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REVIEW

Blood-based and urinary prostate cancer biomarkers: a review and comparison of novel biomarkers for detection and treatment decisions

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BACKGROUND: The diagnosis of prostate cancer (PCa) is currently based on serum PSA testing and/or abnormal digital rectal examination and histopathologic evaluation of prostate biopsies. The main drawback of PSA testing is the lack of specificity for PCa. To improve early detection of PCa more specific biomarkers are needed. In the past few years, many new promising biomarkers have been identified; however, to date, only a few have reached clinical practice.
METHODS: In this review, we discuss new blood-based and urinary biomarker models that are promising for usage in PCa detection, follow-up and treatment decision-making. These include Prostate Health Index (PHI), prostate cancer antigen 3 (PCA3), four-kallikrein panel (4K), transmembrane protease serine 2-ERG (TMPRSS2-ERG), ExoDx Prostate Intelliscore, SelectMDx and the Mi-Prostate score. Only few head-to-head studies are available for these new fluid-based biomarkers and/or models. The blood-based PHI and urinary PCA3 are two US Food and Drug Administration-approved biomarkers for diagnosis of PCa. In the second part of this review, we give an overview of published studies comparing these two available biomarkers for prediction of (1) PCa upon prostate biopsy, (2) pathological features in radical prostatectomy specimen and (3) significant PCa in patients eligible for active surveillance.
RESULTS: Studies show opposing results in comparison of PHI with PCA3 for prediction of PCa upon initial and repeat prostate biopsy. PHI and PCA3 are able to predict pathological findings on radical prostatectomy specimen, such as tumor volume and Gleason score. Only PHI could predict seminal vesicle invasion and only PCA3 could predict multifocality. There is some evidence that PHI outperforms PCA3 in predicting significant PCa in an active surveillance population.
CONCLUSIONS: Future research should focus on independent validation of promising fluid-based biomarkers/models, and prospective comparison of biomarkers with each other.

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INTRODUCTION

Prostate cancer (PCa) is the second most frequently diagnosed malignancy in male worldwide, with 1.1 million estimated new cases in 2012.[1] Early detection of PCa is based on serum PSA testing and/or abnormal digital rectal examination (DRE), and histopathologic evaluation of prostate biopsies. In the past decades, the incidence of PCa increased because of prolonged life expectancy, the use of PSA testing as a detection method and a larger number of men undergoing prostate biopsy. PSA is a kallikrein serine protease encoded by the *KLK3* gene. PSA can be elevated because of PCa and also because of a large prostate volume, BPH or prostatitis. The main drawback is this lack of specificity leading to unnecessary (repeat) biopsies and the diagnosis of indolent PCa, and therefore a high risk for over-diagnosis and overtreatment. The incidence of metastatic disease has decreased since the use of PSA, whereas the incidence of local regional disease has increased. Today clinical stage T1c tumors represent 40–50% of newly diagnosed cases.[2]

For the individual patient, the PSA level does not correlate directly with clinical and pathological tumor stage. PSA levels of $>4.0\ \text{ng ml}^{-1}$ are commonly used as a threshold value for prostate biopsy. However, PSA has a 25–40% positive predictive value to detect PCa, and eventually 65–70% of men presenting with increased PSA between 4.0 and 10.0 ng ml^{-1} have a negative prostate biopsy.[3,4] Additionally, up to 15% of men with PCa have PSA levels below 4.0 ng ml^{-1}, and therefore many cases will be left undetected.[5]

To improve the early diagnosis of PCa and to reduce the overdiagnosis and overtreatment of insignificant tumors, there is an urgent need for a specific test to detect clinically significant PCa.

BIOMARKERS IN PCA DIAGNOSTICS

The increasing knowledge of molecular biology considering carcinogenesis and PCa has led to the identification of new biomarkers. The challenges in developing ideal early detection markers for PCa are widespread. First of all the biomarkers should be specific for PCa and should not be altered or expressed in other tissues or tumors. The method of collection should be non-invasive. In terms of preservation, analytical procedures needed and costs, the biomarkers should possibly be applicable for use in large-scale screening programs. Besides, it is desirable that these biomarkers should not only distinguish patients with and without PCa but also differentiate between clinically significant tumors and indolent disease. For PCa several body fluids would be adequate

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for testing in a noninvasive manner, including prostate serum, semen, plasma and urine.[6]

In this review, we discuss new blood-based and urinary biomarker models that are promising for usage in PCa diagnostics (see Table 1). Moreover, we give an overview of the published studies in which a comparison was made of two well-studied commercially available biomarkers, the blood-based Prostate Health Index (PHI) and urinary prostate cancer antigen 3 (PCA3).

