Chapter

The Clinical Usefulness of Prostate Cancer Biomarkers: Current and Future Directions

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Abstract

Worldwide, prostate cancer (PCa) is the leading cause of morbidity and cancer-related mortality in men. The pathogenesis of PCa is complex and involves abnormal genetic changes, abrogation of cell growth with heterogeneous progression and predictive subgroups. In the last two decades there have been the exploration and development of molecular and genetic biomarkers for PCa due to limitations of traditional serum biomarkers such as prostate specific antigen (PSA) in screening and diagnosis. These biomarkers could possibly differentiate between PCa and benign prostatic hyperplasia (BPH) patients, and healthy controls as well as assist with prognosis, risk stratification and clinical decision-making. Such molecular biomarkers include serum (PHI and 4K score), urine (PCA3 and SelectMDx), and tumor tissue (Oncoytype DX, Decipher and Prolarix). microRNAs (miRNAs) deregulation where there is increased or decreased expression levels, constitute prospective non-invasive molecular biomarkers for the diagnosis and prognosis of PCa. There are also other emerging molecular biomarkers such as exosomal miRNAs and proteins that are in various stages of development and clinical research. This review is intended to provide a wide-ranging appraisal of the literature on current and emerging PCa biomarkers with robust evidence to afford their application in clinical research and by extension routine clinical practice.

Keywords: prostate, cancer, biomarkers, diagnosis, prognosis, molecular, emerging, clinical

1. Introduction

Prostate cancer (PCa) is a complex condition characterized by varying clinical behaviors ranging from indolence to metastatic disease states. Globally, PCa was

the second most prevalent cancer and the fifth major cause of cancer-related deaths among men in 2020. Strikingly, about 1.4 million new cancer cases and 375,000 deaths were attributable to PCa in 2020 [1]. Approximately one in nine men will be diagnosed with PCa in their lifetime [2]. Increased widespread screening using prostate specific antigen (PSA) mirrors the epidemic rise of PCa with geographic variability. However, since the advent of PSA screening, mortality rates have significantly declined [3]. The incidence of PCa in countries with low Human Development Index (HDI) was about three times lower than those with high HDI, 11.3 vs. 37.5 per 100,000 persons respectively [1]. The highest incidences were reported in the Caribbean, Northern and Western Europe, Australia/New Zealand, Southern Africa and North American regions [1]. The Caribbean and Sub-Saharan Africa accounted for the highest mortality rates [1]. There is mounting evidence that PCa disproportionately affects men of African ancestry. In the United States, African American men are 58% more likely to be affected by PCa with a 144% higher risk of PCa-specific mortality than their Caucasian counterparts [4]. The established risk factors of the disease include: increasing age, race and family history of PCa [5]. PSA is currently the most widely used screening tool for PCa indication, but a number of studies have highlighted its failure to discriminate between indolence and more aggressive forms of the disease. The low positive predictive value (PPV) of PSA has led to over-diagnosis of low-grade cancer and complications from unnecessary biopsies [6] as no cancer is detected on approximately 50% of biopsies [7].

Total prostate specific antigen (tPSA) is not very sensitive in detecting early PCa, and being cancer-specific as there are elevated levels in prostatitis, urogenital infections, BPH and transurethral manipulations. False positive results leads to increased rate of over-diagnosis, further expensive diagnostic evaluations and invasive procedures, and possibly over-treatment [8]. There are also false negatives particularly in the 'gray zone' (with tPSA values 4–10 ng/mL) resulting in undiagnosed PCa [9]. Furthermore, there is the absence of a linear correlation between serum tPSA and metastatic PCa as well as staging [10].

In order to increase the diagnostic utility of PSA for PCa, new biomarker such as Prostate Health Index (PHI), four K (4K) score and prostate cancer antigen 3 (PCA3) have become available. These tests decrease the number of needless prostate biopsies, provide valuable information on tumor aggressiveness and aid in the selection of PCa patients for radical therapy or active surveillance [11]. Genomic techniques have permitted the accessibility of novel genetic biomarkers such as transmembrane serine protease 2 (TMPRSS2:ERG fusion gene), Oncotype DX, Decipher and ProMark which stratify the risk of aggressive PCa and aid decision-making by providing information on diagnosis, prognosis and treatment [12].

This article seeks to review current advances in the development and availability of PCa biomarkers and their precise indications for diagnostic, prognostic, predictive and use in monitoring therapeutic response. Current and emerging biomarkers are appraised including their possible integration into medical practice and enhancing the clinical management of PCa.

2. Method of article selection

2.1 Study eligibility criteria

A literature search and review of recent publications in PubMed, Google Scholar, Embase and Cochrane library relating to the clinical utility of PCa biomarkers were

conducted. The search examined all relevant published studies up to January 20, 2022. The studies included in this review were published in peer-reviewed journals, written in English, and reported medical as well as scientific findings. Also, pertinent data that were extracted from published studies include: authors, year of publication and study design and population. Information from the articles concerning molecular biomarkers of PCa used for screening, diagnosis, prognosis, risk stratification and therapeutic tools were reviewed and documented.

3. Results

3.1 Blood-based prostate cancer biomarkers

3.1.1 Prostate specific antigen

Currently, the diagnosis of PCa is based on the results of digital rectal examination (DRE), trans-rectal ultrasound guided biopsy and PSA assay [13]. The aim of utilizing the least invasive methods has led to increased use of PSA as a biomarker. However, the use of PSA is limited by its low specificity, which in turn results in over-diagnosis of PCa followed by unnecessary biopsies and associated potential complications [14]. In the detection of PCa, serum total PSA (tPSA) > 4.0 ng/mL has a 94% sensitivity but only a 20% specificity, which makes the test unsuitable for screening [15]. There are also several limitations to PSA testing in the detection of high-grade PCa, with data from the Prostate Cancer Prevention Trial (PCPT) indicating that in order to achieve a sensitivity of 83.4%, a tPSA threshold of 1.1 ng/mL is required. However, the corresponding specificity was only 39.9% [16]. Since tPSA may be elevated in conditions other than PCa, only about 25% of men with elevated tPSA will be diagnosed, while there is still a 10% chance of patients with tPSA <1.0 ng/mL developing the disease [17]. In light of these limitations, several variations to the PSA assay have been implemented to increase specificity of the test. These includes: free PSA (fPSA), PSA velocity, free-to-total PSA (fPSA/tPSA) ratio and PSA density (PSAD). In addition to these PSA variation tests, prostate specific antibodies may also be useful in selecting men for biopsies especially since localized PCa may not present with symptoms [18].

PSA is typically found in the bound or free form. Bound PSA refers to PSA found complexed to protease inhibitors like alpha-1-antichymotrypsin, while fPSA which is found unbound is mostly inactive and associated with BPH as opposed to PCa [19]. A fraction of fPSA can however be active as such tPSA, refers to any of the active or inactive forms found in serum [20]. In light of these variations in PSA, specific assays targeting precise fractions have been developed. Bound or complexed PSA is found to be elevated in malignancies while the calculation of fPSA/tPSA ratio gives values that are normally lower in men with PCa [21, 22]. The fPSA/tPSA ratio is indicative of %fPSA and has been shown to increase the accuracy of PSA use in detection of PCa [23] (**Table 1**).

