REVIEW

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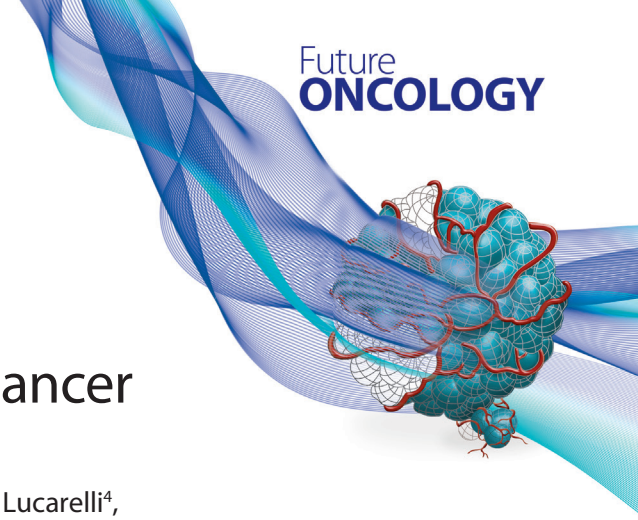

Biomarkers in localized prostate cancer

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Biomarkers can improve prostate cancer diagnosis and treatment. Accuracy of prostate-specific antigen (PSA) for early diagnosis of prostate cancer is not satisfactory, as it is an organ- but not cancer-specific biomarker, and it can be improved by using models that incorporate PSA along with other test results, such as prostate cancer antigen 3, the molecular forms of PSA (proPSA, benign PSA and intact PSA), as well as kallikreins. Recent reports suggest that new tools may be provided by metabolomic studies as shown by preliminary data on sarcosine. Additional molecular biomarkers have been identified by the use of genomics, proteomics and metabolomics. We review the most relevant biomarkers for early diagnosis and management of localized prostate cancer.

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• biomarkers • prostate cancer

The European randomized study of screening for prostate cancer showed that prostate-specific antigen (PSA)-based screening in senior men was associated with a statistically significant 29% prostate cancer mortality reduction [1]. Nevertheless, as many as 40% of those screened are at risk to be treated for a biologically indolent disease that will not affect their life expectancy [1], which results in a 23% negative impact on the life-years gained [2]. The conclusion of one recently published cost–effectiveness analysis was that PSA-based screening should be limited to two or three screens between ages 55 and 59 years because in men older than 63 years overdiagnosis is responsible for loss of quality-adjusted life-years [3]. Several clinical, pathological and biological variables [4] were reported to improve the modest accuracy of PSA as a screening test, although their impact in a community-based setting is yet to be assessed in large trials. An analysis of six externally validated PSA-based models that incorporated readily available variables such as age, race, digital rectal examination, previous biopsies, family history, free PSA, transrectal ultrasonography prostate volume showed that five of these were able to double the sensitivity of PSA testing (44 vs 21%) without loss of specificity [5]. Furthermore, the Prostate Cancer Prevention Trial model can also predict the presence of Gleason ≥7 versus Gleason <7 prostate cancer [6]. Many novel biomarkers also provide valuable prognostic information that can have important therapeutic implications and serve as

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selection criteria for patients eligible for active surveillance or candidates for radiotherapy/surgery [7]. In this review, we will focus on the most relevant biomarkers potentially useful for prostate cancer early diagnosis and for assessment of the prognosis of localized disease. Evidence supporting the use of the biomarkers discussed is presented along with a critical analysis of their potential impact on future research and clinical practice.

