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What Can Be Expected from Prostate Cancer Biomarkers? A Clinical Perspective

Keywords

Biomarkers · Molecular tests · Prostate cancer · Screening ·
Panel tests

Abstract

Along with significant advances in prostate cancer biology research, we also observe the rapid development of modern diagnostic tests. New biomarkers are derived to detect disease while it is organ-confined to stratify the risk and to aid clinical decision-making. Majority of these tools have already been validated clinically, but only a few have received premarket clearance and administration approval. Superiority of novel tests is visible not only in improved detection accuracy but predominantly in the assessment of tumour aggressiveness and selection of patients eligible for conservative management. Two factors limiting the clinical implementation of validated biomarker candidates are costs and local availability. For these reasons, currently, their true clinical role starts after routine screening with prostate-specific antigen test. With this review of prostate cancer biomarkers, we attempted to draw general conclusions on clinical perspectives of these novel tools.

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Introduction

Prostate Cancer Screening – Benefits and Harms

Prostate cancer (PCa) is the most common non-skin cancer in elderly males in Europe. Although it is more frequently found in northern and western Europe (>200 per 100,000), in eastern and southern Europe, PCa is showing a constant growing tendency and in upcoming years, it may reach the rates that are prevalent in western and northern parts of the country [1]. The asymptomatic presentation of localized PCa constitutes a strong rationale for screening. Prostate-specific antigen (PSA) testing in men with lower urinary tract symptoms as well as in asymptomatic men resulted in a strong increase of detection rates and incidence of this neoplasm in European population. Although large European studies confirm 21% reduction in disease-specific fatalities after 13 years of follow-up [2], the survival benefit of PSA screening has been questioned in the US population [3]. PSA level of 4 ng/mL has been challenged even earlier, as 20–25% men diagnosed with PCa including those with poorly differentiated one (Gleason Score [GS] ≥ 8) have PSA below this threshold [4]. Moreover, although it shows high organ specificity, PSA is not cancer-specific, which results in el-

evated serum concentration among patients with benign hyperplasia, urogenital infections or transurethral manipulations. As PSA enzymology seems to evolve during oncogenesis and many PSA variants might be secreted depending on mutated cells population [5], the old diagnostic approach to PSA as a “solo” biomarker slowly fades out. These concerns as well as the significant over-detection and over-treatment trends generated with PSA screening [3, 6] resulted in extensive research for PSA replacement or its improvement with panel tests or novel nomograms. Recent efforts focus on finding biomarkers that could distinguish aggressive forms of cancer from indolent ones, as both PSA screening and pathological grading with GS fail to assess tumour aggressiveness with satisfactory accuracy. Similar to the use of multiparametric MRI for targeting prostate biopsy [7], some modern tests seem to have the potential to limit the detection of indolent disease both in patients with a prior negative biopsy and men who have never been biopsied before.

Discussion

PSA and Its First Derivatives – Does It Really Make a Difference?

According to guidelines of European Association of Urology [8], PSA testing should be offered as a part of risk-adapted strategy for early detection of PCa based on age, race, previous PSA levels and life expectancy. Further risk-assessment with risk calculators/tables, additional serum- or urine-based test or imaging is advised in asymptomatic individuals with PSA between 2 and 10 ng/mL prior to prostate biopsy. The value of PSA measurements repeated in time (PSA velocity – PSAV, PSA doubling time-PSADT) is disputable and does not seem to overcome standard PSA test [9], although PSAV might be of value in PCa significance stratification in particular individuals [10, 11]. In selecting patients eligible for active surveillance (AS) PSA serum levels divided by the TRUS – or MRI – determined prostate volume (PSA density [PSAD]) might be of particular value [12]. However, what remains controversial about placing PSAD within AS criteria is setting cut-offs.

