Prostate Int 4 (2016) 130–135

Contents lists available at ScienceDirect

Prostate International

journal homepage: http://p-international.com

Review Article

Prostate cancer biomarkers: Are we hitting the mark?

Shannon McGrath [1, ☆], Daniel Christidis [1, ☆], Marlon Perera [1], Sung Kyu Hong [2], Todd Manning [1], Ian Vela [3, 4], Nathan Lawrentschuk [1, 5, *]

[1] Department of Surgery, University of Melbourne, Austin Health, Melbourne, Australia
[2] Department of Urology, Seoul National University Bundang Hospital, Seoul, South Korea
[3] Department of Urology, Princess Alexandra Hospital, Brisbane, Queensland, Australia
[4] Queensland University of Technology, Australian Prostate Cancer Research Center-Queensland, Brisbane, Australia
[5] Department of Surgical Oncology, Peter MacCallum Cancer Centre, Melbourne, Australia

ARTICLE INFO

Article history:
Received 5 June 2016
Received in revised form
13 July 2016
Accepted 24 July 2016
Available online 29 July 2016

Keywords:
Biomarker
Diagnosis
Prostate cancer
Prostate specific antigen

ABSTRACT

Purpose: Localised prostate cancer diagnosis and management is increasingly complex due to its heterogeneous progression and prognostic subgroups. Pitfalls in current screening and diagnosis have prompted the search for accurate and invasive molecular and genetic biomarkers for prostate cancer. Such tools may be able to distinguish clinically significant cancers from less aggressive variants to assist with prostate cancer risk stratification and guide decisions and healthcare algorithms. We aimed to provide a comprehensive review of the current prostate cancer biomarkers available and in development.

Methods: MEDLINE and EMBASE databases searches were conducted to identify articles pertaining to the use of novel biomarkers for prostate cancer.

Results: A growing number of novel biomarkers are currently under investigation. Such markers include urinary biomarkers, serology-based markers or pathological tissue assessments of molecular and genetic markers. While limited clinical data is present for analysis, early results appear promising. Specifically, a combination of serum and urinary biomarkers (Serum PSA + Urinary PCA3 + Urinary TMPRSS2-ERG fusion) appears to provide superior sensitivity and specificity profiles compared to traditional diagnostic approaches (AUC 0.88).

Conclusion: The accurate diagnosis and risk stratification of prostate cancer is critical to ensure appropriate intervention. The development of non-invasive biomarkers can add to the information provided by current screening practices and allows for individualised risk stratification of patients. The use of these biomarkers appears to increase the sensitivity and specificity of diagnosis of prostate cancer. Further studies are necessary to define the appropriate use and time points of each biomarker and their effect on the management algorithm of prostate cancer.

1. Introduction

Prostate cancer (PCa) is among the commonest newly diagnosed cancers in the Western world, with projections for increased incidence over the following decades.[1] Prostate specific antigen (also known as PSA or human kallikrein-3) remains the first line and most commonly used serum biomarker for the detection of PCa. The introduction of PSA has resulted in the increased diagnosis of men with localized, early stage PCa.[2] In current practice, controversy remains over its suitability and efficacy as a screening tool for increasing early detection of PCa and lowering mortality.[3] An inherent limitation to PSA testing relates to lack of specificity in the setting of PCa screening.[4] There is great clinical need for accurate screening for PCa to decrease unnecessary prostate biopsies.

Additionally, PSA provides poor differentiation of PCa aggressiveness.[4] PCa presents a difficult entity to accurately risk stratify due to its highly variable clinical course. Prostate biopsy

* Corresponding author. Department of Surgery, University of Melbourne, Austin Health, Studley Road, Heidelberg, Victoria 3088, Australia.
E-mail address: lawrentschuk@gmail.com (N. Lawrentschuk).
☆ These two authors contributed equally.

http://dx.doi.org/10.1016/j.prnil.2016.07.002

is the gold standard for PCa diagnosis, however it has diagnostic limitations and its invasive nature increases the risk of adverse events. Further, the PCa risk stratification by PSA, prostate biopsy Gleason score, or pTNM cancer stage may lead to understaging.[5] Accurate staging and risk stratification is critical, particularly when considering active surveillance. As such, there is a critical need for improved PCa biomarkers that are noninvasive and have improved accuracy and risk stratification properties.

This has led to the search for aids in the decision-making algorithm of PCa that may give information on prognosis, add diagnostic specificity, or act as screening tools. Over recent decades, the development of molecular biomarker assays and genetic assays has provided an avenue for PCa biomarker development. Considerable research has resulted in a new panel of tests that may improve determination of cancer presence, aggressiveness, and prognosis. Emerging biomarkers include those utilizing serum, urinary, or tissue samples as a test substrate. In clinical practice, the utility of these biomarkers is variable and may be used at different time points throughout the care of a patient with suspected or diagnosed PCa. Specifically, these biomarkers assist in diagnosis, guiding definitive treatment options, determine the risk of ongoing monitoring versus intervention, or provide risk stratification in the setting of negative initial biopsy.