BLOOD-BASED BIOMARKERS

PSA-based assays

In the past decades, tests with molecular isoforms of PSA have been developed. Part of PSA in the blood is complexed (with proteins) and the greater part circulates in an unbound form. The unbound form is called freePSA (or fPSA) and the free-to-total PSA ratio significantly improves differentiation between PCa and benign conditions in the PSA 'grey area', 4.0–10.0 ng ml^{-1}.[7] More recently, PCa-specific fPSA isoforms, proPSAs, have been identified to improve specificity in detection.[8] Especially [−2]proPSA (p2PSA) is associated with PCa and has been demonstrated to significantly outperform the use of total PSA and fPSA alone. Besides, p2PSA seemed to be related to the risk of aggressive disease.[9,10]

Prostate Health Index

In 2011 Catalona *et al.*[11] published the results of a large multicenter trial on the PHI for PCa detection. PHI combines total PSA, fPSA and p2PSA, and is calculated using the following formula: $(2pPSA/fPSA) \times \sqrt{PSA}$. In other words, men are more at risk of having significant PCa when they have a higher total PSA and p2PSA, and a lower fPSA.[12] The score can be used in decision-making regarding prostate biopsies, and in the PSA 'grey area'. PHI is now commercially available, and has been approved by the US Food and Drug Administration (FDA) for use in the 4.0–10 ng ml^{-1} PSA range.[11] In the study of Catalona *et al.*,[11] 892 patients with PSA levels of 2.0–10 ng ml^{-1} and a normal DRE underwent prostate biopsies. For discrimination of PCa on prostate biopsies, PHI had a sensitivity of 80–95% and greater specificity than total PSA or fPSA. Besides, PHI had shown an association with biopsy Gleason score (GS) $\geqslant$ 7. Moreover, the PHI test may also have a role in monitoring men under active surveillance (AS).[12]

URINARY BIOMARKERS

Several cancer products are found to be released directly into urine through prostate ducts as cell-free markers or carried in prostate cells.[6]

Prostate cancer antigen 3

PCA3, formerly known as differential display code 3 (DD3), was discovered by Bussemakers *et al.*[13] in 1999. It is a prostate-specific noncoding messenger RNA (mRNA). PCA3 was found to be highly overexpressed in 95% of PCa tissue compared with normal prostate tissue of the same patient and in PCa metastasis.[14] In 2003, Hessels *et al.*[15] reported a median of 66-fold upregulation of PCA3 in PCa tissue compared with normal prostate tissue. Unlike PSA, PCA3 expression appears to be less influenced by patient age, prostate volume, inflammation, trauma or prior biopsies.[4,6] Although PCA3 does not encode a protein, PCA3 mRNA transcripts originating from prostate cells are detectable and quantifiable in urine.[4]

PCA3 was the first possible option for molecular diagnostics in clinical urological practice.[16] In 2006, Groskopf *et al.*[17] developed a quantitative PCA3 urine test for use in clinical settings. The Progensa PCA3 test (Hologic, Marlborough, MA, USA) is a commercially available test and has been approved by the US FDA for men with a previous negative biopsy and a persistently

Table 1. Overview blood-based and urinary prostate cancer biomarkers

Test	*Marker description*	*Biomaterial*	*Indication*	*Clinical value*	*Available as*
PHI	Total PSA, fPSA, p2PSA	Blood serum	Diagnosis/prognosis	Reduction in the number of prostate biopsies and prediction of high-grade prostate cancer	CE-IVD, FDA
Progensa PCA3	PCA3, PSA mRNA	Post-DRE urine	Diagnosis: re-biopsy setting	Reduction in the number of prostate biopsies	CE-IVD, FDA
TMPRSS2-ERG	TMPRSS2-ERG	Post-DRE urine	-	-	RUO
ExoDx Prostate (Intelliscore)	Exosomal RNA (ERG, PCA3, SPDEF)	Urine	Diagnosis/prognosis	Reduction in the number of prostate biopsies, prediction of high-grade prostate cancer	CLIA-approved LDT (end 2016)
4K score	Total PSA, fPSA, intact PSA, hK2	Blood plasma	Diagnosis/prognosis	Reduction in the number of prostate biopsies, prediction of high-grade prostate cancer	CLIA-approved LDT
SelectMDx	HOXC6, DLX1, KLK3 mRNA	Post-DRE first void urine	Diagnosis/prognosis	Reduction in the number of prostate biopsies, prediction of high-grade prostate cancer	CLIA-approved LDT, CE-IVD (end 2016)
MiPS	PCA3 and TMPRSS2-ERG mRNA, serum PSA	Post-DRE first void urine	Diagnosis/prognosis	Reduction in the number of prostate biopsies, prediction of high-grade prostate cancer	CLIA-approved LDT

Abbreviations: 4K, four-kallikrein panel; CE-IVD, European Conformity-*In Vitro* Diagnostics; CLIA-approved LDT, Clinical Laboratory Improvement Amendments-based clinical laboratory-developed test; DRE, digital rectal examination; FDA, US Food and Drug Administration; hK2, human kallikrein-related peptidase 2; fPSA, freePSA; MiPS, Mi-Prostate score; mRNA, messenger RNA; PCA3, PCa antigen 3; PHI, Prostate Health Index; p2PSA, [−2]proPSA; SPDEF, SAM Pointed Domain Containing ETS Transcription Factor; RUO, Research Use Only; TMPRSS2-ERG, transmembrane protease serine 2-ERG.