3.1.1.1 PSA use in detection of prostate cancer

Initial studies indicated that PCa was more likely with <25% fPSA at a sensitivity of 95%. However, subsequent similar studies have yielded unreliable results owing to the instability of fPSA making it a somewhat an unreliable marker on its own [54, 55]. This could be as a result of storage conditions as subsequent studies indicated that the stability of tPSA and fPSA levels in serum did not depreciate

Biomarker tests	Molecular markers	Specimen	Outcome	References
Prostate specific antigen (PSA)	tPSA, fPSA, %fPSA	Serum/ plasma	Screening, diagnosis	[13, 20, 24, 25]
Prostate Cancer Antigen-3 gene (PCA3)	Ratio PCA3 mRNA/ PSA mRNA × 1000	Urine	Diagnosis, prognosis	[13, 26–28]
Select MDx	HOXC6, DLX1, tPSA, clinical parameters	Urine	Diagnosis, prognosis, risk stratification	[29, 30]
K score	tPSA, fPSA, intact PSA, human kallikrein-related peptidase 2	Serum/ plasma	Diagnosis, prediction	[31, 32]
The Decipher	22 RNA genes	Biopsy tissue	Risk stratification, therapy decision making	[33–44]
Oncotype DX Genomic Prostate Score (GPS)	12 cancer related genes, & 5 reference genes	Biopsy tissue	Prediction, risk stratification, therapy decision making	[41, 43–48]
ConfirmMDX	DNA methylation of GSTP1, APC, & RASSF1 gene	Biopsy prostate cores	Prediction (repeat biopsies)	[49–51]
The ProMark	8 proteomic biomarkers	Biopsy tissue	Prediction, risk assessment	[52, 53]

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

significantly after 10 years storage at −80°C [29]. Other studies on tPSA and %fPSA have shown conflicting data as seen in the case of a 2009 study by Omar et al. [24]. Here, %fPSA values were high in patients diagnosed with PCa compared with BPH, while tPSA was found to be a better serum marker for diagnosing PCa in that cohort. However, a Chinese study highlighted that for men in the PSA gray zone, the inclusion of %fPSA will improve the diagnostic accuracy for PCa, and high grade PCa compared with only tPSA [25].

Clinical usefulness of PSA variations appears to be age-specific as a study comprising patients over 60 years old showed that tPSA outperformed %fPSA and therefore had the higher predictive value for detecting high grade carcinoma [20]. Moreover, an Asian study suggested that age-specific ranges for tPSA, fPSA and %fPSA could be considered in the diagnostic workup of PCa. Results from that study highlighted that there was a gradual increase in reference interval for tPSA and fPSA peaking at 7.73 and 2.41 ng/mL respectively for those over 80 years old. The researchers also reported that reference intervals for %fPSA were ≥16.0 for 21–50 years and ≥13.0 for males over 50 years old [56]. In a South American study of over 17,000 men, %fPSA value of <15 as an indication for biopsy was shown to increase the rate of PCa detection in patients with normal DRE results and serum tPSA of 2.5–4.0 ng/mL [17].

Although the results vary with different studies, the PSA parameters mentioned seem to have significant predictive values in PCa screening and should be utilized based on the cohort along with other clinical factors in assessing PCa. This is in line with the conclusion by some researchers that especially within the gray zone, a

multivariate model is a useful tool in diagnosing PCa, and clinically significant PCa while reducing unnecessary biopsies in the process [18].

3.1.2 Prostate cancer antigen-3 gene

The thrust to discovering other biomarkers of PCa is based on the limitations that exist with regard to utilization of PSA or other invasive investigatory methods. Given the complications associated with PCa detection and treatment, the ultimate goal from a clinical perspective appears to be discovery of biomarkers that more accurately predict PCa and clinically significant disease prior to biopsy in an attempt to reduce the number of unnecessary biopsy requests [57].

Over time, some molecular biomarkers have been developed with emphasis placed on those that are capable of predicting disease aggressiveness that will lead to improved guidance for treatment modalities. Some of these markers are serum while others are urine-based.

To date, the most commonly used urinary biomarker of PCa is PCA3 otherwise referred to as Differential Display clone 3 (DD3). It is conventionally assessed in urine post prostate massage to derive the maximum number of prostatic cells, and was the first urinary RNA biomarker approved by the Food and Drug Administration (FDA) in 2012 [58]. Discovered around 1995 by researchers in the USA and The Netherlands, PCA3 is non-coding messenger RNA from chromosome 9q21-22, consisting of four exons and three introns and is over-expressed in most tested PCa tissues [59–61]. Additionally, PCA3 is over-expressed in prostate tumor tissue compared with other benign prostate disorders [13, 26]. It therefore aids in improving the accuracy of PSA with regards to management of early PCa and as such, approval was granted by some developed countries for its use as a molecular marker in the diagnostic workup of PCa owing to improved specificity for PCa over PSA [62, 63]. In fact, the use of PCA3 in tandem with other molecular markers including transmembrane serine protease 2 (TMPRSS2)-ERG, human kallikrein 2, and miRNA-141 was found to have significant clinical utility by way of increasing specificity, and in predicting PCa especially in the PSA gray area of 4–10 ng/mL [13].

Assessment of urinary PCA3 can be done by way of the quantitative real-time PCR (qRT-PCR) reaction followed by generation of a PCA3 score utilizing the ratio of PCA3 to tPSA [64, 65]. In utilizing this method, a negative biopsy result is 4.5 times more likely in men with a score of <25 compared with those with a score >25 [66]. Importantly, low-volume and low-grade disease are seen in men with low PCA3 scores [67]. A study on 407 high risk PCa patients indicated that PCA3 has clinical usefulness as a formidable prognostic indicator of tumor aggressiveness and was associated with higher PCA3 scores [27]. A high PCA3 score was also shown to be a good predictor of a positive PCa diagnosis and displayed greater accuracy than fPSA and tPSA in this regard (**Table 1**) [27, 28]. These results are in contrast to other studies indicating that the PPV of tPSA for diagnosing PCa was only 25% which directly increases the chances of obtaining false positive results in a quarter of cases and consequently a high chance of unnecessary biopsy in 75% of cases [57, 68, 69].

3.1.3 Prostate health index

The prostate health index (PHI) is a modern test that utilized PSA in accelerating the diagnosis of PCa. This particular biomarker has been sanctioned and approved in the United States, Europe and Australia. Studies conducted globally have depicted the reliably of PHI as a biomarker that outclasses its individual components for the

prognostication of overall and high-grade PCa on biopsy [70]. This represent PCa with Gleason score (GS) that is ≥7 [71].

In utilizing PHI as a biomarker, it allows for the combination of the tPSA, fPSA and [−2]proPSA (p2PSA) into a formula to produce a single result that can be used to assist in clinical decision-making of PCa. Such PHI formula computation is ([−2] proPSA/fPSA) × $\sqrt{}$PSA and demonstrates the possibility of clinically significant PCa in men with elevated tPSA and p2PSA while the fPSA is lower [70].

In men that possesses a tPSA between 4 and 10 ng/mL, PHI may be useful in establishing PCa in conjunction with a prostate biopsy. Interestingly, a low possibility of PCa outcome on biopsy is supplementary to small PHI results, while an elevated possibility of PCa outcome on biopsy is directly related to elevated PHI. Different medical considerations or family history of PCa are dominant factors in the management process as it relates to the appropriate PHI value [70, 72, 73].

A huge study was conducted in the USA in 2011 by Catalona et al. The study aimed to demonstrate the diagnostic capability of PHI for PCa recognition in a populace of 892 men with normal DRE, tPSA levels between 2 and 10 ng/mL and a prostate biopsy [73]. Interestingly, based on the PHI reference intervals, a value of 49 was obtained for prostate biopsies that were positive, while 34 was obtained for biopsies that were considered negative. This demonstrates a superior sensitivity and specificity in the diagnosis of PCa, and also differentiating PCa on biopsy compared with tPSA. Although the PHI test has been approved by the FDA only in the tPSA range of 4–10 ng/mL, PHI performed well in the 2–10 ng/mL range [74].

A large multicenter research involving approximately 5543 participants using the bivariate mixed-effect model was conducted by Zhang et al. from 2011 to 2019 to assess the medical significance of PHI in detecting PCa. The results obtained showed the likely sensitivity of PHI for diagnosing PCa of 0.75 and a specificity of 0.69. A value of 0.78 was obtained for the pooled area under the curve (AUC) and the diagnostic odds ratio (OR) was 6.73. The researchers also found that the diagnostic accurateness of PHI for PCa was greater in Asian compared with Caucasian populations (0.83 vs. 0.76). Based on the overall results the authors suggested that PHI has a modest diagnostic accurateness for detecting PCa [75].

