

Review

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Biomarkers for prostate cancer: prostate-specific antigen and beyond

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Abstract: In recent years, several new biomarkers supplementing the role of prostate-specific antigen (PSA) have become available for men with prostate cancer. Although widely used in an *ad hoc* manner, the role of PSA in screening asymptomatic men for prostate cancer is controversial. Several expert panels, however, have recently recommended limited PSA screening following informed consent in average-risk men, aged 55–69 years. As a screening test for prostate cancer however, PSA has limited specificity and leads to overdiagnosis which in turn results in overtreatment. To increase specificity and reduce the number of unnecessary biopsies, biomarkers such as percent free PSA, prostate health index (PHI) or the 4K score may be used, while Progenesa PCA3 may be measured to reduce the number of repeat biopsies in men with a previously negative biopsy. In addition to its role in screening, PSA is also widely used in the management of patients with diagnosed prostate cancer such as in surveillance following diagnosis, monitoring response to therapy and in combination with both clinical and histological criteria in risk stratification for recurrence. For determining aggressiveness and predicting outcome, especially in low- or intermediate-risk men, tissue-based multigene tests such as Decipher, Oncotype DX (Prostate), Prolaris and ProMark, may be used. Emerging therapy predictive biomarkers include AR-V7 for predicting lack of response to specific anti-androgens (enzalutamide, abiraterone), BRAC1/2 mutations for predicting benefit from PARP inhibitor and PORTOS for predicting benefit from radiotherapy. With the increased availability of multiple biomarkers, personalised treatment for men with prostate cancer is finally on the horizon.

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Introduction

Although prostate-specific antigen (PSA) is the most widely used biomarker for prostate cancer [1], several other biomarkers have recently become available for this disease. As a blood-based cancer biomarker, PSA is unique in that it is used in all the main phases of prostate cancer detection and patient management, i.e. in screening, risk stratification for recurrence, surveillance following diagnosis and monitoring therapy [1–3]. To enhance the diagnostic accuracy of PSA for prostate cancer, several new biomarkers have become available in recent years. These newer biomarkers include prostate health index (PHI) and the 4K score which can help in reducing the number of biopsies performed in men with border-line low PSA levels and multigene signatures for helping to differentiate between indolent and aggressive prostate cancers [4, 5]. The aim of this article is to provide an updated and critical review on the role of PSA in prostate cancer screening, risk stratification, follow-up and monitoring therapy. In addition, I also discuss new and emerging blood, urine and tissue biomarkers for prostate cancer. Most of the emphasis, however, will be devoted to the use of PSA in screening asymptomatic men for prostate cancer.

Use of PSA in screening for prostate cancer

Three large randomized prospective trials have now evaluated the benefit of PSA screening in asymptomatic men for prostate cancer, i.e. the Prostate, Lung, Colorectal and Ovarian (PLCO) trial which was carried out in the US, the European Randomised Study for Screening of Prostate Cancer (ERSPC) trial which was performed in eight European countries and the Cluster Randomised Trial of

PSA Testing for Prostate Cancer (CAP) which was carried out at 573 primary care practices across the UK (Table 1) [6–12]. Two of these trials, i.e. PLCO and CAP found no benefit of screening for reducing mortality from prostate cancer [7, 12]. In contrast, the ERSPC found a significant benefit for screening in reducing prostate cancer-specific mortality in men aged 55–69 years [8–11].

The impact of PSA screening on prostate cancer-specific mortality in the ERSPC trial has now been investigated after four different follow-up periods, i.e. after 9 years, 11 years, 13 years and 16 years. At each of these follow-up periods, screening with PSA resulted in a relative mortality reduction of 20% [8–11]. However, the number of men needed to be invited (NNI) for screening in order to prevent one death declined with longer follow-up, i.e. it was 570 following 16 years of follow-up vs. 742 at 13 years [10, 11]. The number of men needed to be diagnosed (NND) to prevent one death was also reduced with the increased follow-up, i.e. 18 at 16 years vs. 23 at 13 years. In one of the pilot study arms of the ERSPC trial (Rotterdam Pilot 1) which randomised 1134 men to screening or no screening, analysis after 19 years of follow-up showed an overall relative risk of metastatic disease of 0.46 and a relative risk of prostate-specific deaths of 0.48, in favour of screening [13].

Although all the three trials mentioned mostly investigated asymptomatic men in their 50s and 60s, they differed widely in design (Table 1). Furthermore, all the trials had limitations, the most serious of which occurred in the PLCO trial. Indeed, the PLCO trial did not strictly compare screening with no screening, as up to 90% of men in the “control group” were estimated to having undergone PSA testing at least once, either prior to screening starting or during the screening period [14, 15]. This trial has thus been

described as a comparison between frequent and sporadic PSA screening or between organised and opportunistic screening [16]. A further limitation of the PLCO trial was that only about 30%–35% of the men in the screening arm with a PSA concentration $>4 \mu\text{g/L}$, underwent a biopsy for confirmation of a definitive diagnosis [6].

