ISSN: 2754-4923

Journal of Diagnosis & Case Reports

SCIENTIFIC Research and Community

Review Article

Open Access

Diagnostic and Risk Stratification Molecular Markers of Prostate Cancer Beyond PSA

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ABSTRACT

Prostate cancer still represents the most common urinary malignancies and the second most common cancer in adult men after skin cancer. Prostate specific antigen (PSA) represents a milestone in the diagnosis and screening of prostate cancer since its introduction even considering its limitations in term of sensitivity and specificity.

The widespread use of PSA often led to unnecessary biopsies and to the diagnosis of indolent cancers that do not require treatment, therefore in the era of tailored personalized medicine there is a strong need for new markers that overcome PSA and that can help to identify the patients that have clinically significant disease that must be treated.

To date different urinary and serum biomarkers have been proposed in the diagnostic setting with promising results in terms of sensitivity and specificity, however, none of them have been routinely introduced in clinical practice.

In this review we reported the latest evidence for prostate cancer diagnosis in terms of urinary and blood biomarkers.

Considering all the available markers, it is highly unlikely that one single assay could fit all the requirements and it seems appropriate to use the combination of different urinary and serum markers together with clinical parameters in order to guarantee a good diagnostic performance and to identify only clinically significant disease.

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Received: April 01, 2021; **Accepted:** April 06, 2021; **Published:** April 09, 2021

Keywords: Prostate Cancer Diagnosis, Psa, Molecular Markers, Urinary Markers

Introduction

Prostate cancer (PCa) remains the second most common cancer in men in Europe after skin cancer. The incidence of clinically diagnosed prostate cancer is highest in northern and western Europe (>200 per 100 000 men per year) [1]. The story of prostate cancer has been radically changed since the clinical introduction of the measurement of Prostate-Specific Antigen (PSA) levels. The first clinical application of this protein was as a prognostic marker for patients with a prostate cancer diagnosis and with this aim was approved by FDA (Food and Drug Administration) in 1986; In 1994, the FDA approved the serum PSA test, in combination with a digital rectal exam (DRE), for diagnostic screening of prostate cancer in the clinic [2].

However, the PSA should be considered as a tissue specific marker rather than a cancer specific assay and despite its value in detecting early-stage cancer and in the follow-up of patients with PCa it has some limitations in detecting clinically significant cancers leading to avoidable biopsies and treatment for indolent tumours [3].

For these reasons, the use of PSA as a diagnostic marker for prostate cancer and in the screening is still under debate worldwide [4]. Moreover, the indication for prostate biopsy according to elevated PSA led to a significant number of unnencessary biopsies, in particular in the so-called "grey zone" of PSA between 2 and 10 ng/ml in which PSA derivatives such as PSA density and Free/Total ratio are used. It has been estimated that 65-75% of men with PSA level between 3 and 10 ng/ml did not have a biopsy detectable cancer [5].

A continued and unselected use of PSA represents a significative increased cost for healtcare system with a high risk of over diagnosis and over treatment. Furthermore, prostate a biopsy is an invasive procedure with a not trascurable rate of complications and remain a significant burden for patients. It has been estimated that from 2006-2009, Medicare spent $450 million annually on PSA screening and subsequent diagnostic procedures [6]. Current efforts focus on the development of non-invasive biomarkers to distinguish between PCa and benign prostatic hyperplasia, aggressive and indolent forms of the disease, with the aim of reducing the number of biopsies performed.

The detection and upgrading rate of PCa has already been improved by the implementation of mpMRI-targeted combined with systematic biopsy. As mpMRI has many pitfalls such as cost, availability, reliability, access and generalizability from centres

of excellence, possibly, a predictive cost- efficient marker might assist with the identification of men who require further evaluation. However, available studies suggest a rather complementary effect of biomarkers in combination with mpMRI or clinical risk stratification tools to further improve the predictive accuracy.

Hence there is an unmet need for new biomarkers in prostate cancer, remembering that the ideal biomarker would be non-invasive, easily accessible, inexpensive and able to identify high risk, clinically relevant disease rather than an indolent cancer. In this review, we focus the attention on diagnostic biomarkers for prostate cancer and risk stratification markers used after the first diagnosis in order to select the right treatment for all the patients.

We conducted a literature search on pubmed and scopus including "prostate cancer markers" and "prostate cancer molecular biomarkers" "prostate cancer diagnosis" as key words. We decided to focus the attention on blood and urinary markers excluding tissue biomarkers that are less accessible, more expensive and not applicable in a diagnostic setting.

Serum Biomarkers

Early studies on PSA have shown that in blood it exists in different forms, mostly complexed with serum protease inhibitors such as α1-antichymotripsin and ten to thirty percent exist in a free state. Indeed, the inactive form preproPSA after a 17 amminoacids cut at the amino-terminal end became proPSA that is inactive but can be found in prostate lumen. After a second cut of 7 amminoacid the mature enzyme, PSA, is generated and it is released in the bloo. Among the inactive fragments form proteolytic degradation of the mature enzyme the most represented are free PSA and the precursor proPSA [7].

The precurosr proPSA can be found in different forms containing a 7 ammino-acid pro-peptide and other various truncated pro-peptide leader sequences such as the form with 5, 4 or 2 ammino-acid (-2 proPSA). The isoform -2 pro-PSA is preferentially produced by malignant cells possibily for the alteration of these enzymatic reactions in tumor cells [8]. Therefore, the first adjunctive biomarkers to go beyond PSA have been developed from the possibility of identifiy in blood different isoform of PSA with a higher sensitivity and specificity for cancer. Serum markers are easy to obtain, inexpensive and ideal for screening and eary diagnosis.

