Cancer Management and Research

Dovepress

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REVIEW

Biomarkers That Differentiate Benign Prostatic Hyperplasia from Prostate Cancer: A Literature Review

This article was published in the following Dove Press journal:
Cancer Management and Research

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Abstract: Prediction of prostate cancer in primary care is typically based upon serum total prostate-specific antigen (tPSA) and digital rectal examination results. However, these tests lack sensitivity and specificity, leading to over-diagnosis of disease and unnecessary, invasive biopsies. Therefore, there is a clinical need for diagnostic tests that can differentiate between benign conditions and early-stage malignant disease in the prostate. In this review, we evaluate research papers published from 2009 to 2019 reporting biomarkers that identified or differentiated benign prostatic hyperplasia (BPH) from prostate cancer. Our review identifies hundreds of potential biomarkers in urine, serum, tissue, and semen proposed as useful targets for differentiating between prostate cancer and BPH patients. However, it is still not apparent which of these candidate biomarkers are most useful, and many will not progress beyond the discovery stage unless they are properly validated for clinical practice. We conclude that this validation will come through the use of multivariate panels which can assess the value of biomarker candidates in combination with clinical parameters as part of a risk prediction calculator. Implementation of such a model will help clinicians stratify patients with prostate cancer symptoms in primary care, with tangible benefits for both the patient and the health service.

Keywords: prostate cancer, benign prostatic hyperplasia, biomarkers, differentiation, transrectal ultrasound-guided biopsy

Introduction

Serum prostate-specific antigen (PSA) was deemed a viable tumor marker for the detection of prostate cancer (PCa) allowing clinicians to track patient response to cancer treatment.[1] However, initial investigations into PCa usually involve a combination of digital rectal examination (DRE) and measurement of PSA levels. Based on these measurements a referral for Transrectal Ultrasound (TRUS)-guided biopsy may be made. Whilst PSA has helped identify many more patients with PCa, one of the main obstacles for clinicians is to differentiate PCa from non-malignant conditions. One of these conditions, benign prostatic hyperplasia (BPH), can also present with raised levels of PSA. With no universally agreed way to stratify suspected cases of PCa to help inform diagnostic process at this point, many men undergo unnecessary biopsy or further procedures that they may not require.[2]

It is therefore clear that there is a need for more accurate methods to risk stratify men who present with symptoms of PCa, to prevent the over-diagnosis and unnecessary treatment of patients with benign conditions.[3] Successfully implemented in

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DovePress
http://doi.org/10.2147/CMAR.S250829

primary care, this would greatly reduce the over-diagnosis rates of patients, as well as relieving financial and management pressures on healthcare providers. Recent research efforts suggest that this stratification is likely to be based on the identification of reliable biomarkers which can improve on the current use of PSA measurement to diagnose PCa. This review aims to present and evaluate data from studies published over the past decade that have proposed biomarkers which may be useful for differentiating between BPH and PCa.

BPH is a non-malignant enlargement of the prostate caused by cellular hyperplasia that occurs within the transitional zone.[3] BPH is associated with age, with around 50% of men aged 50, 70% of men aged 70, and 90% of men aged 80 being affected.[4,5] The proliferation of prostatic cells leads to an increase in the size of the prostate as well as urethral obstruction and lower urinary tract infections (LUTS). Risk factors for BPH include age, decreased testicular function, metabolic syndrome, family history of BPH and obesity.[4] Several studies have investigated an association between BPH and PCa, although the underlying pathophysiology between the two conditions remains unclear.[6–8] A meta-analysis of 19 studies involving 15,899 patients determined that BPH was associated with an increased risk of PCa, risk ratio (RR) 2.93, (95% CI = 1.88–4.56), $P < 0.0000$.[9] The authors demonstrated that the association between BPH and PCa was stronger within Asian populations when compared to Caucasians; RR 6.09 and 1.54, respectively. The authors also suggested that hormones, inflammation, and metabolic syndrome likely play a role in the pathophysiology of BPH.[9] There is also evidence that the homeostasis between prostate cell proliferation and cell death supported by dihydrotestosterone (DHT) and estrogen is often disrupted in BPH patients.[10] Additionally, aggressive BPH is associated with an elevated risk of developing PCa and the subsequent cancer can be high grade compared to individuals without fast-growing BPH.[11] Although the majority of findings are hypothesis-generating rather than hypothesis-confirming, the evidence does support the theory that BPH is a risk factor involved in the pathogenesis of PCa.[12–14]

However, despite this evidence, it is not inevitable that BPH will progress to PCa in any given individual. Therefore, it is important to be able to distinguish BPH patients at an early stage to prevent further invasive and unnecessary tests in these individuals. PSA testing alone is not able to make this differentiation, so new biomarkers are required to improve the risk-stratification of patients at this stage.

