A validation study also demonstrated its good assay performance [67]. A predetermined cutoff point (15.6) yielded an NPV of 91% and a sensitivity of 92%, with 27% of patients having an EPI score below the cutoff point. Applying a cutoff point from the training cohort to serve as a threshold for biopsy in the validation cohort decreased unnecessary biopsies by 27% (138 of 519) while missing only 8% (12 of 148) of GS ≥7 cancers. Recently, it was shown that the test can improve the identification of patients with higher grade disease and reduce the total number of unnecessary biopsies in a phase II study [68]. Thus, the ExoDx panel in combination with PSA, age, race, and family history has

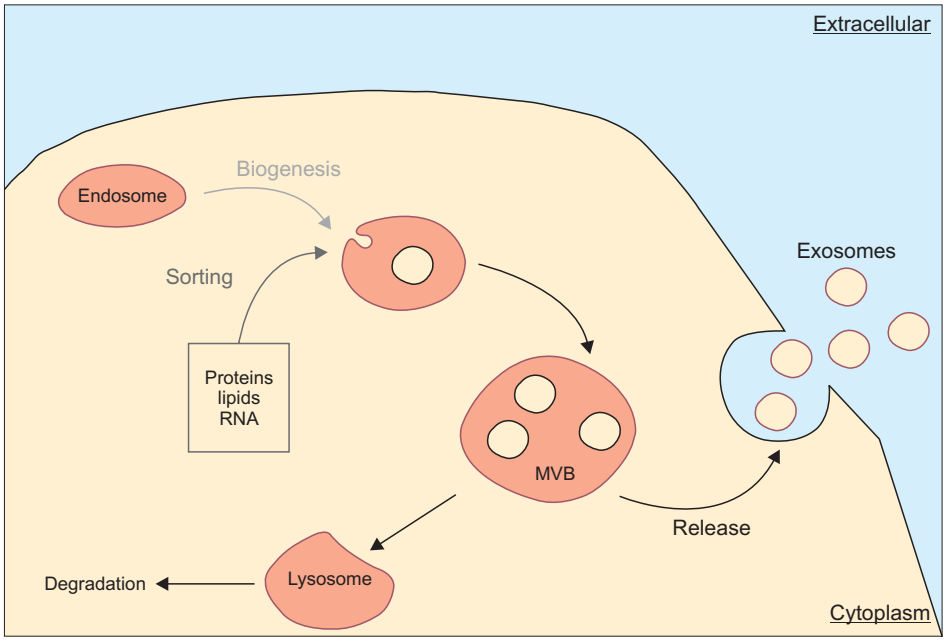

Fig. 5. Exosomes are extracellular vesicles secreted from cells. Exosomes encapsulate a portion of parent cell cytoplasm and are shed into various biofluids, including blood and urine. MVB, multivesicular body. Adapted from Shurtleff MJ, et al. Elife 2016;5:e19276 [64].

been successfully validated in over 1,000 patients across two prospective validation trials. However, this test is only available from the manufacturer through a CLIA-approved (Clinical Laboratory Improvement Amendments) laboratory. It has not been approved by the FDA. In current National Comprehensive Cancer Network (NCCN) guidelines, EPI is mentioned as an investigational biomarker only [69]. The NCCN panel states that additional evidence will be reviewed as it becomes available.

SelectMDx®

SelectMDx test (MDx Health, Irvine, CA, USA) is a gene expression assay performed with post-DRE urine that measures the mRNA levels of a two-gene panel (*DLX1* and *HOXC6*) using reverse-transcription PCR. The expression of *KLK3* is used as an internal reference. Leyten et al. [70] originally found that a validated urinary three-gene panel (*HOXC6*, *TDRD1*, and *DLX1*) showed higher accuracy for the detection of csPCa than PSA and the Progensa PCA3 test (AUC, 0.77 vs. 0.72 vs. 0.68, respectively). *DLX1* and *HOXC6* might be involved in the onset of PCa. They are also associated with PCa aggressiveness [70].

