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REVIEW ARTICLE

Screening for prostate cancer: moving forward in the molecular era

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Summary

Prostate specific antigen (PSA) is the most widely known screening test to detect prostate cancer (PCa). However, PSA testing has been recently put under the microscope mainly due to its weak correlation with prostate malignancy. In several clinical trials the PSA-screening validity for the diagnosis of PCa was evaluated. PSA lacks the ability to define the progression potential of the disease usually resulting in overdiagnosis and overtreatment of patients. Therefore, the development of new "multivariate" prediction models for PCa that would combine the PSA screening marker (and probably PSA metrics) with better biomarkers and imaging techniques has become an evolving field. New screening tests and/or methods with increased specificity could reduce the number of men undergoing prostate biopsy - thus alleviating patients from the anxiety and the distress experienced by an unnecessary (negative) biopsy- and minimizes the healthcare cost. Herein, we reviewed the information on PSA and other novel tests that can assist in diagnosing clinically meaningful prostate cancer.

Key words: *prostate, kallikreins, screening, cancer*

Introduction

PSA is a member of the kallikrein-related peptidase family (kallikrein-3, KLK3). It is secreted exclusively by the prostate gland. In 1994 PSA test received FDA approval as a screening aid for diagnosis of PCa. Up until then digital rectal examination (DRE) was used for the diagnosis of PCa, but its poor sensitivity, limited specificity and intra-observer variability necessitated the introduction of a more sensitive diagnostic method [1].

In the following decades until today, PSA use as a tumour marker continues to receive criticism. In most cases, the argument is about its lack of ability to define the progression potential of the disease, which usually results in overdiagnosis and overtreatment of patients. It is reasonable to assume that a sensitive marker would not only reflect the current status but also the rate of change of the disease. For example, a sensitive marker could

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identify a small, rapidly growing tumour from a larger and indolent tumour.

PSA era – threshold for biopsy

A commonly used PSA threshold for recommending prostate biopsy is set at 4.0 ng/ml and has remained unchanged over time. For PSA between 2-4 ng/ml the patient is screened regularly (usually yearly). Two hundred and one subjects with an initial PSA level of 4.1 to 10.0 ng/ml were followed for a period of 1 to 12 years. From the 201 men, 53 were diagnosed with prostate cancer at last follow-up (detection rate 26%). The initial serum PSA was 6.2±1.5 for men diagnosed with PCa and 5.4±1.4 for men without PCa. Serum PSA at the last follow up was 42.8±210.0 and 5.0±2.9 ng/ml for men with and without PCa, respectively [2]. In 2005 Schroder et al. in the framework of ERSPC evaluated the predictive value of PSA increase to PSA 3.0 ng/ml or greater in a 4-year period in 5.771 men who presented low PSA values (less than 3.0 ng/ml) at first screen. Four years later 662 men had PSA 3.0 ng/ml or greater (11.5%) and from them 578 (87.3%) underwent biopsy. According to the results, the positive predictive value (PPV, the ratio of men with prostate cancer detected and the number of men who underwent biopsy) was between 19 and 28% for men with baseline PSA less than 2.9 ng/ml. The PPV for men with PSA higher than 3.0 ng/ml at baseline that underwent biopsy was between 24 and 30.0% for 3≤PSA≤9.9 ng/ml and 29% for PSA 10 ng/ml or greater [3]. At higher PSA values (over 20 ng/ml) the predictive value verified by tissue biopsy is greater than 87% [4].

In a recent retrospective study of patients over 50 year old, with PSA level of 4-10 ng/ml (range 4-9.5 ng/ml, mean 6.3 ng/ml), the total PSA (tPSA) area under curve (AUC) was only 0.547, indicating that PSA is a poor predictor of the prostatic biopsies results [5].

Guidelines

Since PSA was widely adopted, several studies have been carried out to accurately evaluate the benefit of PSA screening. The recommendations vary by organization (Table 1).