There is still a need for a clear understanding of the role of each of these tests in the diagnosis, management, and prognosis of patients with PCa. This review explores the current literature on biomarkers used in PCa screening. We have reviewed the contemporary literature pertaining to different PCa biomarkers including: ProPSA and PHI, the 4K score test, PCa antigen 3, transmembrane serine protease protein 2 (TMPRSS2)-Erythroblastosis virus E26 Oncogene Homolog (ERG), ExoDx Prostate Intelliscore, Second Chromosome Locus Associated with Prostate-1 (SChLAP1), SelectMDx, ConfirmMDx, Oncotype DX PCa assay, Prolaris, Decipher, and Embryonic Lethal, Abnormal Vision, Drosophila-Like 1 (ELAVL1). We aim to objectively review current biomarkers in PCa in order to further define the utility of these tests and their role in PCa management. Fig. 1 is a guide to the use and appropriate timing of the discussed biomarkers in the role of a patient with suspected or proven PCa.

2. Serum-based PCa biomarkers

2.1. ProPSA and PHI

PSA is derived from an inactive precursor enzyme that contains a pro leader sequence of seven amino acids, known as [-7]proPSA. Activation occurs through posttranslational cleavage of its ≥ 7 amino acid (AA) pro leader sequence by human kallikreins 2 and 4 to form the mature 237 amino acid PSA molecule. Partial cleavage of this leader sequence produces isoforms of proPSA depending on how many amino acids remain attached to the PSA molecule, most commonly [-4]proPSA, [-5]proPSA, and [-2]proPSA. The [-2]proPSA variant has been found to be the most prevalent in PCa extracts.[6] The Prostate Health Index (PHI, Beckman Coulter Inc., Brea, CA, USA) is a mathematical formula which relies on the differing proportions of the specific biomarkers (fPSA, tPSA, -2proPSA). This formula provides additional information to assist in delineating between benign prostatic conditions and PCa in men with suspected PCa. Validation was provided by a multicenter, case