Van Neste et al. [71] collected post-DRE urine samples of 905 patients from two independent prospective clinical trials and evaluated the diagnostic value and clinical utility of the two-gene panel in prostate biopsy specimens for clinical validation. The assay was developed with an initial training set of 519 patients. It was then validated in a separate set of 386 patients from these trials. Using the expression of *DLX1* and *HOXC6* alone resulted in an AUC of 0.76, a sensitivity of 91%, a specificity of 36%, an NPV of 94%, and a PPV of 27% for the prediction of high-grade PCa. When gene expression was combined with PSA, PSA density, DRE, previous negative prostate biopsy results, age, and family history in a multimodal model, the overall AUC was 0.90 in the training set and 0.86 in the validation set. A total reduction of biopsies by 42% and a decrease of unnecessary biopsies by 53% were observed in this model, with an NPV of 98% for high-grade PCa. This means that a low-risk SelectMDx score is correlated with a 90% probability that a man does not have PCa and a 98% probability that he does not have high-grade PCa. Recently, Hendriks et al. [72] found that SelectMDx scores are significantly higher in patients with suspicious lesions in mpMRI ($p<0.01$), with an AUC of 0.83 for the prediction of mpMRI outcome. In current NCCN guidelines, SelectMDx is also mentioned as an investigational biomarker [69]. The NCCN panel states that additional evidence will be reviewed as it becomes available.

ConfirmMDx®

Sampling errors inherent with the random prostate biopsy procedure can result in a false-negative rate of approximately 25% for standard-of-care histopathology [73]. Previous studies on repeated biopsy procedures have shown that initial prostate biopsy histopathology has a false-negative rate of 20% to 30% [74,75]. Repeat biopsies are common in men with previous histopathologically negative findings in an attempt to detect missed PCa.

https://doi.org/10.4111/icu.20200395

ConfirmMDx (MDx Health) is a tissue-based multiplex epigenetic assay that aims to evaluate cancer-negative men being considered for repeat prostate biopsy due to still high PSA. The assay is commercially available. It uses multiplex methylation-specific PCR to measure the epigenetic status of PCa-associated genes *GSTP1*, *APC*, and *RASSF1* in residual cancer-negative prostate biopsy core tissue samples [76,77]. By detecting epigenetic abnormalities in a halo around the tumor, which is shown to be associated with oncogenesis, these biomarkers aid in finding evidence of occult PCa unseen by histopathology [76-78]. This field effect on adjacent benign-appearing biopsy core tissues is a strong independent predictor for diagnosing PCa in a subsequent biopsy, with a negative epigenetic result providing a higher NPV of approximately 90% than standard histopathology alone [75-77]. Test results from this epigenetic assay help guide decision-making for repeat biopsy for patients with a previous negative biopsy result who are still considered to be at risk for PCa.

ConfirmMDx has been validated in two blinded multicenter studies that show the superior NPV of this epigenetic test over standard histopathology for cancer detection in prostate biopsies [76,77]. The European MATLOC study [76] blindly tested this assay using archived prostate biopsy needle core tissue samples of 498 subjects with histopathologically negative prostate biopsies followed by positive or negative repeat biopsy within 30 months. The NPV was 90% (95% confidence interval [CI], 87%–93%). In multivariate analysis, ConfirmMDx was a significant independent predictor of patient outcome (OR, 3.17; 95% CI, 1.81–5.53). A similar validation study was performed in the United States using archived cancer-negative prostate biopsy core tissue samples of 350 subjects who had repeat biopsy within 24 months from a total of five urological centers [77]. The NPV was 88% (95% CI, 85%–91%). The test was again found to be the most significant independent predictor of patient outcomes on multivariate analysis (OR, 2.69; 95% CI, 1.60–4.51). These two independent trials showed that the negative findings of this test could be used to decrease concerns about unsampled cancer and effectively avoid unnecessary repeat biopsies [76,77]. ConfirmMDx is also available from one CLIA-certified laboratory. It is not FDA-approved. ConfirmMDx can be considered as an option for men contemplating repeat biopsy because the assay may identify individuals at higher risk for PCa on repeat biopsy [69].