Table 1. Prostate specific antigen screening guidelines by organization

Organization	*Year*	*Cut-offs for biopsy (ng/ml)*	*Screening interval (ng/ml)*	*Reference*
National Comprehensive Cancer Network	2016	3.0	Age 45-75 PSA <1.0 → every 2-4 yrs PSA 1.0-3.0 → every 1-2 yrs PSA >3.0 → every 0.5-1 yrs Age >75 PSA >3.0 → every 1-4 yrs (select patients)	[12]
Melbourne Consensus Statement	2014	ND	ND	[13]
American College of Physicians	2013	ND	PSA ≥2.5 → every 1 yr	[14]
American Urological Association	2013	ND	every 2 yrs	[15]
European Association of Urology	2013	ND	PSA >1.0 → every 2-4 yrs PSA ≤1.0 → every 8 yrs	[16]
U.S. Preventive Services Task Force	2012	ND	ND	[17]
American Society of Clinical Oncology	2012	ND	ND	[18]
Canadian Urological Society	2011	ND	ND	[19]
American Cancer Society	2010	2.5 (select patients) 4.0 (most patients)	PSA ≥2.5 → every 1 yr PSA <2.5 → every 2 yrs	[20]
Japanese Urological Association	2010	4.0 Age specific 50-64 yrs → 3.0 65-69 yrs → 3.5 >70 yrs → 4.0	PSA ≤1.0 → every 3 yrs PSA 1.1-4.0 → every 1 yr	[21]
American College of Preventive Medicine	2008	ND	ND	[22]

ND: not defined

The two largest randomized screening trials were conducted by the American Urological Association and the European Association of Urology in order to accurately evaluate the benefit of PSA screening: the United States Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial [6] and the European Randomised Study of Screening for Prostate Cancer (ERSPC) [7], respectively.

Briefly, a total of 76,693 men, aged 55-74, participated in the PLCO trial that lasted 8 years, from 1993 to 2001. After randomization in the control and screening arm, men were offered an annual PSA test for 6 years and DRE for 4 years. No difference in mortality was recorded between the two arms of the trials after 13 years of follow up [8,9].

The ERSPC trial was initiated in 1994. A total of 182,160 men, aged 50-74 years, from eight different European countries participated. Screening was performed every 2-4 years and men underwent prostate biopsies when PSA was over 3.0 ng/ml. The relative PCa mortality after 11 and 13 years was significantly reduced in the screening arm by 21% [10,11].

The limitations of the PLCO trial include the substantial level of "contamination" (use of PSA and DRE) of the control group and the lack of definition of a PSA threshold for biopsy. On the contrary, cut-offs of 2.5 and 3.0 ng/ml were used in the ERSPC trial but the participation of multiple centres using different screening intervals and PSA thresholds for biopsy increased the heterogeneity of data.

Discrepancy of the results

The discrepancy of the results could be attributed to: **(a)** Important differences in design and conduct of the screening trials, including the protocol of the study, the number of participants, the definition of the groups, the frequency of PSA screening etc. **(b)** Biopsy threshold. Controversy exists over the threshold level for performing a biopsy at whether it should be lowered to improve the test sensitive, since only 20 to 30% of biopsied men with a PSA value greater than 4.0 ng/ml are detected with prostate cancer. A lower threshold would inevitably lead to a reduced specificity and an increased number of unnecessary biopsies. **(c)** Body weight of the patients. Both stable obesity and weight gain are correlated with a decreased PSA velocity, and thus making it harder to detect changes in circulating PSA [23]. Total, free, and complexed PSAs were lowest in 3251 obese men ≥40 years in the 2007–2010 National Health and Nutrition Examination Survey [24]. Obesity results in greater body mass index (BMI) values and the study of Vidal et al., in 2015 contradicts the above since the authors concluded that BMI doesn't affect the accuracy of prebiopsy PSA to predict PCa in men with a previous negative biopsy [25]. **(d)** Medications. Aspirin users have significantly lower baseline PSA levels than non-users [26]. **(e)** Ethnicity of the patients. According to Tang et al. (2012), Chinese men 50 years-old or younger had a lower baseline PSA level and higher PSA velocity (PSAV) compared with African American and white American men [27]. Non-Hispanic blacks have higher total PSA and complexed PSA than Hispanic and non-Hispanic white men in the USA [24].