Prostate Specific Antigen

Currently, PSA is one of the most widely used biomarkers for the detection and management of PCa. Prior to PSA measurement, PCa was mainly predicted by DRE. However, DRE as a diagnostic tool has low sensitivity and specificity, as well as user subjectivity between clinicians performing the examination.[15] PSA testing was a clear diagnostic improvement and was introduced in the United States in 1987 to determine if patients were responding to curative therapy. Soon after, PSA was used for screening patients at risk of PCa, leading to an increase in disease detection and a decline in mortality.[16] PSA is a kallikrein-like serine protease produced by the epithelial cells of the prostate to help liquefy ejaculate and aid sperm motility. Extraprostatic production of PSA is provided mainly by the periurethral glands, which in turn leads to measurable levels of PSA in the serum[17] The San Diego-based company Hybritech Inc. was the first company to propose a serum PSA threshold of 4.0 ng/mL after a study on healthy men.[17] Subsequently, a PSA value of >4.0 ng/mL became the industry standard for recommending a prostate biopsy. Since the introduction of this threshold, studies have shown that PSA testing has a sensitivity of 67–80% and has helped diagnose a large number of patients with PCa since it was first introduced.[17]

However, although PSA is organ-specific, it is not a cancer-specific biomarker. BPH and other conditions, for example, prostatitis, inflammation of the prostate, can raise serum PSA levels. Conversely, PCa has been shown in males who present with normal PSA levels.[16] Hence, there is a lack of specificity with PSA which can lead to over-diagnosis of PCa and unnecessary treatment. Data gathered from the Surveillance, Epidemiological and End Results (SEER) registry estimates that screening for PCa using PSA has resulted in 28% of over-diagnosed cases in the USA.[18] Likewise, the European Randomised Study of Screening for Prostate Cancer (ERSPC) trial estimated that using PSA as a screening tool for PCa led to 50% of patients being over-diagnosed.[19] Actively diagnosing a clinically insignificant tumor can lead to unnecessary treatment, such as radical prostatectomy or radiotherapy. To avoid this, healthcare providers are utilizing the strategy of active surveillance, where regular PSA and DRE testing is used alongside biopsies over a period of time to minimize the risk of over-diagnosis. However, regular check-ups and repeated prostate biopsies

are invasive and can be very painful for the patient. This unsurprisingly can cause high levels of anxiety and stress and may discourage the patient to seek medical attention at all.[16]

It is known that serum PSA levels increase with age. This is most likely due to the contribution of an enlarged prostate associated with old age as well as the decreased retention of the prostatic epithelium. Age-specific PSA has been shown to increase the detection of PCa in younger men (50–59 years) by 15% but also shown to increase the number of biopsies performed by 45%.[20] To address these issues, various studies have investigated more nuanced measurements of PSA expression to help improve its usefulness for PCa diagnosis. Significant research has been performed on free PSA, rather than total PSA (tPSA), demonstrating the ratios of free-to-total PSA in serum may improve the diagnostic specificity by 15–20%.[21] This type of test is recommended for patients who present with PSA levels within the "grey-zone" of 4.0 to 10.0 ng/mL. Initially, it was proposed that high levels of free PSA were associated with benign prostate tissue and a decreased probability of PCa.[21] However, this test was not widely implemented as a screening tool due to inconsistencies in later studies.[17] The [−2] isoform of proPSA has emerged as a promising biomarker due to its ability to differentiate between PCa and BPH, where levels appear raised in PCa. One large prospective study of patients with PCa showed that the percentage of [−2] proPSA improved the specificity to 44.9% in comparison to total and free PSA which was 30.8% and 34.6% respectively whilst also achieving a sensitivity of 80% for detecting PCa.[22]

The Prostate Health Index (PHI) can also be considered a biomarker, calculated using the following formula: PHI= ([−2]proPSA/freePSA)×√PSA. In one study, both the [−2] proPSA (AUC = 0.76) and the PHI test (AUC = 0.77) outperformed the tPSA test when used to detect PCa between the ranges of 2.5–10 ng/mL.[23] These studies have shown the predictive superiority of these two tests compared to that of just tPSA based screening alongside a significant improvement in accuracy. However, other studies do not agree with these results and indicated that when the goal is to detect at least 95% of the aggressive tumors, PHI does not seem to be much more effective than the %free PSA and the PSA density.[24]

PSA density is calculated as the tPSA in ng/mL divided by prostate volume (mL). Nordström et al in 2018 suggested that PSA density might inform clinicians more on biopsy decisions after determining that a cut-off of 0.10 ng/mL^2 resulted in a detection rate of 77% of Gleason score ≥7 tumors compared to tPSA alone, 64% (n = 947).[25] Additionally, both Verma et al in 2014 and Sebastianelli et al in 2019 both suggested that PSA density could be used to reduce unnecessary biopsies after determining the marker was significant for the detection of aggressive PCa.[26,27] However, Liu et al in 2015 concluded in their paper that if a patient has PSA levels of <2.0 ng/mL, PSA density does not differentiate the PCa effectively enough (n = 343).[28]

PSA velocity is the measure of the rate of PSA increase over time. Serum PSA velocity increases significantly in the presence of a prostate tumor compared to that of a benign disease alone. However, no evidence was found to support the recommendation that men with high PSA velocity should be biopsied in the absence of other indications.[29]

Despite these varied approaches to PSA measurement, there are still many limitations to current PSA testing for PCa. The PSA test possesses a negative benefit-to-harm ratio based on population-based estimates.[16] The future of individualized PSA-based screening seems to lie as a component of multivariate risk stratification, carried out by using various nomograms and prediction risk tools.[16] Therefore, the current challenge is to identify other biomarkers that can be used in combination with PSA in primary care to differentiate BPH from PCa.