CONCLUSIONS

PSA screening has been controversial, leading to an intensive search for alternative PCa biomarkers with better diagnostic and predictive potentials. In particular, there is a quest for biomarkers that can distinguish between aggressive and indolent tumors, thereby leading to better treatment decisions. Several FDA-approved and clinical laboratory-based tests have been developed. These tests show improved sensitivity and specificity over PSA. Nevertheless, serum PSA is still being used in conjunction with other parameters, highlighting the fact that PSA remains an indispensable tool in the clinical management of men with PCa. Emerging alternative biomarkers may continue to supplement or possibly replace PSA over time. These novel biomarkers appear to have better detection and diagnostic values or better prognostic abilities and predictive values. Careful validation of emerging biomarkers may fulfill the so far unmet clinical challenges and guide clinicians to better diagnoses and better treatment options for PCa. In the future, more high-level evidence is needed to answer whether the rational use of the currently available novel biomarkers can effectively decrease biopsy rates and thus decrease costs and morbidity. Such high-level evidence is needed to justify and advocate the broader use of these novel biomarkers.

CONFLICTS OF INTEREST

The authors have nothing to disclose.

AUTHORS' CONTRIBUTIONS

Research conception and design: Jeong Hyun Kim and Sung Kyu Hong. Data acquisition: Jeong Hyun Kim. Statistical analysis: Jeong Hyun Kim. Data analysis and interpretation: Jeong Hyun Kim. Drafting of the manuscript: Jeong Hyun Kim. Critical revision of the manuscript: Sung Kyu Hong. Administrative, technical, or material support: Jeong Hyun Kim. Supervision: Sung Kyu Hong. Approval of the final manuscript: Sung Kyu Hong.

REFERENCES

1. Mistry K, Cable G. Meta-analysis of prostate-specific antigen and digital rectal examination as screening tests for prostate carcinoma. J Am Board Fam Pract 2003;16:95-101.
2. Postma R, Schröder FH. Screening for prostate cancer. Eur J Cancer 2005;41:825-33.
3. Thompson IM, Ankerst DP, Chi C, Goodman PJ, Tangen CM, Lucia MS, et al. Assessing prostate cancer risk: results from the Prostate Cancer Prevention Trial. J Natl Cancer Inst 2006;98:529-34.