Moreover, in a small study of 58 Asian patients with tPSA of 4–10 ng/mL who undertook transrectal ultrasound-guided prostatic biopsy, 18 cases had PCa and the AUC for this biomarker was 0.774. The sensitivity of PHI was 90% with a specificity of 27.5% which was the highest among the group of biomarkers including tPSA, PSAD and %fPSA. The authors suggested that PHI improves the accuracy of predicting PCa and decrease avoidable prostate biopsy [76]. These results are in consonance with another recent study involving 140 Korean patients that underwent prostate biopsy of which there were 63 cases of PCa. The AUC for PHI in the overall group was 0.76 (which was higher than tPSA, fPSA, %fPSA) and in the sub-group with GS ≥ 7, a value of 0.87 was obtained. PHI was a strong independent prognosticator of PCa particularly for the presence and aggressiveness (GS ≥ 7) of the disease, and its application could prevent a substantial amount of unnecessary prostate biopsies [77]. Furthermore, there are other recent studies that have demonstrated that PHI is more specific than tPSA in PCa detection [78] and a better predictor than %fPSA in detecting PCa at prostate biopsy [79].

3.1.4 Four K score

4K score test incorporates the measurement of four biomarkers: tPSA, fPSA, intact PSA, and human kallikrein-related peptidase 2 combined in an algorithm with the

patient's clinical information such as age, previous biopsy and DRE to generate the percentage risk (<1% to >95%) for aggressive metastatic PCa with GS ≥ 7 on biopsy [31]. The 4K score test is usually conducted following a previous abnormal DRE or tPSA [31] and supports clinical decision making to determine whether a biopsy should be done. The 4K score has been found to predict high-grade metastatic PCa and estimates an individual's risk of having the disease that spreads to distant organs within 10 years [32]. This was supported by a multi-institutional prospective trial conducted in the United States among 144 men which sought to determine the association between previous 4K score, staging and grading of PCa at radical prostatectomy. It found that higher 4K scores were significantly associated with worse grades and aggressive histology. There was a higher median 4K score among PCa patients with cancer not confined to the prostate when compared with organ-confined cancer [36% (IQR 19, 58)] vs. [19% (IQR 9, 35)], (p = 0.002) [80].

In a clinical study involving 611 patients the 4K score test led to a 65% reduction in unnecessary biopsies [31]. There was also a strong association between high risk 4K score and a greater possibility of having a prostate biopsy. Similarly, another study with a population of 1012 men reported that the 4K score was useful in identifying candidates for biopsies with GS ≥ 7 and could narrow the gap of unnecessary biopsies by 30–58% [7]. However, the researchers highlighted that about 1.3–4.7% of men with aggressive disease may experience a delay in diagnosis using the 4K score [7]. Moreover, a retrospective study performed on 946 men of different racial ethnicities with elevated tPSA levels and a previous biopsy demonstrated that the 4K score had a higher discriminatory index for high-grade PCa compared with the conventional tPSA, DRE, age and PCPT calculator [31]. The researchers showed that among African American men, the detection of metastatic PCa using the 4K score test was significantly enhanced over the use of tPSA with an AUC of 0.80 versus 0.67. Additionally, it was found that the 4K score test would be able to identify 88% of aggressive cancers while reducing 42% of unnecessary biopsies [81].

3.2 Urine-based prostate cancer biomarkers

3.2.1 TMPRSS2-ERG fusion and PCA3

In a similar way to PCA3, a transmembrane serine protease 2 (TMPRSS2)-ERG fusion gene can be detected in urine post-DRE [82]. Various biomarkers are released in urine which may become enriched with prostate material upon manipulation of the prostate gland during a DRE [83]. A TMPRSS2-ERG gene fusion score is comparable to PSA mRNA quantity, in which the latter is currently being used as the gold standard test for PCa. TMPRSS2-ERG fusion gene is a genetic rearrangement of the androgen-regulated trans-membrane protease, serine 2 (TMPRSS2) gene and the ERG (erythroblast transformation-specific; ETS)-related gene. ERG is an oncogene and is a part of the family of transcription factors. TMPRSS2-ERG gene fusion is expressed specifically in PCa and is the most prevalent known type of PCa-specific gene alterations [84, 85]. There are two mechanisms by which TMPRSS2-ERG gene fusion may occur, either by chromosomal translocation or interstitial deletion; the latter being the more predominant mechanism in which approximately 2.8 Mb genetic material may be lost due to occurrence of this event [86–89]. A study reported that the deletion type fusion was found to be highest among African American patients, followed by Caucasians and no significant differences have been seen in Asian populations regarding either type [89]. Targeted inhibition of the TMPRSS2-ERG fusion

gene or its gene fusion transcripts could possibly serve as a treatment strategy in the future, thereby resulting in favorable outcomes for PCa patients.

Gene fusion of TMPRSS2 and ERG has been shown to result in an overexpression of ERG. ERG has been shown to play a vital role in cell growth, differentiation, and apoptosis [90]. It is possible that overexpression of ERG triggers a downstream cascade of events that lead to the onset and progression of PCa. Hence, TMPRSS2-ERG gene fusion maybe seen as an early phenomenon that takes place in the development of PCa. TMPRSS2-ERG gene fusion has been detected in 40–70% of PCa patients [88]. The frequency of TMPRSS2-ERG gene fusion has been reported to be greatest among Caucasian Americans (50%), followed by African Americans (31%) and Asians (18.5%) respectively [89].

TMPRSS2-ERG and PCA3 are two of the most studied urine biomarkers with PCA3 having received FDA approval. TMPRSS2-ERG and PCA3 in post-DRE urine for the detection of PCa at biopsy showed significant improvement over PSA [85, 91, 92]. When a Mi-Prostate Score for post-DRE urine, was utilized it was reported that TMPRSS2-ERG had a low sensitivity of 24.3–37.0%. However, the fusion gene had a specificity of 93% and a PPV of 94%. Combination of TMPRSS2-ERG with serum tPSA (a cut-off value of 10 ng/mL) and urinary PCA3, greatly improves the accuracy of diagnosing PCa, from a study that reported a sensitivity of 80% and a specificity of 90% [93]. TMPRSS2-ERG gene fusion test also gives information about a risk assessment for aggressive PCa [94].

It was reported in a study that TMPRSS2-ERG gene fusions showed a significant association with a GS ≥7 and PCa-related deaths. When TMPRSS2-ERG and PCA3 were combined with the PCPT risk calculator, the information provided may aid physicians in deciding whether a patient with high serum tPSA will need urgent biopsy [91, 94]. Analyzing ERG mRNA in post-DRE urine in a study cohort of 237 men, a predictive accuracy for AUC of 0.80 was reported for PCa diagnosis in Caucasian men having tPSA levels ≤4.0 ng/mL [92]. Studies have supported the combined use of TMPRSS2-ERG and PCA3 in clinical practice to help in reducing the number of prostate biopsies [95]. With this in mind, Kohaar et al. concluded that the data from cumulated studies suggest that when TMPRSS2-ERG and PCA3 are combined along with serum tPSA there were improvements in the detection of aggressive PCa (GS ≥7) on initial biopsy with a 42% reduction in unwarranted biopsies [85, 91].

3.2.2 SelectMDx (DLX1, HOXC6)

The SelectMDx test is a urine-based gene expression assay. This assay measures the mRNA levels of two biomarkers, distal-less homeobox 1 (DLX1) and homeobox C6 (HOXC6) using qRT-PCR in post DRE urine [96]. Kallikrein serine protease (KLK3) gene which codes for PSA is used as an internal control for this assay. The test was performed on patients who have risk factors for PCa and were being considered for prostate biopsy. DLX1 and HOXC6 are believed to be involved in the onset of PCa and are both associated with high grade disease [95]. In a study using PCa cell lines, it was found that HOXC6, a transcriptional factor when suppressed, caused a reduction in cell viability and induced apoptosis [97, 98]. In another study investigating the role of DLX1, a protein coding gene, it was reported that DLX1 promoted growth, migration and colony formation of cancer cells [97, 99].