A limitation of the ERSPC trial was the lack of a standardised screening strategy in the eight different countries in which the trial was performed. Thus, the strategies used in the different countries varied with respect to frequency of PSA testing, age-range of subject at entry to trial, follow-up tests and PSA cut-off concentrations [8]. These variations may have contributed to the different impacts of screening observed at the different sites, i.e. a decrease in mortality was found in only two of the countries in which the trial was carried out, i.e. in Sweden and the Netherlands [17].

The most recently reported randomised screening trial, i.e. CAP, investigated the effect of a single PSA measurement on prostate cancer-specific mortality [12]. This trial included 419,582 men aged 50–69 years and had a median follow-up of 10 years. As with the PLCO trial, men randomised to PSA screening had a similar outcome to the control group without screening. The key limitation of this trial was that only a single PSA measurement was performed which is unlikely to be the optimum screening strategy. A further limitation was that only 34% (64,436/189,386) of men randomised to the screening group underwent PSA testing with a valid result.

In an attempt to reconcile the different outcomes in the ERSPC and the PLCO trials, Tsodikov et al. [18] recently used mathematical modelling to correct for screening intensity in the two studies. After accounting

Table 1: Key characteristics of major randomised trials addressing PSA screening.

Parameter	PLCO	ERSPC	CAP
Location	USA	Europe	UK
No of participants	77,000	162,000	>400,000
Age range	55–74 years	55–69 years	50–69 years
Proportion tested in screening arm ^a	85%	64%	34%
Contamination in control group ^b	>80%	15%	10%–15%
PSA test used	Beckman	Beckman	NS
PSA cut-off point used	4 $\mu\text{g/L}$	2–4 $\mu\text{g/L}$	3 $\mu\text{g/L}$
fPSA used	No	Yes, at one site	No
Screening interval	Yearly for 6 years	Every 2–4 years ^c	One off
Mean follow-up	13 years	13 years	10 years
PC mortality (RR)	1.04	0.79	0.96

^aProportion of men in intervention group who were tested for PSA. ^bEstimated values for subjects in control group who underwent at least one PSA test. NS, not stated. ^cEvery 4 years apart from Sweden which used 2-yearly intervals. Data summarised from Refs. [6–12].

for protocol adherence, contamination in control arms, and intensity of post-screening diagnostic procedures, the two trials were found to give essentially similar results, i.e. screening was found to be associated with a 25%–31% lower risk of death from prostate cancer in the European trial and a 27%–32% lower risk in the PLCO trial when compared to the respective control groups. In a further modelling study, de Koning et al. [19] concluded that if the PSA testing in the PLCO study had been performed as efficiently as in the ERSPC trial, the American trial would likely have resulted in a modest reduction in mortality (6%–8%). It is important to state that both of these screening trials included mostly White men. Thus, their conclusions may not be applicable to non-White men.

Based on the available data, it would appear that if PSA screening reduces mortality from prostate cancer, the impact is likely to be modest and confined to men aged 55–69 years. However, as screening as well as any subsequent biopsies and treatments can also result in harms, it is not clear if the practice is beneficial overall [16]. Potential harms from the screening process include false positive results due to lack of specificity of PSA for prostate cancer. Thus, in the ERSPC trial which used a PSA cut-off concentration of 3 µg/L, approximately 75% of the men who underwent biopsy were not found to have cancer [10]. Furthermore, approximately 70% of the cancers detected were found to have low grade (i.e. likely to be indolent) [8]. Overall, the low specificity of PSA resulted in a biopsy being performed in approximately 20% of the men screened in the ERSPC trial [20–22].

Complications that may result from biopsy include infection, pain, bleeding, urinary retention and haematuria [23]. A positive biopsy may in turn result in overdiagnosis (i.e. detecting cancers that are unlikely to cause future morbidity and mortality) and overtreatment in men with indolent disease. Although it is difficult to determine the exact extent of overdiagnosis, it was estimated to occur in between 16% and 50% of cases in the randomised trials mentioned [24]. A positive biopsy can lead to treatment such as radical prostatectomy or radiotherapy, resulting in an increased risk of complications such as impotence, incontinence and bowel problems [25].

Clearly, therefore, if population-based screening is to be introduced for prostate cancer, it must be implemented in a manner that maximises benefit and minimises overdiagnosis and overtreatment. Strategies that may achieve these ends include the use of baseline PSA testing during early mid-life [26] (see below), genetic testing to identify

men at increased risk [27], reducing or eliminating screening in asymptomatic men >70 years or those with a life expectancy of <10 years, employing active surveillance for men diagnosed with indolent disease (assessed by clinical stage, tumour grade, PSA concentration and possibly a gene signature test), (see below for discussion of gene signatures) [28]. In addition, the employment of risk calculators [29], measurement of other biomarkers (Prostate Health Index, 4K score, genetic signature) [30, 31] and use of multiparametric magnetic resonance imaging (MRI) [32], could aid in differentiating between aggressive and indolent cancers, thus reducing the number of unnecessary biopsies performed.