Transrectal Ultrasound-Guided Biopsy

Grey-scale TRUS-guided biopsy is the gold standard for prostate imaging and is essential for achieving a histological diagnosis of a prostatic carcinoma through a guided biopsy.[30] The TRUS-guided biopsy is also the main procedure recommended by the National Institute for Health and Care Excellence (NICE) guidelines to diagnose PCa.[31] Although the procedure is considered safe, the number of post-biopsy complications are on the rise, and often reported in up to 50% of cases. Complications include pain, haematuria, haematospermia, urinary retention as well as infection.[32] The pain reported from a TRUS-guided biopsy is relative to the number of cores removed.[33] In a study performed on patients after a sextant biopsy (six-core removal), 94% of patients found the procedure painful and 24% of patients reported the pain as moderate to severe. The extended 12-core biopsy can result in higher levels of pain, subsequent inadequate sampling and eventual abandonment of the procedure.[33] Many patients have refused when asked if they would undergo a repeat prostate biopsy. Pain is subjective, it is difficult to quantify. However, it is clear that both psychosocial factors and physical attributes

play an important role when patients require a prostate biopsy.[34] Aside from immediate surgical pain, complications and infection can occur. UTIs were reported at an occurrence rate of 6% post-TRUS biopsy, with 30–50% of patients within that 6% going on to develop bacteremia. Approximately 0.1% - 2.2% of TRUS biopsy patients will develop severe sepsis.[35] One study reported that 1 in 4 post-TRUS biopsy patients hospitalized due to *E. coli* bacteremia had severe sepsis that required them to be admitted to the intensive care unit (ICU).[32] Interestingly, there are reports to suggest that the rate of infectious complications after TRUS biopsy is on the rise. A study based in Ontario, Canada reported an increase in the rate of hospitalization within 30 days following a TRUS biopsy, from 1.0% of patients in 1996 to 4.1% in 2005 ($P < 0.0001$).[36]

Although the frequency of complications is relatively low, the problem is still a substantial one, given the number of biopsies performed and the associated economic burden from treating biopsy-related complications.[32] A new test that has high specificity and could confidently stratify patients into groups that require a biopsy and those who could be stratified to watch and wait, would significantly reduce the number of post-TRUS biopsy complications.

The rapidly growing use of multiparametric magnetic resonance imaging (mp-MRI) offers a much safer alternative to the TRUS-guided biopsy. The use of MRI continues to show increased accuracy for the detection, localization, risk stratification and staging of PCa for patients.[37–40] The largest benefit will come from the reduced number of unnecessary biopsies being performed, which in turn will significantly reduce levels of overdiagnosis within patients displaying signs of PCa.[41] However, although mp-MRI has shown promising results, it is still not perfect. There is a risk that 5–20% of index lesions are missed. However, in combination with standard TRUS-guided biopsy, this can be improved.[42–44] Additionally, current MRI technology also lacks the resolution to detect tumors with a smaller volume and a lower Gleason score, making it less reliable at detection early-stage PCa.[43,44] Nevertheless, the benefits of mp-MRI are clear and offer an attractive alternative to TRUS-guided biopsies, although access to the required instrumentation and resources may still be a clinical barrier to widespread implementation.

The Search for New Biomarkers

The most ideal biomarkers are ones that can be measured accurately and reproducibly in a minimally invasive manner.[45] Biomarkers can be measured in tissue, blood, urine and/or semen, but standardized collection and analysis of these samples can present some unique challenges. As a result, there is no consensus on the best samples to use, or the optimal biomarkers to measure once the sample has been collected. This review aims to provide a comprehensive overview of research being performed that purport to identify genomic and/or proteomic biomarkers that can differentiate between BPH and PCa. The following search criteria were employed:

- Article obtained using either PubMed or Google Scholar
- Manuscripts published within the last 10 years
- Articles are in English
- Biomarkers must be analyzed from either blood, urine, tissue or seminal fluid
- Analyzed specifically and separately both PCa and BPH specimens in the study
- Attempted to differentiate between PCa and BPH

Using this filtered approach, we retrieved 104 published papers for blood and urine-based biomarkers. Their findings are reported in Tables 1–4, with similar data for tissue and seminal fluid biomarkers gathered from 49 published papers presented in Supplementary Tables 1-3.

Blood-Based Biomarkers

Genomic Blood Biomarkers

Table 1 provides a list of blood-based biomarkers from 19 papers that have been investigated by researchers using genomic techniques to identify patterns that could potentially differentiate BPH and PCa.

Proteomic Blood Biomarkers

Table 2 provides a list of blood-based biomarkers from 44 papers that have been investigated by researchers using proteomic techniques to identify patterns that could potentially differentiate BPH and PCa.

Urine-Based Biomarkers

Genomic Urine Biomarkers

Table 3 provides a list of urine-based biomarkers from 21 papers that have been investigated by researchers using genomic techniques to identify patterns that could potentially differentiate BPH and PCa.

