

# Modern biomarkers in prostate cancer diagnosis

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**Introduction** The most common malignant neoplasm of the urinary tract is prostate cancer (PCa), which is a heterogeneous disease, ranging from very slowly developing and slightly benign to progressing, aggressive, metastatic and fatal, even when properly treated. Existing, imperfect diagnostic methods often lead to over-diagnosis and over-treatment of PCa. That is why new, better PCa biomarkers are being developed.

**Material and methods** This review summarizes the current results of the most promising and clinically used PCa biomarkers, as well as having the potential to create new diagnostic and prognostic tools, based on the Web of Science ([www.apps.webofknowledge.com](http://www.apps.webofknowledge.com)) and Scopus ([www.scopus.com](http://www.scopus.com)) databases.

**Results** Limited specificity of the prostate-specific antigen (PSA) test brings a need to develop new and better diagnostic tools. In the last few years, new approaches for providing significantly better biomarkers, an alternative to PSA, have been introduced. Modern biomarkers show improvement in being used as not only a diagnostic procedure, but also for staging, evaluating aggressiveness and managing the therapeutic process. We describe the methods recommended in the diagnosis of PCa and new PCa molecular diagnostics technologies. Individual biomarkers are used in various stages of the PCa diagnostic process, which was presented on the developed diagnostic flowchart describing the role of biomarkers in prostate cancer management.

**Conclusions** Given the diverse nature of PCa, one diagnostic test will not answer all questions, so the use of several diagnostic methods will allow physicians to provide patients with better, personalized clinical advice.

**Key Words:** prostate cancer ◊ biomarker ◊ risk stratification ◊ diagnosis

## INTRODUCTION

Prostate cancer (PCa) is the most common malignant neoplasm of the urinary tract. PCa ranks second in incidence and fifth in mortality among all malignancies. The life risk of PCa diagnosis is reported as one in nine men, but the risk of death may be as low as 2% [1]. The current recommendations for PCa diagnosis are based on the guidelines of the European Association of Urology (EAU-ESTRO-SIOG), which consist in analyzing the concentration of prostate-specific antigen (PSA), as well as conducting a digital rectal examination (DRE) for abnormalities [2]. However, DRE has low sensitivity [3], while PSA is rather organ-, but not tumor-specific (low specificity), and has a low positive predictive value (~30%) [4]. Thus

far, the final recognition depends on the histopathological report of adenocarcinoma in the core biopsy of the prostate gland. False positive PSA test results, in patients with benign prostatic hyperplasia (BPH) and/or prostatitis, may result in systematic transrectal ultrasonography (TRUS) – controlled prostate biopsy (Bx). Additionally, PSA – based screening may lead to over-diagnosis and potentially over-treatment of PCa, which would never be of clinical relevance. There is a clinically unmet need to develop biomarkers that will help control PCa treatment strategies. Several diagnostic tools are available on the PCa laboratory market. There are new biomarkers for serum, urine and even tissue samples [5]. New biological markers, such as TMPRSS2-ERG fusion gene, and the non-coding RNA (PCA3) [6]

or kallikrein included in basic PHI (prostate health index) or 4K tests [7], have been shown to increase sensitivity and specificity PSA, potentially avoiding biopsy and reducing over-diagnosis. Modern biomarkers used in prostate diagnosis have been listed in Table 1.

The guidelines recommend using these tests, additionally to standard methods, as an effective diagnostic tools for cancer diagnosis. Irregularities resulting from the mentioned tests are an indication for prostate biopsy [2]. Finally, risk calculators can be helpful in determining (individually) the potential risk of cancer, thus reducing the number of unnecessary biopsies [8].

This review is based on current knowledge about available diagnostic and prognostic molecular markers of PCa, contained in the databases of Web of Science ([www.apps.webofknowledge.com](http://www.apps.webofknowledge.com)) and Scopus ([www.scopus.com](http://www.scopus.com)). It describes (Table 1) biomarkers serum-, urine-, tissue-based using diagnostic values such as: AUC – area under the ROC (receiver operating characteristic) curve, sensitivity, specificity and the ability to avoid unnecessary biopsy.