Because of this close balance between potential good and harm, most published guidelines are opposed to mass screening but recommend screening in men 55–69 years of age following the practice of shared decision making and informed consent [33–37]. This shared decision making should include a discussion between the man and his health professional about the potential harms and benefits of the screening process and the likely ensuing impact on the man's health and quality of life. Furthermore, most expert panels are opposed to or discourage screening in men with <10–15 years of life expectancy.

In a deviation from their original guidelines, the European Association of Urology (EAU) recently recommended that well-informed men with a life-expectancy of ≥ 10 years should have a baseline PSA measurement for risk stratification at 45 years of age [38]. It was suggested that men with a PSA concentration <1 µg/L might then undergo further testing at intervals up to 8 years. However, for men with a PSA level ≥ 1 µg/L, screening at intervals of 2–4 years was proposed [38]. The panel also suggested that multiparametric MRI as well as risk calculators based on family history, ethnicity, digital rectal examination and prostate volume should be considered to triage the need for biopsy, thus reducing the risk of overdiagnosis.

The Memorial Sloan Kettering Cancer Center guidelines also recommends that PSA screening should start at 45 years of age [39]. According to this organisation, if PSA levels are ≥ 3 µg/L, a biopsy should be considered. If PSA levels are ≥ 1 but <3 µg/L, a return for PSA testing every 2–4 years was recommended. If, however, PSA levels are <1 µg/L, a return for PSA testing at 6–10 years was recommended. Although these recommendations to begin screening at mid-life would appear to be a rational approach to reduce overdiagnosis, there is no published evidence that it would reduce mortality from prostate cancer.

Use of PSA in risk stratification (prognosis)

In addition to its use in screening, PSA levels in combination with specific clinical and pathological factors are widely used in assessing risk of recurrence (prognosis) in patients with newly diagnosed prostate cancer [40–42]. In general, an approximate linear relationship exists between PSA levels at initial diagnosis and outcome, i.e. the higher the PSA level, the worse the outcome [43–46]. This relationship is especially found in patients with low or intermediate grade (i.e. Gleason score [GS] ≤ 7) disease. However, in some patients with high grade disease (GS, 8–10), low levels of PSA (≤ 2.5 $\mu\text{g/L}$) may predict a particularly poor outcome [43]. Overall, approximately 6% of men with high grade disease have low levels of PSA [43]. Although PSA levels at initial diagnosis broadly correlates with outcome, it has limited prognostic accuracy if used alone. In practice therefore, PSA levels are combined with clinical and tumour histological factors for predicting outcome [33, 47].

The cut-off concentrations used for PSA in assessing risk of recurrence are different from those used in the screening. For example, recent joint guidelines published by the American Urological Association, American Society for Radiation Oncology and Society of Urologic Oncology [33] state that men with PSA concentrations of <10 $\mu\text{g/L}$ should be classified as very low or low risk. Men with PSA concentration $10 - <20$ $\mu\text{g/L}$ should be regarded as being at intermediate risk of recurrence, while those with values ≥ 20 $\mu\text{g/L}$ should be placed at high risk of recurrence. As mentioned, for each of these categories, PSA is not used alone but is combined with specific clinical and pathological features [33]. Essentially similar criteria are recommended by the National Comprehensive Cancer Network (NCCN) for risk stratification in patients with newly diagnosed prostate cancer [47]. However, the NCCN recommend use of the newer International Society of Urological Pathology (ISUP) grading system rather than the older Gleason grading system [47].

Use of PSA in follow-up following initial diagnosis

Management options for patients with newly diagnosed localised prostate cancer include radical prostatectomy, radiotherapy with or without androgen deprivation therapy (ADT), brachytherapy and active surveillance (the

latter only for patients deemed to be at low risk of recurrence). Irrespective of the option chosen, serial concentrations of PSA are generally measured during follow-up. The optimum frequency for PSA testing in follow-up has not been established and indeed is likely to vary depending on aggressiveness of the primary cancer. Generally, however, following initial definitive therapy, measurement of PSA is recommended every 6–12 months for the first 5 years after diagnosis and annually thereafter [3]. For men at high risk for recurrence (i.e. $\geq \text{T3A}$ disease or GR 8–10 or PSA >20 $\mu\text{g/L}$) PSA testing may be performed more frequently (e.g. every 3 months).