In addition to PSAD and PSA kinetics based on standard assay, some analytical variants of PSA test might be implemented. PSA detected in serum is either bonded with macromolecules or in its free form – free PSA (fPSA). Although the ratio of free to total PSA (tPSA) is known to be lower in men with PCa [13] and can reduce unnecessary biopsies with mild loss in PCa detection sensitivity

[14], fPSA molecule is highly unstable when compared to tPSA, which results in variable analytic outcome [15]. Despite advances in this matter in recent years, detection rates might greatly differ depending on the assay used, and further standardization analyses are still required [16]. Taking this into account, the use of fPSA for primary screening is not advised, although in particular individuals’ fPSA seems to be a useful adjunct to standard PSA test, both the diagnostic and the prognostic one. The cut-off level of %fPSA ≤ 15 as a biopsy indication improves PCa detection rates in men with normal digital rectal examination (DRE) and PSA 2.5–4.0 ng/mL [17]. In patients with clinically localized PCa and PSA 4–10 ng/mL, reduction in fPSA/tPSA ratio may be a predictor of extracapsular tumour extension and biochemical recurrence (BCR) [18].

Another PSA-derived biomarker, pro-PSA, is the pro-enzymatic, zymogen form of PSA, cleaved by human kallikrein (hK) enzymes (mainly hK-2 and hK-4) to its mature form. The measurement of isoform of pro-PSA ([–2] pro-PSA) in combination with free and tPSA was used to construct Prostate Health Index (PHI) panel test approved by Food and Drug Administration (FDA) in 2012. Pro-PSA and PHI achieve higher detection accuracy than tPSA, %fPSA or PSAD especially in men with PSA ranging from 2–10 ng/mL [19, 20]. Pro-PSA and PHI can also reduce the number of negative biopsies improving the cost-effectiveness of PCa detection [21] and seem to be a specific tool in biopsy-naïve population [22]. proPSA-derivatives have emerged as successful tools to predict tumour aggressiveness and distinguish PCa from BPH or prostatitis as well as differentiate nonneoplastic lesions [23–26]. Preoperative PHI may predict BCR and pathological features with even better prognostic accuracy than other FDA-approved biomarkers [27–29]. Although due to obvious reasons proPSA is no replacement for imaging diagnostics in predicting PCa [30, 31], combining MRI with proPSA seems to improve its predictive values in biopsy-naïve men [32]. Whether the use of PHI is associated with the benefit for clinical decision-making is yet to be established [29].

4K Score – Does More Mean Better?

The 4K score test (Opko Health Inc.) includes the panel of 4 kallikrein proteins (fPSA, tPSA, iPSA, and hK2) plasma levels and has also been developed to differentiate between patients with aggressive PCa eligible for biopsy and those with low risk and benign lesions [33, 34]. Similarly to PHI, the potential of kallikreins combination to improve detection specificity and thus reduce costs has

already been recognized [35]. Although in comparison with PCA3, 4K test seems to possess a less encouraging predictive value, both of them may improve detection with the European Randomised Study of Screening for Prostate Cancer risk calculator in prescreened men [36]. In another recent study, 4K score was as accurate and specific in predicting PCa and $GS \geq 7$ PCa as PHI [37]. Implementation of 4K test was recently proposed in men >50 years of age in top 10–25% of PSA values to detect metastatic disease as well as to provide biopsy decision support in uncertain cases with elevated PSA [38]. Although 4K score test is yet to be approved by FDA, it is already offered as a Laboratory Developed Test by CLIA-Certified and CAP-Accredited laboratories.

Prostate Cancer Antigen 3 – Replacing Protein Detection with Transcript Tests?