Phi Index

The Prostate Health Index (PHI) is an FDA-approved serum test combines total PSA (tPSA), free PSA (fPSA), and the [-2] proPSA isoform using the formula (-2 proPSA/free PSA) × √total PSA (Beckman Coulter, Inc.) in order to create a score for selecting men at risk of having prostate cancer, particularly high-grade cancer (Gleason 7 or higher) [9]. After its introduction, the validity of the test has been confirmed by several studies [10-12]. A recent meta-analysis of 24 clinical studies reported good diagnostic results for PHI with a pooled specificity 0.34, sensitivity 0.89, AUC 0.76 to detect prostate cancer [13]. Moreover, regarding the possibility of detecting high grade disease the study reported a specificity of 0.34, a sensitivity 0.93, AUC 0.82 confirming a higher capacity to identify aggressive prostate cancer. Another metanalysis confirmed that PHI outperforms its single components in detecting high grade disease, with an overall specificity of 0.17 and sensitivity of 0.90 to detect Gleason disease of grade 3 + 4 and greater [14].

In particular in patients with PSA in the "grey zone" in another metanalysis PHI was found to have overcome PSA in terms of accuracy for detecting prostate cancer in another metanalysys [15]. Recently, the concept of "PHI density" was introduced with promising results by Tosojan et al the authors analysed a group of patients with PSA levels > 2.0 ng/ml, negative digital rectal examination (DRE) who underwent PHI testing and consecutive prostate biopsy [16].

Median PHI density was 0.70 for patients with significant PCa according to biopsy results [interquartile range (IQR) 0.43–1.21] compared to 0.53 (IQR 0.36–0.75) for insignificant or negative biopsies ($p < 0.001$).

Therefore, the authors described a threshold of 0.43 and found a sensitivity of 97.9% and a specificity of 38.0% for detection of clinically significant PCa. Sensitivity for Gleason ≥7 PCa was 100.0%. The diagnostic accuracy for detection was higher for PHI density (AUC = 0.84) compared to tPSA (AUC = 0.52), %fPSA (AUC = 0.75), and Phi alone (AUC = 0.76).

Moreover, the possibility of adding PHI as an adjunctive tool to stratify patients that underwent multiparametric MRI have been esplored with promising results in terms of predictive value [17]. Added showed that PHI density outperforms PSA and PHI itself in detecting clinically significant cancer in a group of 241 patients with elevated PSA and negative digital rectal examination. Moreover, considering 104 patients that underwent mpMRI, the combination of Pi-RADS ≥ 3 with PHID ≥ 0.44 identified nearly 100% of clinically significant cancer. The authors concluded that using a threshold of 0.44 for PHI density, 35.3% of unnecessarty biopsies could be avoided [18].

In a systematic review for biomarkers in prostate cancer management, PHI reached a level of evidence = 1 for discriminating between indolent and aggressive prostate cancer, confirming his additional utility compared to classical parameters such PSA [3]. Finally, data from two different studies suggest a role for PHI as an adjunctive tool to be included in multivariable standard risk calculators for men with elevated PSA candidates for prostate biopsy: the predictive accuracy of the Prostate Cancer Prevention Trial [PCPT] and the European Randomised Study of Screening for Prostate Cancer [ERSPC] risk calculators was improved including PHI score [19,20].

4 K Score

The 4K score is a test that evaluate the levels of four different kallikrein markers (tPSA, % fPSA, intact PSA, hK2), combined with clinical features such as age and digital rectal examination findings in order to define the risk of high-grade disease. These panel of proteins was firstly introduced by Vickers et al. and in men with elevated PSA showed an improved diagnostic accuracy compared to PSA alone, leading the authors to conclude that the application of the test in 1000 men with elevated PSA would reduce the number of biopsies by 513 avoiding the risk on an unnecessary procedure [21]. These results have been confirmed even in another a population-based case control study that confirmed the role of 4K score in reducing the number of unnecesessary biopsies in patients with elevated PSA [22].

Another study with a multi-institutional cohort of 1012 men undergoing biopsy in the USA showed an AUC of 0.82 (95% CI: 0.79–0.85) for high-grade cancer, showing excellent diagnostic performance for the test with a possible reduction of more than 30 % of prostate biopsies with delayed diagnosis in only 1.3-4.7% of Gleason ≥7 PCa cases [23]. Together with PHI the 4k score is mentioned in the EAU guidelines as a potential marker to be used in patients with PSA in the grey zone as a useful risk assessment.

In a study on 513 patients with elevated PSA who underwent prostate biopsy, Nordstrom showed that both these tests outperform PSA level alone with a similar AUC both for predicting any grade PCA [69.0 (4K score) vs. 70.4 (PHI)] as well as high-grade PCa (71.8 vs. 71.1). The authors defined a cut off value for PHI at 39 and for 4K score at 10 % and found that 29 % of biopsies could be avoided if the scores would have been used [24].