elevated PSA level to aid in decision-making regarding repeat biopsies.[18] This molecular diagnostic assay quantitatively detects PCA3 mRNA expression in whole urine after DRE using transcription-mediated amplification.[19] The PCA3 score was developed to determine the likelihood of PCa detection on prostate biopsy. To generate this quantitative PCA3 score, the ratio PCA3 mRNA/PSA mRNA × 1000 is used, meaning that PCA3 expression is normalized with PSA expression.[4,6] In 2003, Hessels *et al.*[15] showed a sensitivity of 67% and a specificity of 83% for PCA3 in 108 voided post-DRE urine samples for the presence of a tumor using prostate biopsies as the gold standard. Moreover, this test had a negative predictive value of 90%, which indicated that the quantitative determination of PCA3 mRNA transcripts in urinary sediments had the potential in reducing the number of biopsies. In men undergoing repeated biopsy, PCA3 was superior to PSA in predicting whether PCa was present upon prostate biopsy.[20] Studies on the value of PCA3 in the prediction of clinical–pathological features of PCa, including GS, tumor volume (TV), stage and extraprostatic extension, are contradictory.[18,21]

Transmembrane protease serine 2-ERG gene fusion

Gene fusions are most often caused by genomic chromosomal rearrangements. These gene fusions are thought to be an initiating event in oncogenesis and have a role in the development of certain tumor types. In 2005, Tomlins *et al.*[22] used a new biostatistical method to identify gene fusions in PCa. These chromosomal rearrangements included transmembrane protease serine 2 (TMPRSS2) that can be fused to several ETS transcription factor genes (erythroblastosis virus E26 transformation-specific transcription factor family), including *ERG*, *ETV1*, *ETV4*, *ETV5* and *ELK4*. ETS transcription factors have an important role in several biological processes, including cell growth and proliferation, apoptosis, stress responses, angiogenesis and invasiveness. TMPRSS2-ERG gene fusions are the most common variant in ~50% of patients with PCa.[23] The genes for TMPRSS2 and ERG are both located on the same chromosome, 21q22.3.[4]

TMPRSS2-ERG gene fusion seemed to be specific for PCa in tissue-based studies,[23] and can also be detected in urine after prostate massage.[24] According to Hessels *et al.*,[24] this gene fusion has a 93% specificity and 94% positive predictive value for detection of PCa in post-DRE urine samples in a cohort of 108 men undergoing prostate biopsy. TMPRSS2-ERG gene fusions are not yet approved as a PCa biomarker to predict the prostate biopsy outcome. Regarding the predictive value for aggressive disease, there still is a lot of uncertainty. In 2007, Rajput *et al.*[25] found a higher frequency of TMPRSS2-ERG gene fusions in moderate to poorly differentiated tumors compared with well-differentiated PCa. There was a positive correlation found between TMPRSS2-ERG fusion transcripts in urine and a high PSA level, pathological stage and GS.[26] This was not confirmed by a large study of 1180 men in which overexpression of TMPRSS2-ERG gene fusion was found in 49% of patients, and no significant correlation with GS or tumor grade.[27] The combination of gene fusions with other markers in a risk algorithm is discussed later in this review.

ExoDx Prostate Intelliscore

In 2009, Nilsson *et al.*[28] showed that urinary exosomes are a promising substrate for PCa biomarkers. Exosomes are small vesicles that are secreted from (tumor) cells containing cellular protein and RNA, and are highly representative for their cell origin.[28,29] Donovan *et al.*[30] used exosomal RNA and developed the EXO106 score (the sum of normalized PCA3 and ERG exosomal RNA), which had negative and positive predictive values for prediction of high-grade PCa of 97.5% and 34.5%, respectively. McKiermann *et al.*[29] showed that the combination of exosomal PCA3 and ERG with normalization of RNA levels with SPDEF (SAM pointed domain-containing ETS transcription factor) derived from non-DRE urine samples could predict high-grade PCa upon initial biopsy with an area under the curve (AUC) of 0.73 (95% confidence interval (CI): 0.68–0.77). This is called the ExoDx Prostate Intelliscore (ExosomeDx) and aims to reduce the number of unnecessary biopsies.