Serum biomarkers

• Prostate-specific antigen

PSA is a glycoprotein serine protease that promotes sperm motility via degradation of the semenogelin I and II proteins [8]. In a landmark study conducted by Catalona [9] and colleagues involving 1653 healthy men, the combined use of serum PSA measurement with a positive cutoff of 4 ng/ml in combination and digital rectal examination, with ultrasonography performed in case of abnormal findings, yielded significantly better accuracy than rectal examination alone. The inexpensiveness and the noninvasiveness of PSA testing made it widely available as a screening tool for prostate cancer, in spite of its unsatisfactory specificity [10]. Serum PSA can also rise in several nonmalignant prostatic diseases, such as benign prostatic hyperplasia [11], which shows a histological prevalence at autopsy of 50% in men aged 50–60 years and of 90% in men over 80 years old [12]. Conversely, among 2950 men enrolled in the Prostate Cancer Prevention Trial showing PSA levels lower than or equal to 4 ng/ml and normal digital rectal examination, prostate cancer was detected in 449 patients (15.2%), with 67 of them showing a Gleason score of 7 or higher. The specificity and sensitivity of PSA alone ranges from 20 to 40% and 70 to 90%, respectively, while the AUC metric of the receiver operating characteristic curve is 0.55 and 0.70 for the accuracy, depending on the cutoff values employed (e.g., 4 vs 3 ng/ml). PSA positive predictive value is only 25–40% [13]. Furthermore, PSA levels are dependent on age and race. In one study assessing a total of 541 evaluable prostate cancer patients (408 white and 133 black) with pretreatment PSA assessment, the mean PSA value for black men was 14.00 ng/ml compared with 8.29 ng/ml for white men ($p < 0.001$). This difference remained statistically significant after adjusting for stage, grade and age. Black men also had tumor volumes 1.3–2.5-times greater than those of white men, depending on tumor stage [14]. In another study evaluating 471 men without evidence of prostate cancer serum PSA concentration was correlated with patient age ($r = 0.43$; $p < 0.0001$) and prostatic volume ($r = 0.55$; $p < 0.0001$), with mean serum PSA concentration increasing by approximately 0.04 ng/ml per year in a healthy 60-year-old man [15]. In view of these findings, age-specific total PSA cutoff shave has been suggested in order to account for the physiological age-dependant increase in prostatic volume [15], and the American Urology Association recommended to discontinue PSA screening in men older than 70 years with a PSA below 3 ng/ml [16]. PSA levels were also associated with polymorphisms of the PSA promotor and of the kallikrein gene, among other genes. In one study involving 409 healthy white men, the analysis of the promoter region of the PSA genes showed that each of the -4643G/A, -5412C/T and 5429T/G single nucleotide polymorphisms were reported in approximately 20% of the population and were significantly associated with increases in serum PSA levels and PSA promoter activity. In another study conducted in 303 male military conscripts, PSA levels were found to be strongly associated with the rs1058205 kallikrein polymorphism [17].

Given the limitations of PSA testing alone, a number of PSA derivatives have been investigated in order to improve PSA diagnostic accuracy. PSA density is computed by dividing PSA by prostate volume and is a strong predictor of adverse pathological features and biochemical recurrence after radical treatment [18,19]. Furthermore, PSA density can be particularly useful for the evaluation of patient candidates for active surveillance, since it is associated with Gleason score upgrade in patients with biopsy Gleason score of 6 and 3 + 4 = 7 [20,21]. In this regard, it must be noted that PSA density is an established prognostic factor and serves as an inclusion criterion for active surveillance [22].

Measures of PSA kinetics, such as PSA velocity (PSAV) and PSA doubling time, were predictive of biopsy results in patients with suspicion of prostate cancer and disease recurrence and survival after radical treatment, although it is not clear whether these measures can improve the accuracy of models based on PSA only [23,24]. One study conducted in 32 patients with high-risk prostate cancer, 606 men without prostate cancer and 79 patients with prostate cancer who were alive or dead of another

cause, assessed the PSAV risk count, defined as the number of times PSAV exceeded a certain threshold. A higher PSAV risk count was significantly associated with the onset of high-risk prostate cancer after adjusting for known predictors (relative risk: 1.41; 95% CI: 1.25–1.59 for a PSAV cutpoint 0.2 ng/ml/year; relative risk: 1.49; 95% CI: 1.29–1.71 for a PSAV cutpoint of 0.4 ng/ml/year; $p < 0.001$) [25]. Moreover, a PSAV risk count of 2 was associated with a significantly higher risk of aggressive disease in men undergoing biopsy after accounting for age and PSA [26], while in another study a risk count of 3 or 2 was associated with a significantly increased risk of diagnosis of high-grade disease on re-biopsy in a surveillance cohort [27]. The PSAV risk count has the potential to overcome the limitation associated with PSA kinetics due to the physiologic fluctuations of PSA.

• Prostate health index

'Free PSA' is a part of circulating PSA that is not protein bound and it had been reported as a promising biomarker of biopsy and re-biopsy findings [26,28–30] before subsequent studies failed to show such an association [31,32]. Precursor forms of PSA (pPSA) are a component of free PSA and were detected at higher levels in prostate cancer tissue with respect to benign prostatic hyperplasia or healthy prostate tissue [33]. In a pioneering study conducted in five patients with prostate cancer and three controls, the truncated form of PSA precursor, namely [-2]pPSA, was estimated to range from 25 to 95% of the free PSA in prostate cancer patients but only 6–19% of the free PSA in the biopsy-negative men [34]. [-2]pPSA presents a serine-arginine pro leader peptide and is sufficiently stable to be measured reliably using the Beckman Coulter assay in order to compute phi by using the formula ([-2]proPSA/free PSA) × √PSA [35]. In one study including 736 serum samples of patients screened for prostate cancer, the use of phi yielded a significantly better accuracy, with an AUC of 0.71–0.75, with respect to PSA (AUC = 0.53–0.58) and %free PSA (AUC = 0.57–0.67). Also %p2PSA showed a better accuracy than PSA and %free PSA [35]. In men aged 50 years and older with a negative digital rectal examination and a serum PSA between 4 and 10 ng/ml phi and%[-2]proPSA have the highest accuracy for the detection of cancer of any grade [36]. Furthermore, phi and %[-2]proPSA are useful tools for the diagnosis of Gleason ≥7 prostate cancer and outperform PSA and free PSA in men with a positive family history [37] and in men with PSA levels between 2 and 10 ng/ml [38]. In an analysis of 158 men with a first-degree relative with prostate cancer, p2PSA,%p2PSA and phi values were significantly higher in 71 men with prostate cancer (44.9%) than in those without prostate cancer. At multivariable analysis,%p2PSA and phi were independent predictors of positive biopsy and significantly improved the accuracy of PSA and prostate volume by 8.7 and 10%, respectively [37]. The diagnostic accuracy of phi was confirmed by a meta-analysis performed by Bruzzese and colleagues in 2969 men undergoing first biopsy with PSA serum levels in the range of 2–10 ng/ml. Biopsy-confirmed prostate cancer was detected in 1287 (43.3%) men. The AUC of phi and%fPSA were 0.74 (95% CI: 0.70–0.77) and 0.63 (95% CI: 0.58–0.67), respectively, with a superiority of phi compared with %fPSA and a relative diagnostic odds ratio of 2.81 (95% CI: 2.19–3.6; $p < 0.0001$) [39]