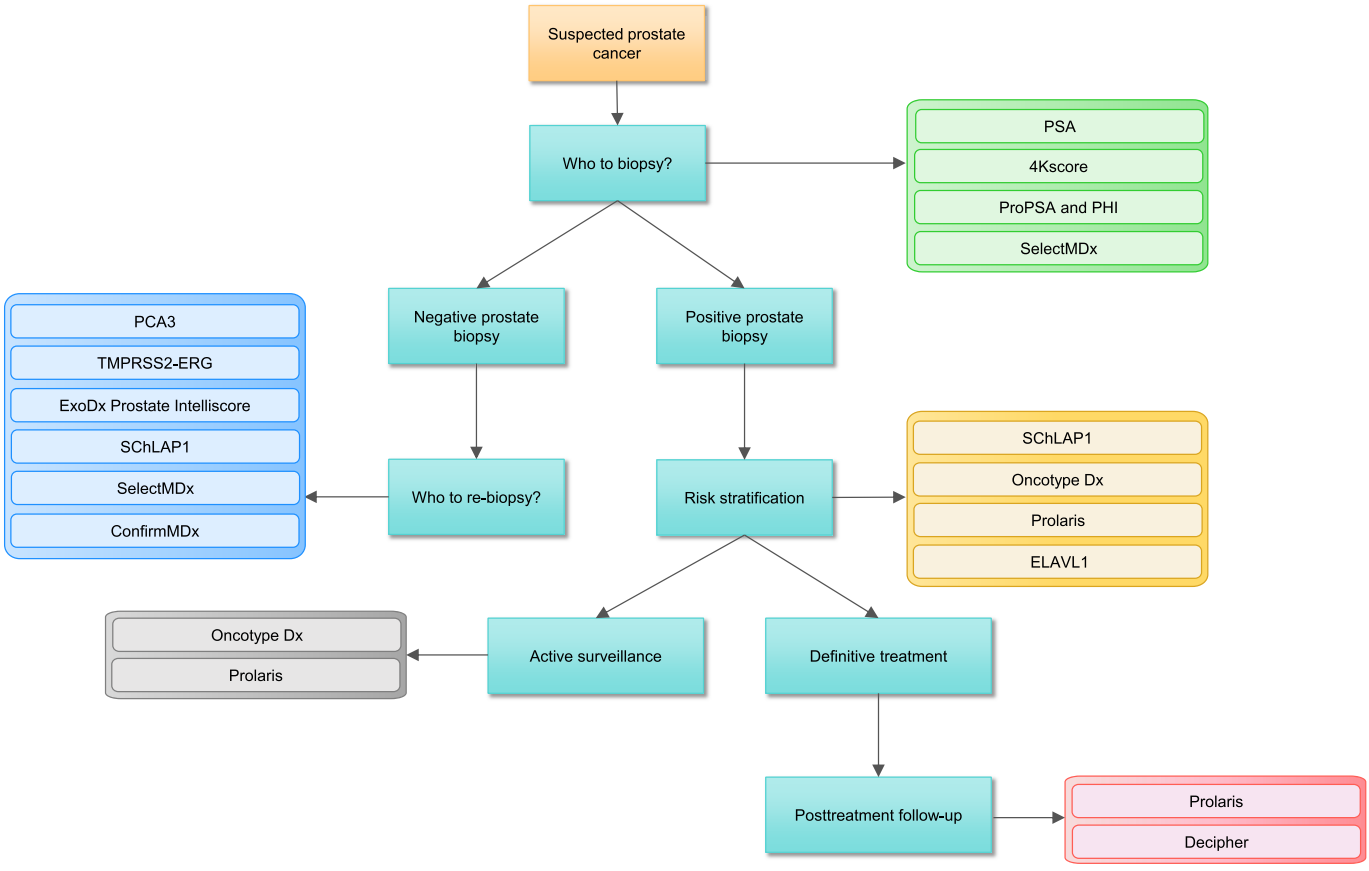

Fig. 1. Appropriate timing of the use of these biomarkers in the care of a patient with suspected or proven prostate cancer.

controlled clinical trial in which 892 patients with no history of PCa, benign digital rectal examination, and PSA between 2 ng/mL and 10 ng/mL underwent prostate biopsy. PHI was found to have greater specificity (AUC 0.73) than PSA alone or other combinations of pro PSAs.[7] Furthermore, the study displayed that increasing PHI values were associated with detection of clinically significant PCa of Gleason Grade 7 or higher. These findings were corroborated by Fossati et al,[6] who reported an association with PHI score and poorer pathological outcomes on 489 patients treated with radical prostatectomy. Whilst data regarding this test is immature, PHI appears to be a promising, noninvasive biomarker that may improve detection and provide prognostic information.

2.2. The 4Kscore test

Similar to PHI, the 4Kscore (OPKO Lab, Miami, FL, USA) test is determined on serum levels of four human kallikreins: total PSA, free PSA, intact PSA, and human kallikrein 2. These values are used in combination with clinical information (age, Digital Rectal Examination (DRE) findings, and history of previous negative biopsy result). These variables are placed into an algorithm and a patient-specific percentage risk of having Gleason score 7 or more on subsequent biopsy is provided.[8] This recent prospective study by Parekh et al[8] examined the intervention of the 4K test on 1,012 men referred for prostate biopsy for clinical suspicion of PCa regardless of PSA. The predictive accuracy of a biopsy result Gleason $\geq 3+4$ was significantly higher with the inclusion of the 4K test (compared to PSA alone), with an AUC 0.821 [95% confidence interval (CI) 0.790–0.852] versus 0.751 (95% CI 0.714–0.789), respectively.[8] In this diagnostic setting, the 4K score has been reported to have reduced the number of prostate biopsies in a multicenter study of 611 patients.[9] Additionally, there is evidence that the 4K score may be able to identify higher risk PCas. Stattin et al[10] conducted a case-control study from a population based cohort over > 15 years using Swedish Cancer registry data and cryopreserved blood from men aged 40 years, 50 years, and 60 years. Improved prediction of risk of metastasis over a 20 year period was found to correlate with increasing 4K score.[10] Similar to PHI, the 4K score is a noninvasive biomarker that improves diagnostic accuracy for clinically significant PCa compared to PSA alone.

3. Urinary biomarkers

3.1. PCa antigen 3

PCa antigen 3 (PCA3 – Progensa Test Kit, Hologic, Marlborough, MA, USA) involves noncoding RNA sequences from prostate specific genes that are highly expressed in PCa cells. It is overexpressed in PCa cells in comparison to normal or benign prostate cells.[11] PCA3 scores reflect the ratio of PCA3 RNA molecules to PSA RNA molecules detected in a patient's urinary specimen following a digital rectal examination. The current indication for the use of Progensa is in men aged 50 years or older who have had one or more previous negative prostate biopsies in whom a repeat biopsy would be recommended as standard of care. A PCA3 score < 25 is associated with a decreased chance of PCa on subsequent repeat biopsy. PCA3 has been shown to have a variable sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) depending on the cutoff score chosen (PCA3 score of 25 or 35). A PCA3 score of 35 was associated with a sensitivity of 58–82%, specificity of 58–76%, PPV of 67–69%, and NPV of 87%, and an AUC of 0.68–0.87 (95% CI for 0.87: 0.81–0.92).[12–14] Leyten et al[14] further demonstrated a difference in a PCA3 score of 25 and 35 with sensitivity and specificity of 82.5% and 50.8%, respectively, for a PCA3 score of 25, and 68.4% and 58.3% with a PCA3 score of 35. Furthermore, higher PCA3 scores have been correlated to tumor aggressiveness, suggesting the PCA3 test has prognostic validity.[15] PCA3 when used in conjunction with PSA and DRE screening can lower the number of unnecessary prostate biopsies in patients considered for initial prostate biopsy or in men considered for repeat biopsy.[16]