4. Peyromaure M, Ravery V, Messas A, Toublanc M, Boccon-Gibod L, Boccon-Gibod L. Pain and morbidity of an extensive prostate 10-biopsy protocol: a prospective study in 289 patients. J Urol 2002;167:218-21.
5. Draisma G, Etzioni R, Tsodikov A, Mariotto A, Wever E, Gulati R, et al. Lead time and overdiagnosis in prostate-specific antigen screening: importance of methods and context. J Natl Cancer Inst 2009;101:374-83.
6. Schröder FH, Hugosson J, Roobol MJ, Tammela TL, Ciatto S, Nelen V, et al. Screening and prostate-cancer mortality in a randomized European study. N Engl J Med 2009;360:1320-8.
7. Tsodikov A, Gulati R, Heijnsdijk EAM, Pinsky PF, Moss SM, Qiu S, et al. Reconciling the effects of screening on prostate cancer mortality in the ERSPC and PLCO trials. Ann Intern Med 2017;167:449-45.
8. Klotz L. Low-risk prostate cancer can and should often be managed with active surveillance and selective delayed intervention. Nat Clin Pract Urol 2008;5:2-3.
9. Lawrentschuk N, Klotz L. Active surveillance for low-risk prostate cancer: an update. Nat Rev Urol 2011;8:312-20.
10. Heijnsdijk EA, de Carvalho TM, Auvinen A, Zappa M, Nelen V, Kwiatkowski M, et al. Cost-effectiveness of prostate cancer screening: a simulation study based on ERSPC data. J Natl Cancer Inst 2014;107:366.
11. Heidenreich A, Abrahamsson PA, Artibani W, Catto J, Montorsi F, Van Poppel H, et al. Early detection of prostate cancer: European Association of Urology recommendation. Eur Urol 2013;64:347-54.
12. Murphy DG, Ahlering T, Catalona WJ, Crowe H, Crowe J, Clarke N, et al. The Melbourne Consensus Statement on the early detection of prostate cancer. BJU Int 2014;113:186-8.
13. Louie KS, Seigneurin A, Cathcart P, Sasieni P. Do prostate cancer risk models improve the predictive accuracy of PSA screening? A meta-analysis. Ann Oncol 2015;26:848-64.
14. Anceschi U, Tuderti G, Lugnani F, Biava PM, Malossini G, Luciani L, et al. Novel diagnostic biomarkers of prostate cancer: an update. Curr Med Chem 2019;26:1045-58.
15. Ahmed HU, El-Shater Bosaily A, Brown LC, Gabe R, Kaplan R, Parmar MK, et al. Diagnostic accuracy of multi-parametric MRI and TRUS biopsy in prostate cancer (PROMIS): a paired validating confirmatory study. Lancet 2017;389:815-22.
16. Carroll PR, Parsons JK, Andriole G, Bahnson RR, Castle EP, Catalona WJ, et al. NCCN Guidelines Insights: prostate cancer early detection, version 2.2016. J Natl Compr Canc Netw 2016;14:509-19.
17. Osses DF, Roobol MJ, Schoots IG. Prediction medicine: biomarkers, risk calculators and magnetic resonance imaging as risk stratification tools in prostate cancer diagnosis. Int J Mol Sci 2019;20:1637.
18. Mikolajczyk SD, Marks LS, Partin AW, Rittenhouse HG. Free prostate-specific antigen in serum is becoming more complex. Urology 2002;59:797-802.
19. Filella X, Fernández-Galan E, Fernández Bonifacio R, Foj L. Emerging biomarkers in the diagnosis of prostate cancer. Pharmgenomics Pers Med 2018;11:83-94.
20. Mikolajczyk SD, Millar LS, Wang TJ, Rittenhouse HG, Marks LS, Song W, et al. A precursor form of prostate-specific antigen is more highly elevated in prostate cancer compared with benign transition zone prostate tissue. Cancer Res 2000;60:756-9.
21. Mikolajczyk SD, Grauer LS, Millar LS, Hill TM, Kumar A, Rittenhouse HG, et al. A precursor form of PSA (pPSA) is a component of the free PSA in prostate cancer serum. Urology 1997;50:710-4.
22. Hori S, Blanchet JS, McLoughlin J. From prostate-specific antigen (PSA) to precursor PSA (proPSA) isoforms: a review of the emerging role of proPSAs in the detection and management of early prostate cancer. BJU Int 2013;112:717-28.
23. Catalona WJ, Partin AW, Sanda MG, Wei JT, Klee GG, Bangma CH, et al. A multicenter study of [-2]pro-prostate specific antigen combined with prostate specific antigen and free prostate specific antigen for prostate cancer detection in the 2.0 to 10.0 ng/ml prostate specific antigen range. J Urol 2011;185:1650-5.
24. Jansen FH, van Schaik RH, Kurstjens J, Horninger W, Klocker H, Bektic J, et al. Prostate-specific antigen (PSA) isoform p2PSA in combination with total PSA and free PSA improves diagnostic accuracy in prostate cancer detection. Eur Urol 2010;57:921-7.
25. Sokoll LJ, Sanda MG, Feng Z, Kagan J, Mizrahi IA, Broyles DL, et al. A prospective, multicenter, National Cancer Institute Early Detection Research Network study of [-2]proPSA: improving prostate cancer detection and correlating with cancer aggressiveness. Cancer Epidemiol Biomarkers Prev 2010;19:1193-200.
26. Guazzoni G, Nava L, Lazzeri M, Scattoni V, Lughezzani G, Maccagnano C, et al. Prostate-specific antigen (PSA) isoform p2PSA significantly improves the prediction of prostate cancer at initial extended prostate biopsies in patients with total PSA between 2.0 and 10 ng/ml: results of a prospective study in a clinical setting. Eur Urol 2011;60:214-22.
27. Lazzeri M, Briganti A, Scattoni V, Lughezzani G, Larcher A, Gadda GM, et al. Serum index test %[-2]proPSA and Prostate Health Index are more accurate than prostate specific antigen and %fPSA in predicting a positive repeat prostate biopsy. J Urol 2012;188:1137-43.
28. Lazzeri M, Haese A, Abrate A, de la Taille A, Redorta JP, McNicholas T, et al. Clinical performance of serum prostate-specific antigen isoform [-2]proPSA (p2PSA) and its derivatives, %p2PSA and the prostate health index (PHI), in men