New diagnostic markers for PCa

The quest for the identification of next generation PCa biomarkers is in progress. The recent years several new biomarkers have been investigated, including urine prostate cancer gene 3 (*PCA3*) score, [-2]-isoform of proPSA, Prostate Health Index (PHI) and the four-kallikrein panel [28-30].

PCA3 gene is a non-coding RNA (ncRNA) that is highly expressed in PCa cells. PCA3 is strongly overexpressed in malignant prostate tissue compared to benign or normal adjacent prostatic tissue. Studies performed during the last 3 years have shown that PCA3 test has a better diagnostic ability than PSA, total PSA and free/total PSA (f/tPSA) in predicting a positive biopsy result [31,32], although in 2013 Goode et al. pointed out that PCA3 is not superior to PSA in the repeat biopsy population [33]. The prognostic value of PCA3 increases significantly when the PCA3 results are combined with PCa risk factors such as age, PSA, DRE and prostate volume [34] or PSA density (PSAD) [32].

Free PSA is found in several subforms, including a precursor form of PSA (proPSA). All forms of proPSA are enzymatically inactive [35] and the most stable form is *p2PSA* [36]. According to Stephan et al. (2013), p2PSA had better clinical performance for predicting PCa - as indicated by the largest area under the ROC curve- than the percent fPSA and tPSA (AUC=0.63 vs 0.61 and 0.56, respectively) but inferior than PHI and the percent p2PSA (AUC=0.63 vs 0.74 and 0.72, respectively) [37]. Similar AUCs and results were reported by Filella et al., in 2014 [38]. The superiority of percent p2PSA and PHI over tPSA and percent fPSA in patients with a tPSA range of 2-10 ng/ml was also demonstrated by Lazzeri et al. in 2013 [39] and over PSAD by Guazomni et al. in 2011 [40].

Prostate Health Index is a mathematical formula (PHI=([-2]proPSA/freePSA) x √PSA) that combines total PSA, free PSA and [-2] proPSA [41]. The

PHI was approved by the FDA in 2012 for men older than 50 years with a tPSA between 4 and 10 ng/ml and negative DRE. In a large multicenter trial of PHI for PCa detection, PHI showed greater specificity for distinguishing prostate cancer on biopsy compared with PSA or percent fPSA [42] with an AUC of 0.70 compared with 0.65 for percent fPSA and 0.53 for PSA [43]. Similar findings were also recorded in a previous study of "grassroot" population [44]. The superiority of the PHI and p2PSa to predict biopsy outcome over total and free PSA was also established in the PRO-PSA Multicentric European Study [39].

The four-kallikrein panel (4Kpanel) is a model that incorporates human kallikrein type 2 (hk2), tPSA, fPSA, and intact PSA (iPSA) [28]. Multiple kallikrein panel measured in blood of men with PSA>3.0 ng/ml improved AUC from 0.68 to 0.83 and from 0.72 to 0.84 for the laboratory (laboratory sending blood results to a doctor) and clinical (clinical consultation between a patient and a doctor) models, respectively. Implementation of this multivariable model would have reduced the number of biopsies by 57% [29]. The higher accuracy of 4Kpanel model was also confirmed by a latter study [45]. According to Stattin et al. (2015) the kallikrein panel could be used as an aid to assist biopsy decision making in men with elevated PSA [46]. In a recent meta-analysis in 2014 by Voigt et al., the use of the kallikrein panel not only improves patient outcomes but also reduces costs. Almost 48 to 56% of current prostate biopsies could be avoided, resulting in annual US savings approaching up to $1 billion [47].