• 4K score

The four kallikrein panel, namely the 4K score, is an extensively investigated test that is based on four kallikrein blood markers, that is total PSA, free PSA, intact PSA and the human kallikrein-related peptide 2. In a series of 161 men undergoing prostatectomy, serum levels of human glandular kallikrein 2 were significantly higher in patients with organ versus nonorgan-confined disease [40]. In a study [41] involving 1501 men with elevated PSA, the 4K score showed a significantly higher predictive accuracy for cancer than a model based on PSA, age and digital rectal examination only, with an AUC of 0.71 versus 0.58. These results were consistent with those obtained in an analysis of 11,063 Swedish men enrolled in the Malmö Diet and Cancer study during 1991–199, which showed that the 4K test predicted a subsequent diagnosis of clinically significant cancer, with improved concordance index compared with total PSA and age (0.75 vs 0.65; $p < 0.001$) [41]. Several studies showed that the 4K test is able to reduce the number of biopsies at the cost of missing only few high-grade cancers [42,43]. One study conducted in 262 men undergoing prostate biopsy because of elevated PSA (≥3 ng/ml) showed that the 4K test was able to reduce biopsies by approximately 50%, with only 1% of aggressive cancer diagnoses missed [44]. Similar findings were obtained in another cohort of 740 screened men undergoing biopsy for elevated PSA [11]. In

a cohort of 925 men with negative findings on a previous biopsy, the use of the 4K test improved the accuracy of a model based on PSA and digital rectal examination alone (AUC = 0.68 vs 0.58 for any cancer and AUC = 0.87 vs 0.76 for high-grade cancer) [41]. Finally, a prospective evaluation of the 4K score as a predictive factor of Gleason ≥7 prostate cancer was conducted in 1012 men scheduled for prostate biopsy and showed that the 4K score presented higher discrimination (AUC = 0.82) and net benefit compared with a modified Prostate Cancer Prevention Trial Risk Calculator 2.0 model, with a possible reduction of 30–58% in the number biopsies and delayed diagnosis in only 1.3–4.7% of Gleason ≥7 prostate cancer cases [45]. Finally, in one study involving 1423 cases of prostate cancer, with 235 patients with distant metastasis among men with PSA >2 ng/ml, a prespecified model based on four kallikrein markers significantly enhanced the prediction of metastasis compared with PSA alone [46]. Overall, there is sufficient evidence to consider the 4K score as a valuable diagnostic and prognostic tool for the management of early prostate cancer.

• miRNAs

miRNAs are small, noncoding RNAs with important functions as regulators of gene expression at the post-transcriptional level [47]. Both tissue and circulating miRNAs have the potential to be employed as diagnostic and prognostic markers for various cancers including prostate cancer [48]. Similarly to mRNA [48], miRNA expression signatures in prostate cancer tissues predict both Gleason score and prognosis of localized prostate cancer [49–51]. Such information is remarkably useful in order to avoid overtreatment.

Mahn *et al.* [52] studied a sample including 37 prostatectomy patients with localized prostate cancer, 18 patients with benign prostate hyperplasia treated with retropubic adenomectomy or transurethral resection of the prostate, eight patients with metastatic prostate cancer as well as 20 healthy volunteers. Four oncogenic miRNAs (miR-32, miR-26a, miR-let7i and miR-195) plus miR-16 were assessed in the serum of men included in the study sample. For the differential diagnosis of benign prostate hyperplasia versus prostate cancer, the use of miR-26a alone level was moderately accurate, with an AUC of 0.703, while the combined use of oncogenic miRNAs was associated with an AUC of 0.758. Of note, the Gleason score was associated with miR-195 and miR-let7i expression levels. In a study sample of 82 prostate cancer patients, Shen *et al.* [53] showed that prostate cancer patients with high-risk CAPRA scores had higher miR-20a and miR-21 levels. Furthermore, miR-21 and miR-221 levels were able to differentiate patients with low versus intermediate risk CAPRA scores (AUC = 0.801; p = 0.002). These findings confirm that miRNAs are associated with different levels of cancer aggressiveness in prostate cancer patients.