Prostate cancer antigen 3 (PCA3 or DD3) was one of the first long non-coding RNA PCa-specific biomarkers developed and marketed [39]. PCA3 possesses some interesting involvement in PCa pathomechanism and has been therefore widely studied to date. Recently, it has been shown that PCA3 RNA expression is under the strong influence of androgens [40]. Progenza PCA3 urine detection assay (Hologic) based on in-vitro nucleic acid quantitative amplification (reverse transcription polymerase chaining reaction [RT-PCR]) [41] has been approved by FDA in 2012 for men with negative PSA/biopsy/DRE. The ratio of PCA3 mRNA molecules to PSA mRNA molecules in urine specimens is calculated. In Progenza assay, specimens can be obtained after immediate DRE; however, recently proposed novel molecular PCA3/ERG signature can also be derived from non-DRE urine after extracting RNA from exosomes (EXO106 score – discussed later) [42]. Another technical problem of RNA instability has also been addressed recently, as RNA extraction efficiency can be potentially improved with novel RNA sequence-specific capture resulting in better sensitivity and specificity [43]. In a majority of studies performed with Progenza, PCA3 score of <25 implies low risk of PCa [34, 44–46]. The meta-analysis on multi-centre European prospective studies confirmed however that clinical aid brought by PCA3 does not end with detection of cancer but also includes stratification of its significance [47]. In combination with traditional tools like Epstein criteria, PCA3 emerged as an attractive test to guide clinical decisions on AS or prostatectomy. Similarly to PHI and 4K-score test, the implementation of PCA3 has shown potential to lower the number of unnecessary biopsies and reduce costs [48]. The choice of PCA3

as a leading biomarker for screening has its own problems. Although in a double-blind PCA3 vs. PHI study, PCA3 seems to outperform PHI in cancer prediction accuracy, PHI remains the most accurate predictor when screening exclusively for significant PCa; thus, in population screening, authors recommend the use of PHI rather than PCA3 [49]. In a repeated biopsies environment, multiparametric MRI grading combined with PCA3 score are associated with better PCa detection rates [50, 51] and in men eligible for AS, PCA3 can improve the prognostic performance of PRIAS and Epstein criteria for insignificant PCa [52]. Hansen et al. [53] proved that the use of PCA3 within nomogram might improve the accuracy of the clinical model by 4.5–7.1% when predicting the risk of harboring PCa at initial biopsy. After its promising initial performance, the nomogram combining established clinical risk factors (age, DRE, prostate volume, PSA) with PCA3 has been successfully validated externally [54]. With 40% probability threshold set in the validation cohort, almost half (49.1%) of initial biopsies would have been saved at a cost of missing only 16% of HGPCa (high-grade PCa). Recently, internal validation of a novel PCA3-based nomogram in initial biopsy setting was performed [55]. In addition to previously analyzed variables (age, digital rectal exam, prostate volume, PSA), the new model incorporated race and family history of PCa. The nomogram yielded high predictive accuracy with a concordance index of 0.742 and 0.768 for probability of PCa and HGPCa respectively. These and other similar studies support the concept of using PCA3 and clinical data combination as a powerful decision support tool.

Oncotype DX and Genomic Prostate Score – Novel Tools from Breast Oncology

Oncotype DX developed by Genomic Health is a quantitative RT-PCR test performed on paraffin-embedded biopsy specimens detecting the panel of 12 cancer genes involved in 4 fields crucial for oncogenesis. Gene-panel was derived from signaling pathways relevant to stromal response (BGN, COL1A1 and SFRP4), proliferation (TPX2), androgen receptor pathway (AZGP1, KLK2, SRD5A2, and RAM13C) and cellular organization (FLNC, GSN, TPM2, and GSTM2) – all normalized to 5 reference genes [34, 56]. Oncotype DX has been originally developed as a valuable predictive tool in detecting breast cancer, but to date it has also been analyzed in cohorts of men diagnosed with low or low-intermediate risk PCa who might be candidates for AS [57, 58]. Algorithmic combination is used to calculate Genomic Prostate Score (GPS) that ranges from 0 to 100, with higher scores

validated to be associated with high-grade PCa and higher staging (OR per 20 GPS units: 2.3; and 1.9 respectively) [57]. High GPS predicts adverse pathology and also BCR and time to metastases (OR per 20 GPS units 3.3, 2.9 and 3.8 respectively) [58].