3.2. TMPRSS2-ERG

TMPRSS2 is a prostate specific and androgen regulated gene that codes for a fusion protein. This has been identified as an oncogene for PCa with overexpression in > 50% of PCa cases. In PCa, the TMPRSS2 gene fuses with the transcriptional factors and regulator ERG (TMPRSS2-ERG) in ~48% of clinically localized PCa, but only 30% of hormone naïve metastases.[17] Acting alone, TMPRSS2-ERG has a low sensitivity of 24.3–37%, high specificity of 93%, and a subsequent PPV of 94% after a DRE.[11,14,18,19] However, in combination with serum PSA (cutoff of 10 ng/mL) and urinary PCA3, TMPRSS2-ERG provides an improved accuracy in diagnosing PCa with a sensitivity and specificity of 80% and 90%, respectively, and AUC of 0.88 (95% CI 0.75–0.98).[20]

3.3. ExoDx prostate Intelliscore urine exosome assay

ExoDx (Exosome Diagnostics Inc., Cambridge, MA, USA) prostate Intelliscore urine exosome assay used in conjunction with the current standard of care is a novel marker produced to determine the risk of Gleason 6, Gleason 7, and benign disease on initial biopsy. ExoDx uses a urinary substrate, utilizing a three exosome gene assay signature (ERG, PCA3, SPDEF) to construct a score. McKiernan et al[21] assessed 519 patients and determined that ExoDx was predictive of high grade PCa (Gleason score > 7) on subsequent initial biopsy with a sensitivity of 92% and specificity of 34%, an NPV of 91%, and a PPV of 36% with an AUC = 0.77 (95% CI 0.71–0.83).[21] This test again may reduce the number of potentially unnecessary prostate biopsies.

3.4. SChLAP1

SChLAP1 (GenomeDx Biosciences, San Diego, CA, USA) expression has been identified as a potential biomarker for the risk of metastatic progression of PCa. Prensner et al[22] using a multivariate analysis of 1,008 patients showed that SchLAP1 expression (high or low) was a predictor of metastatic progression of PCa within 10 years (odds ratio 2.45, CI 95% 1.70–3.53, $P < 0.0001$). Prensner et al[22] similarly reported that SChLAP1 is expressed in ~25% of PCas and may aid in the discrimination of aggressive and indolent tumors. High SChLAP1 expression was associated with a higher risk of biochemical recurrence, metastases, death from PCa, and death from any cause at 10 years post prostatectomy. They also found that it may be viable as an independent prognostic risk factor for metastasis. Adding the SChLAP1 assay to the Decipher assay led to a significant increase in the prognostic potential of Decipher: from 0.77 (95% CI 0.72–0.81) to 0.79 (95% CI 0.74–0.83), $P = 0.048$.[23] Prensner et al[23] also reported preliminary studies on post DRE urinary SChLAP1 expression in a cohort of 230 patients. SChLAP1 was integrated with PCA3 and TMPRSS2-ERG fusion expression with SChLAP1 was able to detect some cancers not detected by the other two methods (8%). SChLAP1 however had a lower sensitivity than PCA3 and TMPRSS2-ERG fusion.[23]

3.5. SelectMDx

SelectMDx (MDxHealth, Irvine, CA, USA) is a urinary two gene assay (HOXC6 and DLX1) used for the detection of high grade PCa

(Gleason score ≥ 7). Current recommendations suggest its use in patients who are considered for prostate biopsy or have previously had negative biopsies, despite high risk factors for PCa (abnormal digital rectal examination, family history of PCa, or high serum PSA levels). Leyten et al[24] validated a urinary three gene panel (HOXC6, TDRD1, and DLX1) and demonstrated a higher accuracy (AUC 0.77; 95% CI 0.71–0.83) for detection of clinically significant PCa when compared to PSA and PCA3 (AUC 0.72; CI 0.68–0.78 and AUC 0.68; 95% CI 0.62–0.75, respectively). Recently, Van Neste et al[25] further validated these findings and compared them to other PCa gene markers detected on urinalysis. This group performed two independent prospective clinical trials ($n = 905$) and compared the performance of individual and combination urinary biomarkers to predict high grade PCa on biopsy. Their findings illustrated that the strongest performers were HOXC6 and DLX1 with a sensitivity of 91%, a specificity of 36%, an NPV of 94%, a PPV of 27%, and an AUC of 0.76.[25]

4. Prostatic tissue biomarkers

4.1. ConfirmMDx

ConfirmMDx (MDxHealth, Irvine, Ca, USA) is an epigenetic test that uses DNA methylation in the detection of significant PCa after a negative prostate biopsy. It uses formalin-fixed, paraffin-embedded (FFPE) prostate tissue from biopsy. Three epigenetic biomarkers are evaluated in ConfirmMDX - GSTP1, APC, and RASSF1.[26] While not FDA approved, current evidence suggests ConfirmMDx predicts the risk of PCa on subsequent biopsy, with an NPV of 90%.[26,27] A recent study by Van Neste et al,[28] assessed 803 patients from two previous cohort studies that underwent two consecutive prostate biopsies within a 30 month period. This group created an algorithm utilizing patient age, histopathology of the first cancer negative biopsy, and the EpiScore (a DNA methylation intensity score). Low DNA methylation in PCa-negative biopsies led to an NPV of 96% for high grade cancer and AUC 0.762 (95% CI 0.68–0.84). Further to this, they compared the clinical utility of this algorithm with PSA and PCa prevention trail risk calculator for deciding to repeat biopsy. Using a decision curve analysis, they demonstrated the largest reduction in unnecessary repeat biopsies with the suggested algorithm.[28]

