Identification and evaluation of clinically significant prostate cancer: a step towards personalized diagnosis

DOI:
10.1097/MOU.0000000000000385

Document Version
Accepted author manuscript

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Abstract

Purpose of review:

Prostate cancer diagnostics are evolving rapidly. The quest to differentiate "clinically significant" from "clinically insignificant" disease has gathered momentum, leading to substantial change in traditional diagnostic approaches. Herein we review the relevant information on currently available biomarkers and assess their ability to help physicians and patients in making a shared and personalised decision based on their individual risk of harbouring clinically significant disease.

Recent findings:

Serum, urine, tissue and imaging biomarkers have been evaluated to improve the identification of clinically significant disease and this international effort has yielded promising, but not always consistent results. Changes in MR imaging technology have realized a quantum change and this facility is now becoming more widely incorporated into diagnostic and disease risk-stratification protocols. However, standardization and further validation is required.

Summary:

Acceptance and widespread adoption of serum, urine and genetic markers is awaited but novel and promising techniques alone and in combination have emerged. With validation and further focus these may be adopted more widely.

Keywords

Prostate cancer, biomarkers, genomics, multiparametric magnetic resonance imaging

Keypoints

1. Personalized risk stratification should be used when considering testing for, and biopsy of the prostate to detect clinically significant prostate cancer.

2. Although multiparametric magnetic resonance imaging (mpMRI) is beneficial in biopsy negative patients suspected of harbouring prostate cancer, it's utility in avoiding prostate biopsy needs further evaluation. The use of concurrent novel biomarkers may strengthen its use in diagnosis and risk stratification in the future.

3. Newer tissue based molecular prognostic tests have to be interpreted cautiously keeping in mind the multifocal and heterogeneous nature of prostate cancer.

4. Newer blood based biomarkers such as the 4kscore and PHI are promising, but clear cut-offs are lacking for diagnosis of significant disease.

Introduction

The importance of cancer biomarkers is exemplified by the development and clinical use of prostate specific antigen (PSA) testing in prostate cancer (PCa). Since its discovery PSA's use became internationally ubiquitous within 10 years and its use in screening and case finding is mainly responsible for the increased detection, and documented clinical incidence of this disease, particularly in the developed world (1). Whilst early detection may be important, the PSA story highlights another problem, that of over-detection and overtreatment (2). The classic paper by Franks showed that "indolent" PCa was commonly present and with increasing frequency as men aged (3). It has taken the publication of 2 large trials nearly 60 years later to reaffirm the notion that many patients diagnosed have "clinically insignificant" disease which can be observed quite safely without the need for intervention with surgery or radiotherapy (2, 4). Thus, the concept of clinically "insignificant" versus "significant" PCa has been "rediscovered"; but in modern practice are we sufficiently confident that we can discriminate between the two? Various criteria for defining clinically significant and insignificant PCa exist (5) but can they identify clinically significant PCa accurately? In this review, we discuss the *status quo* regarding the ability of serum, urine, tissue and imaging biomarkers to make this distinction.

Risk stratification

The decision to pursue identification of potentially curable PCa in age-eligible men is complex and controversial. It involves consideration of a person's age, race, family history, personal history, comorbidities, life expectancy and personal preferences. Therefore, the first step in identifying clinically significant PCa in age-eligible asymptomatic men should be to counsel them prior to PSA testing (6-8), thereby informing them of the possibility of detection of low-grade cancer in relation to their life expectancy and risk of disease progression. Tools are available for this (9) and in elderly, co-morbid patients unnecessary biopsies can be avoided, particularly with the use of adjunctive tests (10). Similarly, where there is high risk, more detailed investigation can be encouraged. This is particularly important in men with inherited high risk, such as African-Americans and men with a family history of a first-degree relative with PCa. African-American men have a higher incidence and mortality compared with Caucasian men (11) and men with first-degree relatives with PCa have a substantially increased risk (12-14).

Men from BRCA gene mutation affected families are also susceptible to the development of aggressive, clinically progressive disease. Germline mutations in BRCA1, BRCA2 or DNA mismatch repair genes are known to be associated with increased PCa risk, with a 6.3 and 3.67 fold chances of developing PCa respectively (15, 16). Those with BRCA mutations have a more aggressive phenotype and significantly reduced survival (17-19).