https://doi.org/10.4111/icu.20200395

with a family history of prostate cancer: results from a multicentre European study, the PROMEtheuS project. BJU Int 2013;112:313-21.
29. Stephan C, Vincendeau S, Houlgatte A, Cammann H, Jung K, Semjonow A. Multicenter evaluation of [-2]proprostate-specific antigen and the prostate health index for detecting prostate cancer. Clin Chem 2013;59:306-14.
30. Park H, Lee SW, Song G, Kang TW, Jung JH, Chung HC, et al. Diagnostic performance of %[-2]proPSA and prostate health index for prostate cancer: prospective, multi-institutional study. J Korean Med Sci 2018;33:e94.
31. Mottet N, Bellmunt J, Bolla M, Briers E, Cumberbatch MG, De Santis M, et al. EAU-ESTRO-SIOG guidelines on prostate cancer. Part 1: screening, diagnosis, and local treatment with curative intent. Eur Urol 2017;71:618-29.
32. Loeb S, Lilja H, Vickers A. Beyond prostate-specific antigen: utilizing novel strategies to screen men for prostate cancer. Curr Opin Urol 2016;26:459-65.
33. Boegemann M, Stephan C, Cammann H, Vincendeau S, Houlgatte A, Jung K, et al. The percentage of prostate-specific antigen (PSA) isoform [-2]proPSA and the Prostate Health Index improve the diagnostic accuracy for clinically relevant prostate cancer at initial and repeat biopsy compared with total PSA and percentage free PSA in men aged ≤65 years. BJU Int 2016;117:72-9.
34. de la Calle C, Patil D, Wei JT, Scherr DS, Sokoll L, Chan DW, et al. Multicenter evaluation of the prostate health index to detect aggressive prostate cancer in biopsy naïve men. J Urol 2015;194:65-72.
35. Lughezzani G, Lazzeri M, Larcher A, Lista G, Scattoni V, Cestari A, et al. Development and internal validation of a Prostate Health Index based nomogram for predicting prostate cancer at extended biopsy. J Urol 2012;188:1144-50.
36. Foley RW, Gorman L, Sharifi N, Murphy K, Moore H, Tuzova AV, et al. Improving multivariable prostate cancer risk assessment using the Prostate Health Index. BJU Int 2016;117:409-17.
37. Loeb S, Shin SS, Broyles DL, Wei JT, Sanda M, Klee G, et al. Prostate Health Index improves multivariable risk prediction of aggressive prostate cancer. BJU Int 2017;120:61-8.
38. Zhang K, Bangma CH, Roobol MJ. Prostate cancer screening in Europe and Asia. Asian J Urol 2017;4:86-95.
39. Vickers AJ, Cronin AM, Aus G, Pihl CG, Becker C, Pettersson K, et al. A panel of kallikrein markers can reduce unnecessary biopsy for prostate cancer: data from the European Randomized Study of Prostate Cancer Screening in Göteborg, Sweden. BMC Med 2008;6:19.
40. Vickers A, Cronin A, Roobol M, Savage C, Peltola M, Pettersson K, et al. Reducing unnecessary biopsy during prostate cancer screening using a four-kallikrein panel: an independent replication. J Clin Oncol 2010;28:2493-8.
41. Vickers AJ, Cronin AM, Roobol MJ, Savage CJ, Peltola M, Pettersson K, et al., A four-kallikrein panel predicts prostate cancer in men with recent screening: data from the European Randomized Study of Screening for Prostate Cancer, Rotterdam. Clin Cancer Res 2010;16:3232-9.
42. Gupta A, Roobol MJ, Savage CJ, Peltola M, Pettersson K, Scardino PT, et al. A four-kallikrein panel for the prediction of repeat prostate biopsy: data from the European Randomized Study of Prostate Cancer screening in Rotterdam, Netherlands. Br J Cancer 2010;103:708-14.
43. Parekh DJ, Punnen S, Sjoberg DD, Asroff SW, Bailen JL, Cochran JS, et al. A multi-institutional prospective trial in the USA confirms that the 4Kscore accurately identifies men with high-grade prostate cancer. Eur Urol 2015;68:464-70.
44. Braun K, Sjoberg DD, Vickers AJ, Lilja H, Bjartell AS. A four-kallikrein panel predicts high-grade cancer on biopsy: independent validation in a community cohort. Eur Urol 2016;69:505-11.
45. Lin DW, Newcomb LF, Brown MD, Sjoberg DD, Dong Y, Brooks JD, et al. Evaluating the four kallikrein panel of the 4Kscore for prediction of high-grade prostate cancer in men in the Canary Prostate Active Surveillance Study. Eur Urol 2017;72:448-54.
46. Zappala SM, Scardino PT, Okrongly D, Linder V, Dong Y. Clinical performance of the 4Kscore Test to predict high-grade prostate cancer at biopsy: a meta-analysis of US and European clinical validation study results. Rev Urol 2017;19:149-55.
47. Nordström T, Vickers A, Assel M, Lilja H, Grönberg H, Eklund M. Comparison between the four-kallikrein panel and prostate health index for predicting prostate cancer. Eur Urol 2015;68:139-46.
48. Bussemakers MJ, van Bokhoven A, Verhaegh GW, Smit FP, Karthaus HF, Schalken JA, et al. DD3: a new prostate-specific gene, highly overexpressed in prostate cancer. Cancer Res 1999;59:5975-9.
49. de Kok JB, Verhaegh GW, Roelofs RW, Hessels D, Kiemeney LA, Aalders TW, et al. DD3(PCA3), a very sensitive and specific marker to detect prostate tumors. Cancer Res 2002;62:2695-8.
50. Cui Y, Cao W, Li Q, Shen H, Liu C, Deng J, et al. Evaluation of prostate cancer antigen 3 for detecting prostate cancer: a systematic review and meta-analysis. Sci Rep 2016;6:25776.
51. Marks LS, Fradet Y, Deras IL, Blase A, Mathis J, Aubin SM, et al. PCA3 molecular urine assay for prostate cancer in men undergoing repeat biopsy. Urology 2007;69:532-5.
52. van Gils MP, Hessels D, Hulsbergen-van de Kaa CA, Witjes JA, Jansen CF, Mulders PF, et al. Detailed analysis of histopatho-