Proteomic Urine Biomarkers

Table 4 provides a list of urine-based biomarkers from 23 papers that have been investigated by researchers using

Table 1 Blood-Based Biomarkers Derived from Genomic Techniques

Biomarkers	BPH (n)	PCa (n)	Method	All Significant ($p<0.05$)	Reference
miR-15a↓,	35	35	RT-PCR	Yes	46
miR-126↓,					
miR-192↓, miR-377↓					
miR-18a↑	24	24	RT-PCR	Yes	47
let-7c↓, let-7e↓,	60	64	qPCR	Yes	48
let-7i↓,					
miR-26a-5p↓,					
miR-26b-5p↓,					
miR-18b-5p↓,					
miR-25-3p↓					
Retinoic Acid Receptor β2 (RARβ2) ↑	94	91	qPCR	Yes	49
Cell-Free DNA↑	112	96	qPCR	Yes	50
let-7a↑, miR-210↑, miR-562↑, miR-616↑	13	31	RT-PCR	Yes	51
let-7c↓, miR-30c↓,	16	59	qPCR	Yes	52
mirR-141↓,					
miR-375↓					
miR-708↓,	39	76	qPCR	Yes	53
miR-221↓,					
miR-518d-5p↓,					
miR-675↑,					
miR-1180↑,					
miR-1225-5p↑,					
miR-659↑					
miR-26a↑,	18	37	qPCR	Yes	54
miR-195↑, let7i↑					
Glutathione S-Transferase Pi 1 (GSTP1)↑	34	31	MS-PCR	Yes	55
miR-499,	353	355	PCR-RFLP	Yes	56
miR-196a2,					
miR-27a variants					
Growth Arrest and DNA Damage Inducible Alpha (GADD45a) Methylation↑	48	34	PyroSequencing	Yes	57
Glutathione S-Transferase Pi 1 (GSTP1)↑, Ras Association Domain Family 1 Isoform A (RASSF1A)↑	103	83	MS-PCR	Yes	58
Cell-Free DNA↑	76	50	Spectrophotometry	Yes	59
miR-375↑	35	146	qPCR	Yes	60

(*Continued*)

Table 1 (Continued).

Biomarkers	BPH (n)	PCa (n)	Method	All Significant ($p<0.05$)	Reference
miR-410-5p↑	121	149	qPCR	Yes	61
SAP30L Antisense RNA 1 (SAP30L-AS1)↓, SWI/SNF Complex Antagonist Associated With Prostate Cancer 1 (SChLAP1)↑	46	34	qPCR	Yes	62
MD-miniRNA↑	32	63	qPCR	Yes	63
miR-15a↓, miR-16-1↓	70	70	qPCR	Yes	64

Note: ↓/↑: Expression levels in PCa group compared to that of BPH group.
Abbreviations: miRNA, microRNA, Let-7, lethal-7 gene family, RT-PCR, reverse transcription-polymerase chain reaction, qPCR, quantitative polymerase chain reaction, MS-PCR, methylation specific-polymerase chain reaction, PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism.

proteomic techniques to identify patterns that could potentially differentiate BPH and PCa.

Tissue- and Semen-Based Biomarkers

Supplementary Tables 1 and 2 provide a list of tissue-based genomic and proteomic biomarkers, respectively, retrieved from 46 published papers, which are proposed as differentiating between BPH and PCa.

Supplementary Table 3 provides a list of biomarkers from seminal fluid retrieved from 3 published papers which are proposed as differentiating between BPH and PCa.

Discussion

The data presented demonstrate clear global research interest in finding biomarkers that can differentiate between PCa and BPH. It is also notable that major advances in both genomic and proteomic technologies have helped facilitate the identification and analysis of many novel biomarkers. The challenge now is to determine which biomarkers, or combination of biomarkers, provides the most effective way to risk stratify PCa patients in primary care. This will require careful, robust analysis to ensure the most useful candidates are selected.

It is interesting to note that the majority of biomarkers listed are all different, with very little overlap between different groups investigating the same biomarkers. One set of biomarkers that did receive attention from multiple groups were PSA glycoforms, again emphasizing the importance of PSA-related measurements for an accurate diagnosis. This is an umbrella term reflecting the various glycosylation profiles that PSA can possess, yet very few of the same PSA glycoforms were investigated by separate groups. The investigators used carbohydrate-binding proteins called lectins to detect these glycolytic changes in an immunoassay format.[152] All of the groups investigating various PSA glycoforms reported successful results in some form in both serum and urine. However, within other cancers, lectins typically only detected late-stage malignancies and a select few of the papers referenced here that reported noteworthy results did not supply the Gleason score. Hence, PSA-related measurements remain likely to be an important factor for PCa prediction and biopsy referral, but the information contained in the tables demonstrate that many other candidate biomarkers offer clear potential for improving PCa diagnosis, prognosis, and management. For example, Filamin-A and Filamin-B are mentioned in our tables but are also well cited as significant contributors to the differentiation of PCa from non-cancer patients. The two proteins play a major role in cell migration, vascular development, extracellular signaling and activity of integrins.[95,153] Filamin-A and androgen receptor (AR) association play a role in nerve growth factor (NGF) induced cell migration where it is known PCa is associated with the synthesis of large amounts of NGF which then stimulates tyrosine receptor kinase A (TrkA).[154] This is just one example of potential successful PCa biomarkers that have the possibility of identifying cancer quicker or preventing overdiagnosis within patients.