In a recent metanalysis by Russo et al. Twenty-eight studies including 16,762 patients have been evaluated. The pooled data showed a sensitivity of 0.89 and 0.74 for PHI and 4K panel, respectively, for PCa detection and a pooled specificity of 0.34 and 0.60 for PHI and 4K panel, respectively. The derived area under the curve (AUC) from the hierarchical summary receiver operating characteristic (HSROC) showed an accuracy of 0.76 and 0.72 for PHI and 4K panel respectively. For high-grade PCa detection, the pooled sensitivity was 0.93 and 0.87 for PHI and 4K panel, respectively, whereas the pooled specificity was 0.34 and 0.61 for PHI and 4K panel, respectively. The derived AUC from the HSROC showed an accuracy of 0.82 and 0.81 for PHI and 4K panel, respectively. Both PHI and the 4K panel provided good diagnostic accuracy in detecting overall and high-grade PCa [13].

Serum Proteins

A recent study evaluating 500 serum specimens reported the development of a novel serum protein panel of three prostate cancer biomarkers, Filamin A, Filamin B, and Keratin-19 (FLNA, FLNB, and KRT19) using multivariate models for disease screening and prognosis [25]. The combination of these prostate biomarkers with PSA testing was better than PSA alone in identifying prostate cancer (AUC for panel of FLNA, FLNB, age, PSA, 0.64; PSA alone AUC, 0.58) and improved the prediction of high risk disease (AUC for panel of FLNB, age, and PSA, 0.81; PSA alone AUC, 0.71), low risk disease (AUC for panel of FLNB, age, PSA, and low Gleason Score, 0.72; PSA alone AUC, 0.63), and the prediction of cancer versus benign prostatic hyperplasia (AUC for panel of FLNA, KRT19, and age with PSA, 0.70; PSA alone AUC, 0.58).

In another retrospective study, two other cancer related proteins have been study such as thrombospondin-1 and cathepsin D, two glycoproteins with increased levels in patients with prostate cancer that are currently being evaluated in combination with the free to total PSA ratio. This panel of proteins was found to be superior to serum PSA alone in predicting a positive biopsy in men with PSA levels of 2–10 ng/ml, a prostate volume of ≥35 ml and negative DRE, reducing the number of unneeded biopsies [26].

Germline Mutations

About 9% of men with PCa have truly hereditary disease, which is associated with an onset 6–7 earlier than nonhereditary cases, but does not differ in other ways, apart from African descent with a more aggressive course of disease as well as for breast cancer predisposition gene 2 (BRCA2) carriers [27].

In a large recent study among 3607 men with a personal diagnosis of prostate cancer who were referred for genetic testing, 620 (17.2%) had positive germline variants, of which only 30.7% were variants in BRCA1/2. Positive variants in HOXB13, a gene associated only with prostate cancer risk, were identified in 30 patients (4.5%). DNA mismatch repair variants with substantial known therapeutic implications were detected in 1.74% of variants in the total population tested [28].

Prospective studies confirmed the association between BRCA2 mutations and aggressive PCa, with the evidence that in patients with this mutation, a specific screening for prostate cancer must be done, considering the higher risk of aggressive disease compared to general population [29]. The prospective IMPACT targeted screening study confirmed a higher incidence of PCa, at a younger age and with more clinically significant tumours only in BRCA2mutation carriers compared with noncarriers [30].

Despite these evidences to date there is no clear indication for germline testing in the diagnostic setting, but it is widely accepted the importance of researching these mutations in patients with metastatic PCa, in patients with a strong family history (brother, father or multiple family members with PCa at age <60 yrs) or patients with more cancers on the same side of the family consistent with hereditary breast and ovarian cancer.

Urinary Biomarkers

Urine has emerged as a promising non-invasive source of biomarkers. Compared with blood, urine contains material that directly originates from the prostate gland. Moreover, after digital rectal examination, the prostatic fluids containing molecules, cells and other substances derived from prostate cancer are exuded into the urine. Using sensitive assay or when these substances are in urine in large amount it is possible to detect prostatic fluids even without manipulation.

PCA3

PCA3 is a prostate-specific long noncoding RNA (lncRNA) biomarker detectable in urine obtained after rectal examination. Compared with benign tissue, PCa cells show a 66-fold upregulation of PCA3 transcript levels, which is measured by qPCR by the Progensa test [31].

After promising results in the validation studies, it has been approved by FDA for men with a prior negative biopsy and no evidence of atypical small acinar proliferation (ASAP) to help clinicians and patients decide whether to forego a repeat biopsy based on a threshold result of 25 [32]. The same threshold was considered in another study by Gittelman et al. in 466 men undergoing repeat biopsy showing the possibility of avoid 48 % of unnecessary biopsies missing only 8 high-grade cancers [33].

In a recently published meta-analysis including 65 studies, the pooled overall diagnostic sensitivity, specificity, positive likelihood and negative likelihood ratio, and 95% CIs for predicting clinically significant were 0.68 (0.64 – 0.72), 0.72 (0.68 – 0.75), 2.41 (2.16 – 2.69), 0.44 (0.40 – 0.49), respectively [34]. However, there were some deficiencies of included studies such as differential verification bias, and a lack of clear inclusion and exclusion criteria leading the authors to the conclusion that more rigorous studies are needed for validate the test in the PCa diagnosis. Contrary to PSA, the PCA3 expression does not seem to be affected by inflammation, trauma or prostate volume, but increases with tumour volume [35].

Even reported a statistically significant correlation between extracapsular extension and higher median PCA3 scores (48.8 vs. 18.7, P 1⁄4 0.02). Using a cut-off of 47, they found a sensitivity of 57%, a specificity of 94%, positive and negative predictive values of 80% and 84%, respectively. PCA-3 has been extensively used for the evaluation of the need of a second biopsy in patients with a first negative biopsy and a clinical suspect of PCa, however even if results in this setting are still uncertain, the FDA approved the Progensa test in this setting of patients [36].