Urine biomarkers

• Sarcosine (N-methyl glycine)

Metabolomic profiling is a powerful tool for the comprehensive analysis of the complete set of metabolic intermediates in normal and cancer cells. This approach can be used to characterize the metabolic fingerprint of a tumor and identify novel markers that may be used for prostate cancer early detection, prognostic stratification and therapy response monitoring [54].

In a proof-of-concept study published in 2009, Sreekumar *et al.* explored the prostate cancer metabolome [55]. This was characterized by an increased amino acid metabolism and a perturbation of nitrogen breakdown pathways, along with high total choline-containing compounds and phosphocholine levels. In addition, androgens had an important role in regulating the prostate cancer amino acid metabolism, and in altering the methylation potential, in accordance with the increased expression of methyltransferases like EZH2 [56].

Among the perturbed metabolites, sarcosine – an N-methyl derivative of glycine – was significantly increased during prostate cancer progression from normal through localized to metastatic disease [55]. Interestingly, the analysis of patients with PSA levels in the range 2–10 ng/ml, showed that urinary sarcosine had a higher predictive value than PSA in differentiating prostate cancer patients from negative controls. Sreekumar *et al.* [55] validated their results in an independent cohort of patients and confirmed their previous results of increased levels of urinary sarcosine in prostate cancer patients. Cao *et al.* evaluated sarcosine levels in urine supernatants and sediments and used prostate cancer antigen 3 (PCA3) and free PSA as comparators. Urinary sarcosine was significantly higher in prostate cancer patients than in controls, and when this marker was combined with PCA3 or free PSA, the combined model had a higher predictive value [57].

Further studies demonstrated that serum sarcosine had a higher predictive value than total PSA and free PSA in detecting prostate cancer in patients with total serum PSA <4 ng/ml. Serum sarcosine had the largest AUC in predicting low-grade, low-PSA prostate cancer, suggesting that this biomarker may be a further tool not only for diagnosis, but also for selecting patients for active surveillance strategies [58–60].

In addition, elevated circulating sarcosine levels have been showed in patients with metastatic castration-resistant prostate cancer compared with patients with nonmetastatic disease [61]. In particular, Kaplan–Meier curve analysis demonstrated differences in overall and progression-free survivals between patients with high versus low sarcosine values. At multivariate analysis, this metabolite remained an independent prognosticator for overall and progression-free survivals. Interestingly, a significant correlation resulted between serum sarcosine levels and the duration of hormone sensitivity. This finding can be explained by considering that glycine-N-methyltransferase gene – that encodes for enzymes that generate sarcosine by transferring a methyl group from S-adenosylmethionine to glycine – has binding sequences for androgen receptor and ETS transcription factor family members. Therefore, these findings directly link activation of the sarcosine pathway to androgen receptor and *TMPRSS2*:*ETS* gene fusion regulation [61]. In the PLCO trial, a positive association was identified between elevated serum sarcosine and prostate cancer. The results of this large prospective study suggested that serum sarcosine could be a potential biomarker for early prostate cancer detection, specifically for nonaggressive disease, and showed a stronger association between sarcosine levels and prostate cancer risk in men with diabetes [62].

• PCA3 score

The DD3 (PCA3) gene is a noncoding RNA mapped to chromosome 9q21–22 that was originally found to be highly specific for prostate cancer tissue with respect to normal prostatic tissue [63]. PCA3 was expressed only in the LNCaP cell line among several human prostate cancer cell lines tested including ALVA-31, DU-145, JCA-1, LNCaP, PC-3, PPC-1 and TSU-pr1. While PCA3 is significantly expressed in androgen receptor positive prostate cancer cells, it is expressed at very low levels in the adjacent non-neoplastic tissue and benign prostate hyperplasia cells [64].

A meta-analysis evaluated the accuracy of PCA3 in predicting re-biopsy outcome in two subgroups respectively including patients with and without either atypical small acinar proliferation or high-grade prostatic intraepithelial neoplasia. If a PCA3 cutoff of 20 or 35 was used, the overall sensitivity values were 0.93 or 0.80 and 0.79 or 0.75, while the specificity values were 0.65 or 0.44 and 0.78 or 0.70, respectively, in the two subgroups. The AUCs of the summary receiver operating characteristic curve were 0.85 or 0.72 and 0.81 or 0.69, respectively. These findings confirm that thePCA3 score is able to avoid unnecessary biopsies by using a cutoff score of 20 [65].