Other molecular markers include the TMPRSS2 fusion genes that occur specifically in PCa [48], the GSTP 1 gene associated with PCa progression [49,50] and histone methyltransferase EZH2 gene which is shown to overexpress in metastatic PCa [51]. Trials that attempted to combine genes with PCA3 in order to increase detection of PCa have also been conducted [52].

Image guided targeted prostate biopsy techniques

A positive DRE and an abnormal PSA are commonly followed by a prostate biopsy. The systematic transrectal ultrasound (TRUS) guided biopsy of the prostate is the gold standard in prostate cancer diagnosis using the sextant method (6-core). In recent years, the number of cores selected for biopsy has increased (up to 18 cores). A recent study showed that although the addition of 4 lateral peripheral samples (10-core) does not increase cancer detection rate (compared to the sextant method), addition of the 4 paramedian peripheral samples (14-core) would detect 16% more tumours in patients with PSA density <0.15 than the 10-core scheme [53].

The introduction of magnetic resonance imaging (MRI) in prostate biopsy is attracting increasing interest among urologists. Multi parametric MRI (mpMRI) has acquired a significant role in prostate cancer surveillance as it is used to exclude PCa in men with elevated PSA and to monitor the disease burden during active surveillance and after focal therapy [54,55]. Superiority of MRI-guided biopsy (MRI-GB) over TRUS-guided biopsy (TRUS-GB) was reported by Pokorny et al. in 2014. According to the authors the MRI-GB had a greater detection rate compared to TRUS-GB (69.7 versus 56.5%). The clinical negative predictive value (NPV) for intermediate/high risk PCa were 71.9 and 96.9%, respectively [56]. In 2014 Itatani et al. also reported a high NPV for mpMRI estimated at 89.6% for significant prostate cancer [57]. Thus, mpMRI could be used before biopsy to eliminate clinically significant prostate cancer. Hoeks et al. (2012) and Vourganti et al. (2012) aimed to address the cancer detection ability of MRI-GB in men that had elevated PSA and one or more negative TRUS-GB session [58,59]. The MRI-GB revealed a significant amount of non detectable cancers by the TRUS-GB (41%) of which 87% of them were clinically significant [58]. A similar number of undetectable cancers by TRUS-GB (37%) were also diagnosed by MRI-GB in the study of Vourganti et al. [59].

In 2014 Quentin et al. compared MRI-guided in-bore biopsy with standard systematic TRUS-GB (12-core) in men with PSA greater than 4 ng/ml (median PSA 6.7 ng/ml). Although the two methods showed similar detection rate (53.1%), the MRI guided in-bore biopsy required significantly fewer cores than the TRUS-GB (5.3 versus 12 per patient) and revealed a higher percent of cancer involvement per biopsy core. Combination of the two methods increases the detection rate up to 60.9% [60].

Magnetic resonance imaging/transrectal ultrasound fusion guided biopsy (MRI/TRUS fusion-guided biopsy) has been proven a significant methodology to improve prostate cancer detection [61], especially for clinically significant tumours [62]. In these two studies the mean PSA of the participants was 9.50 and 9.85 ng/ml and it was clearly shown that the application of MRI to target cores enhances the detection of cancer than the standard biopsy methods (2 to 6-core depending on lesion size in the Kuru et al. study (2013) [62] and 12-core in the Rastinehad et al. study (2014) [61]). Fusion-guided biopsy also exceeds TRUS-GB in detecting anteriorly located PCa [63].

According to the American Urological Association and the Society of Abdominal Radiology's Prostate Cancer Disease-Focused Panel, every patient with a prior negative biopsy should be considered for prostate MRI, especially if there are clinical indications of prostate cancer [64]. The use of mpMRI adds upfront costs so the cost efficacy of MRI should be carefully evaluated [65]. In an earlier study, the total costs of MRI and MRI-GB in men with suspicion of prostate cancer were comparable to the costs of TRUS-GB, but MRI strategy results in a higher improvement in quality of life [66].