4.2. Oncotype DX PCa assay

Oncotype Dx for Prostate (Genomic Health Inc., Redwood City, CA, USA) is a complex genetic-based assay used to further stratify low to low-intermediate risk PCa (Gleason 6 or low volume Gleason $3 + 4$). This assay assesses 12 cancer-related genes, representing four different biological pathways and five reference genes. The relevant results are combined to form the Genomic Prostate Score (GPS). The GPS determines PCa aggressiveness by risk stratifying an individual's underlying tumor biology. This information assists clinicians in assessing a patient's suitability for active surveillance or immediate treatment. A favorable score GPS < 20, improved the confidence of patients remaining on active surveillance, but unfavorable patients should be evaluated for treatment.[29,30] Each 20 point increase in GPS is associated with a 2.3-fold increased risk of high grade disease (95% CI 1.5–3.7) and a 1.9-fold increased risk of nonorgan confined disease (95% CI 1.3–3.0) on final pathology at radical prostatectomy.[31]

4.3. Prolaris

The Prolaris score (Myriad Genomic Inc., Salt Lake City, UT, USA) is a genomic assay that provides a Cell Cycle Progression (CCP) score based on level of expression of mRNA of 31 cell cycle progression genes relative to the level of 15 housekeeping genes.[32] CCP scores have shown a strong correlation on univariate analysis with PCa death in a conservatively managed cohort after biopsy (hazard ratio (HR) 2.02, 95% CI 1.62, 2.53).[33] These findings have been validated by the same group in a cohort of 585 men who underwent needle biopsy with PCa death as the end point. Similar HRs were found for each point increase in the CCP score and showed significance on multivariate analysis (1.76, 95% CI 1.44, 2.14, P 4.2 $\times$ 10^{-7}).[34] Accordingly, the Prolaris score appears to provide information of suitability for enrolment to active surveillance programs. CCP scores have also displayed their usefulness in adding prognostic information to patients diagnosed with localized PCa. In a study of 582 patients who underwent prostate biopsy and had CCP scoring on biopsy samples prior to progressing to prostatectomy, higher CCP scores were associated with biochemical recurrence.[35] This remained significant after adjusting for other prognostic clinical variables such as Gleason score etc. (HR per score unit 1.47, 95% CI 1.23–1.76).[35] In this study a stronger independently associated risk was seen with rising CCP score and metastatic disease on multivariate analysis (HR per score unit 4.19, 95% CI 2.08–8.45).

4.4. Decipher

Decipher (GenomeDx Biosciences) is a 22 gene genomic classifier chosen by statistical selection to predict metastasis among high risk radical prostatectomy patients. Decipher determines a patient's probability of biochemical recurrence within 3 years or clinical metastatic disease from 5 years to 10 years post radical prostatectomy. At present, the reported indications for using decipher include pT3 disease, positive margins, or a PSA rise after radical prostatectomy, with patients given a genomic classifier (GC) score ranging from 0 to 1. Clinically, Decipher has undergone multiple validation studies with a total of > 2,000 patients and an AUC of 0.79. Patients who were deemed high risk with Decipher (GC ≥ 0.4) and received adjuvant therapy had improved survival.[3,36–38] Klein et al[36] evaluated the use of the Decipher GC with the National Comprehensive Cancer Network on 57 patients from a previous Decipher validation study from Cleveland Clinic. Using multivariate analysis, they determined that Decipher predicted the risk of metastasis at 10 years post radical prostatectomy (HR per 10% increase 1.72, 95% CI 1.07–2.81, $P = 0.02$).[36]

4.5. ELAVL1

ELAVL1 (EnVision Kit; Dako, Glostrup, Denmark) is an RNA binding protein expressed in a wide variety of tissues, including the prostate, and may have a role in PCa progression. Melling et al[39] reported that ELAVL1 may be an independent prognostic biomarker in PCa and a predictor of unfavorable tumor phenotype and early PSA recurrence after definitive therapy. This group assessed 12,427 PCas specimens and determined that strong ELVAL1 staining was associated with high Gleason grade, advanced pathological tumor stage, positive nodal status, and PSA recurrence ($P < 0.0001$). ELAVL1 positivity was more frequent in cancers with TMPRSS2-ERG fusions and the presence of known genomic deletions associated with PCa (PTEN, 5q21, 6q21, and 3p13 $P < 0.0001$).

5. Future developments

Developments in the field of PCa biomarkers are ongoing with many new markers currently in preclinical phases. No doubt in the next decade, a considerable armamentarium of biomarkers will be available for use by practicing clinicians. The lack of robust, accurate, noninvasive PCa biomarkers highlights the need for future research.