The purpose of this article is to present the methods currently recommended for the diagnosis of PCa as well as other tests that can potentially create new diagnostic and prognostic tools.

## Serum-based biomarkers

### Prostate-specific antigen

A PSA count >4 ng/ml has a specificity of 94%, but only 20% sensitivity in PCa detection. Such low sensitivity makes the test of little use in PCa screening. Only 1 in 4 men with elevated PSA will be diagnosed with PCa, while patients with PSA <1 ng/ml are still 10% likely to develop the disease [9].

An increased PSA value is observed in both PCa and adenoma patients. Despite the use of several diagnostic models containing clinical data such as patient age, family history of PCa, PSA derivatives (e.g. PSAD – PSA density, PSAV – PSA velocity), PSA as a screening method, leads to over – diagnosis and, consequently, to over – treatment [10]. For this reason, newer and better methods of diagnosing PCa are sought [11].

### Prostate Health Index (PHI Beckman Coulter, Atlanta, GA, USA)

The PHI test was approved by the US Food and Drug Administration (FDA) to diagnose PCa in 2012. It measured three values (total PSA, free PSA, precursor PSA – (-2) proPSA), based on which PCa probability is calculated – the so-called ‘phi result’

(based on the following mathematical formula ‘phi result’ = (-2) proPSA / fPSA) x  $\sqrt{tPSA}$  [12]. First, the PHI test was developed to predict the probability of both any PCa or high-grade cancer. Usually, the use of PHI with cut-off  $\geq 25$  could avoid 40% of biopsies, at the expense of not detecting 5% high-grade cancers. The test is recommended for men whose serum PSA count is between 2 and 10 ng/ml and whose DRE showed no abnormalities. A study by White and colleagues confirmed that PHI tests save up to 40% of unnecessary prostate biopsies [13]. Second, the PHI test also makes it possible to assess the likelihood of PCa progression during active surveillance. Therefore it is used to monitor patients [14].

The 2015 National Comprehensive Cancer Network (NCCN) guidelines recommended the use of PHI for early detection of prostate cancer, but did not recommend its use as a first-line study in all patients. Being a cost-effective blood test, PHI should be used both before prostate biopsy and when determining further course of treatment [15].

### 4KScore (OPKO Lab, Nashville, TN, USA)

The 4K test includes a specially-developed PCa diagnostic algorithm that includes as many as four kallikreins in blood plasma. The analysis includes a 4K panel = total PSA (tPSA), free PSA (fPSA), intact PSA and human kallikrein 2 (hK2). Additionally, the algorithm also takes into account the patient's age, DRE test results, and prostate biopsy history. A calculation of the above data allows to assess the probability of high-grade PCa [16]. Its use is recommended in patients undergoing initial and repeated biopsy. Using 4KScore with a cut-off risk of 9%, the risk calculated would avoid 43% biopsies at the expense of missing 2.4% high-grade PCa [17]. Several prospective multicenter studies have shown, that both PHI and 4KScore tests, performed better prediction of clinically significant PCa (csPCA) than using PSA and free/total PSA in men with PSA between 2–10 ng/ml [13].

According to many experts, 4KScore blood sampling allows the diagnosis of PCa at the early stages of cancer development [18]. Additionally, it helps to assess the risk of occurrence and development of aggressive PCa even in 20 years, with an accuracy of 0.8–0.9 area under the ROC [17] curve (AUC) (Table 1).