Conclusions

The current diagnostic strategy for PCa depends heavily on PSA testing, which results in a problematic overdiagnosis and overtreatment of patients. In the last decade substantial progress has been recorded in the area of developing new tests that can assist in diagnosing prostate disease and facilitating the discrimination of aggressive PCa from non aggressive indolent PCa. So far, none of the novel biomarkers for PCa can stand alone due to the heterogeneous nature of prostate cancer and "multivariate" models (including genetic-based markers, serologic protein markers, urinary-based markers and imaging techniques) seem to become the method of choice in order to overcome each biomarker cons. The improved diagnostic sensitivity and specificity of these models would upgrade the treatment/prevention strategies, reduce the number of men undergoing unnecessary biopsies, relief the patients from the anxiety and the distress and minimize the healthcare cost. In the meantime, large scale clinical studies will continue to evaluate the evolving biomarkers and/or methods and will lead to updated guidelines.

Conflict of interests

The authors declare no conflict of interests.

References

1. Alberts AR, Schoots IG, Roobol MJ. Prostate-specific antigen-based prostate cancer screening: Past and future. Int J Urol 2015;22:524-32.
2. Ito K, Yamamoto T, Ohi M, Kurokawa K, Suzuki K, Yamanaka H. Free/total PSA ratio is a powerful predictor of future prostate cancer morbidity in men with initial PSA levels of 4.1 to 10.0 ng/mL. Urology 2003;61:760-4.
3. Schröder FH, Raaijmakers R, Postma R, van der Kwast TH, Roobol MJ. 4-year prostate specific antigen progression and diagnosis of prostate cancer in the European Randomized Study of Screening for Prostate Cancer, section Rotterdam. J Urol 2005;174:489-94; discussion-493-494.
4. Gerstenbluth RE, Seftel AD, Hampel N, Oefelein MG, Resnick MI. The accuracy of the increased prostate specific antigen level (greater than or equal to 20 ng/ml) in predicting prostate cancer: is biopsy always required? J Urol 2002;168:1990-93.
5. Ng CF, Chiu PK, Lam NY, Lam HC, Lee KW, Hou SS. The Prostate Health Index in predicting initial prostate biopsy outcomes in Asian men with prostate-specific antigen levels of 4-10 ng/mL. Int Urol Nephrol 2014;46:711-7.
6. Prorok PC, Andriole GL, Bresalier RS et al. Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial Project Team. Design of the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial. Control Clin Trials 2000; 21(6 Suppl):273S-309S.
7. Schröder FH, Denis LJ, Roobol M et al. ERSPC. The story of the European Randomized Study of Screening for Prostate Cancer. BJU Int 2003;92 (Suppl 2):1-13.
8. Andriole GL, Crawford ED, Grubb RL et al. Mortality results from a randomized prostate-cancer screening trial. N Engl J Med 2009;360:1310-9.
9. Andriole GL, Crawford ED, Grubb RL et al. Team PP. Prostate cancer screening in the randomized Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial: mortality results after 13 years of follow-up. J Natl Cancer Inst 2012;104:125-32.
10. Schröder FH. Prostate-Cancer Mortality at 11 Years of Follow-up. N Engl J Med 2012;366:981-90.
11. Schröder FH, Hugosson J, Roobol MJ et al. Screening and prostate cancer mortality: results of the European Randomised Study of Screening for Prostate Cancer (ERSPC) at 13 years of follow-up. Lancet 2014;384:2027-35.
12. Carroll PR. NCCN Guidelines® Insights Prostate Cancer Early Detection, Version 2.2016 Featured Updates to the NCCN Guidelines. J Natl Compr Cancer Netw 2016;14:509-19.
13. Murphy DG, Ahlering T, Catalona WJ et al. The Melbourne Consensus Statement on the early detection of prostate cancer. BJU Int 2014;113:186-8.
14. Qaseem A. Screening for Prostate Cancer: A Guidance Statement From the Clinical Guidelines Committee of the American College of Physicians. Ann Intern Med 2013;158:761-9.
15. Carter HB, Albertsen PC, Barry MJ et al. Early detection of prostate cancer: AUA Guideline. J Urol 2013;190:419-26.
16. Heidenreich A, Abrahamsson PA, Artibani W et al. European Association of Urology. Early detection of prostate

cancer: European Association of Urology recommendation. Eur Urol 2013;64:347-54.