The SelectMDx assay is not a "standalone" test and so incorporates clinical factors such as age, tPSA, prostate volume and DRE findings to estimate the percent likelihood of detecting PCa and high-grade (GS ≥ 7) disease upon prostate biopsy. Leyten et al. [30] first identified that a three gene panel of HOXC6, DLX1 and

Tudor domain-containing protein 1 (TDRD1) was able to show higher accuracy (AUC = 0.77) for the detection of clinically significant PCa (csPCa) compared with tPSA (AUC = 0.72) and PCA3 (AUC = 0.68) tests respectively. In follow-up prospective studies, focus was placed on the urinary mRNA levels of HOXC6 and DLX1 genes. Using a large cohort (n = 905), the expression of DLX1 and HOXC6 gave an AUC of 0.76, a sensitivity of 91%, a specificity 36%, a NPV of 94% and PPV of 27% for the prediction of high-grade PCa (**Table 1**) [100]. It was seen that when SelectMDX was combined with clinical factors such as tPSA, PSAD, family history and history of prostate biopsy, the risk stratification of high-grade PCa and biopsy decisions maybe improved as the AUC was 0.90 in identifying high-grade PCa (GS $\geq$ 7) [100].

In a more recent validation study by Haese et al. [101], the data showed high sensitivity and NPV in detecting csPCa when investigating urinary HOXC6 and DLX1 mRNA levels. These results were combined with patient age, DRE and tPSA levels less than 10 ng/mL which gave an AUC of 0.82, sensitivity 89%, specificity 53% and NPV 95% [102]. Haese et al. [102] concluded that the data supported using the SelectMDx test to aid in decision-making around prostate biopsies. Furthermore, Govers et al. [101] conducted a study on the healthcare cost using SelectMDx for PCa in four European countries. From the results of the study, it was reported that quality-adjusted life years (QALYS) could be gained and that the use of SelectMDx may have favorable economic outcomes for patients at initial PCa diagnosis. Currently, SelectMDx is only available through companies that received CLIA (Clinical Laboratory Improvement Amendments)-approval [102].

3.3 Tissue-based prostate cancer biomarkers

3.3.1 Oncotype DX Genomic Prostate Score

Oncotype DX Genomic Prostate Score (GPS) is considered a molecular biomarker that was established to aid in the prognostication of PCa in men with intermediary possibility of the disease. This particular biomarker is based on 17 genes GPS. This can be further explained where 12 of the genes referred to as qRT-PCR genes are responsible in identifying growth linked to PCa, while the remaining five genes are responsible for demonstrating stromal response, androgen signaling, cellular organization, and proliferation, thereby achieving a computational formula system that resulted in the GPS [103]. This particular Oncotype DX GPS assay measures mRNA expression of the 17 genes accountable for neoplasm progression and was established and reviewed in approximately 4500 patients [104].

A study was conducted to identify and authenticate a biopsy-based 17-gene GPS signature by investigating 732 candidate genes in their clinical utility to predict PCa mortality, adverse pathology and clinical recurrence. The GPS predicted high-grade PCa and clinical recurrence notwithstanding multi-focality and heterogeneity. The authors suggested that the GPS test assist patients in making knowledgeable decisions concerning immediate therapy or active surveillance [103]. In another study, Cullen et al. assessed the association of the 17-gene GPS with clinical recurrence in 431 men with clinically low to intermediate risk PCa. GPS results (scale 0–100) were obtained for 402 PCa patients and it predicted time to metastases and biochemical recurrence. GPS was significantly associated with adverse pathology (OR = 3.3 per 20 GPS) and the predictive outcomes were similar for Caucasian and African American men [43]. Recently, the same authors performed a multicenter comparison of 17-gene Oncotype

DX® GPS in Caucasian (n = 1144) and African American (n = 201) men diagnosed with clinically localized PCa. The GPS scores were the same between the two racial groups showing corresponding predictive outcomes, and using a multivariate model, biochemical recurrence and adverse pathology was significantly associated with the GPS assay (**Table 1**) [44]. Supporting evidence of Oncotype DX GPS as an independent predictor of adverse pathology in the two racial groups was provided by Murphy et al. [45] using PCa patients (96 African American and 76 European American men) from two multi-institutional observational studies.

There are other studies such as that performed by Kornberg et al. [46] which found that higher GPS in PCa patients who undertook radical prostatectomy following active surveillance is associated with greater risk of adverse pathology and biochemical recurrence. Lynch et al. [47] reported that GPS testing was a valuable tool in risk stratification among PCa patients and those who are low risk are more likely to make the decision to adopt active surveillance. Notably, Chang et al. posited that the deployment of GPS was worthwhile in guiding decisions regarding therapy in patients with early stage PCa compared with active surveillance [48]. The finding that the GPS test is associated with long-term outcomes such as PCa-specific mortality and distant metastases is also worth mentioning [105]. However, in a recent study there was no significant association of the GPS test with adverse pathology after initial period of neither active surveillance nor improvement in risk stratification for adverse pathology versus the use of only clinical variables [106].

3.3.2 The Decipher

The Decipher, a molecular biomarker is categorized as a genomic assessment, established by GenomeDx Biosciences in Vancouver, Canada. This particular test evaluates the expression signature of approximately 22 RNA genes that demonstrates the prognostication and progression of PCa. Among the various genes group, the Decipher in recent time is considered the most powerful method in that it comprises of a comprehensive transcriptome investigation of a prostatectomy, biopsy, or transurethral resection specimens. Interestingly, the Decipher method for evaluation of PCa prognostication was initially authenticated in radical prostatectomy patients with uncomplimentary specimen characteristics inclusive of positive cancer margins and is however independent of clinical data [33–35, 107]. A study by Den et al. found that the Decipher method demonstrated both biochemical recurrence and cancer spreading in approximate 139 participants after removal of the prostate in addition to radiotherapy [36].

Several authors have done in-depth assessment and evaluation of the clinical utility of the Decipher method in PCa progression. Spratt et al. performed a meta-analysis of 5 studies comprising 855 patients in assessing the performance of the Decipher test in PCa patients who underwent radical post-prostatectomy. The Decipher test classified patients as low, intermediate and high risk for developing metastases and was a significant predictor of metastasis (HR = 1.30, $p < 0.001$). The authors posited that the Decipher test can increase the prognosis of PCa patients after radical prostatectomy including those in the different clinicopathologic and therapy subgroups (**Table 1**) [33]. Similarly, the prognostic potential of the Decipher test was assessed in two high-risk USA and European case-control studies. The median Decipher scores were higher in PCa patients who developed metastases, and multivariate analysis showed that there was a greater risk of distant metastases for each 10% increase in Decipher score within a 10-year follow-up period. Therefore, the Decipher test predicted metastatic recurrence in PCa patients within a follow-up period of 10 years [37]. In a retrospective

multicenter cohort study comprising of 266 PCa patients, the Decipher from prostatectomies from PCa patients with low to intermediate risk predicted the absence of adverse pathologic features thus making these individuals suitable for active surveillance [38]. In a later study involving prostatectomies from 2342 PCa patients, the Decipher score was positively correlated with baseline tumor characteristics such as age, pathologic T-stage and GS [39]. The Decipher score was able to reclassify patients according to tumor aggressiveness and may be valuable in assessing postoperative risk and decision making [39].

There are other studies that have assessed the clinical usefulness of the Decipher test in risk stratification of newly diagnosed PCa patients. In a multicenter prospective study involving 855 persons who underwent Decipher Biopsy testing, a high-risk Decipher score for PCa patients in the active surveillance group was independently associated with time to treatment while the same was related to time to failure in the radical therapy group [40]. Likewise, in a study of 203 PCa cases, the Decipher score enables risk stratification and was significantly associated with time to biochemical recurrence. The Decipher score could assist PCa patients with treatment decision as high-risk values were significantly associated with salvage treatment [40]. Interestingly, a systematic review conducted recently of 42 studies and 3407 patients [localized, post-prostatectomy, metastatic castration resistant PCa (mCRPCa) and metastatic hormone sensitive PCa (mHSPCa)], and metastatic hormone sensitive PCa (mHSPCa), the Decipher test was robust for intermediate-risk PCa and decision-making after radical prostatectomy [41].