## Urine-based biomarkers

### Progenza (Gen-Probe Inc., San Diego, California, USA)

The prostate cancer gene 3 (PCA3) detects long non-coding RNA (lncRNA), which can be detected

in urine sediments obtained after three strokes of prostate massage in DRE. Its expression may be up to 80 times higher in patients with PCa than in benign prostatic hyperplasia. The test was FDA-approved in 2012. The Progenesa PCA3 calculates the ratio of value PCA3 mRNA (messenger RNA) versus PSA mRNA. Commercially available Progenesa PCA3 urine test outweighs the total and percentage of free/total PSA for detecting PCa in biopsy-naïve men [20]. A PCA3 test result <20 indicates a very low probability of significant cancer (<15%), re-examination should be considered after 6–12 months. This test result justifies refraining from prostate biopsy, which undoubtedly improves patient comfort and prevents suffering from unwanted complications that may occur. A PCA3 test value >35 sug-

gests an increased risk of PCa and justifies prostate biopsy. Adopting the 35 threshold to make a biopsy decision makes for the most reliable risk of a positive biopsy. For test results between 20 and 35, it is recommended to retest after 6 months [21]. Numerous studies indicate that the PCA3 result has greater accuracy in general detection of PCa in repeat biopsy settings compared to tPSA and f/tPSA. However, the data on the association of the PCA3 result with clinically significant prostate cancer (csPCa) and its use for monitoring in active surveillance (AS) is contradictory [20, 22].

Currently, the main indication for the Progenesa PCA3 test is whether it is necessary to repeat the biopsy after an initially negative. This indication is only approved by the FDA, because, there is a high

**Table 1.** Modern biomarkers used in prostate cancer diagnosis

	Biomarker test	Molecular markers	AUC	Sensitivity / Specificity	Unnecessary biopsy reduction	Other characteristics
Consideration for initial biopsy	Serum-based biomarkers					
	Prostate-specific antigen (PSA)	PSA	0.55 [20]	60% / 79% [40]	NA	Treatment monitoring
	PHI	total PSA, free-PSA, p2PSA isoform	0.71 [19]	82% / 80% [12]	40% [13]	Reduce unnecessary Bx Prediction of high-grade PCa active supervision monitoring
	4KScore	total PSA, free-PSA, intact PSA, hK2	0.8–0.9 [17]	75% / 63% [19]	43% [18]	Reduce unnecessary Bx PCa metastases risk prediction (up to 20 years later); previous negative biopsy/biopsies
	Urine-based biomarkers					
	ExoDX Prostate IntelliScore	Exosomal RNA (SPDEF, PCA3, ERG)	0.70 [26]	92% / 34% [26]	27% [26]	Improved identification of high-grade PCa
	MiPS Mi(chigan) Prostate Score	PCA3 and TMPRSS2-ERG mRNA	0.69 [25]	N/A/ 93% [25] 93% / 33% [32]	35–47% [25]	Predict the risk of PCa and csPCa
	Progenesa (PCA3)	long non-coding (lnc) RNAs	0.73 [21]	69% / 65% [21]	23–38% [22]	Previous negative biopsy/biopsies
	SelectMDX	HOXC6 and DLX1 mRNA	0.71–0.81 [23]	91% / 36% [23]	53% [23]	Prediction of high-grade PCa
	Confirmation of test results	Tissue-based biomarkers				
ConfirmMDx		DNA hypermethylation GSTP1, APC, RASSF1	0.74 [27]	68% / 64% [27]		Prediction of true negative prostate biopsies
Prolaris		mRNA expression (31 genes)	0.78 [32]	NA		Monitoring of tumor aggressiveness
OncotypeDx		mRNA expression (17 genes)	0.73 [32]	NA	NA	Monitoring of tumor aggressiveness
Decipher		mRNA expression (22 genes)	0.79 [31]	73% / 74% [31]		Treatment monitoring
ProMark	Protein biomarker test (8 proteins)	0.72 [27]	90% / 85% [27]		Monitoring of tumor aggressiveness	

AUC – area under the ROC (receiver operating characteristic) curve; PSA – prostate-specific antigen; NA – not applicable; Bx – biopsy; PCa – prostate cancer; PHI – prostate health index; csPCa – clinical significant prostate cancer

risk of missing high-grade PCa (HGPCa) with low value of PCA3 [21].