logical parameters in radical prostatectomy specimens and PCA3 urine test results. Prostate 2008;68:1215-22.
53. Auprich M, Chun FK, Ward JF, Pummer K, Babaian R, Augustin H, et al. Critical assessment of preoperative urinary prostate cancer antigen 3 on the accuracy of prostate cancer staging. Eur Urol 2011;59:96-105.
54. Seisen T, Rouprêt M, Brault D, Léon P, Cancel-Tassin G, Compérat E, et al. Accuracy of the prostate health index versus the urinary prostate cancer antigen 3 score to predict overall and significant prostate cancer at initial biopsy. Prostate 2015;75:103-11.
55. Cantiello F, Russo GI, Ferro M, Cicione A, Cimino S, Favilla V, et al. Prognostic accuracy of Prostate Health Index and urinary Prostate Cancer Antigen 3 in predicting pathologic features after radical prostatectomy. Urol Oncol 2015;33:163.e15-23.
56. Magi-Galluzzi C, Tsusuki T, Elson P, Simmerman K, LaFargue C, Esgueva R, et al. TMPRSS2-ERG gene fusion prevalence and class are significantly different in prostate cancer of Caucasian, African-American and Japanese patients. Prostate 2011;71:489-97.
57. Hessels D, Smit FP, Verhaegh GW, Witjes JA, Cornel EB, Schalken JA. Detection of TMPRSS2-ERG fusion transcripts and prostate cancer antigen 3 in urinary sediments may improve diagnosis of prostate cancer. Clin Cancer Res 2007;13:5103-8.
58. Tomlins SA, Aubin SM, Siddiqui J, Lonigro RJ, Sefton-Miller L, Miick S, et al. Urine TMPRSS2:ERG fusion transcript stratifies prostate cancer risk in men with elevated serum PSA. Sci Transl Med 2011;3:94ra72.
59. Demichelis F, Fall K, Perner S, Andrén O, Schmidt F, Setlur SR, et al. TMPRSS2:ERG gene fusion associated with lethal prostate cancer in a watchful waiting cohort. Oncogene 2007;26:4596-9.
60. Robert G, Jannink S, Smit F, Aalders T, Hessels D, Cremers R, et al. Rational basis for the combination of PCA3 and TMPRSS2:ERG gene fusion for prostate cancer diagnosis. Prostate 2013;73:113-20.
61. Salami SS, Schmidt F, Laxman B, Regan MM, Rickman DS, Scherr D, et al. Combining urinary detection of TMPRSS2:ERG and PCA3 with serum PSA to predict diagnosis of prostate cancer. Urol Oncol 2013;31:566-71.
62. Leyten GH, Hessels D, Jannink SA, Smit FP, de Jong H, Cornel EB, et al. Prospective multicentre evaluation of PCA3 and TMPRSS2-ERG gene fusions as diagnostic and prognostic urinary biomarkers for prostate cancer. Eur Urol 2014;65:534-42.
63. Tomlins SA, Day JR, Lonigro RJ, Hovelson DH, Siddiqui J, Kunju LP, et al. Urine TMPRSS2:ERG plus PCA3 for individualized prostate cancer risk assessment. Eur Urol 2016;70:45-53.
64. Shurtleff MJ, Temoche-Diaz MM, Karfilis KV, Ri S, Schekman R. Y-box protein 1 is required to sort microRNAs into exosomes in cells and in a cell-free reaction. Elife 2016;5:e19276.
65. Nilsson J, Skog J, Nordstrand A, Baranov V, Mincheva-Nilsson L, Breakefield XO, et al. Prostate cancer-derived urine exosomes: a novel approach to biomarkers for prostate cancer. Br J Cancer 2009;100:1603-7.