Notably, a globally accepted cut-off for PCA3 does not exist and for example in the pre-biopsy setting a role has been showed

with promising results when a cut off value of 60 has been used [37]. Nevertheless, in a head-to-head comparison in biopsy naive patients, the PHI index outperformed PCA 3 in avoiding the diagnosis of indolent prostate cancer [38].

Combining the PCA3 score with mpMRI showed a significantly higher score in patients with suspicious mpMRI findings (52 vs. 21, $P < 0.001$). The combination of these two diagnostic tests improved the predictive accuracy to 91.8% in men with PI- RADS III lesion. Performing repeat biopsy in these men revealed PCa in five out of 15 men. [39-40]. These results confirmed that PCA3, like many of the other biomarkers discussed, performs best when used as part of a model that includes other clinical factors (such as PSA, percent free PSA, prostate volume, age, family history) and it is really useful in men with a negative biopsy who are contemplating a repeat biopsy.

TMPRSS2 – ERG

The fusion of an androgen receptor regulated gene promoter and the N-terminally deleted ERG coding sequence is the most common PCa-specific driver gene alteration detected in urine following a DRE [41,42]. Although being significantly associated with malignancy in biopsy specimens (P 1⁄4 0.0145), especially in men with a PSA of 4 ng/ml or less, no association with clinical stage or Gleason score could be detected [43]. A meta-analysis, including patients treated with radical prostatectomy, could neither prove a significant correlation between TMPRSS2-ERG fusion and biochemical recurrence (BCR) [95% confidence interval (CI), 0.86 – 1.17, relative risk 1.00] nor cancer specific mortality (95% CI, 0.47 – 2.09, relative risk 0.99) [44]. Although TMPRSS2-ERG is the most common alteration, it remains absent in 35 – 50% of white PCa patients, 73% of Asian and 75% of African ancestry PCa patients [45].

Combined Urinary Markers: The MiPS Score

Starting from the evidence for TMPRSS2-ERG fusion gene have a significant predictive value for significant prostate cancer, its role in comination with PCA-3 have been explored with promising results [46]. Showed in 108 prebiopsy post-DRE urine samples (72% diagnosed with PCa) that PCA3 plus TMPRSS2:ERG increase the test sensitivity from 62% for PCA3 alone to 73% for both markers.

The rational basis of this association and the possibility of reducing the false positive rate of PCA-3 using the combination with TMPRSS2-ERG fusion gene have been shown in a study [47]. A clinical algoritm combining serum PSA, PCA3 and TMPRSS2-ERG in urine after digital rectal examination have been proposed in a group of 48 patients by Salami et al. showing the highest sensitivity in predicting prostate cancer for PCA3 (93%) and the highest specificity for TMPRSS2-ERG (87%). Therefore, the authors proposed a clinical agloritm to select patients for biopsy when PSA is < 10 ng/ml in which combining this serum and urinary biomarkers the highest specicifity and sensitivity could be reached [48].

In the same regard that combination of biomarkers such as TMPRSS2:ERG with other clinical parameters might have increased diagnostic value, Tomlins et al. designed and validated logistic regression models based on TMPRSS2:ERG, PSA and PCA3 status and created a score (Mi Prostate score; MiPS). The authors found that the statistical models that incorporated MiPS models had significantly greater AUC than models incorporating only PCA3 plus PSA (AUC 0.751; $p < 0.001$) [49].

In another study the TMPRSS2-ERG and PCA3 were evaluated in addition to the European Randomised Study of Screening for Prostate Cancer (ESPRC) risk calculator has diagnostic tool in the urine samples of 443 men. 196 of the group had a diagnosis of prostate cancer and both PCA 3 and TMPRSS2-ERG showed an increased predictive value compared to EPRS test alone [50].

The combination of TMPRSS2:ERG and PCA-3 have been tested for the prediction of aggressive prostate cancer (Gleason 7 or higher) in a population of 516 men, confirming an improved specificity compared to PSA and high sensistivity for aggressive disease (93%).The authors conclude that Forty-two percent of unnecessary prostate biopsies would have been averted by using the urine assay results to select men for biopsy [51]. However, to date only a statistical significance has been shown for this test without a real impact compared to available clinical features and this is not enough to consider this biomarker clinically valid.

SELECT MDX

SELECTMDX (MDxHealth, Inc, Irvine, California, USA) is a test that analyses mRNA-based biomarkers HOXC6 (urinary homeobox C6) and DLX1 (distal-less homeobox 1) in post DRE urine samples; it has been shown to be a promising tool to identify aggressive cancer even in patients wit low PSA [52]. In 2015 Van Neste et al. developed a multimodal risk score in which select MDX was included in a model with traditional risk factors (DRE, psa, history of familiar prostate cancer) with an AUC of 0.90 (95% CI: 0.87 – 0.93) for prediction of clinically significant PCa. To evaluate the clinical utility of the model, a decision curve analysis was performed, and the model was compared to other decision-making models (e.g., Prostate Cancer Prevention Trial risk calculator with or without PCA3 test). Hereby, a total reduction of biopsies by 42% and a decrease of unnecessary biopsies by 53% could be observed for a model with a negative predictive value of 98% for Gleason ≥7 PCa [53].