Table 1
Summary of currently available biomarkers for use in prostate cancer detection or stratification.

Name	Company	Molecular marker	FDA	Accuracy
Serum-based biomarkers				
ProPSA & Prostate Health Index	Beckman Coultier Inc., Brea, CA, USA	PSA Free PSA [2]proPSA	Yes	AUC 0.703 Spec 16%, Sens 95%
4K score test	OPKO Lab, Miami, FL, USA	Human Kallikrien Assay (fPSA, PSA, tPSA, hK2)	No	AUC 0.82
Urinary biomarkers				
Prostate cancer antigen 3 (Progensa)	Hologic, Marlborough, MA, USA	PCA3	Yes	AUC 0.68–0.87
TMPRSS2-ERG	N/A	TMPRSS2-ERG fusion	No	Sens 24.3–37%, Spec 93%, PPV 94% PCA3 + PSA + TMPRSS2-ERG-AUC 0.88
ExoDx Prostate Intelliscore urine exosome assay	Exosome Diagnostics Inc, Cambridge, MA, USA	ERG, PCA3, & SPDEF	No	Sens 92%, Spec of 34%, NPV 91%, PPV 36%, AUC 0.77
SChLAP1	GenomeDx Biosciences, San Diego, CA, USA	SChLAP1 expression	No	In conjunction with Decipher, AUC 0.79
SelectMDx	MDxHealth, Irvine, CA, USA	HOXC6 & DLX1	No	Sens 91%, Spec 36%, NPV 94%, PPV 27%, AUC 0.76
Prostatic tissue-based biomarkers				
ConfirmMDx	MDxHealth, Irvine, CA, USA	DNA methylation of GSTP1, APC, & RASSF1 genes	No	NPV 96%
Oncotype DX Prostate Cancer Assay	Genomic Health Inc., Redwood City, CA, USA	12 cancer related genes, & 5 reference genes	No	Not reported
Prolaris score	Myriad Genomic Inc., Salt Lake City, UT, USA	31 cell cycle progression genes	No	Not reported
Decipher	GenomeDx Biosciences, San Diego, CA, USA	22 RNA biomarkers	No	AUC 0.79
ELAVL1	EnVision Kit; Dako, Glostrup, Denmark	Embryonic Lethal, Abnormal Vision, Drosophila-Like 1 (ELAVL1) expression	No	Not reported

AUC, area under the curve; DNA, deoxyribonucleic acid; ELAVL1, Embryonic Lethal, Abnormal Vision, Drosophila-Like 1; NPV, negative predictive value; PCA3, prostate cancer antigen 3; PPV, positive predictive value; PSA, prostate specific antigen; RNA, ribonucleic acid; SChLAP1, Second Chromosome Locus Associated with Prostate-1; Sens, sensitivity; Spec, specificity; TMPRSS2-ERG, Transmembrane serine protease protein 2-Erythroblastosis virus E26 Oncogene Homolog.

Emerging biomarkers include micro RNA based assays. Such assays have recently been shown to be elevated in prostate tissues, peripheral blood, and body fluids of PCa patients, but did not reveal the same increase in benign prostatic hypertrophy.[40,41] Their utility also lies in differentiating between patients with raised PSA from benign prostatic hypertrophy and clinically significant PCa, adding to the specificity of diagnosis. In a recent study, this helped clinicians determine a need for prostate biopsy in patients with PSA results in the “gray zone” and reduced biopsy rates by 10–50%.[42]

There currently appears to be a paucity of biomarkers assessing specific timepoints in the care of a PCa patient (See Table 1). There are minimal biomarkers currently for determining the risk of missed cancer after negative transrectal or transperineal biopsy[43] and the risk of progression following focal therapies.[44] Further, with the increasing use of active surveillance,[45] biomarkers accurately determining the risk of progression while on active surveillance are required. The increased use of multiparametric magnetic resonance imaging of the prostate in these clinical scenarios will also potentially impact on the future role of such biomarkers.

6. Conclusion

Each of the tests discussed has a potentially unique role in the screening, diagnosis, surveillance, and treatment of PCa. To date, no formal algorithm has been created to determine the role of each of these tests in PCa. There is a critical need for robust, prospective comparative data to further define the role of each biomarker in clinical practice.

Conflicts of interest

The authors declare no competing interests. The corresponding author is not a recipient of a scholarship. This paper is not based on previous communication to a society or meeting.