The Decipher test has been used in risk stratification of early diagnosed PCa patients and for treatment making decisions [108]. Dalela et al. investigated the use of the Decipher test in a cohort of 512 PCa patients as a valuable risk-stratification tool for identifying those persons who would be received maximum benefit from adjuvant radiotherapy (ART). The Decipher test was one of the parameters used to develop a Multivariable Prediction Model that predicted reduced risk for clinical recurrence. Using the Decipher test, the authors suggested that ART might decrease overtreatment and needless adverse effects [34]. Also, supporting evidence was observed in the Multicenter Prospective PRO-IMPACT study comprising of 150 PCa patients where the Decipher score acts as a guide for making treatment choice, and enhance the effectiveness of the decision-making process for PCa patients considering salvage radiotherapy (SRT) or ART post-radical prostatectomy [35]. The use of the Decipher test has been found to significantly improve therapy decision-making in a study published of two prospective registries of PCa patients [42]. Of particular interest is a study by Lobo et al. which based on the findings suggests that the Decipher test was a cost-effective approach to PCa treatment decisions after radical prostatectomy. This should result in improved clinical outcomes and the potential for the application of the Decipher test for personalized cancer medicine [109].

3.3.3 ConfirmMDx

ConfirmDx is a tissue based biomarker that is used to determine the likelihood of a true negative biopsy versus one that has an occult cancer. The aim of this test is to prevent unnecessary repeat biopsies and also to detect those patients with negative biopsies who in fact, do require repeat biopsies. Unnecessary biopsies put patients at risk for complications from the biopsy procedure and increases morbidity, in addition to increasing economic burden.

Prostate biopsies are done when there is an increase in tPSA level and/or abnormal prostate DRE. When a prostate biopsy is negative and there is a high suspicion of PCa, the residual tissue from the biopsy can be submitted for the ConfirmMDx test. ConfirmMDx is an epigenetic assay that is used to detect DNA hyper-methylation changes. Epigenetic changes such as DNA methylation have been implicated in the molecular pathogenesis of PCa. These changes occur surrounding the tumor foci, called the halo effect. It occurs within a DNA sequence, when a methyl group is added to a cytosine nucleotide that is adjacent to a guanine nucleotide [110]. The three genes tested for DNA hyper-methylation associated with PCa are: GSTP1, APC, and RASSF1 genes [110]. Unlike histopathology of a prostate core biopsy which would have missed the diagnosis of epigenetic changes, these 3 genes have the potential to expose the presence of tumor activity via the use of ConfirmMDx.

From the methylation analysis to locate occult cancer (MATLOC) study conducted in 2013, the rate of false negative results from prostate biopsies was significantly lowered with a 90% NPV when compared with histopathology. Specificity and sensitivity were found to be 64% and 68% respectively [111]. Moreover, another study to substantiate the MATLOC study was the DOCUMENT (detection of cancer using methylated events in negative tissue) trial done in 2013. This resulted in a NPV of 88% (95% Cl 85–91) [49]. This was significant for repeat biopsies as an independent predictor of PCa.

In another study, Waterhouse et al. in 2018 found that ConfirmMDx used for PCa detection had a sensitivity and specificity of 74.1% and 60.0% respectively at repeat biopsies. The study validated the use of ConfirmMDx in African American men, and was significant as most studies were done on a Caucasian population [50]. Further, Wojno et al., found that PCa patients who were managed using ConfirmMDx test had a <5% rate of repeat prostate biopsies. Compared with previous rates, there was a tenfold reduction [51]. ConfirmMDx test has the potential therefore, to reduce healthcare costs by avoiding unnecessary repeat biopsies and to also avoid the morbidity associated with prostate biopsies. Notably, ConfirmMDx is not FDA approved.

3.4 Emerging molecular prostate cancer biomarkers

3.4.1 The ProMark

There are challenges in defining the aggressiveness of PCa as well as its outcome (particularly lethality) using prostate biopsy as there are sample errors and disparities in interpretation. Shipitsin et al. documented a proteomic biopsy-based PCa prognostic advanced test panel called ProMark that is manufactured and distributed by Metamark Genetics Incorporated, USA. They performed and documented a result-orientated method that provides an accurate prognosis of PCa aggressiveness and lethal outcome irrespective of variation in biopsy sampling [112]. Using a large patient cohort, prostatectomy tissue samples were identified and classified as having lowest to the highest Gleason grade. Tissue microarrays were produced comprising of cores from low as well as high Gleason area from each PCa patient. An assessment of 160 known protein biomarkers was carried out by means of using a quantitative multiplex proteomics in situ imaging system and a selection strategy with three types of criteria namely biological, technical and performance-based. Analytical performance and the application of univariate and multivariate analyses resulted in a final set of 12 protein biomarkers which provided prognostic accuracy of tumor behavior [113]. The same researchers conducted further investigations with the subsequent selection

of 8 of these 12 protein biomarkers in a prognostic model that offered "risk scores" predictive of the final post-prostatectomy pathology.

The eight proteomic biomarkers that constitute ProMark are: HSPA9, YBX1, CUL2, PDSS2, pS6, FUS, DERL1 and SMAD4 [52].

Blume-Jensen et al. performed a multicenter 8-protein biomarker assay model investigation involving 381 PCa patient biopsies with corresponding prostatectomy specimens. This was followed by a second blinded study of 276 cases which validated the 8-protein biomarker assay model's capacity to differentiate "non-favorable" versus "favorable" pathology in a manner that was independent and comparative to D'Amico and National Comprehensive Cancer Network (NCCN). The protein biomarker panel of ProMark gives a risk score ranging from 0 to 1 and predicts the aggressiveness of PCa and lethal outcome in patients with GS of 3 + 4 and 3 + 3 on biopsy. There was a false positive rate of 5% that corresponds to a non-favorable protein biomarker assay risk score >0.80 and false negative rate of 10% which relates to favorable protein biomarker assay risk score <0.33 [52]. The ProMark predictive model gave values for favorable pathology (risk score $\leq$ 0.33) of 87.2% for patients in the low-risk D'Amico group, as well as 81.5% and 95.0% for those in the low-risk and very low-risk NCCN groups respectively. These predictive values were higher than those of the up-to-date risk classification groups (70.6%—low-risk D'Amico group; 63.8%—low-risk NCCN; 80.3%—very low-risk NCCN respectively). The ProMark predictive model gave a value for non-favorable pathology (risk score > 0.80) of 76.9% for all the NCCN and D'Amico risk groups. The validation study of the 8-protein biomarker assay predictive model was able to distinguish non-favorable from favorable (AUC = 0.68; $p < 0.0001$; OR = 20.9) and GS 3 + 3 versus GS 3 + 4 (AUC = 0.65; $p < 0.0001$; OR = 12.95) [52]. Overall, with increasing ProMark biomarker risk scores there was reduced frequency of favorable PCa cases across all the D'Amico and NCCN risk groups [52].