Moreover, it has been shown that strategic use of SelectMDx in patients with PSA levels of > 3 ng/ml can lead towards a reduction of overdiagnosis and overtreatment and ultimately reduce total costs per patient and increase health-related quality of life [54]. Considering the primary role of MRI in the diagnostic setting of PCa, correlated this score with high- grade disease on biopsy and mpMRI findings [55]. They found a significant correlation with suspicious lesions on mpMRI ($P < 0.001$). Moreover, a statistical correlation between the SelectMDx score and the PI- RADS score was reported ($P < 0.01$).

The definitive role of SelectMDx might be considered as an additional parameter together with clinical, histopathological features and imaging, but its future application in daily routine remains to be further assessed taking into account public health strategies and resource availability.

EXO DX

EVs are small vesicles of 30–1000 nm in diameter that are secreted from various cell types, normal epithelial cells, immune cells and cancer cells. EVs in urine after prostate massage include exosomes and prostasomes. Exosomes, containing RNAs, DNAs and proteins, have been shown to be involved in tumour progression and a rich potential source of tumour biomarkers, especially for profiling analysis of their miRNAs content [56].

One of the promising potential biomarkers are prostate cancer-related exosomes that can deliver various pro-oncogenic molecules between cancer cells and from cancer to normal cells to induce

malignant transformation [57]. In 2009, Nilsson et al. reported that prostate cancer-derived exosomes, which contain the prostate cancer-specific markers of PCA3 and TMPRSS-ERG, were detected in urine after prostate massage of patients with prostate cancer [58].

EXO106, an algorithm that associates PCA3 and ERG exosomal mRNA levels, demonstrated good clinical performance in predicting high-grade disease (Gleason score 7) with negative predictive value (NPV) and positive predictive value of 97.5% and 34.5%, respectively, in 195 non-DRE urine samples of men undergoing a prostate biopsy [59]. Moreover, the median EXO106 score correlated with histologic grade of prostate cancer.

Exosome Diagnostics Inc. (Cambridge, MA, USA) developed the ExoDx prostate Intelliscore urine exosome assay that is an exosomal RNA-based test to assess ERG, PCA3 and SPDEF expression and calculate a score. All three genes play a significant pro-oncogenic role in prostate cancer. This test predicted high-grade prostate cancer (Gleason≥7 (3+4)) with NPV > 0.9 for men aged≥50 years with PSA of 2–10 ng/ml who underwent the initial biopsy [60]. This test showed an improved identification of patients with higher-grade prostate cancer among men with elevated PSA levels and could reduce the number of patients selected for unnecessary biopsy.

Mir Sentinel Test

The Sentinel PCa Test determines the presence or absence of PCa, the miR Sentinel CS Test aims to distinguish patients with low-risk PCa (GS 6) from intermediate and high-risk disease (Gleason score 7a to 10), and the miR Sentinel HG Test stratifies men within the low and favourable intermediate-risk group (GG1 or GG2) vs. those with high-risk (GG3 – 5) disease. The test is a platform that analyses the small noncoding RNA from urinary exosomes. In a study by Wei-Lin et al. validated the test analysing the urinary exosomes of 235 participants and using a case-control sample of 1,436 subjects. The results published by Wei-Lin were very promising; miR Sentinel PCa shows a sensitivity of 94%, a specificity of 92%, a PPV of 92% and an NPV of 94%. Similar performance characteristics were reported for the two other tests miR Sentinel CS and miR HG [61]. This test might help to distinguish between patients who could benefit from a definitive therapy and those who might be eligible for active surveillance. As necessary for all biomarkers, these assays need to undergo stringent assessment but might lead to important changes in future PCa diagnosis/management.

Conclusion

Promising results have been recently reported on blood and urinary biomarkers for PCa diagnosis and management. Considering the broad area of application for biomarkers, it is highly unlikely that there will be one specific biomarker that meets all demands according to a one fits all principle. In the future, an accurate, personalized PCa management will probably be determined by sequential use of biomarker panels, artificial intelligence and new imaging modalities such as mpMRI, PSMA-PET/CT and contrast-enhanced or high-resolution microultrasound. This approach might make invasive diagnostics redundant, personalize clinical care and drive precision medicine forward for the care of our PCa patients. Therefore, further studies for a concrete clinical application of these biomarkers must be conducted, both for PCa screening both for having adjunctive tools to discriminate indolent cancer from aggressive disease and to tailor the right therapeutic approach for PCa patients.

Table 1: Biomarkers according source, accuracy, mention in EAU (european Association of urology) guidelines, fda approval

	SOURCE	DIAGNOSTIC ACCURACY	EAU	FDA APPROVAL
PHI TEST	Blood	AUCs [24] Any grade 70.4 (95% CI: 66.1–74.8) Gleason ≥7 71.1 (95% CI: 66.0–76.2)	Yes	Yes
4 k Score	Blood	AUCs [24] Any grade 69.0 (95% CI: 64.5–73.4) Gleason ≥7 71.8 ((95% CI: 66.8–76.7)	Yes	Yes
Serum Proteins Panel	Blood	AUCs [25] * Any grade 0.70 [95% CI 0.60, 0.80] Gleason ≥7 0.81 [95% CI 0.70, 0.90]	Not mentioned	No
PCA 3	Urine	AUC [34] Any grade 0.76 (95% CI: 0.72–0.79)	Yes	Yes
MiPS score	Urine	AUC [49] Any grade 0.71 Gleason ≥6 0.72	Yes	Not needed
Select MDX	Urine	AUC [53] ** Any grade 0.90 (95% CI, 0.85–0.95)	Yes	Yes
Exo Dx	Urine	AUCs [59] Any grade 0.71 [95% CI 0.64, 0.78] Gleason ≥7 0.76 [95% CI 0.69, 0.83]	Yes	No
Mir Sentinel Test	Urine	Sensitivity 94% [61] Specificity 92% [61]	No	No