TMPRSS2:*ERG* gene fusion

The *TMPRSS2*:*ERG* gene fusion is a frequent chromosomal rearrangement in prostate cancer. It is the result of the fusion of the *TMPRSS2* gene and the v-ets avian erythroblastosis virus E26 oncogene homolog (*ERG*) gene or other ETS (E26 transformation specific) transcription factors. The *TMPRSS2*:*ERG* gene fusion is regarded as one of the most frequent gene-specific alterations in prostate cancer [66].

Similarly to PCA3, a *TMPRSS2*:*ERG* rearrangement can be detected in urine after digital rectal examination [66] and can also be normalized to the amount of PSA mRNA in order to compute a TMPRSS2:ERG score. *TMPRSS2*:*ERG* is highly specific for predicting clinically significant prostate cancer on biopsy, despite the relatively low sensitivity. Gene fusion test can correctly detect cancer in 50–75% of cases [67,68].

Robert *et al.* provided a rational basis for combining PCA3 and *TMPRSS2*:*ERG* in tissue samples [69]. After the first study on combining PCA3 and *TMPRSS2*:*ERG* reported by Hessels *et al.* [66], several studies [70–72] showed better accuracy of the combination with *TMPRSS2*:*ERG* than PCA3 alone for the prediction of prostate cancer detection and progression.

Tissue biomarkers

• Prolaris

Prolaris (Myriad Genetics, UT, USA) is a commercially available test that evaluates the expression of 31 cell-cycle progression genes, normalized to 15 housekeeper genes. One study of 413 prostatectomized men reported a hazard ratio for recurrence for each unit increase in the Prolaris score of 1.7 (95% CI: 1.3–2.4), after adjusting for a standard postoperative risk assessment (Cancer of the Prostate Risk Assessment Postsurgical score) [73,74].

Combining the Prolaris score and the Cancer of the Prostate Risk Assessment Postsurgical score improved the concordance index for both the overall cohort and low-risk subset [75]. In one study involving 351 biopsies, the prognostic accuracy of the Prolaris score was evaluated along with other known predictors of survival, such as Gleason score, baseline PSA level, age, clinical stage and extent of the disease. At multivariate analysis, the Prolaris score was associated with a hazard ratio for death of 1.65 (95% CI: 1.31–2.09) for each unit increase in the Prolaris score, independently on the Gleason score and PSA levels (p = 0.017) [74]. In another retrospective study, the Prolaris test was performed in 141 men with prostate cancer treated with curative external beam radiation therapy. With 13% of patients showing biochemical recurrence, the hazard ratio for biochemical recurrence was 2.55 for a 1-unit increase in the Prolaris score and remained statistically significant even after adjustment for Gleason score, prostate-specific antigen, percent positive cores and androgen deprivation therapy. Furthermore, the Prolaris score was also associated with prostate cancer-specific mortality (p = 0.013) [75].

• Oncotype DX®

The Oncotype DX® Genomic Prostate Score test (Genomic Health, Inc., CA, USA) is a commercially available test that evaluates the expression of 17 genes and is able to provide prognostic information and assess the risk of adverse pathology, defined as primary Gleason pattern 4 or any pattern 5 and/or pT3 disease, after radical prostatectomy. This RT-PCR-based assay was developed and validated in a discovery prostatectomy study involving 441 subjects, in a biopsy study including 167 biopsies, and in a prospective study of 395 patients with low to intermediate clinical risk who were candidates for active surveillance and whose biopsy was tested retrospectively. Of the 732 candidate genes analyzed, 288 (39%) predicted clinical recurrent disease and 198 (27%) were associated with aggressive disease after adjusting for clinical stage, prostate-specific antigen and Gleason score. Seventeen genes were finally selected to be included in the Oncotype DX test. In the validation study, the Oncotype DX test significantly predicted both high-grade and high-stage at surgical pathology after controlling for established clinical factors [76]. This assay was performed on biopsies taken from 431 men with very low-, low- or intermediate-risk prostate cancer, defined according to the National Comprehensive Cancer Network between 1990 and 2011. Oncotype DX was able to predict time to biochemical recurrence at univariable analysis and also after adjusting for the risk group. The test results were also strongly associated with adverse pathology, after adjusting for the risk group [77].