Blood and urine based biomarkers

PSA is currently the first-line blood based biomarker for PCa detection and risk stratification. It is a continuous biomarker, and prostate, but not PCa specific. There is no value below which the risk of Gleason score (GS) ≥ 7 cancer is zero and the risk of aggressive cancer increases with increasing level (20). Table 1 lists PSA along with other select biomarkers and their sensitivity specificity for detecting clinically significant cancers (21-27). Age specific and race-specific cut-offs have been proposed with some utility by several studies (28, 29) but the role of these specific cut-offs in current practice remains unclear.

PSA kinetics, namely velocity and doubling time are of value. PSA velocity (PSAV) is the absolute annual increase in serum PSA (ng/mL/year), whilst PSA doubling time (PSADT) measures the exponential increase in serum PSA over time. These measurements have been investigated in various studies with conflicting results in their ability to predict the presence of cancer or its aggressiveness (eg identification of Gleason score ≥ 7). Currently, PSAV and PSADT have a prognostic role after treatment but a limited role in diagnosis (30, 31). Another PSA based measurement, PSA density (PSAD) is calculated as serum PSA divided by prostate volume. PSAD is helpful in identifying patients with high PSA secondary to benign prostatic hyperplasia (BPH). It has been found to correlate with aggressiveness (32) but its utility is limited due to lack in the precision of prostate volume measurement.

Free PSA (fPSA) is the unbound fraction of PSA in blood and the ratio of fPSA:total is commonly used in clinical practice. However, it is not a specifically accurate test for diagnosis or biological aggression. Its value is also affected by external factors such as temperature and appropriately reliable and repeatable cut-off thresholds have been difficult to produce (33). fPSA use has therefore been developed further: it forms a part of two recently introduced blood-based markers, the 4Kscore and the prostate health index (PHI). The 4Kscore is a prediction model, which integrates clinical variables (age, prior biopsy, digital rectal examination (DRE) results) and levels of four kallikreins: total PSA, fPSA, intact PSA and human kallikrein 2. It generates a score that predicts the patient's risk of GS ≥ 7 on biopsy. It has been evaluated in multiple studies for prediction of GS ≥ 7 on biopsy, risk of upgrading on RP and risk of development of metastatic disease (22, 34). Another blood-based test is PHI, which combines total PSA, fPSA and serum isoform [-2]proPSA using a mathematical equation: ([-2]proPSA/fPSA) X √(PSA). PHI has also been tested in multiple studies and has been found to predict GS ≥ 7 on biopsy, upgrading/upstaging on prostatectomy and recurrence (23). Both, these tests have shown promising preliminary results in guiding decisions for initial and repeat biopsy. However, these tests rely inherently on the use PSA, and clear cut-offs have not yet been set. Another recently reported test, the "Stockholm-3" test (10) uses fPSA in combination with an array of other PCa risk associated factors (PSA, free PSA, intact PSA, hK2, MSMB, MIC1), genetic polymorphisms (232 SNPs), clinical variables (age, family, history, prostate examination and previous prostate biopsy). This test performed significantly better than PSA alone for detection of cancers with a Gleason score of at least 7 and claimed the potential "to reduce unnecessary biopsies without compromising the ability to diagnose PCa with a Gleason score of at least 7", However, this test needs to be validated in large scale independent studies before its use can be recommended more generally.

Urine based biomarkers include PCA3, TMPRSS2:ERG and the Mi-Prostate Score (MiPS). PCA3 is a non-coding RNA that is measured after digital rectal examination (DRE). It was FDA approved in 2012 for patients with initial negative biopsy and high suspicion of PCa but current studies have reported conflicting results in its association with clinically significant disease (24, 35-37). TMPRSS2:ERG is a gene fusion associated with the early onset of PCa. Its implications in clinical practice remain unclear, with studies demonstrating conflicting data in predicting biochemical recurrence following radical prostatectomy (38, 39). Evaluation of TMPRSS2:ERG within an active surveillance group has shown an association with progression risk (58.6 Vs 21.7%; HR: 2.45)(40) but this remains un-validated. MiPS combines urine PCA3 and TMPRSS2:ERG to serum PSA level to generate a risk score. It predicts the presence of PCa and high-grade PCa on biopsy (41). The uncertainty in using these biomarkers is reflected in their adoption by national and international guidelines for PCa diagnosis and treatment: the NCCN and EAU guidelines recommend PCA3 and PHI usage, whilst NICE has recommended against their use (6, 8, 42).