*in combination with PSA
** in combination with PSA and clinical factors

Funding: This research received no external funding

Aknowledgment: None

References

1. Arnold M, Karim-Kos HE, Coebergh JW, Graham Byrnes, Ahti Antilla, et al. (2015) Recent trends inincidence of five common cancers in 26 European countries since1988: analysis of the European Cancer observatory. Eur J Cancer 51: 1164-1187.
2. Catalona W J, Smith D S, Ratli T L, Dodds K M, Coplen D E, et al. (1991) Measurement of prostate-specific antigen in serum as a screening test for prostate cancer. N. Engl. J. Med 324: 1156-1161.
3. Lamy P J, Allory Y, Gauchez A S, Bernard Asselain, Philippe Beuzeboc, et al. (2018) Prognostic biomarkers used

forlocalised prostate cancer management: a systematic review. EurUrol Focus 4: 790-803.

4. N Mottet, R C N, Van Der Bergh, E Briers, Thomas Van den Broeck, Marcus G Cumberbatch, et al. (2021) EAU-EANM-ESTRO-ESUR-SIOG Guidelines on Prostate Cancer—2020 Update. Part 1: Screening, Diagnosis, and Local Treatment with Curative Intent, 79: 243-262.
5. Schröder F H, Hugosson J, Roobol M J, Tammela T L, Zappa M, et al. (2014) Screening and prostate cancer mortality: results of the European Randomised Study of Screening for Prostate Cancer (ERSPC) at 13 years of follow-up. Lancet 384: 2027-2035.
6. Ma X, Wang R, Long J B, Ross J S, Soulos P R, et al. (2014) The cost implications of prostate cancer screening in the medicare population. Cancer 120: 96-102.
7. M Ferro, O De Cobelli, G lucarelli, Angelo Porreca, Gian Maria Busetto, et al. (2020) Beyond PSA: The Role of Prostate Health Index (phi) International Journal of Molecular Sciences 21: 1184.
8. Sokoll L J, Sanda M G, Feng Z, Kagan J, Mizrahi I A, et al. (2010) 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 19: 1193-1200.
9. Catalona W J, Partin A W, Sanda M G, John T Wei, George G Klee, et al. (2011) 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 185: 1650-1655.
10. G Lughezzani, Massimo Lazzeri, Alexander Haese , Thomas McNicholas, Alexandre de la Taille, et al. (2014) Multicenter European external validation of a prostate health index-based nomogram for predicting prostate cancer at extended biopsy, Eur. Urol 66: 906-912.
11. C de la Calle, Dattatraya Patil, John T Wei, Douglas S Scherr, Lori Sokoll, et al. (2015) Multicenter evaluation of the prostate health index to detect aggressive prostate cancer in biopsy naïve men, J. Urol 194: 65-72.
12. M Boegemann, Carsten Stephan, Henning Cammann, Sébastien Vincendeau, Alain Houlgatte, et al. (2016) 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 117: 72-79.
13. G I Russo, Federica Regis, Tommaso Castelli, Vincenzo Favilla, Salvatore Privitera, et al. (2016) A systematic review and meta-analysis of the diagnostic accuracy of prostate health index and 4-kallikrein panel score in predicting overall and high- grade prostate cancer, Clin. Genitourin. Cancer 15: 429-439.
14. W Wang, Meilin Wang, Li Wang, Tamara S Adams, Ye Tian, et al. (2014) Diagnostic ability of %p2PSA and prostate health index for ag- gressive prostate cancer: a meta-analysis, Sci. Rep 4: 5012.
15. Filella X, Giménez N (2013) Evaluation of [-2] proPSA and Prostate Health Index (PHI) for the detection of prostate cancer: a systematic review and meta-analysis. Clin Chem Lab Med 51: 729-39
16. Tosoian J J, Sasha C Druskin, Darian Andreas, Patrick Mullane, Meera Chappidi, et al. (2017) Prostate health index density improves detection of clinically- significant prostate cancer. BJU Int 120: 793-798.