MOLECULAR RISK CLASSIFIERS

Four-kallikrein panel

To improve the clinical value of PSA-based tests, Vickers *et al.*[31] studied the combination of a four-kallikrein panel (4K) (total PSA, fPSA, intact PSA and human kallikrein-related peptidase 2) in blood samples from 740 men in Goteborg, Sweden, undergoing biopsy as part of the European Randomized study of Screening for Prostate Cancer (ERSPC). This four-kallikrein model was able to predict the biopsy outcome more accurately than total PSA and age alone. The 4K score test (OPKO Health, Miami, FL, USA) combines measurement of the four prostate-specific kallikreins in blood with clinical information in an algorithm that calculates the probability of significant (GS ⩾ 7) PCa before biopsy. To validate these findings, Vickers *et al.*[32] used an independent large, population-based cohort, the Rotterdam section of the ERSPC. In this cohort of 2186 men, the laboratory base model (PSA and age) had an AUC of 0.637, which increased to 0.764 for the full laboratory model (age plus kallikrein panel). The clinical models included DRE findings and the comparison demonstrated a difference between the base model (age, DRE and PSA) with an AUC of 0.695 vs 0.776 for the full model (age, DRE and four-kallikrein panel).[32] This was a confirmation of the previously found predictive value in the Goteborg ERSPC cohort. In terms of predicting aggressive disease, Parekh *et al.*[33] showed in a cohort of 1012 men scheduled for prostate biopsy a good diagnostic performance (AUC 0.82) in detecting significant PCa. Nordstrom *et al.*[34] compared the 4K score with PHI and showed that both similarly increased predictive accuracy for high-grade disease and all PCa. The 4K score could save 44% of the biopsies when using a 15% chance for high-grade PCa, with the risk of missing ~20% high-grade tumors.[34]

SelectMDx

In 2015, Leyten *et al.*[35] described the identification of a novel urinary gene panel for the early diagnosis of PCa. A three-gene panel (*HOXC6*, *TDRD1* and *DLX1*) with an AUC of 0.77 (95% CI: 0.71–0.83) to predict GS ⩾ 7 PCa upon biopsy outperformed PCA3 (AUC 0.68) and PSA (AUC 0.72). Van Neste *et al.*[36] developed a multimodal model, incorporating two of the previously identified biomarkers (*HOXC6* and *DLX1*) and traditional clinical risk factors that could be used to identify patients with high-grade PCa (GS ⩾ 7) upon prostate biopsy. The combination of biomarkers *HOXC6* and *DLX1* had the best performance with an AUC of 0.76 (95% CI: 0.71–0.81) in the training cohort. Using the risk factors age, PSA, PSA density, family history of PCa, DRE, history of prostate biopsy in combination with *HOXC6* and *DLX1* expression levels resulted in an AUC of 0.90 (95% CI: 0.87–0.93) for high-grade PCa.[36] The AUC of the model for predicting high-grade PCa was significantly higher than the AUC of the Prostate Cancer Prevention Trial risk calculator (AUC 0.77) ($P = 0.015$).[36] Moreover, in men with a PSA level of < 10 ng ml^{-1}, the risk score remained the strongest predictor with an AUC of 0.78, compared with Prostate Cancer Prevention Trial risk calculator with an AUC of 0.66.[36] The two-gene risk score, named SelectMDx (MDxHealth, Irvine, CA, USA), could be used in decision-making, reducing the number of unnecessary prostate biopsies and potential over-treatment. At a cutoff with an negative predictive value of 98% for high-grade PCa, a total reduction of biopsies by 42% could be obtained.[36]

Table 2. Comparison of PHI and PCA3 in initial and repeat biopsy setting

Reference	*Population*	*PSA range inclusion*	*Patients with PCa*	*PHI threshold*	*PCA3 threshold*	*AUC PHI*	*AUC PCA3*
Ferro *et al.*[42]	Initial biopsy ($n=151$)	0–20 ng ml^{-1}	48 (31.8%)	Continuous	Continuous	0.77	0.71
Perdona *et al.*[47]	Initial biopsy ($n=160$)	0–20 ng ml^{-1}	47 (29.4%)	43.8 All 39 (30–52)	35.2 All 37 (17–73)	0.71	0.66
Stephan *et al.*[45]	Initial biopsy ($n=136$)	0.5–20 ng ml^{-1}	Overall: 110 (44.7%)	Continuous	Continuous	0.68 (0.60–0.76)	0.70 (0.62–0.78)
	Repeat biopsy ($n=110$)	0.5–20 ng ml^{-1}		Continuous	Continuous	0.69 (0.60–0.78)	0.77 (0.69–0.85)
Ferro *et al.*[48]	Initial biopsy ($n=300$)	2–10 ng ml^{-1}	108 (36.0%)	Continuous	Continuous	0.77 (0.72–0.83)	0.73 (0.68–0.79)
Scattoni *et al.*[44]	Initial biopsy ($n=116$)	4–20 ng ml^{-1}	Initial: 34.4%	Continuous	Continuous	Overall: 0.70 Initial: 0.69	Overall: 0.59 Initial: 0.57
	Repeat biopsy ($n=95$)	4–20 ng ml^{-1}	Repeat: 31.5%	Continuous	Continuous	Repeat: 0.72	Repeat: 0.63
Seisen *et al.*[43]	Initial biopsy ($n=138$)	4–20 ng ml^{-1}	62 (44.9%)	40	35	Overall: 0.65 Sign. PCa: 0.80	Overall: 0.71 Sign. PCa: 0.55
Porpiglia *et al.*[46]	Repeat biopsy ($n=170$) vs mpMRI	Median 6.9 ng ml^{-1} (5.2–9.8 (IQR))	52 (30.6%)	48.9	32.5	Not applicable Decision curve analysis: The most significant improvement in the net benefit was provided by mpMRI. The inclusion of PCA3 and/or PHI to models containing mpMRI did not substantially improve the net benefit	

Abbreviations: AUC, area under the curve; IQR, interquartile range; mpMRI, multiparametric magnetic resonance imaging; PCa, prostate cancer; PCA3, prostate cancer antigen 3; PHI, Prostate Health Index. AUC with 95% confidence interval (within parentheses).