Tissue markers

Gleason grading is an indispensable risk stratification system based on the microscopic appearance of glandular architecture. Initially proposed in 1966, it has been incorporated into various risk stratification algorithms (43). More recently, in 2005 and 2014, the International Society of Urological Pathology (ISUP) consensus conferences updated this PCa grading system. A new five-tiered prognostic grade grouping (PGG) system was adopted with 90% consensus at the 2014 ISUP (44). Five Gleason score groups were considered: ≤6; 3 + 4 = 7; 4 + 3 = 7; 8; and 9–10 as PGG 1 to 5 respectively. This new revision makes distinction between Gleason 3+4 and 4+3 and separates them as two different entities with distinct clinical outcomes. Subsequently, in 2016 different studies have reported and validated that each PGG is associated with a different risk of biochemical recurrence, metastatic free survival and PCa specific death (45-47). Furthermore, Rubin et al. (48*) investigated and reported the genomic correlates to the PGG in a pooled cohort of 426 PCa cases. Of these 426 tumors, none of the 74 belonging to PGG 1 (Gleason ≤ 6) exhibited polyploidy and they harbour very few gene amplifications as compared to PGG 2-5. Interestingly, there was no significant difference in the number of aberrant genes between PGG 4 and PGG 5, suggesting that the reported difference in clinical outcomes in PGG 4 and PGG 5 could be due to epigenetic changes or currently undescribed alterations.

Tissue based molecular prognostication assays

A number of molecular assays have been developed recently to assess the genome, methylation status, transcriptome or proteome for prognostication in PCa. These tests aim to further discriminate newly diagnosed PCa in to low and intermediate risk categories.

Oncotype DX PCa test (Genomic Health Inc., Redwood City, CA, USA) is a RT-PCR gene expression assay. It is used to predict primary Gleason pattern 4, any Gleason 5 pattern, pT3 disease, or BCR in NCCN low to intermediate risk patients (49). It comprises 5 housekeeping genes and 12 genes involved in four tumorigenic pathways: the androgen pathway (AZGP1, KLK2, SRD5A2, and FAM13C), stromal response (BGN, COL1A1, and SFRP4), proliferation (TPX2) and cellular organization (FLNC, GSN, TPM2, GSTM2) (50). Prolaris (Myriad Genetics, Inc.) is another qRT-PCR based gene expression assay which measures the proliferative index and predicts PCa specific death. It evaluates 15 housekeeping genes and 31 cell cycle related genes. ProMark (Metamark Genetics) is an 8 protein based signature (CUL2, SMAD4, PDSS2, HSPA, DEL2L1, pS6, YBOX1 and FUS). It is used for predicting two co-primary end-points: (i) Gleason ≥ 4+3 or non-organ confined disease, (ii) Gleason ≥ 3+ 4 or non-localized disease. It analyses the region of interest using a quantitative multiplex immunofluorescence platform. Currently, Oncotype DX and Prolaris are recommended post diagnostic biopsy as a part of NCCN guidelines for use in patients with very-low to low risk PCa and a 10-20 year life expectancy (8). They are not currently used regularly in general urological practice outside the US.

Imaging as a biomarker

The development of multiparametric MRI (mpMRI) has shifted the focus of MRI from local staging of PCa to the detailed characterisation of primary prostatic lesions. This has generated considerable interest and debate as a non-invasive imaging biomarker with the promise of improving detection of clinically significant disease through targeted biopsies and reduction in the detection of clinically insignificant cancer.

Prostate imaging reporting and data system (PIRADS) scoring

To standardise the acquisition parameters, interpretation, grading and reporting of mpMRI in PCa, PIRADS was initially proposed in 2012. This has now been replaced with the more robust PIRADSv2 guidelines (51). However, there are still potential gaps and concerns regarding interpretation and scoring that need addressing (52). Moreover, the revised PIRADSv2 has only moderate inter-observer agreement and accuracy for detection of clinically significant disease (53). Good quality pictorial atlases from high volume centres enabling further refinement in interpretation guidelines are still required and indeed, some leading PCa research groups have not adopted PIRADS scoring because of concerns about its interpretation and reliability.