Prolaris and Cell-Cycle Progression – A Molecular Player among Risk Calculators

Prolaris (Myriad Genetics) is another molecular-based (RT-PCR) prognostic test measuring the expression of 31 cell-cycle progression (CCP) genes. The test is performed on paraffin-embedded specimens. Analytical outcome is represented by the CCP score correlated with the proliferation index of PCa and serving as the postoperative risk stratification tool [34]. In the first 2 validation studies by Cuzick et al. [59, 60], CCP was the strongest prognostic factor in a multivariate analysis for predicting BCR and time to PCa-specific death. Next, external validation of Prolaris performed by different teams included the comparison of CCP with standard postoperative recurrence risk assessment (Cancer of the Prostate Risk Assessment post-Surgical [CAPRA-S]) and combination of both scores [61]. CCP expressed better prognostic values (HR 2.1 for each unit increase) than CAPRA-S (HR 1.7) and combined CCP/CAPRA-S outperformed individual scores on decision curve analysis. After observing an encouraging outcome of combining CCP with clinicopathological markers in this and similar studies [62, 63], previously mentioned team validated clinical-cell-cycle-risk score in the group of 585 men. Clinical-cell-cycle-risk score was developed as a linear combination of the CCP score and clinical variables included in the CAPRA score [64]. Initial experience suggests that CCP might be even more accurate when combined with 3D-documented biopsy-mapping technology [65]. Finally, recently published meta-analysis provides strong evidence that the use of the CCP score improves treating-decision and constitutes a clinically useful prognostic tool [66]. The economic analysis of CCP implementation is yet to be performed and would be of great value.

Decipher and Genomic Classifier-Know Your Enemy to Change Tactics

Decipher developed by Genome Dx Biosciences and Mayo Clinic is a genomic test based on the panel of RNA biomarkers involved in multiple signaling pathways relevant to prostate oncogenesis (immune modulation, proliferation, adhesion and motility, cell differentiation, CCP and androgen signaling). Based on the expression of 22 genes, genomic classifier (GC) score is calculated [67].

GC was developed as a prognostic tool to assess the probability of metastatic disease development after RP and eventually change secondary treatment recommendations including post-RP adjuvant radiation therapy [34, 67–72]. Based on the PRO-ACT study from 2014, even 60% of post RP high-risk patients might be reclassified with GC, 30% patients might need additional adjuvant treatment and in 40%, adjuvant therapy might be unnecessary in spite of initial recommendations [73]. Moreover, the predictive value of GC is not limited to post-RP patients but can be utilized in recurrent individuals who underwent RP and have already been treated with secondary salvage RT [74]. Similarly to Prolaris, GC scoring adds a significant predictive value to the clinical scoring (CAPRA-S) as well as to the Eggeners risk model, which constitutes the rationale for genomic-clinical classifier integration [72, 73, 75]. In a recent prospective study on a large cohort of 2,342 patients, utilization of the GC score in postoperative decision-making changed initial tumour aggressiveness classification based on clinical parameters alone [76], regardless of the positive correlation between Decipher score and GS, T-staging, CAPRA-S, and age. In fact, the relation between GC and conservative pathological predictors remains under questions as Decipher scoring might be inconsistent with up to 20% GS assessed as 3 + 3. Taking the following into consideration, profiling with GC might improve the patients' selection process for AS in low-grade PCa [77]. After collecting convincing evidence, Decipher tool and GC have been recently included in the National Comprehensive Cancer Network guidelines as postprostatectomy tools, while Prolaris and Oncotype DX Prostate were mentioned as biopsy-based tools [78].