• Methylation assays

Tumorigenesis is associated with changes in DNA methylation, with hypermethylation involving specific gene promoters in the context of a generalized hypomethylation, thus causing silencing of tumor suppressor genes [78]. A number of genes are hypermethylated in prostate cancer, such as FILIP1L isoform 2 [79], a known senescence marker, the pi-class glutathione S-transferase gene (GSTP1) [80]. In the MATLOC study, histopathologically negative prostate biopsy samples with matching subsequent either positive (cases) or negative (controls) repeat biopsy samples taken within 30 months were analyzed via a quantitative methylation-specific PCR assay panel that included GSTP1, APC and RASSF1. This epigenetic assay yielded a negative predictive value of 90% (95% CI: 87–93) [81]. These findings were externally validated in the DOCUMENT study [82], which analyzed cancer-negative prostate biopsy core tissue samples of 350 subjects who did not show (controls) or did show (cases) cancer after repeating biopsy within 24 months. Biopsies were analyzed for *GSTP1*, *APC* and *RASSF1* relative to the *ACTB* reference gene by the use of quantitative methylation-specific PCR. The epigenetic assay resulted in a negative predictive value of 88% (95% CI: 85–91). As a result, this test appears useful to avoid unnecessary re-biopsy [82]. The clinical usefulness of the evaluation of the GSTP1 promoter methylation was confirmed in another study conducted in 42 patients with benign prostatic hyperplasia and in a training and validation cohort of 147 and 71 prostate cancer patients, respectively. The promoter methylation of the *APC*, *CCND2*, *GSTP1*, *PTGS2* and *RARB* genes was evaluated using a quantitative multiplex, methylation-specific PCR assay in formalin-fixed, paraffin-embedded tissue samples. Among the genes considered, only GSTP1 methylation was significantly associated with clinical failure in both independent high-risk prostate cancer cohorts [82]. Patients showing either a low or a high GSTP1 methylation level were at a higher risk for clinical failure, both in the training (hazard ratio [HR]: 3.65; 95% CI: 1.65–8.07) and validation sets (HR: 4.27; 95% CI: 1.03–17.72) and also in

Table 1. Summary of biomarker useful in localized prostate cancer.

Biomarker	Sample	Application	Finding from existing study
PSA	Serum	PCa follow-up and screening marker	PSA cutoff values (>2.5, 3.0 or 4.0 ng/ml) provide a reasonable balance between excessive detection rates and the risk of missing relevant prostate cancer PSA testing alone gives more limitations. Its greatest limitation is the lack of tumor specificity The use of free PSA ratio (%fPSA) and PSAD increase significantly the specificity of the diagnostic test and, the use of derivatives that evaluate time kinetics of PSA, PSAV and PSADT represent a very useful tool for prognosis estimation during treatment and follow-up of the disease
phi	Serum	Distinguishing PCa from benign prostatic conditions in men ≥50 years old	Precursor forms of PSA (p2PSA) are a component of free PSA and were detected at higher levels in prostate cancer tissue with respect to benign prostatic hyperplasia or healthy prostate tissue %p2PSA showed a better accuracy than PSA and %free PSA. phi and%[-2]proPSA are useful tools for the diagnosis of Gleason ≥7 prostate cancer and outperform PSA and free PSA in men with PSA levels between 2 and 10 ng/ml
PCA3 SCORE	Post-DRE first catch urine	Indicated in ≥50 years old men who had prior negative prostate biopsy/s	The DD3 (PCA3) gene is a noncoding RNA mapped to chromosome 9q21–22 that was originally found to be highly specific for prostate cancer tissue with respect to normal prostatic tissue. PCA3 score is able to avoid unnecessary biopsies by using a cutoff score of 20
4K SCORE	Blood plasma anticoagulated with EDTA	Algorithm calculates the risk probability for findings high-grade PCa (Gleason score ≥7)	Test is based on four kallikrein blood markers, that is total PSA, free PSA, intact PSA and the human kallikrein-related peptide 2 In men with high level of PSA, the 4K score showed a significantly higher predictive accuracy for cancer than a model based on PSA, age and digital rectal examination only Several studies showed that the 4K test is able to reduce the number of biopsies at the cost of missing only few high-grade cancers A prespecified model based on four kallikrein markers significantly enhanced the prediction of metastasis compared with PSA alone
Prolaris	FFPE PBx or RP	Markers useful to distinguish aggressive from indolent tumors and decide who to treat or surveil	This test evaluates the expression of 31 cell-cycle progression genes, normalized to 15 housekeeper genes The potential impact of Prolaris was investigated in one study where physicians were surveyed about treatment recommendations in 305 men with newly diagnosed PCa. In 65% of the cases, the treatment recommendation changed after the genetic test, and in 40% there was reduction in treatment burden (interventional treatment changed to noninterventional). Although this study shows genomic tests can have a significant impact on treatment decisions, follow-up data were not reported to determine the long-term impact of these changes in management The gene panel significantly predicted PCa death in a multivariate model
Oncotype DX	FPE PBx (as little as 1 mm cancer)	Predictor of recurrence, PCa death and adverse pathology at RP	The Oncotype DX® Genomic Prostate Score test is a commercially available test that evaluates the expression of 17 genes The test significantly predicted both high grade and high stage at surgical pathology after controlling for established clinical factors The test was significantly associated with adverse pathologic features and also independently predicted time to BCR after adjusting for risk as well as time to metastases
Methylation assay	12-core PBx-FFPE tissue within 24 months	Test useful to avoid unnecessary re-biopsy	A methylation marker genetic test utilizes methylation analysis of *GSTP1*, *APC* and *RASSF1* genes from negative biopsies to estimate the likelihood of a repeat biopsy also being negative. The test achieved a 90% NPV within 30 months of the initial biopsy. In a recent validation trial, 88% NPV was reported, and the test was the most significant predictor of biopsy results. The impact of the epigenetic test on re-biopsy rates was recently surveyed in five centers, and among 138 patients with a negative ConfirmMDx assay, only six patients (4%) underwent repeat biopsies