66. Donovan MJ, Noerholm M, Bentink S, Belzer S, Skog J, O'Neill V, et al. A molecular signature of PCA3 and ERG exosomal RNA from non-DRE urine is predictive of initial prostate biopsy result. Prostate Cancer Prostatic Dis 2015;18:370-5.
67. McKiernan J, Donovan MJ, O'Neill V, Bentink S, Noerholm M, Belzer S, et al. A novel urine exosome gene expression assay to predict high-grade prostate cancer at initial biopsy. JAMA Oncol 2016;2:882-9.
68. McKiernan J, Donovan MJ, Margolis E, Partin A, Carter B, Brown G, et al. A prospective adaptive utility trial to validate performance of a novel urine exosome gene expression assay to predict high-grade prostate cancer in patients with prostate-specific antigen 2-10ng/ml at initial biopsy. Eur Urol 2018;74:731-8.
69. National Comprehensive Cancer Network. NCCN Clinical Practice Guidelines in Oncology. Prostate cancer early detection. 2018 Version 2. Plymouth Meeting: National Comprehensive Cancer Network; 2018.
70. Leyten GH, Hessels D, Smit FP, Jannink SA, de Jong H, Melchers WJ, et al. Identification of a candidate gene panel for the early diagnosis of prostate cancer. Clin Cancer Res 2015;21:3061-70.
71. Van Neste L, Hendriks RJ, Dijkstra S, Trooskens G, Cornel EB, Jannink SA, et al. Detection of high-grade prostate cancer using a urinary molecular biomarker-based risk score. Eur Urol 2016;70:740-8.
72. Hendriks RJ, van der Leest MMG, Dijkstra S, Barentsz JO, Van Criekinge W, Hulsbergen-van de Kaa CA, et al. A urinary biomarker-based risk score correlates with multiparametric MRI for prostate cancer detection. Prostate 2017;77:1401-7.
73. Djavan B, Ravery V, Zlotta A, Dobronski P, Dobrovits M, Fakhari M, et al. Prospective evaluation of prostate cancer detected on biopsies 1, 2, 3 and 4: when should we stop? J Urol 2001;166:1679-83.
74. Naughton CK, Miller DC, Mager DE, Ornstein DK, Catalona WJ. A prospective randomized trial comparing 6 versus 12 prostate biopsy cores: impact on cancer detection. J Urol 2000;164:388-92.
75. Trock BJ, Brotzman MJ, Mangold LA, Bigley JW, Epstein JI, McLeod D, et al. Evaluation of GSTP1 and APC methylation as indicators for repeat biopsy in a high-risk cohort of men with negative initial prostate biopsies. BJU Int 2012;110:56-62.
76. Stewart GD, Van Neste L, Delvenne P, Delrée P, Delga A, Mc-

https://doi.org/10.4111/icu.20200395

Neill SA, et al. Clinical utility of an epigenetic assay to detect occult prostate cancer in histopathologically negative biopsies: results of the MATLOC study. J Urol 2013;189:1110-6.
77. Partin AW, Van Neste L, Klein EA, Marks LS, Gee JR, Troyer DA, et al. Clinical validation of an epigenetic assay to predict negative histopathological results in repeat prostate biopsies. J Urol 2014;192:1081-7.
78. Mehrotra J, Varde S, Wang H, Chiu H, Vargo J, Gray K, et al. Quantitative, spatial resolution of the epigenetic field effect in prostate cancer. Prostate 2008;68:152-60.