Rather than relying on static or absolute levels, the rate of change in serial PSA levels can also be used during follow-up. The rate of change in serial levels is usually determined by the PSA velocity (PSAV) (change in PSA concentration over time) or PSA doubling time (PSADT) (the time required for PSA levels to double their concentration). For follow-up after initial diagnosis, PSADT is more frequently used than PSAV. Although measurement of PSADT after radical prostatectomy, radiotherapy, following salvage therapy after PSA failure or during active surveillance has been found to correlate with patient outcome (for review, see Refs. [48, 49]), its determination has several problems. One of the main problems is the lack of standardised methodology for its measurements. Thus, the methods described to date varied in the number of samples used for calculation, frequency of measurement and the interval over which the measurements were made. In order to standardise methodology for the calculation of PSA-DT, the Prostate Specific Antigen Working Group published guidelines [50]. The main points in these guidelines are summarised:

- All PSA concentrations used in calculating PSA-DT should be >0.2 $\mu\text{g/L}$ and follow an increasing trend.
- All values contained during a maximum period of 12 months should be included in the calculation.
- The maximum period of the last 12 months is recommended to reflect the current disease status.
- Minimum requirements for the PSA-DT calculation are 3 PSA results obtained during 3 months with a minimum of 4 weeks between measurements.
- All PSA results must be obtained using the same method and preferable using the same laboratory.
- PSA results should be recorded with a maximum of two significant digits after the decimal point.
- Serum testosterone should be relatively stable during the period used for calculation.

Following successful radical prostatectomy for men with localised disease, PSA concentrations should decline

to undetectable levels ($<0.1 \mu\text{g/L}$) within 2 months [3]. Biochemical recurrence (BCR) is then defined by two consecutive increasing PSA concentrations $>0.2 \mu\text{g/L}$ [51]. In contrast, following radiotherapy or brachytherapy, PSA levels decrease slowly and generally reach concentrations $<0.5 \mu\text{g/L}$ after about 6 months. However, in contrast to radical prostatectomy, they do not reach undetectable levels. Furthermore, with follow-up after radiotherapy, a transient increase or bounce may occur in up to 40% of treated patients. Transient increases generally occur within the first 3 years following treatment [52, 53]. According to an expert consensus group (Phoenix Consensus Conference), an increase in PSA concentration of $2 \mu\text{g/L}$ or more above the nadir (defined as the lowest PSA level achieved) should be regarded as a biochemical failure following radiotherapy with or without ADT [54]. However, this panel also suggested that an older definition of biochemical failure, i.e. three consecutive PSA increases above a nadir value could be used after radiotherapy or brachytherapy without ADT. Failure was then backdated to a date between the first increasing level and the nadir value.

Management of patients with biochemical recurrence

Although the presence of BCR indicates an increased risk of clinical recurrence, many such patients continue to remain free of symptoms. Thus, in one retrospective study in which 315 men experienced BCR, only 34% subsequently developed evidence of clinical recurrence [55]. The median time between the BCR and evidence of clinical recurrence was 8 years. The median time to death following clinical recurrence was then a further 5 years [55].

To identify factors associated with poor outcome following BCR, Van den Broeck et al. [56] carried out a systematic review of the published literature. Following radical prostatectomy, BCR was associated with poor outcome mostly in patients with a short PSA-DT and high final GS. Due to the heterogeneity in the PSA-DT used in the different studies, the authors were unable to select an optimum cut-off point. Most studies however, found that patients with a PSA-DT of <12 months had an increased risk of recurrence. After radiotherapy, a poor outcome was correlated with a short interval to BCR as well as a high biopsy GS [56]. As with the patients treated with radical prostatectomy, different studies used different cut-off points for PSA-DT in men treated with radiotherapy. Most however, found that an interval to BCR

of <18 months was associated with the development of disease recurrence.

As many men with evidence of BCR never develop clinical evidence of recurrence, it is unclear if or when ADT should be administered, i.e. whether to administer it early or await clinical evidence of disease [57–60]. A recent randomised phase III clinical trial however, suggested that the early administration of ADT may enhance outcome compared to waiting for clinical symptoms to develop [61]. In this trial involving men with evidence of BCR following surgery or radiotherapy or those not considered suitable for these treatments, Duchesne et al. [61] found that immediate administration of ADT improved survival compared with delayed treatment administration. Median follow-up in this trial, however, was relatively short (5 years). Furthermore, only 40 deaths were recorded, of which only 18 were due to prostate cancer. Quality of life was reported to decrease by a “small but clinically notable amount” in men receiving the immediate vs. men in the delayed treatment arm.

Although administration of ADT may be beneficial in some men with evidence of BCR, many receiving this treatment will develop disease progression as evidenced by increasing PSA levels. Despite the increasing PSA levels, some of these men will nevertheless show no evidence of metastasis using conventional imaging. Such men are referred to as having castrate-resistant prostate cancer (CRPC). Castrate-resistant disease is frequently defined as two consecutive PSA increases, at least 1 week apart, with testosterone levels $<1.7 \text{ nmol/L}$ ($<50 \text{ ng/mL}$). Three prospective randomised trials have recently shown the administration of third-generation androgen receptor (AR) antagonists such as enzalutamide, apalutamide or darolutamide enhanced metastasis-free survival in men who had non-metastatic CRPC with PSA doubling times of ≤ 10 months [62–64].