Mi-Prostate score

Considering PCa heterogeneity, combining biomarkers or the use of a panel of biomarkers is likely the way forward. Earlier studies showed that the combined use of PCA3 and TMPRSS2-ERG in urine had additional diagnostic and prognostic value in the prediction of PCa.[24,37] The validated Mi-Prostate score (MiPS) (University of Michigan MLabs, Ann Arbor, MI, USA) combines measurement of PCA3 and TMPRSS2-ERG in post-DRE urine samples together with serum PSA levels.[38] Cornu *et al.*[38] showed that PCA3 score, PSA density and TMPRSS2-ERG score were independently associated with prostate biopsy outcome in multivariable analysis with an AUC of 0.734. In multiple logistic regression model, PCA3 score and PSA density were significantly associated with the presence of Gleason grade 4 upon biopsy and there was a positive trend for TMPRSS2-ERG score. Salami *et al.*[39] combined serum PSA, PCA3 and TMPRSS2-ERG in a multivariable algorithm to predict PCa upon biopsy with an AUC of 0.88 (95% CI: 0.75–0.98). A recent publication of Tomlins *et al.*[40] concluded that the MiPS test could improve prediction of PCa and of high-grade PCa (GS > 6) (AUC 0.772). Decision curve analysis demonstrated a net benefit of the MiPS test together with the multivariate Prostate Cancer Prevention Trial risk calculator. The MiPS test is promising for risk stratification of (high-grade) PCa while avoiding unnecessary biopsies.[38–41]

COMPARISON OF THE PHI AND PCA3 FOR PCA DETECTION

Publications in which new biomarker tests are compared head-to-head are limited. Comparative data is needed to determine the best pathway for detection, prognosis and follow-up of PCa. The two commercially available tests, PHI and PCA3, are both promising to improve overdiagnosis and overtreatment. Up-to-date seven articles have been published comparing PHI with PCA3 in the initial and/or repeat biopsy setting (see Table 2). The first comparison of the two tests was made by Ferro *et al.*[42] in 2012. In 151 men with initial prostate biopsies, the accuracy of PHI and PCA3 was assessed to predict benign, malignant and HG-PIN diagnosis. Receiver operating characteristic (ROC) curve analysis showed that PHI and PCA3 were good indicators of malignancies (AUC 0.77 and 0.71, respectively). PHI had the highest AUC but there was no significant difference with PCA3 ($P=0.368$), indicating comparable ability to discriminate benign for malignant condition. On the contrary, Seisen *et al.*[43] showed PCA3 was the most accurate predictor of PCa in the initial biopsy setting compared with PHI (AUC 0.71 vs 0.65; $P=0.03$). Scattoni *et al.*[44] compared PHI and PCA3 in a cohort of patients in the initial and repeat biopsy setting. In the whole group, ROC analyses revealed that PHI had the highest AUC (0.70, 95% CI: 0.63–0.76) compared with PCA3 (AUC 0.59, 95% CI: 0.52–0.66; $P=0.043$). Moreover, PHI was slightly more accurate than PCA3 in the repeat setting alone (AUC 0.72 vs 0.63).[44] According to the study of Stephan *et al.*,[45] PCA3 was the most accurate predictor of PCa in candidates for repeat biopsy compared to PHI (AUC 0.77 vs 0.69), although the AUCs were not statistically different.

Porpiglia *et al.*[46] evaluated the diagnostic accuracy of PCA3, PHI and multiparametric magnetic resonance imaging (mpMRI) in patients undergoing repeat biopsy. The multivariate logistic regression analysis showed only mpMRI was a significant independent predictor of PCa diagnosis on repeat biopsy. Interestingly, the results of missing PCa were listed as well: mpMRI missed 5 of 52 (9.6%) tumors (3 GS 6 and 2 GS 7). PCA3 missed 22 of 52 (42.3%) tumors (10 GS 6, 10 GS 7 and 2 GS $\geqslant$ 8), whereas PHI missed 30 of 52 (57.7%) tumors (16 GS 6, 12 GS 7 and 2 GS $\geqslant$ 8).