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics. CA Cancer J Clin 2016;66:7–30.
2. Rosser CJ, Parker A. Re: The prostate specific antigen era in the United States is over for prostate cancer: what happened in the last 20 years? J Urol 2005;174:1154–5.
3. Karnes RJ, Bergstralh EJ, Davicioni E, Ghadessi M, Buerki C, Mitra AP, et al. Validation of a genomic classifier that predicts metastasis following radical prostatectomy in an at risk patient population. J Urol 2013;190:2047–53.
4. Cooperberg MR, Broering JM, Carroll PR. Time trends and local variation in primary treatment of localized prostate cancer. J Clin Oncol 2010;28:1117–23.
5. Bolton DM, Papa N, Ta AD, Millar J, Davidson A, Pedersen J, et al. Predictors of prostate cancer specific mortality after radical prostatectomy: 10 year oncologic outcomes from the Victorian Radical Prostatectomy Registry. BJU Int 2015;116:66–72.
6. Fossati N, Buffi NM, Haese A, Stephan C, Larcher A, McNicholas T, et al. Preoperative prostate-specific antigen isoform p2PSA and its derivatives, %p2PSA and prostate health index, predict pathologic outcomes in patients undergoing radical prostatectomy for prostate cancer: results from a multicentric European prospective study. Eur Urol 2015;68:132–8.
7. Catalona WJ, Partin AW, Sanda MG, Wei JT, Klee GG, Bangma CH, 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.
8. Parekh DJ, Punnen S, Sjoberg DD, Asroff SW, Bailen JL, Cochran JS, et al. A multi-institutional prospective trial in the USA confirms that the 4Kscore accurately identifies men with high-grade prostate cancer,. Eur Urol 2015;68:464–70.
9. Konety B, Zappala SM, Parekh DJ, Osterhout D, Schock J, Chudler RM, et al. The 4Kscore(R) test reduces prostate biopsy rates in community and academic urology practices,. Rev Urol 2015;17:231–40.
10. Stattin P, Vickers AJ, Sjoberg DD, Johansson R, Granfors T, Johansson M, 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.
11. Yang Z, Yu L, Wang Z. PCA3 and TMPRSS2-ERG gene fusions as diagnostic biomarkers for prostate cancer. Chin J Cancer Res 2016;28:65–71.
12. Marks LS, Bostwick DG. PCA3 molecular urine assay for prostate cancer in men undergoing repeat biopsy. Urology 2007;69:532–5.
13. Tinzl M, Marberger M, Horvath S, Chypre C. DD3(PCA3) RNA analysis in urine – a new perspective for detecting prostate cancer. Eur Urol 2004;46:182–7.
14. Leyten GH, Hessels D, Jannink SA, Smit FP, de Jong H, Cornel EB, 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.
15. Merola R, Tomao L, Antenucci A, Sperduti I, Sentinelli S, Masi S, 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.
16. Crawford ED, Rove KO, Trabulsi EJ, Qian JQ, Drewnowska KP, Kaminetsky JC, et al. Diagnostic performance of PCA3 to detect prostate cancer in men with increased prostate specific antigen: a prospective study of 1,962 cases. J Urol 2012;188:1726–31.
17. Perner S, Mosquera JM, Demichelis F, Hofer MD, Paris PL, Simko J, et al. TMPRSS2-ERG fusion prostate cancer: an early molecular event associated with invasion. Am J Surg Pathol 2007;31:882–8.
18. Hessels D, Smit FP, Verhaegh GW, Witjes JA, Cornel EB, Schalken JA. Detection of TMPRSS2-ERG fusion transcripts and prostate cancer antigen 3 in urinary sediments may improve diagnosis of prostate cancer. Clin Cancer Res 2007;13:5103–8.
19. Sanguedolce F, Cormio A, Brunelli M, D'Amuri A, Carrieri G, Bufo P, et al. Urine TMPRSS2: ERG fusion transcript as a biomarker for prostate cancer: literature review. Clin Genitourin Cancer 2016;14:117–21.
20. Salami SS, Schmidt F, Laxman B, Regan MM, Rickman DS, Scherr D, et al. Combining urinary detection of TMPRSS2:ERG and PCA3 with serum PSA to predict diagnosis of prostate cancer. Urol Oncol 2013;31:566–71.
21. McKiernan J, Donovan MJ, O'Neill V, Bentink S, Noerholm M, Belzer S, et al. A novel urine exosome gene expression assay to predict high-grade prostate cancer at initial biopsy,. JAMA Oncol 2016:2882–9.
22. Prensner JR, Iyer MK, Sahu A, Asangani IA, Cao Q, Patel L, et al. The long noncoding RNA SChLAP1 promotes aggressive prostate cancer and antagonizes the SWI/SNF complex. Nat Genet 2013;45:1392–8.
23. Prensner JR, Zhao S, Erho N, Schipper M, Iyer MK, Dhanasekaran SM, et al. RNA biomarkers associated with metastatic progression in prostate cancer: a multi-institutional high-throughput analysis of SChLAP1. Lancet Oncol 2014;15:1469–80.
24. Leyten GH, Hessels D, Smit FP, Jannink SA, de Jong H, Melchers WJ, et al. Identification of a candidate gene panel for the early diagnosis of prostate cancer. Clin Cancer Res 2015;21:3061–70.