3.4.2 miRNAs

3.4.2.1 Diagnosis, progression, risk stratification and therapeutic potential of serum or plasma miRNAs

MicroRNAs (miRNAs) are minute single-stranded as well as non-coding sections in RNA comprising of approximately 22 nucleotides that pay a critical role in gene regulation [53]. In the past decade a number of studies have investigated the differential expression and levels of miRNAs in plasma or serum in order to develop non-invasive blood-based biomarkers with the ability to diagnose, detect progression and assess prognosis as well as recurrence of PCa [114]. Jin et al. investigated 10 serum-circulating miRNAs as non-invasive molecular biomarkers in 31 BPH and 31 PCa patients. The expression levels of miR-375, miR-200b, and miR-141 levels were significantly elevated in the PCa patients compared with those in the BPH group, and miR-200b was the most effective diagnostic marker with AUC = 0.923 [115]. An association was found between the three miRNAs and tPSA, as well as miR-200b and GS [115]. The upregulation of miR-141 was also observed in a study by Ibrahim et al. that comprised 80 PCa (30 metastatic and 50 localized), 30 BPH patients and 50 healthy controls (**Table 2**). Plasma miR-141, miR-221, miR-18a and miR-21 levels were significantly higher in PCa patients than healthy controls. miR-18a differentiate PCa from healthy individuals with the highest AUC of 0.966, while miR-221 has a sensitivity of 92.9% and specificity of 100% at differentiating localized from metastatic PCa [116]. Likewise, another study differentiated PCa from BPH as higher significant expressions of the two onco-mRNAs

miRNA Identified	Study type	Specimen	Outcome	References
miR-141, miR-182, miR-200b, and miR-375	31 PCa and BPH patients	Serum	Diagnosis	[115]
miR-21, miR-141, miR-18a and miR-221	80 PCa, 30 BPH and 50 controls	Plasma	Diagnosis	[116]
miR-494	90 PCa and 90 BPH	Serum	Diagnosis	[117]
miR-301a	13 BPH and 28 PCa	Serum and tissue	Diagnosis/ prognosis	[118]
miR-410-5p	149 PCa, 121BPH and 57 controls	Serum	Diagnosis	[119]
miR-320a/-b/-c	145 PCa, 31 BPH and 19 controls	Serum	Diagnosis	[120]
miR-128	129 PCa patients	Serum and tissue	Diagnosis, prognosis	[121]
miR-628-5p	40 PCa patients and 32 controls	Serum	Diagnosis, prognosis	[122]
miR-4286, miR-27a-3p, and miR-29b-3p	78 PCa and 77 BPH	Serum	Diagnosis	[123]
miR-15a, miR-126, miR-192 and miR-377	35 PCa, 35 BPH and 30 controls	Serum	Diagnosis/risk	[124]
let-7b, miR-34a, miR-125b, miR-143, miR-miR-145 and miR-221	2 Prospective cohorts (12 mPCa and 25 controls; 149 PCa patients)	Plasma and tissue	Diagnosis	[125]
miR-210-3p, miR-23c, miR-592 and miR-93-5p	159 PCa fresh tissues and 60 plasma samples	Plasma and tissue	Risk stratification	[126]
miR-141-3p and miR-375-3p	84 mCRPCa patients	Serum	Therapy	[127]
miR-1825, miR-484, miR-205, miR-141, and let-7b	72 PCa and 34 controls	Serum	Prognosis/ therapy	[128]
miRNA-223, miRNA-24 and miRNA-375	196 PCa patients for training and 133 PCa patients for validation	Serum	Surveillance	[129]
miR-200c and miR-200b	102 PCa patients and 50 controls	Plasma	Diagnosis, prognosis	[130]

Table 2.
Summary of miRNA expression studies on plasma or serum samples from prostate cancer patients.

miR-375-3p and miR-182-5p were found in the plasma of PCa compared with BPH patients (specificity = 90.2%) [131]. Similarly, the expressions levels of miR-375-3p and miR-182-5p were evaluated for their diagnostic and prognostic potential in a cohort of 98 PCa and 52 normal controls. The plasma miR-182-5p expression level was significantly higher in PCa patients compared with controls, and it detected the disease with AUC = 0.64 (specificity of 77% and NPV of 99%). The authors also found that the levels of both miRNAs were associated with higher GS, and miR-182-5p was significantly elevated in metastatic PCa [132].

miRNAs exhibited both diagnostic and prognostic ability and these could be considered for possible use in clinical practice [108]. Cai and Peng investigated the diagnostic potential of miR-494 in a study comprising 90 BPH and 90 PCa patients, and 90 healthy controls. The serum expression of miR-494 was significantly higher in PCa compared with BPH patients and healthy controls, and positively correlated with GS, tumor size and stage, and serum tPSA levels. miR-494 was suggested to be a sensitive biomarker of PCa as the AUC was 0.809 [117]. A similar investigation was carried out for miR-301a extracted from serum and tumor samples in a study design involving two cohorts (cohort 1 of 13 BPH and 25 PCa, and cohort 2 of 12 BPH and 13PCa). miRNA-301a expression in serum and tissue was significantly higher in PCa compared with BPH patients, and there was correlation with increased GS for miR-301a in radical prostatectomy specimens [118]. Also, miR-410-5p was investigated as a potential serum biomarker for PCa in a study comprising 149 PCa and 121 BPH patients, and 57 healthy controls. The serum expression of miR-410-5p was significantly elevated in PCa compared with BPH patients or healthy controls. The diagnostic accuracy of serum miR-410-5p indicated by an AUC of 0.810 suggests that it is a potential molecular biomarker for the diagnosis of PCa [119].

Other miRNAs have been overexpressed or under-expressed in circulation thus demonstrating their diagnostic and prognostic potentials. Lieb et al. in a study of 145 PCa and BPH patients, and 19 healthy controls reported that the serum levels of miRNA family members (miR-320a, miR-320b and miR-320c) differed among the three groups been highest in the PCa patients. In addition, the serum levels of all three miRNAs were significantly higher in older patients, high tumor stage and those with tPSA >4 ng/mL [120]. Conversely, decreased miR-128 expression was found in the serum of 128 PCa patients which was associated with disease progression and short biochemical recurrence-free survival (**Table 2**) [121].

miRNA profiling experiments followed by validation showed decreased expression of serum miR-25, miR-628-5p, and miR-101 in African American and Caucasian Americans with PCa compared with healthy controls [122]. Other serum or plasma miRNAs which have been identified as potential non-invasive biomarkers for PCa include: has-miR-101-3p and has-miR-19b-39 (diagnosis and prognosis) [133], miR-940 (diagnosis, AUC = 0.75) [134], panel of miR-27a-3p, miR-424-5p, miR-29b-3p, miR-4286 and miR-365a-3p (detecting early stage PCa) [123], panel of miR-126, miR-377, miR-15a and miR-192 (detection of localized PCa and risk stratification) [124] as well as panel of miR-373, miR-141, miR-21, miR-125b, miR-126, miR143 and let-7b (diagnosis of metastatic PCa) (**Table 2**) [125].

3.4.2.2 Diagnosis, prognosis and therapeutic potential of miRNAs in tissue

The profiling of miRNAs in tissues of PCa patients shows pattern that are different compared with those from healthy controls. These distinct miRNA expressions in PCa tissues could afford tools for improved diagnosis, prognosis and therapeutic approaches for PCa [114]. Huang et al. investigated the clinical utility and prognostic potential of hsa-miR-30c and hsa-miR-203 in tissues of 44 PCa patients. The expressions of the two miRNAs in tumor tissues were significantly different from those in neighboring normal tissue indicating their diagnostic potential. All the PCa patients were followed up for 36 months and the data showed that the mean survival times of high and low expressions of hsa-miR-203 and has-miR-30c respectively were significantly lower, which attest to their possible prognostic utility for PCa [135]. In a study involving tissue samples from 14 BPH and 60 PCa patients (cancerous and noncancerous prostate

samples) and the employment of qRT-PCR followed by validation, the expression levels of 4 miRNAs (miRNA-141-5p, miR-183-5p, miR-32-5p and 187-3p) differed significantly between PCa and BPH samples [136]. The data suggests that these four miRNAs could detect cancer in prostate biopsy as they were able to differentiate between malignant and nonmalignant prostates [136]. Likewise, the expressions of miR-27b were higher in PCa compared with BPH tissues, and correlated with GS and clinical stages in PCa. The researchers further posited that PCa patients with higher expression of miR-27b had worse progression free as well as overall survival [137]. There are other studies that have shown an overexpression of miR-153 in PCa tissue which was associated with worse overall survival (**Table 3**) [145], and the deregulation of miR-30c and miR-29b with decreased expression in PCa tissues and ability to differentiate between PCa and adjacent para-cancerous tissues (AUC = 0.944 for miR-30c and AUC = 0.924 for miR-29b) (**Table 3**) [138].