ConfirmMDx – Cancer Leaves Epigenetic Fingerprints

ConfirmMDx offered by MDxHealth is a quantitative methylation-specific polymerase chain reaction assay panel, measuring GSTP1, APC and RASSF1 derived to detect occult PCa in histopathologically negative biopsies [34]. This methylation assay has been validated in 2 large independent cohorts. In the MATLOC study, the methylation status was a significant independent factor of patient outcome (OR 3.17, 95% CI 1.81–5.53) and its negative predictive value was superior to standard histopathology reaching as high as 90% [79]. Since similar data was collected during validation in the DOCUMENT study, epigenetic profiling for *GSTP1*, *APC* and *RASSF1* emerges as a valuable screening tool especially to reduce unnecessary biopsies [80] and improve biopsy-decision cost-effectiveness. Those capabilities of ConfirmMDx as-

say are due to methylation field or “halo effect,” which is observed in cancer foci: epigenetic DNA changes are present not only in cancer lesions but also in adjacent benign tissues [81, 82]. Epigenetic risk score (EpiScore) for epigenetic aberration in GSTP1, APC and RASSF1 has been recently proposed and combined with traditional clinical risk factors [83] with significant improvement in unnecessary biopsies avoidance and high-grade PCa identification when compared to currently used metrics. In a recently published paper, GSTP1 hypermethylation on a negative biopsy was highly associated with the risk of PCa on a second biopsy, especially GS $\geq 4 + 3$ tumours. In the same study, alternative marker-global methylation (LINE1) had a poor predictive value [84]. Although ConfirmMDx aims mainly at identifying significant PCa by improving sensitivity of detection, DNA methylation assays might also be a valuable tool to distinguish PCa from BPH. In the study by Paziewska et al. [85], DNA methylation levels of APC, TACC2, RARB, DGKZ and HES5 promoter regions provided an excellent discrimination between malignant and benign tissues with AUC reaching 0.95–1. Interestingly, in the same study, standard analysis of gene expression of the best 3 genes (HOXC6, AMACR, and PCA3) yielded only moderate accuracy with AUC >0.8 [85]. Simultaneously with growing interest in invasive epigenetic assays, non-invasive methylation biomarkers were evaluated. Zhao et al. [86] have recently identified a panel of 4 methylation biomarkers (APC, CRIP3, GSTP1, and HOXD8) available from urine collected after DRE. Clinical validation is necessary to introduce it to monitor patients on AS [86].

TMPRSS2-ERG – An Androgen-Regulated Prostatic BCR-ABL

The recurrent TMPRSS2-ERG (T/E) is an androgen receptor-regulated fusion gene (transmembrane serine protease isoform 2-TMPRSS2 fused with ERG-ETS transcription factor family member gene) observed in about 50% PCa, which constitutes it as the most common genetic rearrangement observed in this neoplasm [34, 87]. T/E is speculated to be a result of repair genes failure [88] and it is found to have an influence on a wide spectrum of signaling pathways. T/E has been proved to induce particular molecular mechanisms involved in metastases development [89], PCa castration-resistance [90] and methylation patterns [91]. TMPRSS2-ERG transcript serves as PCa biomarker detected classically with RT-PCR in urine, although T/E detection rate might be improved significantly by performing an assay both in urine and biopsy material [92]. Addressing the fact that the relation be-

tween T/E expression and HGPCa remains unclear [93–96] and its prognostic value is constantly questioned [95, 97], Font-Tello et al. [98] have suggested that instead of its mere presence, it is the T/E expression level that corresponds with tumour aggressiveness or prognosis. Moreover, based on meta-analysis from 2014, T/E detection assay (pooled rates of data obtained with RT-PCR) used alone provides unsatisfactory sensitivity of 49% PCa detection despite high specificity reaching 90% [99]. In addition to the above-mentioned information, T/E seems to be a potent predictor of aggressiveness and BCR when combined with other markers (PCA3) and standard risk calculators [100, 101]. It might also be used for outcome prediction and selection of patients eligible for systemic treatment [102]. As simultaneous T/E implementation is associated with correction of more than 50% false negative results of PCA3 when predicting PCa [103], combination of both tests has become a topic of great interest in recent years. T/E-PCA3 urine assay (Mi-Prostate score) is of additional predictive value when added to serum PSA or Prostate Cancer Prevention Trial risk calculator score [104], which has been recently validated in a large prospective cohort [105]. Combined test has the potential to reduce the number of unnecessary biopsies by over 60% with minimal survival rate change (circa 1%) when restricting biopsies to patients with higher PSA levels or individuals with detectable PCA3 or T/E in urine [106, 107].