Perdona *et al.*[47] evaluated using the combination of PCA3 and PHI in predicting biopsy results in 160 men with initial biopsy. ROC analyses showed that PHI outperformed PCA3 for high specificity level, whereas PCA3 outperformed PHI for high sensitivity level. Multivariable analysis showed that the

Table 3. Comparison of PHI and PCA3 in prediction of pathological features in radical prostatectomy specimen

Reference	*Population*	*End points*	*Base model*	*Key study notes*
Cantiello *et al.*[42]	Clinically localized PCa ($n=156$)	TV, ECE, SVI, GS $\geqslant$ 7, pathologically significant PCa[a]	Patient age, total PSA, fPSA, percentage of positive cores, clinical stage (cT1c vs cT2), prostate volume, body mass index and biopsy GS	Univariate logistic regression analysis: PHI and PCA3 were accurate predictors of TV $>$ 0.5 ml, pathologically confirmed significant PCa and ECE, only PHI predicted pathologic GS $\geqslant$ 7 and SVI. Multivariate analyses: Prediction of TV $>$ 0.5 ml: base model AUC 89.3, addition of PHI increase of 7.9% (AUC 97.2; $P<0.05$), PCA3 did not lead to a significant increase (gain of 2.8%; AUC 92.1). Prediction of ECE: PHI and PCA3 both significantly improved predictive accuracy ($P<0.01$). Prediction of SVI: only PHI led to a significant improvement (AUC 92.2, $P<0.05$ with a gain of 3.6%).
Tallon *et al.*[43]	Clinically localized PCa ($n=154$)	TV, ECE, SVI, GS $\geqslant$ 7, multifocality, positive resection margins, pathological T stage	Age, DRE findings (suspicious vs non suspicious), total PSA and GS at biopsy (6 vs $\geqslant$ 7)	Univariate linear regression analysis: PHI and PCA3 are predictors for TV $\geqslant$ 0.5 ml, only PHI predicted pathologic GS $\geqslant$ 7 and ECE. Only PCA3 predicted multifocality. Multivariate analyses: Prediction of GS $\geqslant$ 7: base model AUC 0.81, addition of PHI increase AUC to 0.86 ($P>0.05$). Prediction of TV $\geqslant$ 0.5 ml: base model AUC 0.69, addition of PHI increase AUC to 0.76 ($P>0.05$), addition of PCA3 increase AUC to 0.74 ($P>0.05$). Addition of PHI and PCA3 significant 12% increase in AUC to 0.81 ($P=0.03$). Decision curve analysis: higher benefit in incorporating PHI to the base model to predict GS $\geqslant$ 7 and TV $\geqslant$ 0.5 ml at RP. Addition of both biomarkers provided the best increase in clinical benefit.
Ferro *et al.*[44]	Clinically localized PCa ($n=78$)	TV, GS $\geqslant$ 7, pathological T stage	No base model used	Predictive accuracy of the single markers: Prediction of TV $\geqslant$ 0.5 ml: PHI AUC 0.94 and PCA3 AUC 0.86. Prediction of GS $\geqslant$ 7: PHI AUC 0.74 and PCA3 AUC 0.78. Prediction of T stage $\geqslant$ 2: PHI AUC 0.85 and PCA3 AUC 0.74. Decision curve analysis: PHI and PCA3 result in greater net benefit in TV $\geqslant$ 0.5 ml and GS $\geqslant$ 7 probability.

Abbreviations: AUC, area under the curve; DRE, digital rectal examination; ECE, extra capsular extension; fPSA, freePSA; GS, Gleason score; PCa, prostate cancer; PCA3, prostate cancer antigen 3; PHI, Prostate Health Index; RP, radical prostatectomy; SVI, seminal vesicles invasion; TV, tumor volume. [a]Using Epstein criteria (organ confined disease, TV $<$ 0.5 ml, and no Gleason pattern 4/5) to exclude pathologically confirmed insignificant PCa.

combination of PHI with PCA3 overall performed better than the single biomarkers. In an initial biopsy cohort in the PSA grey area (2–10 ng ml^{-1}), Ferro *et al.*[48] showed that PHI and PCA3 are the strongest predictors of PCa with no significant differences in pairwise comparison. The combination of the two tests did not further improve diagnostic power in this cohort, in contrast with the results of Stephan *et al.*[45] and Perdona *et al.*[47]

PHI VS PCA3 IN PREDICTION OF PATHOLOGICAL FEATURES OF PROSTATECTOMY SPECIMEN

To evaluate the prognostic accuracy of PCA3 and PHI, these tests were also studied in patients who underwent radical prostatectomy (RP) (see Table 3). Cantiello *et al.*[49] included 156 patients with biopsy-proven, clinically localized PCa and showed that inclusion of PHI significantly increased the accuracy of a base multivariate model (which included age, total PSA, fPSA, rate of positive cores, clinical stage, prostate volume, body mass index and biopsy GS), in predicting TV $>$ 0.5 ml, extra capsular extension (ECE), seminal vesicles invasion (SVI), pathologic GS $\geqslant$ 7 and pathologically confirmed significant PCa. Although both PHI and PCA3 significantly improved accuracy independently (all P's $<$ 0.01) to predict ECE compared with the base model, only PHI led to a significant improvement in the prediction of SVI (AUC 92.2, $P<0.05$). Moreover, in the study of Tallon *et al.*,[50] PHI and PCA3 were both predictors of a TV $\geqslant$ 0.5 ml. Only PHI predicted GS $\geqslant$ 7 and ECE, and multifocality was predicted by PCA3 only. A smaller study of Ferro *et al.*[51] showed that the largest AUC's were obtained with PHI compared with PCA3 for TV $\geqslant$ 0.5 ml (0.94 vs 0.86), GS $\geqslant$ 7 (0.94 vs 0.78) and tumor stage (0.85 vs 0.74). Furthermore, Fossati *et al.*[52] used the PROMETHEUS database to select 489 patients who underwent RP for localized PCa, and to test the correlation between p2PSA, %p2PSA and PHI with pathological features of the RP specimen. For prediction of pT3 disease and/or pathologic GS $\geqslant$ 7, PHI was the most accurate biomarker (AUC 0.74 and 0.69, respectively). Moreover, PHI significantly increased the predictive accuracy of the used base model (PSA, DRE, biopsy GS and percentage of positive biopsy