### SelectMDx (MDx Health, Irvine, CA, USA)

SelectMDx test is a non-invasive examination involving the analysis of a urine sample obtained after strokes of prostate during DRE. The presence of HOXC6 and DLX1 mRNA genes levels is assessed to evaluate the risk of both: the presence of any PCa during biopsy, and the risk of detecting high-grade PCa. At the same time, with a low risk of PCa, it allows to avoid unnecessary prostate biopsy. Van Neste and colleagues, using the SelectMDx test, estimated that 42% of the total number of biopsies and 53% of unnecessary biopsies could be avoided [23].

### TMPRSS2-ERG Fusion

Another gene associated with PCa and detectable in urine after DRE is the fusion of TMPRSS2-ERG. Studies have shown that TMPRSS2-ERG levels were associated with csPCa. Fusion trans-membrane serine protease 2 (TMPRSS2) and ERG gene can be detected in 50% of PCa [24]. However, its low sensitivity reduces its meaning as a standalone test.

### MiPS (University of Michigan, MLabs)

The Michigan Prostate Score (MiPS) was released in 2013 and is a test covering serum PSA, urinary PCA3 mRNA and urinary TMPRSS2: ERG. Using the MiPS test, Tomlins et al. showed the possibility of avoiding from 35 to 47% of biopsies [25].

### ExoDx Prostate IntelliScore urine exosome test (Exosome Diagnostics, Inc., Waltham, MA, USA)

ExoDx Prostate IntelliScore (EPI) uses the exosomal level of RNA expression of three genes (e.g. SPDEF, ERG and PCA3) to predict the probability of high-grade PCa from Grade Group (GG) 2 or higher [26]. EPI is the only test for this indication, except for multi-parametric magnetic resonance imaging (mpMRI), which does not contain PSA or a PSA derivative in the test algorithm. As such, EPI is a 'standalone' test that is not based on any other parameters to calculate the result compared to the exosomal RNA markers measured in a urine sample. This allows clinicians to use the test result in conjunction with other clinical variables, including clinical nomograms, mpMRI or standard care risk calculators. The use of the ExoDx Prostate IntelliScore urine exosome test avoided 27% of unnecessary biopsies compared to standard care [26].

## Tissue-based biomarkers

### ConfirmMDx (MDxHealth, Inc, Irvine, CA, USA)

The ConfirmMDx test is based on the concept that benign prostate tissue near the focus of PCa shows pronounced epigenetic changes. If PCa were omitted during biopsy, demonstration of epigenetic changes in benign tissue would indicate the presence of cancer. The ConfirmMDx test determines the level of methylation of the promoter regions of three genes (APC – adenomatous polyposis coli, RASSF1 – ras association (RalGDS/AF-6) domain family member 1 and GSTP1 – glutathione-transferase pi1) in benign prostate tissue. GSTP1 is involved in DNA detoxification, RASSF1 is involved in cell cycle regulation, and APC is involved in apoptosis, cell migration and cell adhesion [27].

An outcome of the test, comprising DNA-methylation intensity, is the better stratification of patient risk and significantly better score than current risk prediction models such as PCPTRC (prostate cancer prevention trial risk calculator) and PSA. In this way it can help to identify patients' histopathologically negative biopsies containing high – grade PCa.

However, given the limited data available and the widespread use of multi-parametric magnetic resonance imaging (mpMRI) in repeat biopsy settings, no recommendations can be made regarding the routine use of ConfirmMDx [27].

### OncotypeDx (Genomic Health, Redwood, CA, USA)

OncotypeDx Genomic Prostate Score (GPS – genomic prostate score) test is based on RNA determination of 12 carcinogens and 5 reference genes, based reverse transcription polymerase chain reaction, when examining prostate tissue obtained during prostate biopsy or after surgery. This is the only test intended for patients diagnosed with low grade PCa (Gleason score 3 + 3 and 3 + 4). The test result (GPS) is then assessed on a scale of 0 to 100 for tumor aggressiveness. It lets recognize adverse pathology and biochemical recurrence after RP (radical prostatectomy) in men with low and intermediate risk prostate cancer. It can also improve the risk stratification in men with newly diagnosed disease [28].