However, lack of follow-up in terms of validation and clinical trials means many of these candidate biomarkers will not progress beyond the discovery stage. Research efforts need to be improved in terms of validating these candidate biomarkers within larger cohorts to translate the findings to clinical practice.[155] Moreover, the implementation of improved risk stratification approaches for PCa

Table 2 Blood-Based Biomarkers Derived from Proteomic Techniques

Biomarkers	BPH (n)	PCa (n)	Method	All Significant ($p<0.05$)	Reference
Total Cholesterol↑, Triglycerides↑	40	40	Atomic Absorption Spectrophotometry	Yes	65
Free to Total PSA Ratio↓	283	49	Immunoassay	Yes	66
Pigment Epithelium-Derived Factor (PEDF) ↓, Zinc-α2-Glycoprotein (ZAG)	13	37	2D-DIGE, MS, WB, ELISA, IHC	No	67
Glutathione peroxidase 3 (GPx-3)↓, Apolipoprotein A-IV (ApoA-IV) ↓, ApoA-I↓, Coagulation Factor XIII B Chain↑, Antithrombin-III↓, α-1-Antitrypsin↓, α-2-Macroglobin↓, Thrombin↓, Kininogen-1↑	14	32	2D-DIGE, MS, NMR, Gene Ontology Enrichment Analysis	Yes	68
Matrix Metalloproteinase-26 (MMP26)↑	40	80	ELISA	Yes	69
Cyclin B1↑	21	174	ELISA, WB	Yes	70
Prostate Health Index (PHI)↑, α2,3-Sialylated PSA↑	29	50	Glycosylation Immunoassay	Yes	71
Human Growth Factor (HGF)↑, Vascular Endothelial Growth Factor (VEGF)↑, Omentin↑, Leptin↑	40	40	ELISA	Yes	72
Prostate Health Index (PHI)↑	150	113	Immunoassay	Yes	73
α1,2-Fucosylated PSA↑ and β-N-Acetylgalactosaminylated PSA↑	20	20	Lectin Column Chromatography, ELISA	Yes	74
Estradiol↑, Insulin↓, Insulin Growth Factor 1 (IGF-1)↓	70	70	Immunoassay	Yes	75
β-N-Acetylgalactosaminylated PSA↑	184	244	Immunoassay	Yes	76
Apolipoprotein A2 (APOA2)↓, Complement C3 Chain Fragment (C3f)↓, Inter-Alpha-Trypsin Inhibitor Heavy Chain 4 Fragment (ITIH4f)↓, alpha-1-Antitrypsin (AAT)↑, High Molecular Weight Kininogen (KNG)↑, Transthyretin (TTR)↑	8	8	2DE SS, WB, LA Chromatography, MS	Yes	77
tPSA↑, Carbonic Anhydrase 1 (CA1)↑, IL-6 Soluble Receptor (IL-6sR)↓, Spondin-2↓	120	100	Piezoelectric Assay	Yes	78
Platelet to Lymphocyte Ratio↑	110	76	Cell Count	No	79
Prolidase, Malondialdehyde, Superoxide Dismutase	51	30	Spectrophotometry	No	80
60S Ribosomal Protein L7 Clones↑	70	49	Protein Macroarrays	Yes	81
Glypican-1↓	15	15	ELISA, FC, WB	Yes	82
f/tPSA Ratio↓, α2,3-Sialylated PSA↑, Cathepsin D↑	100	75	ELISA	Yes	83
Secreted group IIA phospholipase A2 (sPLA2-IIA), C-reactive protein (CRP)	25	25	ELISA	No	84
Serum Amyloid A (SAA), Secreted Group IIA Phospholipase A2 (sPLA2-IIA), C-Reactive Protein (CRP)	55	55	Immunoassay	No	85
sPSP94/tPSA Ratio↑	44	33	ELISA	Yes	86

(*Continued*)

Table 2 (Continued).