Table 4. Comparison of PHI and PCA3 in active surveillance

Reference	*Population*	*Criteria for active surveillance*	*End points*	*Base model*	*Key study notes*
Cantiello *et al.*[55]	Clinically localized PCa, underwent RP ($n=188$)	PRIAS[a] and Epstein[b] criteria, retrospectively	PHI and PCA3 added to the PRIAS or Epstein criteria in predicting the presence of pathologically insignificant PCa	1[a]: Age, total PSA, PSA density, clinical stage, number of positive cores and biopsy GS. 2[b]: Age, PSA density, number of positive cores, % of core involvement and biopsy GS	Prediction of pathologically insignificant prostate cancer according to PRIAS[a]: the base model 1 AUC 0.58, which significantly increased by 29% with the addition of PCA3 (AUC 0.87; $P<0.05$), and by 39% with the addition of PHI (AUC 0.97; $P<0.01$). Prediction of pathologically insignificant prostate cancer according to Epstein[b]: the base model 2 AUC 0.60, which significantly increased by 17% with the addition of PCA3 (AUC 0.77; $P<0.05$), and by 32% with the addition of PHI (AUC 0.92; $P<0.01$). Decision curve analysis: both PHI and PCA3 added net benefit over Epstein or PRIAS criteria with a threshold $>10\%$ and $>20\%$, respectively.
Porpiglia *et al.*[56]	Clinically localized PCa, underwent RP ($n=120$)	PRIAS criteria,[53] retrospectively	PHI, PCA3, mpMRI	Total PSA, free/total PSA, DRE, age, positive cores and biopsy GS	Prediction of pathologically confirmed significant prostate cancer: the base model AUC 0.71, which significantly increased by 4% with the addition of PHI (AUC 0.75; $P<0.01$), by 1% with the addition of PCA3 (AUC 0.72; $P<0.01$) and by 7% with the addition of mpMRI (AUC 0.78; $P<0.01$). Decision curve analysis: At the threshold of $>20\%$ the prediction models that included mpMRI added value compared with the base model. At the threshold of $>60\%$ the prediction models that included PHI added net benefit compared with base model. The model that included PCA3 did not add value.

Abbreviations: AUC, area under the curve; DRE, digital rectal examination; GS, Gleason score; mpMRI, multiparametric magnetic resonance imaging; PCa, prostate cancer; PCA3, prostate cancer antigen 3; PHI, Prostate Health Index; PRIAS, Prostate Cancer Research International Active Surveillance; RP, radical prostatectomy. *Note*: Same study group, possible overlap between cohorts. [a]PRIAS criteria: Clinical stage T1c or T2 disease, PSA level of $\leqslant 10$ ng ml^{-1}, Gleason score $\leqslant 6$, PSA density <0.2 ng ml^{-1} and one or two positive biopsy cores. [b]Epstein criteria: PSA density $\leqslant 0.15$ ng ml^{-1}, one or two positive biopsy cores, clinical stage T1c, and % core involvement $\leqslant 50$.

cores) with 2.4% ($P=0.01$) when considering pT3 disease and pathologic GS $\geqslant 7$. Models including %p2PSA and PHI, however, did not result in a greater net benefit when plotted against various threshold probabilities in the decision curve analysis.[52]