SChLAP1 – Long Non-Coding RNA Marks Lethal PCa

Along with increasing insight into PCa biology, long non-coding RNA (lncRNA) has been hypothesized to be aberrantly expressed in cancerous prostate tissues. After obtaining in vitro and in vivo evidence on its contribution in cancer cell invasiveness and metastasis by antagonizing tumour-suppressive function of chromatin-modifying protein complex [108], one of lncRNAs – SChLAP1 has been proposed as a marker of lethal PCa. The expression of lncRNA can be easily assessed with in situ hybridization; thus, SChLAP1 can be used as a tissue-based biomarker [34, 109]. SChLAP1, expressed at higher levels, has been proved to correlate with disease progression and poor outcome after radical prostatectomy in patients with localized PCa [109]. The test was validated in multi-institutional studies in predicting both metastases [34, 110] and disease-specific survival [34, 111]. Although clinical evidence on SChLAP1 in situ hybridization is being constantly updated, its prognostic features for prediction of pathologic outcome, metastases, BCR and survival have already been confirmed by meta-analysis [112]. Since the

assay shows particular potential in detecting aggressive forms of PCa, it has recently been proposed as a biomarker of unfavourable subpathologies in localized disease [113]. With these encouraging properties, lncRNA has the potential to serve as a stratification tool for surveillance in patients of highest risk.

Will Urine Exosome Assays Reduce the Number of Unnecessary Biopsies?

What has been a significant limitation of transcript tests is the lability of mRNA outside cellular environment. Simultaneously with advances in RNA purification techniques, a novel method of isolating PCA3 and ERG transcripts encapsulated in exosomes has been developed [42]. The rationale for replacing standard methods with the extraction of exosomal RNA comes from protective properties of exosome and its particular mRNA expression patterns, which are highly representative for the cell of the origin [114, 115]. Initial experience with urine-derived EXO106 score (the sum of normalized PCA3 and ERG transcript levels normalized to SPDEF) has constituted exosomal RNA extraction as a promising tool to discriminate PCa from healthy individuals [42]. With a negative predictive value of 97.5% within “gray zone” serum, PSA in the 2–10 ng/mL range EXO106 score has emerged as a promising tool to aid initial biopsy decision-making [42]. Exosome gene expression assay was then externally validated in the prospective, multisite trial under the name ExoDx Prostate Intelliscore [34, 116]. With expression levels cut-points set in training cohort, 27% biopsies in independent “gray zone” cohort would have been avoided, missing only 5% of patients with HGPCa. With this significant clinical benefit, ExoDx Prostate Intelliscore might not only aid in decision making on initial biopsy but also be supportive in repeated-biopsy population and patients undergoing AS. Thus, full spectrum of clinical utility of exosome expression assays is yet to be established.

NADia ProsVue – to Detect Undetectable PSA

NADia is an immunoassay derived to detect PSA nucleic acid in serum [117]. The test is based on immunopolymerase chain reaction with 2 monoclonal antibodies employed – one conjugated to DNA label and the other to paramagnetic microparticles. After the removal of excess reporter conjugate (magnetic capture) and several re-suspension cycles, PSA concentrations are determined. As limit of quantification drops to 0.65 pg/mL, which is >ten-fold lower than ultrasensitive PSA immunoassays, immuno-polymerase chain reaction can be considered the most sensitive commercial PSA test. NADia might be used to

determine PSA kinetics at very low concentrations and thus constitutes an efficient post-RP stratifying tool. ProsVue PSA slope ≤ 2 pg/mL/month has been proposed as a prognostic factor of PCa clinical recurrence reduced risk that might also be of additional predictive value when used with established risk factors [118, 119]. In reanalysis from 2014, NADia was also predictive of reduced PCa-specific mortality risk [119]. Post-RP stratification with NADia seems to be particularly valuable in individuals in whom secondary therapy is considered, as it may help to avoid unnecessary treatment. Indeed, in multicentre, risk-stratified cohort ProsVue PSA slope ≤ 2 pg/mL/month reduced secondary treatment recommendations (ART or ADT), which in turn implied possible health-care costs reduction [120]. However, in cost-effectiveness analysis, ProsVue impact on costs is speculated to depend on the extent to which ART will be reduced in patients with low risk of recurrence and remains uncertain when related to quality-adjusted life years [121]. Although ProsVue received US premarket clearance in 2011, it is yet to be approved by FDA.