Biomarkers	BPH (n)	PCa (n)	Method	All Significant ($p<0.05$)	Reference
α2,3-Sialylated PSA↑	35	35	Lectin Column Chromatography, ELLA	Yes	87
α1,2 Fucosylated PSA↑	13	13	ELLA	Yes	88
Dihydrotestosterone (DHT)↓	97	60	LC-MS/MS, IHC	Yes	89
TAR DNA-Binding Protein (TARDBP)↑, Talin-1↑, PARK7↑, The Lentiviral Integrase Binding Protein (LEDGF)↑, Caldesmon-1 (CALD1)↑	39	41	Microarrays	Yes	90
Alcohol Dehydrogenase Isoenzyme II (ADH II)↓	34	52	Spectrophotometry	Yes	91
Cluster of differentiation 40 (CD40L)↓	15	15	ELISA	Yes	92
Mac-2 Binding Protein (Mac-2BP)	50	50	ELISA	No	93
Serum Insulin-Like Growth Factor 1, Insulin-Like Growth Factor Binding Protein 3 (IGF1, IGFBP3)	113	36	ELISA, Radioimmunoassays	No	94
Filamin A, Filamin B, Keratin-19 (FLNA, FLNB and KRT19)	122	311	ELISA	Yes	95
Fibronectin 1↑, Afamin↑, α-2-HS-Glycoprotein Chain B, Ceruloplasmin↑, β-2-glycoprotein 1↑	5	5	iTRAQ, IHC, WB, MS	Yes	96
PSA 2-DE Subform F3↓	20	20	ELISA, Immunoadsorption, 2-DE, Immunodetection	Yes	97
Prostate Specific Antigen (PSA)↑, Prostatic Acid Phosphatase (PAP)↓	30	24	ELISA, Kinetic Method	Yes	98
Vascular Endothelial Growth Factor (VEGF)↑	57	44	ELISA	Yes	99
tPSA↑, Mean Platelet Volume↓, and Platelet Distribution Width↑	108	100	Immunoassay, Flux Cytometry	Yes	100
Fetuin ↑	-	-	Nanoelectrode Label Free Detection	Yes	101
Omentin↑, Blood Urea Nitrogen↑, Creatinine↑, Total Cholesterol (TC)↑, Low-Density Lipoproteins (LDL)↑, tPSA↑	30	50	ELISA, Spectrophotometry	Yes	102
tPSA↑, fPSA↑, f/tPSA Ratio↓, Ferritin↑, Triglycerides↑, Total Cholesterol (TC)↑, Low-Density Lipoprotein (LDL)↑, Very Low-Density Lipoprotein (VLDL(↑, Gamma-Glutamyltransferase (GGT)↑	951	2002	Immunoassay, Spectrophotometry	Yes	104
Claudin 3↑	69	15	MS	Yes	105
Testosterone/Prostate-Specific Antigen ratio (T/PSA)↓	92	164	Immunoassay	Yes	106
Serum PF4V1↓, tPSA↑	38	66	iTRAQ, IHC, WB, ELISA	Yes	107
Thioredoxin Reductase (TR)↑	100	120	ELISA	Yes	108

Note: ↓/↑Expression levels in PCa group compared to that of BPH group.

Abbreviations: ELISA, enzyme-linked immunosorbent assay, WB, Western blot, ELLA, enzyme-linked lectin assay, MS, mass spectrometry, FC, flow cytometry, IHC, immunohistochemistry, NMR, nuclear magnetic resonance, LC-MS, liquid chromatography-mass spectrometry, iTRAQ, Isobaric tag for relative and absolute quantitation.

Table 3 Urine-Based Biomarkers Derived from Genomic Techniques

Biomarkers	BPH (n)	PCa (n)	Method	All Significant ($p<0.05$)	Reference
PRCAT17.3↑, PRCAT38↑	19	19	qPCR	Yes	109
let-7e↓, let-7c↓, miR-30c↓, miR-25↓, miR-346↑, miR-622↑, miR-940↑, miR1285↑	17	25	qPCR	Yes	110
PCA3↑	26	22	qPCR	Yes	111
RASSF1↑, GSTP1↑, RARB↑	32	253	qPCR	Yes	112
miR-222-3p↓, miR-24-3p↓, miR-30c-5p↑	29	215	RT-PCR	Yes	113
miR-222-3p↓, miR-24-3p↓, miR-30c-5p↑	289	758	RT-PCR	Yes	114
miR-21-5p↑, miR-141-3p↑, miR-205-5p↑	22	23	qPCR	Yes	115
miR-1825↑, miR-484↓	12	8	WGS	Yes	116
HIST1H4K	29	57	qPCR	No	117
Prostate Cancer Antigen-3 (PCA3)↑	40	24	qPCR	Yes	118
UDP-N-Acetylglucosamine Pyrophosphorylase 1 (UAP1), PDZ and LIM Domain 5 (PDLIM5), Inosine Monophosphate Dehydrogenase 2 (IMPDH2), Heat Shock Protein Family D Member-1 (HSPD1), Prostate Cancer Antigen-3 (PCA3), PSA, Transmembrane Serine Protease 2 (TMPRSS2), ERG, Glyceraldehyde 3-Phosphate Dehydrogenase (GAPDH), Beta-2-Microglobulin (B2M)	60	61	RT-PCR	Yes	119
Prostate Cancer Antigen-3 (PCA3)↑, PSA↑	26	70	qPCR	Yes	120
Whole-Genome Gene Expression	24	25	WGS	No	121
Cadherin 3↓	4	6	WGS	Yes	122
miR-100/200b↑	70	73	qPCR	Yes	123
miR-148a↑, miR-375↑	23	215	RT-PCR	Yes	124
miR-21↓	143	23	qPCR	Yes	125
Exosomal miR-2909↑, miR-615-3p↑	10	90	qPCR	Yes	126
S100A8↓, S100A9↓	363	283	qPCR	Yes	127
miR615-3p↑, hsv1-miR-H18↑, hsv2-miR-H9-5p↑, hsa-miR-4316↑	5	14	miRNA Microarray	Yes	128
PCA3/PSA ratio↑	18	34	RT-PCR	Yes	129

Note: ↓/↑Expression levels in PCa group compared to that of BPH group.