Use of PSA in monitoring treatment in advanced disease

Although most patients diagnosed with localised prostate cancer do not die from the disease, a minority develop distant metastases which are generally incurable. The initial treatments for patients with metastatic prostate cancer is usually hormone therapy (ADT) [65]. Although, serial determinations of PSA are widely used to monitor response to ADT, validated definitions of response or progression have not been established. Most studies, however, have shown that patients with a PSA level

≤ 4 $\mu\text{g/L}$ following approximately 6/7 months of ADT treatment tend to have a better outcome than those with PSA levels >4 $\mu\text{g/L}$. In a large prospective trial involving 1345 patients with advanced prostate cancer treated with ADT (goserelin and bicalutamide), median survival was 13 months for patients with a PSA >4 $\mu\text{g/L}$, 44 months for patients with a PSA of $>0.2 - \leq 4$ $\mu\text{g/L}$, and 75 months for patients with a PSA of ≤ 0.2 $\mu\text{g/L}$ [66]. Furthermore, after controlling for standard prognostic factors, patients with a PSA of >0.2 but ≤ 4 $\mu\text{g/L}$ had less than one third the risk of death compared with those with a PSA of >4 $\mu\text{g/L}$, while patients with PSA levels of ≤ 0.2 ng/mL had less than one fifth the risk of death as patients with a PSA value of >4 $\mu\text{g/L}$. Other studies have also found that the PSA nadir value following ADT is prognostic of patient outcome [67, 68], i.e. the lower the PSA level following ADT, the better the outcome. One of the problems in using PSA to monitor response to ADT in patients with prostate cancer is that as PSA production is controlled by androgens, this therapy can lower PSA levels without necessarily impacting on tumour bulk.

As in the non-metastatic situation mentioned, resistance (CRPC) usually occurs following initial ADT in the metastatic situation. Potential treatments for CRPC include second line ADT (abiraterone plus prednisolone, enzalutamide), ADT plus chemotherapy (docetaxel, cabazitaxel), radium-223 and immunotherapy (sipuleucel-T) [69, 70]. Although PSA is used in assessing response to these therapies in patients with metastatic CRPC, changes in its levels are poor predictors of survival [71, 72]. Indeed, a recent report involving five randomised phase III clinical trials using different treatments showed that a decrease in the number of circulating tumour cells (CTC) from ≥ 1 to zero was superior to PSA for predicting survival [72]. It should be stated however, that unlike PSA, CTC are not believed to be directly under the control of androgens and thus should not be affected by ADT.

Measurement of CTC, however, is more expensive than that of PSA and furthermore is not widely available.

Finally, although immunotherapy with sipuleucel-T or administration of the radiopharmaceutical, radium-223 have been shown to increase overall survival, this improvement was not found to correlate with alterations in PSA levels. [73, 74]. Changes in PSA levels are thus also of limited value for predicting outcome in patients with castrate-resistant metastatic prostate cancer undergoing treatment with sipuleucel-T or radium-223.

Biomarkers for improving the diagnostic accuracy of PSA

One of the main problems in using PSA to screen for prostate cancer is the lack of specificity, especially when values are <10 $\mu\text{g/L}$. The consequence of this poor specificity is that large numbers of men may undergo unnecessary biopsy to confirm or exclude malignancy. Thus, 65%–75% of men with a PSA level in the 3/4–10 $\mu\text{g/L}$ range do not have biopsy-detectable prostate cancer [10]. To improve specificity and thus reduce the number of unnecessary biopsies/repeat biopsies, several additional or adjunct tests have been proposed (Table 2). These include percent free PSA, PHI, 4K score and PCA3.

The key to the development of many of these tests was the discovery that PSA can exist in multiple forms (for review, see Refs. [75, 76]). Early studies showed that PSA existed in different forms in blood. Seventy to ninety percent of the protein is complexed with serum protease inhibitors especially with α_1 -antichymotrypsin. The remainder 10%–30% exists in a free or unbound state (75, 76). The free PSA in serum is composed of three major forms: pro-PSA, BPSA and intact PSA. The preform can in turn exist

Table 2: Serum and urinary biomarkers that may be combined with PSA for enhancing the diagnostic accuracy of prostate cancer detection including high grade disease.

Test	Analytes detected	Fluid	References
Per cent fPSA	PSA and fPSA	Serum	[75–79]
PHI	PSA, fPSA, -2ProPSA	Serum	[80–90]
4K Score	PSA, fPSA, iPSA, kK2	Serum	[91–96]
Progens PCA3	PCA3 ^a , PSA ^a	Urine	[97–107]
MiPS	PCA3 ^a , TMPRSS2-ERG ^a	Urine	[108]
STHLM3	PSA, fPSA, iPSA, HK2, β -microseminoprotein, macrophage inhibitory cytokine, 232 SNP ^b	Serum	[109, 110]
epiCaPture	Methylated GSTP1, SFRP2, IGFBP3, IGFBP7, APC, PTGS2	Urine	[111]

^aMeasured at mRNA level. ^bThese variables are combined with clinical and histological criteria. PSA, total PSA; fPSA, free PSA; iPSA, intact PSA; HK2, human kallikrein 2; PCA3, prostate cancer antigen 3; MiPS, Mi-Prostate Score; TMPRSS2, transmembrane protease, serine 2.

in multiple forms including the native proPSA form containing a 7-amino-acid pro-peptide leader [(7)proPSA] as well as forms with various truncated pro-peptide leader sequences. These truncated proPSA forms consist mostly of proPSA with a 5-amino-acid (-5) proPSA, 4-amino-acid (-4)proPSA or 2-amino-acid (-2)proPSA [112, 113].