17. Hsieh P F, Li J, Lin C, Chang H, Chang CH, et al. (2019) Combining prostate health index and multiparametric magnetic resonance imaging in the diagnosis of clinically significant prostate cancer in an Asian population. World J Urol 2019.
18. Druskin S C, Tosoian J J, Young A, Collica S, Srivastava A, et al. (2018) Combining Prostate Health Index density, magnetic resonance imaging and prior negative biopsy status to improve the detection of clinically significant prostate cancer. BJU Int 121: 619-626.
19. Loeb S, Shin S S, Broyles D L, John T Wei, Martin Sanda, et al. (2017) Prostate health Index improves multivariable risk prediction of aggressive prostate cancer. BJU Int 120: 61-68.
20. Foley R W, Maweni R M, Gorman L, Keefe Murphy, Dara J Lundon, et al. (2016) European randomised study of screening for prostate cancer (ERSPC) risk calculators significantly outperform the prostate cancer prevention trial (PCPT) 2.0 in the prediction of prostate cancer: a multi-institutional study. BJU Int 118: 706-713.
21. Vickers A J, Gupta A, Savage C J, Pettersson K, Dahlin A, et al. (2011) A panel of kallikrein marker predicts prostate cancer in a large, population-based cohort followed for 15 years without screening. Cancer Epidemiol. Biomark. Prevent 20: 255-261.
22. Stattin P, Andrew J Vickers, Daniel D Sjoberg, Robert Johansson, Torvald Granfors et al. (2015) Improving the specificity of screening for lethal prostate cancer using prostate-specific antigen and a panel of kallikrein markers: a nested case-con- trol study. Eur. Urol 2105 68: 207-213.
23. Parekh D J, Punnen S, Sjoberg DD, Scott W Asroff , James L Bailen et al. (2015) A multi-institutional prospective trial in the USA confirms that the 4K score accurately identifies men with high-grade prostate cancer. Eur Urol 68: 464-470.
24. Nordstrom T, Vickers A, Assel M, Lilja H, Gronberg H, et al. (2015) Comparison between the four-kallikrein panel and Prostate Health Index for predicting prostate cancer. Eur Urol 68: 139-46.
25. Ravipaty S, Wu W, Dalvi A, Tanna N, Andreazi J, et al. (2017) Clinical validation of a serum protein panel (flna, flnb and krt19) for diagnosis of prostate cancer. J. Mol. Biomark. Diagn 8: 323.
26. Steuber T, Pierre Tennstedt, Annalisa Macagno, Alcibiade Athanasiou, Anja Wittig, et al. (2019) Thrombospondin 1 and cathepsin D improve prostate cancer diagnosis by avoiding potentially unnecessary prostate biopsies. BJU Int 123: 826-833.
27. Castro E, Goh C, Leongamornlert D, Ed Saunders, Malgorzata Tymrakiewicz, et al. (2015) Effect of BRCA mutationson metastatic relapse and cause-specific survival after radicaltreatment for localised prostate cancer. Eur Urol 68: 186-93.
28. Piper Nicolosi, Elisa Ledet, Shan Yang, Scott Michalski, Brandy Freschi et al. (2019) Prevalence of Germline Variants in Prostate Cancer and Implications for Current Genetic Testing Guidelines JAMA Oncol 5: 523-528.
29. Nyberg T, Frost D, Barrowdale D, D Gareth Evans, Elizabeth Bancroft, et al. (2020) Prostate cancer risks formale BRCA1 and BRCA2 mutation carriers: a prospective cohortstudy. Eur Urol 77: 24-35.
30. Page E C, Bancroft E K, Brook M N, Melissa Assel, Mona Hassan Al Battat, et al. (2019) Interim results from theIMPACT study: evidence for prostate-specific antigen screening inBRCA2 mutation carriers. Eur Urol 76: 831-842.
31. Hessels D, Klein Gunnewiek JMT, van Oort I, Herbert F M Karthaus, Geert J L van Leenders, et al. (2003) DD3 (PCA3)-based molecular urine analysis for the diagnosis of prostate cancer. Eur Urol 44: 6-8.
32. Crawford E D, Rove K O, Trabulsi E J, Junqi Qian, Krystyna