Abbreviations: Let-7, lethal-7 gene family, miRNA, microRNA, qPCR, quantitative polymerase chain reaction, RT-PCR, reverse transcription polymerase chain reaction, MS-PCR, methylation specific-polymerase chain reaction, WGS, whole genome sequencing, miRNA, microRNA.

must be practically and financially feasible, using approaches that return results in a timely manner.

The variety of biomarkers under investigation has inevitably led to the development of several commercially available prostate cancer tests that variously rely upon protein and gene expression measurements in biopsy, blood and urine samples.[156] Each test utilizes a selection of different biomarkers, such as PSA-related measurements, expression of selected genes, the extent of gene methylation or detection of PCa-related gene fusions. Each test is positioned for clinical use in various cohorts of men at different stages of the disease management process, either before or after biopsy or following treatment. More tests are in development by

Table 4 Urine-Based Biomarkers Derived from Proteomic Techniques

Biomarkers	BPH (n)	PCa (n)	Method	All Significant ($p<0.05$)	Reference
48 Protein Groups	9	9	MS	Yes	130
Glypican-1 (GPC-1)↑	37	41	Immunoassay	Yes	131
Maltose-Binding Protein (MBP)↑, Apolipoprotein A1 (APOA1)↓, Fibrinogen Alpha Chain (FGA)↑, Fibrinogen Gamma Chain (FGG)↓, HP↓, Inter-Alpha-Trypsin Inhibitor Heavy Chain 4 (ITIH4)↑, Serpin Family A Member 1 (SERPINA1)↓, Transferrin↓, Transthyretin (TTR)↓	16	16	2-D DIGE, MS	Yes	132
Matrix Metallopeptidase 9 (MMP-9)↓	8	30	Gelatin Zymography	Yes	133
Fibronectin↓, TP53INP2↓	12	8	LC-MS/MS	Yes	134
PSA Glycoforms	61	38	LC-MS/MS	Yes	135
Saposin B↓, Inter-α-Trypsin Inhibitor Light Chain (ITIL) Fragments↑	16	13	2DE, SS	Yes	136
Beta-2-Microglobulin (B2M)↑, Pepsinogen 3↑, and Mucin 3A↑	83	90	iTRAQ LC/LC/MS/MS	Yes	137
PSA Glycoforms	32	30	LC-MS/MS	Yes	138
Furan↑, 2-ethylhexanol↓, 3,5-dimethylbenzaldehyde↓, Santolin Triene↓, 2.6-dimethyl-7-octen-2-ol↓	21	29	GC-MS	Yes	139
56 Intact N-glycopeptides	6	6	HILIC	Yes	140
Fucα1-6/3GlcNAc PSA Glycoforms↑	15	16	Immunoassay	Yes	141
Survivin↑	20	39	ELISA, WB	Yes	142
Osteopontin↓, Prothrombin↓ Peptides	20	28	LC-MS/MS	Yes	143
Engrailed-2 (EN2)↑	76	66	ELISA	Yes	144
Arginine↑, Homoserine↑, Proline↑	50	50	LC-MS/MS	Yes	145
α-Methylacyl-CoA Racemase (AMACR), Hepsin	76	66	ELISA	No	146
Spermine (Spm)↑	88	66	UPLC-MS/MS	Yes	147
PSA Glycoforms	93	74	Capillary Electrophoresis	Yes	148
Urinary Vesicle-Associated PSA Extraction Ratio↑	122	85	TEM	Yes	149
Sarcosine/Creatinine Ratio↑	208	209	Sarcosine Oxidase Method	Yes	150
PF4V1↓, tPSA↑, urinary CRISP3↑	48	86	iTRAQ LC	Yes	107
Ferritin-Creatinine Ratio↑	3	3	2DE, MS, WB	Yes	151

Note: ↓/↑: Expression levels in PCa group compared to that of BPH group.

Abbreviations: MS, mass spectrometry, 2D DIGE, 2-dimensional fluorescence difference gel electrophoresis, LC-MS, liquid chromatography-mass spectrometry, ELISA, enzyme-linked immunosorbent assay, WB, Western blot, ELLA, enzyme-linked lectin assay, IHC, immunohistochemistry, iTRAQ, isobaric tag for relative and absolute quantitation, GC-MS, gas chromatography-mass spectrometry, HILIC, hydrophilic interaction liquid chromatography, UPLC-MS, ultra-performance liquid chromatography-mass spectrometry, TEM, transmission electron microscopy.

various manufacturers and these will be on the market soon. However, there is no consensus about which of these tests is best and it is likely that multiple biomarkers must be considered by clinicians as part of the decision-making process for any given individual.