NPV: Negative predictive value; PCa:Prostate cancer; PCA3: Prostate cancer antigen 3; PSA: Prostate-specific antigen; PSAD: PSA density; PSADT: PSA doubling time; PSAV: PSA velocity.

Table 1. Summary of biomarker useful in localized prostate cancer (cont.).

Biomarker	Sample	Application	Finding from existing study
Circulating miRNAs	Plasma	Predict both the Gleason score and the relative risk of PCa lethality	Specific miRNAs are found, not only in tumor tissue, but also in the plasma of PCa patients; miRNA-375 and miRNA-141 are reported to be associated with advanced PCa. miR-195 correlated with clinic pathological parameters such as surgical margin positivity and the Gleason score, and that miR-let7i also correlated with some specific parameters
Sarcosine (N-methylglycine)	Serum and urine	Evaluation of PCa progression	The analysis of patients with PSA levels in the range 2–10 ng/ml, showed that urinary sarcosine had a higher predictive value than PSA in differentiating PCa patients from negative controls. When sarcosine was combined with PCA3 or free PSA, the combined model had a higher predictive value The serum sarcosine had a higher predictive value than total PSA and free PSA in detecting PCa in patients with total serum PSA <4 ng/ml Elevated circulating sarcosine levels have been showed in patients with metastatic castration-resistant PCa compared with patients with nonmetastatic disease

NPV: Negative predictive value; PCa:Prostate cancer; PCA3: Prostate cancer antigen 3; PSA: Prostate-specific antigen; PSAD: PSA density; PSADT: PSA doubling time; PSAV: PSA velocity.

the combined cohort (HR: 2.74; 95% CI: 1.42–5.27), with respect to the moderate methylation groups [83]. In another study involving 149 prostate cancer patients, the promoter methylation of seven genes was evaluated by methylation-sensitive PCR in tissue and urine samples. *GSTP1* was confirmed to be frequently methylated in prostate cancer, along with *RARB* and *RASSF1* genes. The *RASSF1* gene was methylated in 45% of prostate cancer urine and was significantly associated with biochemical recurrence in patients at multivariate analysis [84].

The most promising methylation markers recently identified as independent predictors of biochemical recurrence include *C1orf114*, *PITX2*, *GABRE~miR-452~miR-224* and the marker panel *AOX1/C1orf114/HAPLN3*. All of these candidate biomarkers have been assessed in tissue specimens only, while their noninvasive evaluation (e.g., in circulating tumor cells) is yet to be explored [85].

Conclusion

Prostate cancer prognosis is highly heterogeneous, with men harboring an indolent disease that can be safely observed and men whose disease is rapidly fatal. The widespread use of PSA as a screening tool has contributed to improve early detection and reduce mortality, but it has also been responsible for overdiagnosis and overtreatment. The complex management of prostate cancer can be improved by the use of diagnostic, prognostic and predictive biomarkers, as discussed in this review and summarized in **Table 1**.

Future perspective

Next-generation sequencing-based clinical assays are likely to play an important role as accurate diagnostic tools of localized prostate cancer. Capture exome/transcriptome sequencing on body fluids or biopsy tissues will enable biological discovery useful for precision cancer medicine activities [86]. Whole-exome sequencing has identified genes that are mutated in prostate cancer. This expanded genetic framework may provide new mechanism tools for patient stratification [87]. Recently, Beltran *et al.* showed the feasibility of performing clinical diagnostics based on deep next-generation sequencing using a novel platform that requires little DNA retrieved from formalin-fixed and paraffin-embedded tissue [88]. Of note, the IMPACT screening network supported the use of targeted PSA screening based on *BRCA* genotype, with a high proportion of aggressive disease [89]. The possibility for the combined use of novel biomarker assays to optimize accuracy is intriguing, but must be weighed against increased costs. There is a compelling need not only for additional well-designed, large, multicenter, prospective trials, but also for economic studies evaluating the cost/benefit ratio of novel biomarkers. Increased attention to reimbursement policies is also required in order to maximize their impact in clinical practice.

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EXECUTIVE SUMMARY

Background

- This review focuses on the most relevant biomarkers potentially useful for prostate cancer early diagnosis and for assessment of the prognosis of localized disease.