Percent free PSA

One of the first adjunct tests developed to enhance the specificity of PSA was percent free PSA (free PSA/total PSA ratio). In general, men with prostate cancer have lower levels of percent free PSA when compared to men without prostate cancer. Although the absolute amount of free PSA in blood has no established clinical value, measurement of the percent free PSA has been shown to increase the specificity of PSA for prostate cancer detection, especially in men with total PSA levels between 2 and 4 and 10 $\mu\text{g/L}$ [75–77]. Because of this enhanced specificity, measurement of percent free has the potential to reduce the number of biopsies performed in men with border-line total PSA levels.

In an early multicentre prospective study, carried out in men with total PSA levels between 4 and 10 $\mu\text{g/L}$, percent free PSA ranged from 2% to 52% [75]. Using a cut-off value of 25, the measurement of percent free PSA was found to detect 95% of cancers and avoid 20% of unnecessary biopsies [75]. The cancers detected in men with $\geq 25\%$ free PSA tended to be of lower grade and smaller volume than those found in men with lower percent free PSA levels. The risk of cancer increased as the free PSA percentage decreased. Thus, for men with per cent free PSA values of 0–10, the risk of cancer was 55% or 56% (depending on man's age) while at levels $>25\%$, the risk of cancer was $<10\%$. Using multivariate analysis that also included total PSA and patient age, percent free PSA was an independent predictor of prostate cancer (odds ratio [OR], 3.2).

These early finding have been essentially confirmed in several subsequent reports [76, 77]. Furthermore, it has been shown that measurement of percent free PSA can increase diagnostic specificity not only in the total PSA range of 4–10 $\mu\text{g/L}$ range but also in the 2–10 $\mu\text{g/L}$ range [76]. However, the diagnostic accuracy of percent free PSA was found to be significantly better for men with total PSA levels of 4–10 $\mu\text{g/L}$ compared to those with total PSA levels of 2–4 $\mu\text{g/L}$ ($p < 0.01$). At a sensitivity of 95%, specificity was 18% for men in the 4–10 $\mu\text{g/L}$ total PSA range but only 6% in the 2–10 $\mu\text{g/L}$ total PSA range.

In practice, measurement of percent free PSA is most useful at its extreme concentration limits, i.e. at low and high levels. Thus, as mentioned above, for men with a total PSA level between 4 and 10 $\mu\text{g/L}$, those with percent free PSA concentrations lower than 10% have a $>50\%$ probability of being diagnosed with prostate cancer, whereas those with levels $>25\%$ have a $<10\%$ chance of having prostate cancer [75–77]. The latter however, may not be of much clinical value, as some men would consider a $<10\%$ probability of being diagnosed with prostate cancer to be insufficiently low not to undergo a biopsy.

Caution is advised when measuring and interpreting percent free PSA levels. Firstly, as increasing prostate volume produces a greater amount of percent free PSA, this parameter has been reported to provide reliable data only in men with a prostate volume <40 mL [78]. A further problem is selecting the optimum cut-off point. Indeed, there is no universally accepted cut-off point for percent free PSA, with values ranging from 8% to 25% described in the literature [79]. Most investigators, however, use cut-off values of between 15% and 20% [79]. A practical problem in determining percent free PSA is that the free PSA molecule is relatively unstable at room temperature [114]. According to the National Academy of Clinical Biochemistry (NACB) guidelines [34], serum for free PSA may be stored at refrigerated temperatures for up to 24 h. Samples not analysed within 24 h of collection should be stored frozen at -20 °C or lower. For long-term storage, freezing at -70 °C was recommended [34].

Currently, percent free PSA has no role in screening for prostate cancer but may be useful as a reflex test for men with total PSA levels between 2 and 10 $\mu\text{g/L}$. Thus, according to the NACB recommendations “the use of percent free PSA is recommended as an aid in distinguishing prostate cancer from benign prostate hyperplasia, when the total PSA levels in serum are between 4 and 10 $\mu\text{g/L}$ and DRE is negative” [34].

Prostate Health Index (PHI)

The PHI involves measurement of -2proPSA, percent free PSA and total PSA. Levels of these three proteins are then combined using the formula $(-2 \text{ proPSA/free PSA}) \times \sqrt{\text{total PSA}}$ (Beckman Coulter, Inc.) [80]. PHI has been shown to be superior to total PSA and percent free PSA in detecting prostate cancer including aggressive prostate cancer [80–83]. Thus, following a meta-analysis of eight studies ($n = 2919$ patients), the pooled sensitivity of PHI for the detection of prostate cancer was 90% while the pooled specificity was 31.6% [83]. In this meta-analysis,

measurement of PHI was found to have superior accuracy for detecting prostate cancer than total or percent free PSA across the eight studies analysed. This superior accuracy was especially evident in men with PSA levels between 2 µg/L and 10 µg/L.