17. Moyer VA, U.S. Preventive Services Task Force. Screening for Prostate Cancer: U.S. Preventive Services Task Force Recommendation Statement. Ann Intern Med 2012;157:120-34.
18. Basch E, Oliver TK, Vickers A et al. Screening for prostate cancer with prostate-specific antigen testing: American Society of Clinical Oncology Provisional Clinical Opinion. J Clin Oncol 2012;30:3020-5.
19. Izawa JI, Klotz L, Siemens DR et al. Prostate cancer screening: Canadian guidelines 2011. Can Urol Assoc J 2011;5:235-40.
20. Wolf AM, Wender RC, Etzioni RB et al. American Cancer Society Prostate Cancer Advisory C. American Cancer Society guideline for the early detection of prostate cancer: update 2010. CA Cancer J Clin 2010;60:70-98.
21. Committee for Establishment of the Guidelines on Screening for Prostate C, Japanese Urological A. Updated Japanese Urological Association Guidelines on prostate-specific antigen-based screening for prostate cancer in 2010. Int J Urol 2010;17:830-38.
22. Lim LS, Sherin K, Committee APP. Screening for prostate cancer in U.S. men ACPM position statement on preventive practice. Am J Prev Med 2008;34:164-70.
23. Rundle AG, Neugut AI. Modeling the effects of obesity and weight gain on PSA velocity. Prostate 2009;69:1573-8.
24. Lacher DA, Hughes JP. Total, free, and complexed prostate-specific antigen levels among US men, 2007-2010. Clin Chim Acta 2015;448:220-7.
25. Vidal AC, Howard LE, Moreira DM, Castro-Santamaria R, Andriole GL, Freedland SJ. Does Obesity Modify the Ability of Prebiopsy Prostate Specific Antigen to Detect Prostate Cancer on Repeat Biopsy? Results from the REDUCE Study. J Urol 2015;194:52-7.
26. Algotar AM, Thompson PA, Ranger-Moore J et al. Effect of aspirin, other NSAIDs, and statins on PSA and PSA velocity. Prostate 2010;70:883-8.
27. Tang P, Du W, Xie K et al. Characteristics of baseline PSA and PSA velocity in young men without prostate cancer: Racial differences. Prostate 2012;72:173-180.
28. Filella X, Foj L. Emerging biomarkers in the detection and prognosis of prostate cancer. Clin Chem Lab Med 2015;53:963-73.
29. Vickers AJ, Cronin AM, Aus G et al. A panel of kallikrein markers can reduce unnecessary biopsy for prostate cancer: data from the European Randomized Study of Prostate Cancer Screening in Goteborg, Sweden. BMC Med 2008;6:19.
30. Morgan T, Palapattu G, Wei J. Screening for Prostate Cancer-Beyond Total PSA, Utilization of Novel Biomarkers. Curr Urol Rep 2015;16:63.
31. Merola R, Tomao L, Antenucci A et al. PCA3 in prostate cancer and tumor aggressiveness detection on 407 high-risk patients: a National Cancer Institute experience. J Exp Clin Cancer Res 2015;34:15.
32. Ochiai A, Okihara K, Kamoi K et al. Clinical utility of the prostate cancer gene 3 (PCA3) urine assay in Japanese men undergoing prostate biopsy. BJU Int 2013;111: 928-33.