PHI VS PCA3 IN AS

Patients are currently selected for AS instead of active treatment based on clinical and pathological characteristics (e.g., total PSA, PSA density, biopsy GS, number of positive cores, percentage of core involvement, clinical stage).[53] Unfortunately, the current stratification risk schemes are not perfect. There is limited accuracy in correctly selecting patients with insignificant PCa and limited tools for predicting progress or need for active treatment during follow-up. Bul *et al.*[53] showed the updated results from the PRIAS (Prostate Cancer Research International Active Surveillance) study, in which 28% of the cohort disease was reclassified (defined as GS >6 and/or >2 positive cores) at the first repeated biopsy during follow-up. Moreover, selection criteria for AS can exclude patients who would actually benefit from expectant management. Therefore, the long-term safety and effectiveness of AS depends on the ability to select appropriate patients and there is an urgent need for better selection and follow-up tools for improving the risk assessment. A recently published systematic review included 30 studies on MRI, serum biomarkers (2pPSA, PHI) and urinary markers (PCA3) for the selection and monitoring of patients on AS.[54] Van den Bergh *et al.*[54] concluded that the addition of a PSA isoform to the current AS criteria could benefit the outcomes. Furthermore, the use of mpMRI is very promising in this domain because of a high negative predictive value with respect to significant PCa. Besides, the mpMRI should be able to guide in decision-making regarding the need for repeat biopsy during AS. There were two retrospective studies published regarding the comparison of PHI and PCA3 in an AS cohort (see Table 4).[55,56] Cantiello *et al.*[55] showed that the outcomes of using the Epstein and PRIAS protocols for selecting patients for AS could be improved by adding PHI or PCA3, with an increase in the predictive accuracy that ranged from 17 to 39%. In a direct comparison and decision curve analysis, PHI outperformed PCA3 performance resulting in higher net benefit. In the study of Porpiglia *et al.*[56] in 55 patients (45.8%) pathologically confirmed reclassification was observed. On multivariate analysis, the inclusion of both PHI and mpMRI significantly increased the accuracy in prediction of significant PCa, whereas PCA3 did not add net benefit.

CONCLUSIONS

To date, many novel promising biomarkers for PCa have been identified, which have been shown to outperform the use of PSA alone. Most studied are the two commercially available biomarkers PHI and PCA3. After reviewing the current literature wherein PHI and PCA are compared head-to-head, we are not able to give a clear recommendation about how and when to use PHI and/or PCA3 in the biopsy setting and treatment selection. In the initial biopsy setting, some studies showed that PHI was a better predictor for PCa and high-grade PCa,[42,44,46] whereas other studies showed that PCA3 was the most accurate predictor.[43,45] In the repeat biopsy setting, there were opposing results as well, and there were no statistically significant differences between PHI and PCA3.[44,45] As for the combination of the two biomarker tests, there is some evidence for improving the diagnostic accuracy,[45,47] although the third study could not confirm these findings.[48] Regarding prediction of pathological features of prostatectomy specimen, PHI and PCA3 both improved the prediction of tumor stage as well as TV.[49–51] Furthermore, only PHI led to a significant improvement for prediction of SVI,[49] whereas PCA3 was the only predictor for tumor multifocality.[50] For selection of eligible patients for AS and follow-up, PHI and/or PCA3 could be used to improve predictive accuracy. According to the decision curve analysis, PHI outperforms PCA3, and the use of mpMRI in this group is very promising.[55,56]

Although many studies have shown that novel biomarkers outperform PSA, they are not yet part of daily clinical practice and guidelines. We would recommend that before using new biomarkers as tools for risk stratification, biopsy decisions and treatment selection in patients with PCa, the biomarkers should be validated and prospectively compared with each other. Especially models in which biomarkers are combined with clinical risk factors should be compared, in particular the 4K score, SelectMDx test and MiPS Score. It should be given the highest priority to compare these risk scores head-to-head in large prospective studies to find out the clinical value and benefit.

Future research should also focus on use of random transrectal ultrasound biopsies as golden standard, because of a high false-negative biopsy rate and the chance of missing clinically significant tumors in the anterior and apical part of the prostate.[57,58] Options to overcome this limitation would be targeted biopsy techniques such as mpMRI-guided biopsies, transrectal ultrasound/MRI fusion biopsies and mapping biopsies, and of course RP specimen. It would also be interesting to study the biomarkers in longer follow-up in the same cohort, to see if there are fluctuations that should be taken into account when interpreting the results. De Luca *et al.*[59] demonstrated that PCA3 scores showed clinically notable changes in ~20% of patients when measured multiple times. Besides the assessment of clinical effectiveness, the cost-effectiveness should be the main focus of future research as well. Nicholson *et al.*[60] performed an economic evaluation of PCA3 and PHI in the diagnosis of PCa for the National Institute for Health Research. Unfortunately, despite a systemic search no published literature met the inclusion criteria for the review for cost-effectiveness. They presented a *de novo* economic model that showed that neither PHI nor PCA3 is likely to be cost-effective when identifying patients for second biopsy compared with clinical assessment alone (e.g., DRE, total PSA, PSA density, age, family history) or clinical assessment plus mpMRI. In addition, the authors suggested that there is a higher risk of identifying more patients as potentially having PCa when PHI or PCA3 are used, compared with if clinicians had only relied on their clinical assessment. On the other hand, PHI and PCA3 could be cost-effective if the tests had higher sensitivity for detecting clinically significant PCa.[60]

To conclude this review, longitudinal studies are required following men from initial investigation through to diagnosis and treatment of PCa to determine clinical effectiveness and cost-effectiveness to guide doctor and patient in decision-making regarding PCa diagnostics and treatment selection.

CONFLICT OF INTEREST

JAS and IMvO have consultancy with honoraria for Astellas, Janssen and Sanofi. The remaining author declares no conflict of interest.

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