### ProMark (Metamark, Cambridge, MA, USA)

The Promark test is a protein-based prognostic assay that predicts the aggressiveness of cancer in patients with biopsy Gleason scores of 3 + 3 and 3 + 4. The test estimates (8 protein signature) eight protein markers and gives a score of 0 to 1. It pre-

dicts adverse pathology during RP and provides a personalized prediction that the cancer can be managed without aggressive treatment, or an indication that aggressive therapy may be appropriate. Blume-Jensen et al., in their study [29] showed that the T-point risk  $\leq 0.33$ , anticipate benign pathology 95%, 81.5% and 87.2% in the D'Amico groups respectively. But for the risk result  $> 0.8$ , the predictive value of adverse pathology was 76.9% in all risk groups.

### Decipher (GenomeDx, San Diego, CA, USA)

The Decipher genomic classifier measures the levels of RNA expression of 22 different genes. These signatures are available for both RP and prostate biopsy samples. The test is presented as a score from 0 to 1.0 (a higher score means a higher probability of clinical metastasis). The Decipher test calculates the likelihood of clinical metastases within 5 years of RP and 10-year mortality specific for prostate cancer in men with high risk pathology or high risk clinical features after RP. It could be a useful tool at diagnosis and for local therapy planning for newly PCA patients [30].

The Decipher test is not the only independent predictor of clinical metastases in patients with biochemical recurrence after surgery. But it also determines the time of postoperative radiotherapy [31].

### Prolaris (Myriad Genetics, Inc., Salt Lake City, UT, USA)

Prolaris is a novel prognostic assay that measures tumor biology (cell-cycle progression score), in order to improve accuracy of risk stratification for men with localized prostate cancer.

The Prolaris test combines the RNA expression levels of 31 genes involved in cell cycle progression and 15 housekeeping genes to generate a Prolaris Score™. It has been shown to be a strong predictor of oncologic outcomes, and adds information that is new and independent of standard clinicopathologic features, such as PSA and Gleason score. Test can be safely used to guide patient selection for active surveillance or definitive treatment [32].

### Risk calculators

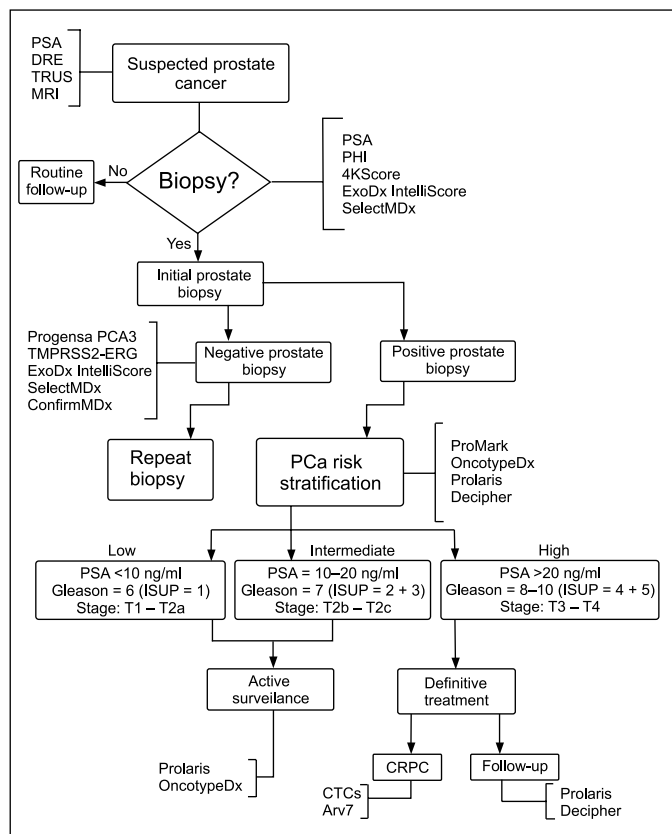
The aim of all biomarkers is to increase anticipation of prostate cancer risk in the initial and repeat biopsy setting. Risk calculators (RCs) use the combination of biomarkers and clinical parameters. A systematic review shows that there are numerous risk stratification tools RCs ( $n = 127$ ), but only six RCs have been confirmed in study populations [33].