33. Goode RR, Marshall SJ, Duff M, Chevli E, Chevli KK. Use of PCA3 in detecting prostate cancer in initial and repeat prostate biopsy patients. Prostate 2013;73:48-53.
34. Salagierski M, Mulders P, Schalken JA. Predicting prostate biopsy outcome using a PCA3-based nomogram in a Polish cohort. Anticancer Res 2013;33:553-7.
35. Abrate A, Lughezzani G, Gadda GM et al. Clinical use of [-2]proPSA (p2PSA) and its derivatives (%p2PSA and Prostate Health Index) for the detection of prostate cancer: a review of the literature. Korean J Urol 2014;55:436-45.
36. Filella X, Gimenez N. 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 2013;51:729-39.
37. Stephan C, Vincendeau S, Houlgatte A, Cammann H, Jung K, Semjonow A. Multicenter evaluation of [-2]pro-prostate-specific antigen and the prostate health index for detecting prostate cancer. Clin Chem 2013;59:306-14.
38. Filella X, Foj L, Alcover J, Auge JM, Molina R, Jimenez W. The influence of prostate volume in prostate health index performance in patients with total PSA lower than 10 mug/L. Clin Chim Acta 2014;436:303-7.
39. Lazzeri M, Haese A, de la Taille A et al. Serum isoform [-2]proPSA derivatives significantly improve prediction of prostate cancer at initial biopsy in a total PSA range of 2-10 ng/ml: a multicentric European study. Eur Urol 2013;63:986-94.
40. Guazzoni G, Nava L, Lazzeri M et al. Prostate-specific antigen (PSA) isoform p2PSA significantly improves the prediction of prostate cancer at initial extended prostate biopsies in patients with total PSA between 2.0 and 10 ng/ml: results of a prospective study in a clinical setting. Eur Urol 2011;60:214-22.
41. Loeb S, Catalona WJ. The Prostate Health Index: a new test for the detection of prostate cancer. Ther Adv Urol 2014;6:74-7.
42. Catalona WJ, Partin AW, Sanda MG et al. A multicenter study of [-2]pro-prostate specific antigen combined with prostate specific antigen and free prostate specific antigen for prostate cancer detection in the 2.0 to 10.0 ng/ml prostate specific antigen range. J Urol 2011;185:1650-5.
43. Loeb S, Sokoll LJ, Broyles DL et al. Prospective multicenter evaluation of the Beckman Coulter Prostate Health Index using WHO calibration. J Urol 2013;189:1702-6.
44. Le BV, Griffin CR, Loeb S et al. [-2]Proenzyme prostate specific antigen is more accurate than total and free prostate specific antigen in differentiating prostate cancer from benign disease in a prospective prostate cancer screening study. J Urol 2010;183:1355-9.
45. Benchikh A, Savage C, Cronin A et al. A panel of kallikrein markers can predict outcome of prostate biopsy following clinical work-up: an independent validation study from the European Randomized Study of Prostate Cancer screening, France. BMC Cancer 2010;10:635.
46. Stattin P, Vickers AJ, Sjoberg DD et al. Improving the Specificity of Screening for Lethal Prostate Cancer Using Prostate-specific Antigen and a Panel of Kallikrein Markers: A Nested Case-Control Study. Eur Urol 2015;68:207-13.