25. Van Neste L, Hendriks RJ, Dijkstra S, Trooskens G, Cornel EB, Jannink SA, et al. Detection of high-grade prostate cancer using a urinary molecular biomarker-based risk score. Eur Urol 2016. Available from: http://dx.doi.org/10.1016/j.eururo.2016.04.012.
26. Wojno KJ, Costa FJ, Cornell RJ, Small JD, Pasin E, Van Criekinge W, et al. Reduced rate of repeated prostate biopsies observed in ConfirmMDx clinical utility field study. Am Health Drug Benefits 2014;7:129–34.
27. Partin AW, Van Neste L, Klein EA, Marks LS, Gee JR, Troyer DA, et al. Clinical validation of an epigenetic assay to predict negative histopathological results in repeat prostate biopsies. J Urol 2014;192:1081–7.
28. Van Neste L, Partin AW, Stewart GD, Epstein JI, Harrison DJ, Van Criekinge W. Risk score predicts high-grade prostate cancer in DNA-methylation positive, histopathologically negative biopsies. Prostate 2016. Available from: http://dx.doi.org/10.1002/pros.23191.
29. Knezevic D, Goddard AD, Natraj N, Cherbavaz DB, Clark-Langone KM, Snable J, et al. Analytical validation of the oncotype DX prostate cancer assay – a clinical RT-PCR assay optimized for prostate needle biopsies. BMC Genomics 2013;14:690.
30. Leapman MS, Nguyen HG, Cooperberg MR. Clinical utility of biomarkers in localized prostate cancer. Curr Oncol Rep 2016;18:30.
31. Klein EA, Cooperberg MR, Magi-Galluzzi C, Simko JP, Falzarano SM, Maddala T, et al. A 17-gene assay to predict prostate cancer aggressiveness in the context of Gleason grade heterogeneity, tumor multifocality, and biopsy undersampling,. Eur Urol 2014;66:550–60.
32. Szulkin R, Whitington T, Eklund M, Aly M, Eeles RA, Easton D, et al. Prediction of individual genetic risk to prostate cancer using a polygenic score. Prostate 2015;75:1467–74.
33. Cuzick J, Berney DM, Fisher G, Mesher D, Moller H, Reid JE, et al. Prognostic value of a cell cycle progression signature for prostate cancer death in a conservatively managed needle biopsy cohort. Br J Cancer 2012;106:1095–9.
34. Cuzick J, Stone S, Fisher G, Yang ZH, North BV, Berney DM, et al. Validation of an RNA cell cycle progression score for predicting death from prostate cancer in a conservatively managed needle biopsy cohort. Br J Cancer 2015;113:382–9.
35. Bishoff JT, Freedland SJ, Gerber L, Tennstedt P, Reid J, Welbourn W, et al. Prognostic utility of the cell cycle progression score generated from biopsy in men treated with prostatectomy. J Urol 2014;192:409–14.
36. Klein EA, Haddad Z, Yousefi K, Lam LLC, Wang Q, Choeurng V, et al. Decipher genomic classifier measured on prostate biopsy predicts metastasis risk. Urology 2016;90:148–52.
37. Zhao SG, Evans JR, Kothari V, Sun G, Larm A, Mondine V, et al. The landscape of prostognostic outlier genes in high-risk prostate cancer,. Clin Cancer Res 2016;22:1777–86.
38. Ross AE, D'Amico AV, Freedland SJ. Which, when and why? Rational use of tissue-based molecular testing in localized prostate cancer. Prostate Cancer Prostatic Dis 2016;19:1–6.
39. Melling N, Taskin B, Hube-Magg C, Kluth M, Minner S, Koop C, et al. Cytoplasmic accumulation of ELAVL1 is an independent predictor of biochemical recurrence associated with genomic instability in prostate cancer. Prostate 2016;76:259–72.
40. Al-Kafaji G, Al-Naieb ZT, Bakhiet M. Increased oncogenic microRNA-18a expression in the peripheral blood of patients with prostate cancer: a potential novel non-invasive biomarker,. Oncol Lett 2016;11:1201–6.
41. Roberts MJ, Richards RS, Chow CWK, Gardiner RA. Prostate-based biofluids for the detection of prostate cancer: a comparative study of the diagnostic performance of cell-sourced RNA biomarkers. Prostate Int 2016;4:97–102.
42. Salido-Guadarrama AI, Morales-Montor JG, Rangel-Escareno C, Langley E, Peralta-Zaragoza O, Cruz Colin JL, et al. Urinary microRNA-based signature improves accuracy of detection of clinically relevant prostate cancer within the prostate-specific antigen grey zone. Mol Med Rep 2016;13:4549–60.
43. Perera M, Manning T, Finelli A, Lawrentschuk N. Management of men with previous negative prostate biopsy. Curr Opin Urol 2016;26:481–7.
44. Perera M, Krishnanathan N, Linder U, Lawrentschuk N. An update on focal therapy for prostate cancer. Nature Rev Urol 2016 [in press].
45. Weerakoon M, Papa N, Lawrentschuk N, Evans S, Millar J, Frydenberg M, et al. The current use of active surveillance in an Australian cohort of men: a pattern of care analysis from the Victorian Prostate Cancer Registry. BJU Int 2015;115:50–6.