Can mpMRI avoid biopsy in insignificant prostate cancer?

A recent meta-analysis concluded that mpMRI detects clinically significant cancers with a sensitivity, specificity and negative predictive value (NPV) of 58-96%, 23-87% and 63-98% respectively (25). This meta-analysis only included studies using PIRADS scoring and therefore only one study which used patient specific whole mount post-RP analysis as a reference standard was included. Another recent study, which used PIRADSv2 as scoring criteria, reported correct identification of 94-95% PCa foci ≥ 0.5ml. While, this study was an unblinded retrospective analysis, an important caveat demonstrated was the study's inability to detect 75% of lesions with GS ≥ 4+3 which were <0.5ml (54). This highlights a significant limitation of the use of mpMRI for screening and AS. Other studies used post-RP cross-sectional analysis as a reference standard and evaluated mpMRI in a blinded manner using the Likert scale(26, 27). These data highlight the fact that although mpMRI has a high NPV depending on the prevalence of PCa in the screened population, it also misses significant cancers, particularly small, high-grade lesions (Table 1).

mpMRI is now widely incorporated within diagnostic protocols for its role in guiding diagnostic intervention, particularly in patients with previous negative biopsy and high suspicion of PCa. It has the potential to improve detection of clinically significant disease through targeted biopsies whilst reducing the detection of clinically insignificant cancer. mpMRI has also seen widespread incorporation within AS protocols, but the current data about its long-term accuracy is lacking and robust evaluation studies are required to define radiographic progression in patients on surveillance programmes (55). A recent study evaluated 207 men undergoing MRI fusion biopsy during AS. The authors reported that MRI-ultrasound fusion biopsy increased the upgrading in AS patients and in a small subset of patients missed by systematic biopsy, upgrading also occurred in areas outside MRI targeted biopsy fields, suggesting that systematic sampling should also be offered (56). Understanding of the utility of mpMRI has improved with the publication of the PROMIS trial (57, 58*). It reported the accuracy of mpMRI in differentiating between insignificant and significant PCa, defined as Gleason ≥4+3 and/or maximum cancer core length ≥6mm, proposing that this methodology might enable the avoidance of "unnecessary" prostate biopsies in men with mpMRI defined insignificant disease. The results have generated significant debate. The study evaluated 576 men with a raised PSA up to 15ng/mL. Each underwent an mpMRI which was followed by TRUS guided and template guided core prostate biopsies. The results for mpMRI published a sensitivity of 93% (95%CI 88-96), specificity of 41% (36-46), positive predictive value 51% (46-56) and negative predictive value of 89%. TRUS biopsy demonstrated significantly less sensitivity that mp-MRI; 48% (95%CI 42-55) (p≤0.0001 and had a significantly worse negative predictive value than mp-MRI; 74% (69-78) vs 89 (83-94) (p≤0.0001) (although TRUS biopsies were performed immediately after template biopsies in this study, potentially reducing their accuracy). It was concluded that the TRUS biopsy alone is no longer an appropriate method for diagnosing "significant disease", and the authors proposed two

strategic approaches: using mpMRI alone as a triage tool to avoid biopsies in 27% of men (with 2% fewer clinically significant cases being detected) and using biopsy targeted to mpMRI findings to achieves the sensitivity of template mapping biopsies. According to the authors, triage with mpMRI could avoid primary biopsy in 27% of cases but would detect 3% more cases of clinically significant cancer than standard TRUS biopsy for all pathways. Discussion following publication of the PROMIS trial revolves around the use of mpMRI in patients with clinically significant but low volume disease, defining Gleason 3+4 as "insignificant disease" (particularly in young patients), questioning the trial definition of significant disease in routine clinical practice and accepting a 10% "miss rate" for Gleason 3+4. There is also the issue of applicability of mpMRI results in departments where specialist radiological expertise is not always of the standard seen in a high volume cancer centre. A considerable strength of the study however was its negative predictive value in excluding insignificant disease which might safely be observed.