There are a few studies that have investigated the therapeutic potential of miRNAs for PCa treatment because of their ability to bind targets using prostate tissue and cell lines [149]. Wang et al. explored the prognostic potential and possible use of miR-1231 as a therapeutic tool by measuring its expression levels in PCa tissues. The

miRNA Identified	Study type	Specimen	Outcome	References
miR-128	128 PCa cases	Tissue	Prognosis	[121]
hsa-miR-203 and hsa-miR-30c	44 PCa patients	Tissue	Diagnosis, prognosis	[135]
miR-187-3p, miR-183-5p, miR-32-5p, and miR-141-5p	14 BPH and 60 PCa tissue samples	Tissue	Diagnostics	[136]
miR-30c and miR-29b	187 cases of PCa	Tissue	Diagnosis	[138]
miR-424-3p	Prostatectomy specimens 535 PCa patients	Tissue	Therapy	[139]
miR-20b	127PCa patients	Tissue	Prognosis	[140]
miR-17-5p	535 PCa patients	Tissue	Prognosis	[141]
miR-148b-3p	PCa and BPH samples	Tissue	Diagnosis	[142]
miR-1231	PCa tissues and cell lines	Tissue	Prognostic, therapy	[143]
miR-615-3p	239 PCa patients	Tissue	Prognosis	[144]
miR-153	143 pairs of PCa tissues	Tissue	Prognosis	[145]
miR-130b	PCa tissue from African Americans	Tissue	Prognosis and race disparity	[146]
miR-27b	28 BPH and 63 PCa tissues	Tissue	Diagnosis, prognosis	[137]
miR-1207-3p	404 post-prostatectomy prostate tumor tissue samples	Tissue	Prognosis	[147]
miR-301a	75 formalin fixed paraffin embedded localized PCa tissue, 4 mPCa tissue and 13 BPH tissue	Tissue	Prognosis	[148]

Table 3.
Summary of miRNA expression studies on tissue from prostate cancer patients.

miR-1231 expression was decreased in PCa tissues and significantly associated with shorter overall survival, higher TNM stage, lymph node metastasis and higher clinical stage. The data also showed that epidermal growth factor receptor (EGFR) is a target of miR-1231 and its over-expression reduced migration, proliferation and invasion of PCa cell lines in vitro. Based on the evidence, the researchers suggested that miR-1231 has a tumor-suppressive role, may be a prognostic biomarker, and a therapeutic tool for PCa treatment in the future (**Table 3**) [143]. In another study involving naïve radical prostatectomy specimens from 535 PCa patients, decreased expression of miR-424-3p was significantly associated with the aggressive phenotype of PCa and clinical failure-free survival. Based on the evidence, the authors posited that miR-424-3p could be a possible target for treatment of PCa (**Table 3**) [139].

3.4.3 Exosomes

Exosomes are minute membrane-bound extracellular vesicles with diameters of 30–150 nm. They are released from many cell types into body fluids and the extracellular environs after the amalgamation of multi-vesicular bodies fuse with the plasma membrane [150]. Exosomes comprised of a number of cytoplasmic biomolecules such as lipids, glycol-conjugates, proteins, DNA and RNA including miRNAs surrounded by a lipid bilayer membrane. They are found in blood (plasma and serum), urine, saliva and semen and cell culture medium [140, 151]. During PCa development and metastasis, exosomes are secreted by tumor cells and have been reported to play a critical role in initiation, promotion, immuno-regulation, angiogenesis, annexation and metastasis [152]. As exosomes play a role in PCa development via a number of mechanisms of actions, they are valuable biomarkers for PCa diagnosis, prognosis and monitoring [153, 154].

3.4.3.1 Exosome-contained microRNAs

Exosomal miRNAs are small-stranded non-coding RNA (about 17–25 nucleotides in length) and changes in their concentration in body fluids make them useful molecular biomarkers of PCa progression [155]. There is increasing interest in exosomal miRNAs in serum and plasma samples due to their stability and as non-invasive molecular biomarkers for PCa diagnosis and recurrence [156]. Li et al. conducted a study comprising 31 PCa patients and 19 healthy persons and found that plasma exosomal miR-125a-5p levels were significantly decreased in the former compared with the latter. The researchers also found that plasma exosomal miR-141-5p levels mildly increased in PCa patients, and the miR-125a-5p/miR-141-5p ratio was able to distinguish these patients from healthy controls (AUC = 0.793) [157].

In a recent study, Guo et al. examined the predictive potential of 6 plasma exosomal miRNAs in a first validation prospective cohort of 42 CRPCa and 108 treatment-naive PCa patients, and found that miR-423-3p was associated with CRPCa (AUC = 0.784). In a second validation study reported by the same authors, plasma exosomal miR-423-3p expression was significantly higher in 30 CRPCa patients undergoing androgen depletion treatment compared with 36 non-CRPCa patients (AUC = 0.879). The authors suggested that plasma exosomal miR-423-3p may serve as a useful molecular biomarker for early diagnosis and prognosis of castration resistance in PCa patients [153]. Moreover, in an earlier study that examined the overall survival in a prospective cohort of 23 CRPCa patients, significantly elevated levels of

plasma exosomal miR-375 and miR-1290 were associated with worse clinical outcome and overall survival in CRPCa patients [158].

There are serum exosomal miRNAs that are investigated for potential clinical utility for PCa. In an investigation by Li et al., the expression of serum exosomal miR-141 was significantly higher in PCa patients compared with those of BPH patients and healthy individuals. Serum exosomal miR-141 expression was also significantly higher in PCa patients with metastasis compared with localized disease. The authors suggested that serum exosomal miR-141 could be a valuable molecular biomarker for the diagnosis of metastatic PCa [159]. In preclinical vivo and in vitro studies, exosomal miR-1246, a tumor suppressor was downregulated in PCa cell lines, and clinical tissues correlated with the presence of metastasis, poor prognosis and increasing GS [160].

Urinary exosomal miRNAs have been found to be valuable noninvasive and diagnostic molecular biomarkers of PCa [161]. Shin et al. investigated the clinical utility and profiles of urinary exosomal miRNA expressions in 149 PCa cases and the identification of those associated with metastasis. Urinary exosomal miR-21 and miR-451 expressions were upregulated and miR-636 downregulated in metastatic PCa patients compared to those with localized disease. These three exosomal miRNAs were used to develop a Prostate Cancer Metastasis Risk Scoring (PCa-MRS) model for PCa patients with high scores showing significantly worse biochemical recurrence-free survival [162]. Similarly, in a recent study comprising of a next-generation sequencing cohort (6 PCa patients and 3 healthy individuals) and use of qRT-PCR (28 BPH patients, 47 PCa patients and 25 gender- and age-matched healthy controls), urinary exosomal miR-486-5p, miR-486-3p, miR-375, miR-486-5p and miR-451a expressions discriminated PCa patients from healthy controls with AUCs ranging from 0.704 to 0.796. The researchers found that urinary exosomal miR-375 differentiated metastatic PCa from localized (AUC = 0.806), and miR-451a along with miR-375 distinguished localized PCa from BPH (AUC = 0.726) [155]. In an earlier study comprising 90 PCa patients, 10 BPH and 50 healthy controls, urinary exosomal miR-2909 was reported to be a non-invasive diagnostic molecular biomarker of PCa and disease aggressiveness [163]. Preclinical studies also revealed that urinary exosomal miR-574-3p and miR-375 were detected by molecular beacons in PCa cells such as PC-3 and DU145 [164]. Furthermore, urinary exosomal miR-532-5p expression was upregulated in 26 PCa patients with biochemical recurrence and exhibit poor prognosis in those with intermediate-risk disease [165].

The findings from these observational studies indicated that circulating exosomal miRNAs in serum, plasma or urine may assist in the diagnosis, prognosis and outcomes of PCa and could be adopted into routine clinical practice in the near future.

3.5 Potential prostate cancer biomarkers in development

3.5.1 Prostarix

Prostarix is another PCa biomarker that has potential value in screening/diagnosis. It is a post-DRE urine test that is used to predict the likelihood of having a negative prostate biopsy versus one with occult PCa. This is done using a risk score. The risk score is generated using an algorithm which ranges from 0% to 100%. One hundred percent equates to a 100% chance of having PCa on a prostate biopsy. Therefore, it is used to determine which men will undergo prostate biopsy. Prostarix is used when there is an elevated tPSA and normal DRE, and in some cases, for men who are candidates for a repeat prostate biopsy [166, 167].