Future Perspectives

It is clear that there is a move towards the use of multiple biomarkers in a risk prediction model, which could include a combination of proteomic, genomic and clinical measurements. These multivariate models

should result in a stronger degree of accuracy compared to that of a single marker for the prediction of PCa. The standardized use of such risk stratification is recommended by the recent NICE guidelines.[157] Several different models have been developed and are currently employed, each demonstrating their superiority to the use of tPSA measurement alone. The 4Kscore is a multivariate model that is used to identify the risk of aggressive PCa. Using a panel of 4 protein biomarkers, combined with clinical information, such as age and DRE results, the model achieved an AUC of 0.90 in one trial (n=1012).[158] Similarly, the Stockholm-3 risk-based model (S3M) incorporates several plasma protein biomarkers (PSA, free PSA, intact PSA, hK2, MSMB, and M1C1) together with clinical data and individual genetic information to predict the likelihood of PCa. Using a validation cohort comprising of 47,688 men, the S3M was estimated to reduce the number of men biopsied by 53%, as well as avoiding 76% of negative biopsies when compared to the standard tPSA method.[159] Other risk stratification tools include the European Randomized Study of Screening for Prostate Cancer (ERSPC) risk calculator, which uses PSA, DRE, prostate volume and previous biopsy status to predict PCa risk.[160] In North America, the Prostate Cancer Prevention Trial (PCPT) risk calculator uses PSA, DRE, family history, previous biopsy status, age, and race to do the same.[161] Interestingly, one Irish study which directly compared these two models concluded the EPSRC was superior for the prediction of PCa in an Irish population (n=2001) and advised combining it with the PHI model to improve accuracy.[162] A study by Murphy et al in 2018 investigated a combination of data from DNA methylation, transcripts, protein and glycosylation biomarkers for use in a single PCa biomarker panel. Using modeling techniques on almost 200 variables the authors achieved an AUC of 0.91 when differentiating various stages of PCa severity from indolent to aggressive (n=158). This article is the first to incorporate data from five omic platforms retrieved from tissue and serum and clearly shows that the accuracy and predictive power of PCa models come from this multi-platform approach.[163] Others have illustrated how technology can incorporate the measurements for ease of use. The Rotterdam Prostate Cancer Risk Calculator (RPCRC) utilizes a smartphone app with up to 11 clinical parameters used in combination to predict the risk of PCa and stratify those patients in need of a prostate biopsy. The algorithm has been used on around 6500 patients so far with AUC 0.72–0.81 for the prediction of PCa.[164] Although there is no consensus yet about which of these models is best, it is clear that many men presenting with symptoms of PCa could benefit from the use of these multivariate models to help determine their subsequent treatment.

Together, these studies demonstrate that the use of PSA screening can be improved to develop a more sophisticated method for the diagnosis and risk prediction of PCa. With such a vast majority of biomarkers being investigated, it is unfortunate that none have found clinical utility. The pathogenesis of PCa is complex and it is unlikely a single biomarker will arise as a replacement to PSA. Nonetheless, there is clear scope for further novel discoveries in this area, particularly in identifying new biomarkers that can be included in multivariate models that can aid PSA in identifying PCa patients earlier.

There is also an emerging role in the use of exosomes to aid in the diagnosis of PCa. Exosomes are small vesicular bodies released from a whole array of cells, they contain miRNA, mRNAs, and proteins that possess the potential to regulate signaling pathways in cells.[165] Nilsson et al described in 2009 how they showed the presence of two known PCa biomarkers (PCA3 and TMPRAA2:ERG) within exosomes of urine patients, which in turn shows the potential for diagnosis and monitoring of patients in cancer care.[166] One review states that exosomes have the potential of predicting the prognosis of castration-resistant PCa, inducing PCa drug treatment sensitivity as well as being used as a marker for PCa drug response. In addition, exosomes can be used as a delivery vector to target malignant cells, as well as being utilized in tumor vaccination.[165] However, with no gold standard in place for exosome isolation to validate these promising findings, it will not be possible until a uniformly defined method of isolation and characterization is in place.[167] Nevertheless, with the accumulation of evidence, exosomes may prove to be a valuable source of biomarker material for informing PCa diagnosis, prognosis and therapy in the very near future.

Summary

Management of PCa in primary care presents several challenges for clinicians. Current diagnosis, based primarily on DRE and PSA measurements, can result in patients being sent for invasive biopsies that they do not require. A diagnostic test that can more accurately distinguish BPH

from PCa would help alleviate clinical uncertainty in primary care, with tangible benefits for both the individual patients and the healthcare system. The use of multivariate biomarker measurement is required to differentiate BPH from PCa. Combining selected biomarkers with clinical risk factors could be used to build a robust risk stratification model that could effectively triage patients within primary care; low and high risk. However, this clearly depends on the identification and validation of an optimal combination of biomarkers to use, so continued research is required to identify the combination of biomarkers with the sensitivity and specificity to direct clinical management.

Disclosure

Dr Mark W Ruddock is an employee of Randox Laboratories Ltd and holds no shares in the company. Mr Christopher McNally is a PhD student who Dr Ruddock jointly supervises with Dr Declan McKenna and Professor Tara Moore (both Ulster University). Dr Ruddock is the industrial supervisor at Randox Laboratories Ltd. Dr McKenna and Professor Moore are the academic supervisors at Ulster University. The PhD studentship is funded by the Randox-Ulster UniversityPhD Academy. The authors report no other conflicts of interest in this work.

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