MRI/Ultrasound Fusion guided prostate biopsies

Image guided biopsies are commonly practised in many other solid urological and non-urological tumours. Unlike other solid tumours, prostate biopsies traditionally rely upon randomly sampling the whole organ. The development of mpMRI technology has enabled the identification of prostatic lesions susceptible to targeted biopsy. Fusion technology superimposes mpMRI images onto TRUS software enabling real time dynamic identification of lesions. This greatly assists the clinician in focal biopsy of a prostatic lesion either using standard TRUS, or trans-perineal guided biopsies, depending on lesion location and clinician preference. Studies evaluating this technology have commented that fusion biopsies can detect 30% more significant PCa when compared to standard biopsies and with 17% fewer insignificant cancers (59). The addition of standard biopsies to fusion-targeted biopsies simply increased the insignificant disease pick up rates. Furthermore, similar clinically significant PCa detection rates have been reported irrespective of mpMRI guided biopsy technique (60).

Implications of intra-tumor and inter-tumor heterogeneity

Up to 60-90% cases of PCa are multi-focal in nature (61). Moreover, recent genomic studies have reported inter-tumour and intra-tumour heterogeneity in PCa (62, 63). Liu et. al. using a cohort of 30 men with metastatic disease from a rapid-autopsy program also suggested that metastatic clones may share a monoclonal origin (64). However, the origin of metastatic disease from index lesions is not yet proven conclusively (65, 66). Non-index lesions have also been reported to invade locally and to metastasise (67-69). More recently, Wei et. al. evaluated 26 non-contiguous foci from 4 patients (70*). CNA, WES and RNA-sequencing of these 26 foci revealed considerable intra-tumoral and inter-tumoral heterogeneity. Furthermore, using the gene expression data, the authors generated Oncotype DX, Prolaris and Decipher scores and demonstrated intra-prostatic heterogeneity. The authors concluded that there was widespread genetic diversity between the different PCa foci and a molecular prognostication test from a single biopsy is not sufficient to dictate treatment. Nevertheless, this study had limitations: first it included patients with intermediate to high risk disease that don't accurately reflect the type of patients requiring prognostication; and second, the authors only quantitated genes used in the commercial tests and didn't use the commercial tests themselves. Nonetheless, these studies highlight a critical limitation of using a single core or foci for prognostication using tissue based molecular and imaging tests. Imaging tests might miss high-grade small foci of high malignant potential, and basing a molecular prognostication test based on a mpMRI targeted biopsy may lead to false negative results. Further studies are required exploring and linking the two.

Perfecting imperfect biomarkers

While the quest for elusive "perfect" diagnostic biomarker continues, physicians currently have to rely on sequencing of emerging biomarkers in certain "grey areas". Due to limited literature on biomarker sequencing, an optimal strategy requires an implicit understanding of sensitivity, specificity and limitations of each individual test to personalize the diagnostic pathway based on clinical priorities. Currently only a few studies have reported on sequencing PCA3 and PHI with mpMRI whilst similar data for 4Kscore is awaited (71-74). A 2015 clinical and cost-effectiveness analysis conducted by the National Institute of Health Research (NIHR) evaluated PCA3 and PHI along with mpMRI (74). This report concluded that the addition of PCA3 or PHI to mpMRI did not have a noticeable impact on diagnosis of prostate cancer in men who are suspected of PCa and in whom the results of an initial biopsy were negative or equivocal. However, a few limitations highlighted by this report were (i) lack of information on sequencing of these tests, (ii) insufficient evidence on appropriate threshold values of PCA3 and PHI for clinical use and (iii) issues due to uncertainty in defining clinically significant and insignificant PCa. More recent studies have attempted to address these concerns and suggest that PCA3 and PHI would primarily benefit in patients with a negative or equivocal mpMRI (PIRADS 1-3) (72, 73, 75). For instance, a study modelled four sequencing combinations of PCA3 and mpMRI in biopsy naïve men (72). The diagnostic pathway with mpMRI followed by PCA3 testing in patients with low to equivocal mpMRI was reported to be the best. This pathway avoided 36.3% biopsies whilst missing 4.9% Gleason ≥ 7 cancers. Cohort studies evaluating the performance of PHI along with mpMRI have also suggested its benefit in patients with negative or equivocal mpMRI (73, 76). Furthermore, in repeat biopsy scenario, a joint consensus statement by the American Urological Association (AUA) and Society of Abdominal Radiology (SAR) have strongly advocated initial mpMRI when optimal imaging is possible, while reserving ancillary markers (PCA3, PHI and 4Kscore) for patients with PIRADSv2 score 1-2 (77). Table 2 summarizes their potential role in evolving diagnostic pathway.