Prostarix measures four metabolites using liquid chromatography mass spectrometry. These metabolites are sarcosine, alanine, glycine and glutamate. Sarcosine especially, has been linked with PCa progression [168]. A study conducted by McDunn et al. 2013, showed that these metabolites improved prediction of organ confinement (AUC, 0.53–0.62) and 5-year recurrence (AUC, 0.53–0.64) [166]. However, a study done by Sroka et al. in 2017 that evaluated various amino acids as PCa biomarkers found that sarcosine was not a definitive indicator of PCa when analyzed in pre and post-prostate massage urine samples. Also, the amino acids arginine, homoserine, and proline were mostly seen in the urine samples of PCa patients as opposed to patients with a benign prostatic disease [169]. It is evident that more studies need to be done on Prostarix, and of note, it is not FDA approved.

3.5.2 Prostate Core Mitomic Test

Prostate Core Mitomic Test (PCMT) is a tissue based biomarker for detecting PCa. It is an RT-PCR test that detects large-scale mitochondrial deletions in prostate biopsy samples. It is able to identify tumor activity at the molecular level and detects molecular changes that occur at the mitochondrial DNA (mDNA) level. The large-scale deletion of 3.4 kb of the mitochondrial genome has been known to occur as a part of the prostate "cancerization" field effect [170]. Cancerization field effect occurs when cells adjacent to primary tumors become transformed [171]. This takes place at the start of oncogenesis. Cancerization field effect is seen in some type of cancers including PCa. Histopathology would have otherwise labeled these prostate biopsy samples with cancerization field effects as "normal" appearing tissue.

PCMT is used to predict repeat biopsy outcomes with an initial negative biopsy. This test can therefore, aid in reduction of the number of unnecessary biopsies and also reduce health care costs. According to Robinson et al. in 2010, the sensitivity and specificity of this mitochondrial deletion in predicting repeat biopsy outcomes were found to be 84% and 54% respectively. It also showed a NPV of 91% and an AUC of 0.749 [172]. Legisi et al. performed a multicenter observational study to assess the use of the PCMT in the decision making of repeated biopsy among patients with a strong suspicion of PCa. Using two independent query language databases, the PCMT addressed sampling errors related to prostate biopsy and gave more evidence concerning the clinical uncertainty surrounding an initial negative prostate biopsy [173]. Notably, the PCMT is not FDA approved.

4. Discussion

Globally, PCa is regarded as the most predominant malignancy in males and the principal cause of cancer-related mortality. Given the negative impact of PCa as it relates to morbidity and quality of life as well as mortality, early detection of the disease is of critical importance. Presently, tPSA and DRE are used in the diagnosis of PCa but there are a number of limitations which include low specificity and sensitivity leading to over-diagnosis and subsequent unnecessary prostate biopsies. In the early stage of PCa development there are no symptoms so there is a need for biochemical and molecular diagnostic tests for accurate and prompt detection. This review documents a number of PCa diagnostic and prognostics tests that have been discovered in the last two decades due to improvements in genomic technologies.

Among them are serum PHI and 4K score, urine PCA3 and SelectMDx, and tumor tissue Oncotype DX, Decipher and Prolarix. These biomarkers are used in clinical decision making for PCa such as suspected patients who are required to undertake an initial prostate biopsy, or those who need a second biopsy given that the initial one was negative for the disease. These tests have generated new prospects for advancing PCa diagnosis, prognosis such as the prediction of metastatic disease recurrence and decisions regarding therapy. In particular, Oncotype DX afford information regarding risk stratification which aid in identifying PCa patients that should receive treatment after a positive prostate biopsy, and Decipher those individuals to be treated post-surgery [174].

There have been a substantial number of miRNA-based assays and evidence in the literature demonstrates increased or decreased expression levels of miRNAs in peripheral blood (serum and plasma), prostate tissues and body fluids (urine and seminal) that differentiate between PCa and BPH patients, as well as healthy controls. This review presented findings on the clinical utility of miRNAs as diagnostic and prognostic biomarkers, and possible therapeutic targets for PCa. The findings are encouraging particularly the downregulation or upregulation of miRNAs expressions in plasma, serum or urine which facilitates the non-invasive nature, fast and cost-effectiveness of the tests. However, significant work is warranted if the miRNAs biomarkers are to translate from bench to routine clinical use. Also, larger observational prospective studies are needed with the intent of substantiating the validity of miRNAs and determining precise stratification based on the expression levels of miRNAs in PCa at different stages along the continuum of the disease. Furthermore, there are issues to be addressed such as the absence of guidelines for miRNAs development including isolation protocols, differences in study designs, pre-analytical variables such as specimen collection issues and tumor heterogeneity, need for validation and the selection of the best detection method (qRT-PCR vs. microarray) [175].

The presence of proteins and miRNAs in exosomes of prostate tissues makes them a valuable molecular diagnostic biomarker and therapeutic tool for PCa treatment. The development of these biomarkers is still in its early stages, but the results are very encouraging. The evidence presented in this review suggests that serum, plasma and urine exosomal miRNAs are useful for early detection as it differentiate metastatic from localized PCa together with prognosis of mCRPCa. There is a paucity of studies on exosomal proteins such as serum claudin 3 and survivin as well as urine-based LAMTOR1, TMEM256 and PARK7. This is an exciting area and research is continuing. However, there are challenges such as the complex process of obtaining appropriate samples, the lack of suitable isolation protocols and the need to standardized purification and quantification methods [176]. Overcoming these obstacles in preclinical research could result in these exosomal biomarkers being applied in the clinical setting for risk stratification, prognosis and the monitoring of PCa.

Moreover, PCa biomarkers employ diverse types of samples such as blood (serum or plasma), urine, prostate tissue (specimen from transurethral resection, biopsy and radical prostatectomy) and seminal fluid [177]. Assays comprising these PCa biomarkers are evaluated as their clinical use involved improving early diagnosis and risk stratification of localized tumor, reducing the number of needless biopsies with subsequent saving on use of expensive intervention strategies [177].

Traditionally blood, a minimally invasive and easily obtained sample is the chief source of PCa biomarkers for example serum tPSA and in recent years emerging biomarkers such miRNA has been developed. Panels for new PCa biomarkers will permit

fingerprinting of the biologic behavior of the tumor with possibly personalized therapy and monitoring [178]. The collection of urine sample for the assessment of PCa biomarkers is a simple, non-invasive approach and assays can be used to monitor PCa with heterogeneous tumor foci [179]. Currently some of the emerging protein and non-protein non-invasive urinary PCa biomarkers comprise TMPRSS2, PCA3, miRNAs and SelectMDx [180]. Genomic studies and biotechnological advancement have improved the sensitivity and specificity of these urinary PCa biomarkers thus enhanced clinical outcomes relating to early diagnosis and better selection of treatment approaches [181].

Tissue-based PCa biomarkers are invasive and fewer, and in the area of molecular diagnostics there is more focus on assays for blood and urine-based biomarkers with diagnostic and prognostic potential. However, there is growing interest on tissue-based PCa biomarkers such as ProMark, Oncotype DX and Decipher as well as miRNA and exosomal miRNA [182].

Finally, there are emerging molecular biomarkers that are at different phases of development, and many are in the preclinical phase. It is hope that in the next decade or so a significant collection of biomarkers with excellent diagnostic good sensitivity and specificity together with significant prognostic potential will be available for use by physicians in the clinical setting [183–185].

5. Conclusion

This review provides evidence of the use of established and emerging biomarkers detected in body fluids as diagnostic and prognostic tools for PCa. Despite the promising findings in preclinical and clinical research among the increasing body of investigations, there are challenges which delay the translation of a number of biomarkers from bench to bedside. Nonetheless, the considerable prospect of the biomarkers such as miRNAs and exosomal miRNAs in clinical practice as therapeutic tools for PCa is widely acknowledged and hopefully will be a reality in the not too distant future.

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