47. Voigt JD, Zappala SM, Vaughan ED, Wein AJ. The Kallikrein Panel for prostate cancer screening: its economic impact. Prostate 2014;74:250-9.
48. Leyten GH, 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;65:534-42.
49. Maldonado L, Brait M, Loyo M et al. GSTP1 promoter methylation is associated with recurrence in early stage prostate cancer. J Urol 2014;192:1542-8.
50. Wu T, Giovannucci E, Welge J, Mallick P, Tang WY, Ho SM. Measurement of GSTP1 promoter methylation in body fluids may complement PSA screening: a meta-analysis. Br J Cancer 2011;105:65-73.
51. Yang YA, Yu J. EZH2, an epigenetic driver of prostate cancer. Protein Cell 2013;4:331-41.
52. Cornu JN, Cancel-Tassin G, Egrot C, Gaffory C, Haab F, Cussenot O. Urine TMPRSS2:ERG fusion transcript integrated with PCA3 score, genotyping, and biological features are correlated to the results of prostatic biopsies in men at risk of prostate cancer. Prostate 2013;73:242-9.
53. Cormio L, Scattoni V, Lorusso F et al. Prostate cancer detection rates in different biopsy schemes. Which cores for which patients? World J Urol 2014;32:341-6.
54. Lawrentschuk N, Fleshner N. The role of magnetic resonance imaging in targeting prostate cancer in patients with previous negative biopsies and elevated prostate-specific antigen levels. BJU Int 2009;103:730-3.
55. Kirkham AP, Emberton M, Allen C. How good is MRI at detecting and characterising cancer within the prostate? Eur Urol 2006;50:1163-74.
56. Pokorny MR, de Rooij M, Duncan E et al. Prospective study of diagnostic accuracy comparing prostate cancer detection by transrectal ultrasound-guided biopsy versus magnetic resonance (MR) imaging with subsequent MR-guided biopsy in men without previous prostate biopsies. Eur Urol 2014;66:22-9.
57. Itatani R, Namimoto T, Atsuji S et al. Negative predictive value of multiparametric MRI for prostate cancer detection: outcome of 5-year follow-up in men with negative findings on initial MRI studies. Eur J Radiol 2014;83:1740-5.
58. Hoeks CM, Schouten MG, Bomers JG et al. Three-Tesla magnetic resonance-guided prostate biopsy in men with increased prostate-specific antigen and repeated, negative, random, systematic, transrectal ultrasound biopsies: detection of clinically significant prostate cancers. Eur Urol 2012;62:902-9.
59. Vourganti S, Rastinehad A, Yerram NK et al. Multiparametric magnetic resonance imaging and ultrasound fusion biopsy detect prostate cancer in patients with prior negative transrectal ultrasound biopsies. J Urol 2012;188:2152-7.
60. Quentin M, Blondin D, Arsov C et al. Prospective evaluation of magnetic resonance imaging guided in-bore prostate biopsy versus systematic transrectal ultrasound guided prostate biopsy in biopsy naive men with elevated prostate specific antigen. J Urol 2014;192:1374-9.
61. Rastinehad AR, Turkbey B, Salami SS et al. Improving detection of clinically significant prostate cancer: magnetic resonance imaging/transrectal ultrasound fusion guided prostate biopsy. J Urol 2014;191:1749-54.
62. Kuru TH, Roethke MC, Seidenader J et al. Critical evaluation of magnetic resonance imaging targeted, transrectal ultrasound guided transperineal fusion biopsy for detection of prostate cancer. J Urol 2013;190:1380-6.
63. Volkin D, Turkbey B, Hoang AN et al. Multiparametric magnetic resonance imaging (MRI) and subsequent MRI/ultrasonography fusion-guided biopsy increase the detection of anteriorly located prostate cancers. BJU Int 2014;114:E43-49.
64. Rosenkrantz AB, Verma S, Choyke P et al. Prostate MRI and MRI-Targeted Biopsy in Patients with a Prior Negative Biopsy: A Consensus Statement of the American Urological Association and the Society of Abdominal Radiology's Prostate Cancer Disease-Focused Panel. J Urol 2016; pii: S0022-5347(16)30659-0.
65. Hutchinson RC, Costa DN, Lotan Y. The economic effect of using magnetic resonance imaging and magnetic resonance ultrasound fusion biopsy for prostate cancer diagnosis. Urol Oncol 2016;34:296-302.
66. de Rooij M, Crienen S, Witjes JA, Barentsz JO, Rovers MM, Grutters JP. Cost-effectiveness of magnetic resonance (MR) imaging and MR-guided targeted biopsy versus systematic transrectal ultrasound-guided biopsy in diagnosing prostate cancer: a modelling study from a health care perspective. Eur Urol 2014;66:430-6.