In addition to having superior accuracy for detecting prostate cancer in general, PHI also has higher predictive accuracy for clinically-significant/aggressive disease compared with PSA or percent free PSA [84, 85]. Thus, in a large multicentre study, PHI was found to be significantly associated with high grade disease ($GR \geq 7$) with an area under the curve (AUC) of 0.815 [85]. At 95% sensitivity, the PHI specificity was 36.0% compared to 17.2% for total PSA and 19.4% for percent free PSA. At 95% sensitivity for detecting aggressive prostate cancer, the optimal cut-off value for PHI was 24. At this cut-off point, measurement of PHI could potentially avoid 36%–41% of unnecessary biopsies and 17%–24% of overdiagnosed indolent cancers.

The main clinical use of PHI is in reducing the number of unnecessary biopsies in men with border-line PSA levels. Depending on the cut-off point used, this can vary from about 15% to 45% [86]. Avoiding biopsy, however, may result in missing a small number of cancers (usually <10% at a cut-off point of 25) [86].

In addition to reducing the number of unnecessary biopsies, other potential uses of PHI include predicting biochemical recurrence following radical prostatectomy [87, 88] and enhancing the predictive value of multi-parametric MRI [89, 90]. PHI at present is not recommended in primary screening for prostate cancer. However, in the future, its value in this setting should be considered for evaluation as a reflex test in patients with PSA values between 2 and 10 µg/L.

Finally, regarding the measurement of PHI levels, it is important to state that the optimum conditions for pre-analytical handling and storage of samples remain to be determined. Indeed, further studies are needed to assess whether plasma or serum is the best matrix for its measurement.

4K score

The 4K score tests measure total PSA, free PSA, intact PSA (a form of free PSA) and human kallikrein 2 (hK2) [91]. Levels of these biomarkers are combined in an algorithm together with patient age, digital rectal exam status and any prior biopsy findings for predicting the risk of a man having high grade prostate cancer. Similar to the situation with PHI, several studies have also shown that the 4K score provides superior diagnostic accuracy for detecting

prostate cancers in general as well as high grade prostate cancer, compared to PSA or percent free PSA [92–96]. Thus, using 4765 patients participating in a prospective, randomised trial (ProtecT trial), the AUC for the 4K score was 0.719 compared with 0.634 for PSA for all cancers and 0.820 vs. 0.738 for high-grade cancers [92]. As regards comparison with PHI, a meta-analysis of published studies concluded that both tests had essentially similar diagnostic accuracy for high grade prostate cancer [96]. As with percent free PSA and PHI, one of the main clinical uses of the 4K score is its potential to reduce the number of unnecessary biopsies. Depending on the cut-off point selected, this has been reported to vary from 41% to 57% [86].

PCA3

PCA3 (prostate cancer gene 3) which is also known as DD3, is a prostate-specific non-coding mRNA. In an early study, 53/56 specimens of prostate cancer tissue investigated were found to contain 10–100-fold greater levels of *PCA3* than that found in adjacent non-malignant prostate tissue [97]. Although *PCA3* was also found in both normal prostate and benign prostate hypertrophy, expression was undetectable in several normal and malignant non-prostatic tissues [97]. These results when combined with the finding of *PCA3* in prostate cells in urinary sediments [98] suggested that this molecule might be a new marker for prostate cancer.

The best characterised *PCA3* assay (ProgenSA *PCA3*, Hologic Inc, Marlborough, MA, USA) detects mRNA for *PCA3* and PSA in first-catch urine following digital rectal examination [99]. Measurement of PSA mRNA is necessary to control for the number of prostate epithelial cells in the urine. In one of the largest reports to evaluate the diagnostic potential of *PCA3* for prostate cancer, Cui et al. [100] combined the data from 46 studies involving 12,295 men investigated for possible prostate cancer. Pooled sensitivity, specificity, positive likelihood ratio (+LR), negative likelihood ratio (–LR), diagnostic odds ratio (DOR) and AUC for prostate cancer were 0.65 (95% confidence interval [CI]: 0.63–0.66), 0.73 (95% CI: 0.72–0.74), 2.23 (95% CI: 1.91–2.62), 0.48 (95% CI: 0.44–0.52), 5.31 (95% CI: 4.19–6.73) and 0.75 (95% CI: 0.74–0.77), respectively.