Several complex models have been developed to assess the risk of PCA GS  $\geq 7$  as biopsy outcome, including e.g.: The Prostate Cancer Prevention Trial (PCPT) model, Stockholm Model 3 (STHLM3) [34], Rotterdam Prostate Cancer Risk Calculator (RPCRC) [35]. Lately, a lot of research has been done on risk prediction models connecting new, emerging biomarkers (e.g.: 4K panel [36] SelectMDx [37] PCA3 [22] PHI [13, 19]) and standard clinical data. The results show a biopsy reduction of 40–55%, with only 2% csPCa missing.

The possibilities of using modern, well-studied biomarkers incorporated in RC of PCA management have been shown in Figure 1, which was developed on the basis of the drawing provided in [38].

### Development studies

Development work is ongoing in the field of PCA biomarkers, and many new markers are currently in the preclinical phase. Emerging biomarkers in-



**Figure 1.** Role of biomarkers in prostate cancer management.

PSA – prostate-specific antigen; DRE – digital rectal examination; TRUS – transrectal ultrasonography; MRI – magnetic resonance imaging; PHI – prostate health index; PCa – prostate cancer; CRPC – castration-resistant prostate cancer; ISUP – International Society of Urological Pathology; CTCs – circulating tumor cells

clude: circulating tumor cells (CTCs) are studied for use as mCRPCa (metastatic castration-resistant prostate cancer) prognostic biomarker and for predicting treatment efficacy, micro-RNA-based tests, a 4-metabolite panel (sarcosine, alanine, glycine and glutamate) in the post-urine period derived from DRE called - Prostarix Risk Test (Boswick Laboratories) and other biomarkers based on long non-coding RNA (lncRNA) (test – MALAT1, SchLAP1, TINCR, CCAT1) or exosomes [39]. One mitochondrial DNA test, Mitomics (Broomfield, CO), is currently available. Mitomics offers the Prostate Core Mitomics Test which measures mitochondrial DNA variants in an initial negative prostate biopsy to determine whether a patient should undergo repeat biopsy. The test is performed on the initial negative prostate biopsy tissue [38].

## CONCLUSIONS

New technologies for detecting prostate cancer are appearing regularly. There is widespread agreement on the need for the stratification tools to improve early detection of clinically significant prostate cancer and to reduce over-diagnosis and over-treatment. However, head-to-head comparisons of biomarkers and risk calculators (RCs) are

necessary, to get the best assay for personalized treatment.

It should be noted that the main areas of application of molecular biomarkers in the context of clinical management of PCa are:

- a) diagnostics (e.g.: PHI, 4KScore, PCA3, Select-MDx),
- b) prognostics (e.g.: Prolaris, OncotypeDx, Decipher).

This review presents commercially available tests and new genetic biomarkers in risk stratification, especially in patients not previously treated with biopsy. The search for new prostate cancer (PCa) diagnostic biomarkers is a hot topic in the modern cancer diagnosis community.

Given the complex and heterogeneous nature of PCa, one diagnostic test will not answer all questions, so the use of several diagnostic methods will allow clinicians to give patients better, personalized clinical advice.

For this reason, it is necessary to conduct further clinical research on new genetic markers that may be used in diagnosis and prognosis of prostate cancer. These innovative tools can thus change and improve the PCa diagnostic pathway.

## CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

## References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. *CA Cancer J Clin.* 2018; 68: 7-30.
2. Mottet N, Cornford P, Van den Bergh RCN, et al. EAU-EANM-ESTRO-ESUR-SIOG Guidelines on prostate cancer. *Eur Ass Urol.* 2020; 1-182. <http://uroweb.org/guidelines/compilations-of-all-guidelines/>
3. Dall'era MA, Albertsen PC, Bangma C, et al. Active surveillance for prostate cancer: a systematic review of the literature. *Eur Urol.* 2012; 62: 976-983.
4. Tokudome S, Ando R, Koda Y. Discoveries and application of prostate specific antigen, and some proposals to optimize prostate cancer screening. *Cancer Manag Res.* 2016; 8: 45-47.
5. Hendricks RJ, Van Oort IM, Schalken JA. Blood-based and urinary prostate cancer biomarkers: a review and comparison of novel biomarkers for detection and treatment decisions. *Prostate Cancer Prostatic Dis.* 2017; 20: 12-19.
6. Leyten GHJM, Hessels D, Jannink SA, 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: 534-542.
7. Boegemann M, Stephan C, Cammann H, 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: 72-79.
8. Osses DE, 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: 1-19.
9. Hayes JH, Barry MJ. Screening for prostate cancer with the prostate-specific antigen test: a review of current evidence. *JAMA.* 2014; 311: 1143-1149.
10. Catalona WJ, Richie JP, Ahmann FR, et al. Comparison of digital rectal examination and serum prostate specific antigen in the early detection of prostate cancer: results of a multicenter clinical trial of 6,630 men. *J Urol.* 1994; 151: 1283-1290.
11. Klotz L. Prostate cancer overdiagnosis and overtreatment. *Curr Opin Endocrinol Diabetes Obes.* 2013; 20: 204-209.
12. Al Saidi SS, Al Riyami NB, Al Marhoon MS, et al. Validity of Prostate Health Index and Percentage of [-2] Pro-Prostate-Specific Antigen as Novel Biomarkers in the Diagnosis of Prostate Cancer: Omani Tertiary Hospitals Experience. *Oman Med J.* 2017; 32: 275-283.
13. White J, Shenoy BV, Tutrone RF, et al. Clinical utility of the Prostate Health Index (phi) for biopsy decision management in a large group urology practice setting. *Prostate Cancer Prostatic Dis.* 2018; 21: 78-84.
14. Becerra MF, Bhat A, Mouzannar A, et al. Serum and urinary biomarkers for detection and active surveillance of prostate cancer. *Curr Opin Urol.* 2019; 29: 593-597.