However, the issues of prioritizing in the initial biopsy setting remain unresolved. Prioritizing blood and urine based biomarkers without mpMRI, risks limitation relating to imprecise biopsy techniques whilst "scan all, biopsy only visible, avoid invisible" scenario risks of missing aggressive PCa. Therefore, a two-tiered approach in patients with PSA 3-10 ng/mL might be more appropriate to strike a balance. Current NCCN guidelines state that 4Kscore and PHI may be beneficial in "grey areas" but also points out that mpMRI may maximize detection of aggressive disease in the initial biopsy setting (with reservations about its routine usage) (8). Sequencing these biomarkers to maximize clinically significant PCa detection, minimize insignificant PCa findings in a cost-effective, reliable population based setting still requires further study before a consensus on their use can be agreed.

Conclusion

The future of PCa diagnostics will continue focus on improving the yield of clinically significant disease whilst minimising the invasive nature of tests which often result in the diagnosis of biologically insignificant disease. For these changes to occur a consensus needs to be sought regarding the definition of clinically significant PCa to drive these changes. This consensus is currently some way off.

Traditional diagnostic protocols have begun to incorporate modern diagnostic techniques including the widespread adoption of mpMRI into routine PCa diagnostics, with increasing investment and availability of mpMRI/Ultrasound fusion guided technology within urological units.

However, the urological community is still unable to dispense safely with prostatic biopsy, although imaging for gland/lesion characterization and targeted sampling are clearly beneficial. Continued refinements of mpMRI technique, with standardization of image interpretation are still required. The addition of genetic/serum markers may also help to reassure the clinician and the patient that their disease is truly low-risk but large-scale validation of these tests are needed. Ultimately, the acid test for future diagnostics will be their ability to define the “grey area” between the diagnostic categories more clearly, thereby turning the art of diagnosis and risk stratification into a more exact science.

Acknowledgements

None.

Financial support and sponsorship

This work was supported by the Belfast-Manchester Movember Centre of Excellence (CE013_2-004), funded in partnership with Prostate Cancer UK to N.W.C and by start-up fund from CRUK to E.B.

Conflicts of interest

There are no conflicts of interest.

Table 1: Select biomarkers with their sensitivity and specificity for distinguishing clinically significant prostate cancer.

Test name (reference) and cut-offs if applicable	Number of patients	CS cancer definition	Sensitivity (%)	Specificity (%)
PSA (21)	5575	Gleason ≥ 7 on biopsy		
1.1			92.8	37
3.1			57.6	82.3
4.1			40.4	90
6.1			13.2	97.8
4KScore Panel (22)	1012	Gleason ≥ 7		
≥6			94.4	39.3
≥15			79.2	75.7
PHI (23)	1157	Gleason ≥ 7	90	17
PCA3 (24)	305	Gleason ≥ 7		
>24			78.3	46.4
>35			63.8	57.1
mpMRI (25-27)	1981 (25)	Meta-analysis comprising different definitions of clinically significant cancer	58-96	23-87
	297 (26, 27)	Tumor size ≥ 0.5cc or ≥1cm diameter	77.85	72.95
		Gleason ≥ 7	79.76	71.36

CS- Clinically significant, PSA – Prostate specific antigen, PHI – Prostate health index, mpMRI – multiparametric magnetic resonance imaging, RP- Radical prostatectomy

Table 2: Potential role of different biomarkers in evolving diagnostic pathway of prostate cancer.

Pre-diagnosis		Post-diagnosis
Initial biopsy	**Re-Biopsy**	**Prognostic markers**
PSA or mpMRI or percent free PSA or PCA3 or PHI or 4Kscore	**mpMRI and/or PSA, 4Kscore, PHI, PCA3, percent free PSA**	**Oncotype DX or Prolaris**
mpMRI – multiparametric magnetic resonance imaging, PHI- prostate health index		

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