Circulating Cell-Free Tumour DNA – Next Generation Sequencing for “Liquid Biopsy”

Next Generation Sequencing (NGS), an extremely efficient sequencing approach, provides an opportunity to transfer the outcomes of research on mutational changes in PCa directly to the clinic. The main rationale for seeking cell-free tumour DNA (cfDNA) in serum lies in heterogeneity of regional chromosomal DNA ploidy between cancer patients and healthy individuals, and has been raised long ago [122]. Although it is present also in healthy individuals’ serum [123], the amount of cfDNA released during cancerogenesis differs significantly [124]. qRT-PCR for cfDNA can be helpful in differentiating PCa from BPH within particular PSA levels [125], but the analytic process is long and toilsome. cfDNA NGS, especially massive parallel sequencing, provides great statistical power, which could also be successfully utilized to distinguish between PCa and prostatitis or healthy individuals [126]. NGS measurements use copy number variation converted into copy number instability score as accurate hallmark of malignant transformation [127]. Currently, Chronix Biomedical is validating NGS-based blood test using copy number instability on cfDNA in the prospective clinical trial on 1,500 men referred for a prostate biopsy after abnormal PSA test or DRE. The study is expected to complete its patient accrual in mid-2017 and announce results in early 2018 (<https://clinicaltrials.gov/ct2/show/NCT02771769>).

Table 1. Characteristics of selected clinically validated PCa biomarkers – summary

Biomarker	Brand name	Material	Primary indication	FDA-approved by EAU guidelines	Recommended by EAU guidelines	External validation studies available	Cost per patient
PSA, fPSA and pro2PSA – protein-based test	PHI	Blood	First biopsy decision	Yes	Yes	Yes	USD 500
tPSA, fPSA, iPSA and hK2 – protein-based test	4K-score	Blood	First biopsy/rebiopsy decision	No	Yes	Yes	USD 1,200
PCA3 – qRT-PCR	Progensa	Urine (post DRE)	First biopsy/rebiopsy decision	Yes	Yes	Yes	USD 300–500
8 proteins prostate expression	ProMark	Biopsy specimen (FFPE)	Eligibility for AS	No	No	No	USD 3,900
17 (12 + 5) genes – qRT-PCR	Oncotype Dx	Biopsy specimen (FFPE)	Eligibility for AS	Yes	Yes	Yes	USD 4,200
46 (31+15) genes – qRT-PCR	Prolaris	Biopsy specimen (FFPE)	Eligibility for AS	Yes	No	Yes	USD 3,400
22 RNA biomarkers prostate expression	Decipher	Tissue (post RP specimen)	Eligibility for post-RP RT	No	No	Yes	USD 3,400
GSTP1, APC and RASSF1 – methylation assay	ConfirmMDx	Biopsy specimen (FFPE)	Rebiopsy decision	No	Yes	Yes	USD 3,300
4 metabolites (aminoacids) LC-MS assay	Prostarix	Urine (post DRE)	First biopsy/rebiopsy decision	No	No	No	Discontinued
Immuno-PCR (IPCR) for pg/mL PSA levels	NADia ProsVue	Blood	Eligibility for post-RP secondary treatment	Yes	No	Yes	No data obtained
NGS in cfDNA	Developed by chronix biomedical	Blood	First biopsy decision	No	No	Recruiting participants for clinical trial (NCT02771769)	1,300 USD
2 mRNA biomarkers combined with clinical variables	SelectMDx	Urine	Eligibility for AS	No	No	No	300 USD
SChLAP1 expression (ISH or qRT-PCR)	Developed by genomeDx biosciences	Tissue (post RP specimen) or Urine (post DRE)	Risk stratification in patients after RP	No	No	Yes	No data obtained
PCA3 and ERG exosome-derived gene expression (qRT – PCR)	ExoDx prostate intelliscore	Urine	First biopsy/rebiopsy decision	Yes	No	Yes	600 USD