15. Loeb S, Sanda MG, Broyles DL, et al. The Prostate Health Index selectively identifies clinically significant prostate cancer. *J Urol.* 2015; 193: 1163-1169.
16. Voigt JD, Dong Y, Linder V, Zappala S. Use of the 4KScore test to predict the risk of aggressive prostate cancer prior to prostate biopsy: overall cost savings and improved quality of care to the us healthcare system. *Rev Urol.* 2017; 19: 1-10.
17. Zappala SM, Scardino PT, Okrongly D, et al. 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: 149-155.
18. Parekh DJ, Punnen S, Sjoberg DD, 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: 464-470.
19. Nordström T, Vickers A, Assel M, et al. Comparison between the four- kallikrein panel and Prostate Health Index (PHI) for predicting prostate cancer. *Eur Urol.* 2015; 68: 139-146.
20. Auprich M, Bjartell A, Chun FKH, et al. Contemporary role of prostate cancer antigen 3 in the management of prostate cancer. *Eur Urol.* 2011; 60: 1045-1054.
21. Nicholson A, Mahon J, Boland A, et al. The clinical effectiveness and cost- effectiveness of the PROGENSA® 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.* 2015; 19: 1-191.
22. Muñoz-Rodríguez SV, García-Perdomo HA. Diagnostic accuracy of prostate cancer antigen 3 (PCA3) prior to first prostate biopsy: a systematic review and meta- analysis. *Can Urol Assoc J.* 2020; 14: E214-219.
23. Van Neste L, Hendriks RJ, Dijkstra S, et al. Detection of high- grade prostate cancer using a urinary molecular biomarker- based risk score. *Eur Urol.* 2016; 70: 740-748.
24. Tomlins SA, Rhodes DR, Perner S, et al. Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer. *Sci.* 2005; 310: 644-648.
25. Tomlins SA, Day JR, Lonigro RJ, et al. Urine TMPRSS2:ERG plus PCA3 for individualized prostate cancer risk assessment. *Eur Urol.* 2016; 70: 45-53.
26. McKiernan J, Donovan MJ, O'Neill V, et al. A novel urine exosome gene expression assay to predict high- grade prostate cancer at initial biopsy. *JAMA Oncol.* 2016; 2: 882-889.
27. Van Neste L, Partin AW, Stewart GD, et al. Risk score predicts high- grade prostate cancer in DNA- methylation positive, histopathologically negative biopsies. *Prostate.* 2016; 76: 1078-1087.
28. Van Den Eeden S, Zhang N, Shan J, et al. A diagnostic biopsy- based genomic prostate score as an independent predictor of prostate cancer death and metastasis in men with localized prostate cancer. *J Urol.* 2017; 197: 240-241.
29. Blume-Jensen P, Berman DM, Rimm DL, et al. Development and clinical validation of an in- situ biopsy- based multimarker assay for risk stratification in prostate cancer. *Clin Cancer Res.* 2015; 21: 2591-2600.
30. Klein EA, Haddad Z, Yousefi K, et al. Decipher genomic classifier measured on prostate biopsy predicts metastasis risk. *Urology.* 2016; 90: 148-152.
31. Ross AE, Feng FY, Ghadessi M, et al. A genomic classifier predicting metastatic disease progression in men with biochemical recurrence after prostatectomy. *Prostate Cancer Prostatic Dis.* 2014; 17: 64-69.
32. Gene-based tests for screening, detection, and/or management of prostate cancer. *Medical Policy Manual Genetic Testing.* 2020; Policy No. 17 <http://www.policy.asuris.com/geneticTesting/gt17.pdf>
33. 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: 848-864.
34. Grönberg H, Adolfsson J, Aly M, et al. Prostate cancer screening in men aged 50-69 years (STHLM3): a prospective population- based diagnostic study. *Lancet Oncol.* 2015; 16: 1667-1676.
35. Roobol MJ, Verbeek JM, Van der Kwast T, et al. Improving the Rotterdam European Randomized Study of Screening for prostate cancer risk calculator for initial prostate biopsy by incorporating the 2014 International Society of Urological Pathology Gleason grading and cribriform growth. *Eur. Urol.* 2017; 72: 45-51.
36. Verbeek JFM, Bangma CH, Kweldam CF, et al. Reducing unnecessary biopsies while detecting clinically significant prostate cancer including cribriform growth with the ERSPC Rotterdam risk calculator and 4KScore. *Urol Oncol.* 2019; 37: 138-144.
37. Dijkstra S, Govers TM, Hendriks RJ, et al. 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.* 2017; 120: 659-665.
38. Kohaar I, Petrovics G, Srivastava S. A rich array of prostate molecular biomarkers: opportunities and challenges. *Int J Mol Sci.* 2019; 20: 1813.
39. Szeliski K, Adamowicz J, Gastecka A, Drewa T, Pokrywczyńska M. Modern urology perspectives on prostate cancer biomarkers. *Cent European J Urol.* 2018; 71: 420-426.
40. Oto J, Fernández-Pardo Á, Royo M, et al. A predictive model for prostate cancer incorporating PSA molecular forms and age. *Sci Rep.* 2020; 10: 2463. ■