Serum biomarkers

- Among the serum biomarkers/tests considered, prostate-specific antigen (PSA) has a number of limitations for diagnosis and prognostic assessment of localized prostate cancer, but its widespread use is justified because of its inexpensiveness and organ specificity.
- A number of PSA derivatives, such as PSA density, which can be particularly useful for the evaluation of patient candidates for active surveillance, and measures of PSA kinetics, such as PSA velocity, have shown limited usefulness.
- The assessment of PSA-based variables, such as the prostate health index and four kallikrein (4K) panel, has been shown to increase the diagnostic accuracy of the combined use of PSA with clinical variables. As an example, in one study involving 1501 men with elevated PSA, the 4K score showed a significantly higher predictive accuracy for cancer than a model based on PSA, age and digital rectal examination only, with an AUC of 0.71 versus 0.58.
- The use of miRNA has also been proven useful for the differential diagnosis of prostate cancer versus benign prostatic hyperplasia and for the evaluation of the disease aggressiveness.

Urine biomarkers

- Among urine biomarkers, sarcosine, prostate cancer antigen 3 (PCA3) and the *TMPRSS2*:*ERG* gene fusion have a promising diagnostic potential.
- Sarcosine is an N-methyl derivative of glycine that was significantly increased during prostate cancer progression from normal through localized to metastatic disease. Urinary sarcosine was found to be significantly higher in prostate cancer patients than in controls.
- Sarcosine can be measured both in the urine and in the serum. Serum sarcosine had a higher predictive value than total PSA and free PSA in detecting prostate cancer in patients with total serum PSA <4 ng/ml. In addition, elevated circulating sarcosine levels have been showed in patients with metastatic castration-resistant prostate cancer compared with patients with nonmetastatic disease.
- Sarcosin may be employed in combination with PCA3. The PCA3 gene is a noncoding RNA mapped to chromosome 9q21–22. Its usefulness relies in its ability to predict re-biopsy outcome in patients with and without either high-grade prostatic intraepithelial neoplasia or atypical small acinar proliferation.
- The *TMPRSS2*:*ERG* gene fusion is a frequent chromosomal rearrangement in prostate cancer. It is the result of the fusion of the transmembrane protease, serine 2 (*TMPRSS2*) gene and the v-ets avian erythroblastosis virus E26 oncogene homolog (*ERG*) gene or other ETS (E26 transformation specific) transcription factors. *TMPRSS2*:*ERG* is highly specific for predicting clinically significant prostate cancer on biopsy, despite the relatively low sensitivity.

Tissue biomarkers

- Prolaris (Myriad Genetics, UT, USA) is a commercially available test that evaluates the expression of 31 cell-cycle progression genes, normalized to 15 housekeeper genes. One study of 413 prostatectomized men reported a hazard ratio for recurrence for each unit increase in the Prolaris score of 1.7 (95% CI: 1.3–2.4), after adjusting for a standard postoperative risk assessment (Cancer of the Prostate Risk Assessment Postsurgical).
- The Oncotype DX® Genomic Prostate Score test (Genomic Health, Inc., CA, USA) is a RT-PCR-based assay that evaluates the expression of 17 genes. It is able to provide prognostic information and assess the risk of adverse pathology, defined as primary Gleason pattern 4 or any pattern 5 and/or pT3 disease, after radical prostatectomy.
- Hypermethylation involving specific gene promoters in the context of a generalized hypomethylation can cause silencing of tumor suppressor genes and thus promote tumor growth.
- In the MATLOC study, histopathologically negative prostate biopsy samples with matching subsequent either positive (cases) or negative (controls) repeat biopsy samples taken within 30 months were analyzed via a quantitative methylation specific PCR assay panel that included *GSTP1*, *APC* and *RASSF1*. This epigenetic assay yielded a negative predictive value of 90% (95% CI: 87–93).

EXECUTIVE SUMMARY (CONT.)

Tissue biomarkers (cont.)

- Recently, promising prognostic methylation marker candidates have been identified, the most prominent being *PITX2*, *GABRE~miR-452~miR-224*, *C1orf114* and the marker panel *AOX1/C1orf114/HAPLN3* which have all been reported as independent predictors of biochemical recurrence after radical prostatectomy.

Conclusion

- Prostate cancer prognosis is highly heterogeneous, with men harboring an indolent disease that can be safely observed and men whose disease is rapidly fatal.
- The widespread use of PSA as a screening tool has contributed to improve early detection and reduce mortality, but it has also been responsible for overdiagnosis and overtreatment.

Future perspective

- The possibility for the combined use of novel biomarker assays to optimize accuracy is intriguing, but must be weighed against increased costs.
- There is a compelling need not only for additional well-designed, large, multicenter, prospective trials, but also for economic studies evaluating the cost/benefit ratio of novel biomarkers.
- Increased attention to reimbursement policies is also required in order to maximize their impact in clinical practice.

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