P Drewnowska, et al. (2012) Diagnostic performance of PCA3 to detect prostate cancer in men with increased prostate specific antigen: a prospective study of 1,962 cases. J Urol 188: 1726-1731.

33. Gittelman MC, Hertzman B, Bailen J, Thomas Williams, Isaac Koziol, et al. (2013) PCA3 molecular urine test as a predictor of repeat prostate biopsy outcome in men with previous negative biopsies: a prospective multicenter clinical study. J Urol 190: 64-69.
34. Qin Z, Yao J, Xu L, Zheng Xu, Yuzheng Ge, et al. (2019) Diagnosis accuracy of PCA3 level in patients with prostate cancer: a systematic review with meta-analysis]. Int Braz J Urol 45: 10.
35. Whitman E J, Jack Groskopf, Amina Ali, Yongmei Chen, Amy Blase, et al. (2008) PCA3 score before radical prostatectomy predicts extracapsular extension and tumor volume. J Urol 180: 1975-1978.
36. Nicholson A, James Mahon, Angela Boland, Sophie Beale, Kerry Dwan, et al. (2015) The clinical effectiveness and cost-effectiveness of the PROGENSA(R) prostate cancer antigen 3 assay and the Prostate Health Index in the diagnosis of prostate cancer: a systematic review and economic evaluation. Health Technol Assess 19: 1.
37. Wei J T, Ziding Feng, Alan W Partin, Elissa Brown, Ian Thompson, et al. (2014) Can urinary PCA3 supplement PSA in the early detection of prostate cancer? J. Clin. Oncol 32: 4066-4072.
38. Seisen T, Roupret M, Brault D, Priscilla Léon, Géraldine Cancel-Tassin, et al. (2015) 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 75: 103-111.
39. Leyten G H, Wierenga E A, Sedelaar J P, Inge M van Oort, Jurgen J Futterer, et al. (2013) Value of PCA3 to predict biopsy outcome and its potential role in selecting patients for multiparametric MRI. Int J Mol Sci 14: 11347-11355.
40. Kaufmann S, Bedke J, Gatidis S, J Hennenlotter, U Kramer, et al. (2016) Prostate cancer gene 3 (PCA3) is of additional predictive value in patients with PI-RADS grade III (intermediate) lesions in the MR-guided re-biopsy setting for prostate cancer. World J Urol 34: 509-515.
41. Petrovics G, Liu A, Shaheduzzaman S, B Furusato, C Sun, et al. (2005) Frequent overexpression of ETS- related gene-1 (ERG1) in prostate cancer transcriptome. Oncogene 24: 3847-3852.
42. Sanguedolce F, Cormio A, Brunelli M, Alessandro D'Amuri, Giuseppe Carrieri, et al. (2016) Urine TMPRSS2: ERG fusion transcript as a biomarker for prostate cancer: literature review. Clin Genitourin Cancer 14: 117-121.
43. Rice KR, Chen Y, Ali A, Eric J Whitman, Amy Blase, et al. (2010) Evaluation of the ETS-related gene mRNA in urine for the detection of prostate cancer. Clin Cancer Res 16: 1572-1576.
44. Pettersson A, Graff RE, Bauer SR, Michael J Pitt, Rosina T Lis, et al. (2012) The TMPRSS2:ERG rearrangement, ERG expression, and prostate cancer outcomes: a cohort study and meta- analysis. Cancer Epidemiol Biomarkers Prev 21: 1497-1509.
45. Zhou C K, Young D, Yeboah E D, Sally B Coburn, Yao Tettey, et al. (2017) TMPRSS2:ERG gene fusions in prostate cancer of west African men and a meta-analysis of racial differences. Am J Epidemiol 186: 1352-1361.
46. Hessels D, Smit F P, Verhaegh G W, Witjes J A, Cornel E B, et al. (2007) Detection of TMPRSS2-ERG fusion transcripts and prostate cancer antigen 3 in urinary sediments may improve diagnosis of prostate cancer. Clin Cancer Res 13: 5103-5108.
47. Robert G, Jannink S, Smit F, Tilly Aalders, Daphne Hessels, et al. (2013) Rational basis for the combination of PCA3 and TMPRSS2:ERG gene fusion for prostate cancer diagnosis. Prostate 73: 113-120.
48. Salami S S, Schmidt F, Laxman B, Meredith M Regan, David S Rickman, et al. (2013) Combining urinary detection of TMPRSS2:ERG and PCA3 with serum PSA to predict diagnosis of prostate cancer. Urol Oncol 31: 566-571.
49. Tomlins S A, Day J R, Lonigro R J, Daniel H Hovelson, Javed Siddiqui, et al. (2016) Urine TMPRSS2: ERG plus PCA3 for individualized prostate cancer risk assessment. Eur Urol 70: 45-53.
50. Leyten GHJM, Hessels D, Jannink S A, Smit F P, de Jong H, et al. (2014) Prospective multicentre evalua- tion of PCA3 and TMPRSS2-ERG gene fusions as diagnostic and prognostic urinary biomarkers for prostate cancer. Eur Urol 65: 534-542.
51. Sanda MG, Feng Z, Howard D H, Scott A Tomlins, Lori J Sokoll, et al. (2017) Association between combined TMPRSS2: ERG and PCA3 RNA urinary testing and detection of aggressive prostate cancer. JAMA Oncol 3: 1085-1093.
52. Gisele H J M Leyten, Daphne Hessels, Frank P Smit, Sander A Jannink, Hans de Jong, et al. (2015) Identification of a candidate gene panel for the early diagnosis of prostate cancer. Clin. Cancer Res 21: 3061-3070.
53. Van Neste L, Hendriks R J, Dijkstra S, Geert Trooskens, Erik B Cornel, et al. (2016) Detection of high-grade prostate cancer using a urinary molecular biomarker – based risk score. Eur Urol 70: 740-748.
54. Dijkstra S, Tim M Govers, Rianne Hendriks, Jack A Schalken, Wim Van Criekinge, et al. (2017) Cost-effectiveness of a new urinary biomarker-based risk score compared to standard of care in prostate cancer diagnostics – a decision analytical model. BJU Int 120: 659-665.
55. Hendriks R J, Marloes M G van der Leest, S Dijkstra, Jelle Barentsz, Wim Van Criekinge, et al. (2017) A urinary biomarker-based risk score correlates with multi- parametric MRI for prostate cancer detection. Prostate 77.
56. J E McDunn, Zhen Li, Klaus-Peter Adam, Bruce P Neri, Robert L Wolfert, et al. (2013) Metabolomic signatures of aggressive prostate cancer, Prostate 73: 1547-1560.
57. C A Franzen, Robert H Blackwell, Kimberly E Foreman, Paul C Kuo, Robert C Flanigan et al. (2016) Urinary exosomes: the potential for biomarker utility, intercellular signaling and therapeutics in urological malignancy, J. Urol 195: 1331-1339.
58. Nilsson J, Skog J, Nordstrand A, V Baranov, L Mincheva-Nilsson, et al. (2009) Prostate cancer-derived urine exosomes: a novel approach to biomarkers for prostate cancer. Br. J. Cancer 100: 1603-1607.
59. Donovan MJ, Noerholm M, Bentink S, S Belzer, J Skog, et al. (2015) A molecular signature of PCA3 and ERG exosomal RNA from non-DRE urine is predictive of initial prostate biopsy result. Prostate Cancer Prostatic Dis 18: 370-375.
60. McKiernan, Michael J Donovan, Eric Margolis, Alan Partin, Ballentine Carter, et al. (2016) A novel urine exosome gene expression assay to predict highgrade prostate cancer at initial biopsy, JAMA Oncol 2: 882-889.
61. Wang W-LW, Sorokin I, Aleksic I, Hugh Fisher, Ronald P Kaufman, et al. (2020) Expression of small noncoding RNAs && in urinary exosomes classifies prostate cancer into indolent and aggressive disease. J Urol 204: 466-475.