FDA, Food and Drug Administration; EAU, European Association of Urology; tPSA, total PSA; fPSA, free PSA; iPSA, intact PSA; PHI, Prostate Health Index; qRT-PCR, quantitative reverse transcription polymerase chain reaction; DRE, digital rectal examination; FFPE, formalin-fixed paraffin-embedded; AS, active surveillance; LC-MS, liquid chromatography–mass spectrometry; NGS, next generation sequencing; cfDNA, cell-free DNA; ISH, in situ hybridization.

SelectMDx – Simple Transcript Panel, Complicated Algorithm and Non-Stand-Alone Test

SelectMDx is the algorithm combining qRT-PCR for 2 mRNA molecules (HOXC6 and DLX1) with clinical parameters (PSA, PSAD, DRE, age and family history of PCa) in order to predict the presence of significant PCa. Both analyzed transcripts were chosen out of 39 biomarkers identified in the previous gene expression profiling study [128]. Interestingly, in the same study combination of HOXC6, DLX1 (used in SelectMDx) and TDRD1 was suggested to outperform (AUC = 0.81) Progenesa PCA3 assay (AUC = 0.68) and serum PSA (AUC = 0.72) when predicting GS ≥ 7 PCa. Implementation of HOXC6 and DLX1 into the algorithm resulted in improving HG PCa predictive accuracy with AUC reaching 0.90 in the validation cohort [129]. In this recent multi-centre prospective study, SelectMDx outperformed Progenesa as well as Prostate Cancer Prevention Trial risk calculator. In fact, some controversies that arose around the PCA3 assay as single transcript marker [100] seem to catalyze the evaluation of transcript panel tests like SelectMDx. Based on the preliminary data, SelectMDx can emerge as a supportive prostate biopsy decision tool. Although biomarker-based risk score might be promising, some concerns should be named. First clinical validation has been performed, yet further evaluation and external validation are greatly expected, as our recent knowledge is limited practically to 2 studies. The fact that SelectMDx becomes predictively valuable only after combining with other parameters, might be considered a limitation in itself. Although the algorithm outperforms PCA3 analyzed exclusively, one should notice that the construction of nomogram based on PCA3 and clinical data has also been undertaken in some already-mentioned studies [52, 55]. Comparison between these tools and SelectMDx is yet to be performed.

Conclusion

In recent years, there has been an increased in PCa biomarker research activities, which was backed by numerous new validation studies (Table 1). Together with emerging prospective updates, more often, the question about PSA replacement or its supplementation is being raised. Most of the tests presented here aim at outperforming markers, nomograms and calculators used to date or supply additional value to them. Based on current knowledge, not every described assay is eligible for population screening, but most show at least interesting po-

tential as stratifying, differentiating and prognostic tools. There are also factors that are limiting further progress. Price and availability are some such factors. With an average cost of 500 USD per measurement, now they do not meet one of the most important conditions of a screening test. Utility of biomarkers as decision-supporting tools often seems to be limited by lack of clear indications on further clinical proceeding. Defining cut-offs in large validation cohorts and calibrating test-based scores are not helpful in solving the issue. Similar problems are faced by clinicians when using nomograms – stratifying patients as exhibiting high risk of reaching a particular end-point is not equal to diagnosing this end-point. At the same time, there is also growing competition from multiparametric MRI, which provides binary and usually straightforward detection of PCa both in primary and rebiopsy settings. Having considered the above factors, it seems that PSA will stay with us in the immediate future, but the future of these new technologies remains unclear.

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