In a large multicentre prospective validation study (n = 859) published subsequent to the above meta-analysis, Wei et al. [101] reported that *PCA3* had positive predictive value (PPV) of 80% (95% CI, 72%–86%) for men presenting for their initial biopsy. In those with a *PCA3* score >60 units, the specificity for prostate cancer was 0.91 (95% CI, 0.87–0.94) and the sensitivity was

0.42 (0.36–0.48). For men presenting for a second biopsy, *PCA3* had a negative predictive value (NPV) of 88% (95% CI, 81%–93%). For men with a *PCA3* score of <20 units at repeat biopsy, the sensitivity for prostate cancer was 0.76 (95% CI, 0.64–0.86) and the specificity was 0.52 (95% CI, 0.45–0.58). Importantly, Wei et al. [101] found that adding of the *PCA3* score to individual risk estimation models that included patient age, race/ethnicity, prior biopsy finding, PSA and digital rectal examination result, improved the stratification of any prostate cancer as well as high-grade cancer. Based on these findings, the authors concluded that measurement of *PCA3* helps minimise underdetection of high grade disease in initial biopsies and overdetection of low grade malignancy in repeat biopsies [101]. Other studies have suggested that the diagnostic accuracy of *PCA3* for prostate cancer was superior to that of the total PSA, percent free PSA and PSA velocity [102, 103]. However, *PCA3* does not appear to add to PHI in predicting cancer at an initial or repeat biopsy [104]. Finally, conflicting data has been published regarding a possible role for *PCA3* in predicting prostate cancer aggressiveness [105, 106].

Although *PCA3* is unlikely to replace PSA as the front-line biomarker for prostate cancer, the combined measurement of both should result in enhanced specificity for prostate cancer diagnosis. Assaying of *PCA3* however, may be of particular value in patients with elevated PSA levels but who have histologically-negative biopsies [107]. In this situation, *PCA3* can provide information in deciding whether or not to repeat a biopsy. In 2012, The Food and Drug Administration (USA) approved the PROGENSA *PCA3* assay for use in conjunction with other patient information to aid in the decision for repeat biopsy in men ≥ 50 years who have had one or more previous negative prostate biopsies and for whom a repeat biopsy would be recommended by a urologist based on the current standard of care.

Other biomarkers for enhancing the specificity of PSA

Other reflex biomarkers that may be used for enhancing decision making with respect to performing a biopsy in men with borderline PSA levels are listed in Table 2.

Tissue-based biomarkers for determining prognosis and clinical decision making

In recent years, several tissue biomarker tests have become available for determining aggressiveness and predicting outcome in patients with newly diagnosed prostate cancer (Table 3). Some of these are multi-analyte tests, measure multiple molecular species, especially mRNAs. Of the tests listed in Table 3, the best validated include Decipher, Oncotype DX (Prostate), Prolaris and ProMark. Although these tests have been evaluated in different settings and used different end points, they all essentially provide information on tumour aggressiveness and patient outcome.

Although multigene signatures can potentially fulfil an unmet need in the management of patients with prostate cancer, several problems are currently limiting their use. Firstly, none have yet been prospectively validated for clinical utility using a randomised clinical trial. Secondly, compared to the blood biomarkers discussed above, tissue-based multigene signatures are expensive, costing \$3000–\$4000. Thirdly, it is unclear which test (if any) is best for predicting a specific endpoint or indeed how they compare with the simple and less expensive blood-based tests discussed above (PHI, 4K score). Finally, due the heterogeneity of prostate cancer tissue, different results can be found depending on the location of sample

Table 3: Tissue-based assays reported to identify prostate cancer aggressiveness and/or predict patient outcome.

Test	No. of analytes detected	Type of analyte	Outcome/endpoint provided by test	References
Confirm	3	Methylated genes	Deciding on repeat biopsy if PSA is high and initial biopsy is negative	[115, 116]
Decipher	22	mRNA	High-grade disease, metastasis, prostate-cancer-specific mortality	[117, 118]
Ki67	1	Protein	Formation of metastasis	[119]
Oncotype DX	17 ^a	mRNA	Tumour aggressiveness and outcome	[120, 121]
Prolaris	46 ^b	mRNA	Tumour aggressiveness and prostate-specific mortality	[122, 123]
ProMark	8	Protein	Aggressive disease in patients with GS 3 + 3 and 3 + 4	[124]
PTEN	1	Protein	Aggressive disease and patient outcome	[125]
Select MDX	2	mRNA	Tumour aggressiveness	[126]

^a12 test genes and five control genes; ^b31 test and 15 control genes. GS, Gleason score.

biopsied [127]. Despite these limitations, the current NCCN guidelines state that Decipher, Oncotype DX, Prolaris and ProMark may be considered for risk stratification in men with low or favourable intermediate-risk disease [47].

Therapy predictive biomarkers

The tissue-based multigene tests discussed are essentially prognostic and provide little information regarding response or resistance to different therapies. Recently, however, a small number of therapy predictive biomarkers for prostate cancer treatments have begun to emerge. These include the AR splice variant, AR-V7 measured in CTC for predicting resistance to enzalutamide and abiraterone [128–130], mutant *BRCA1/2* in prostate cancer tissue for predicting response to the PARP inhibitor, olaparib [131], the PORTOS test (measured in prostate cancer tissue) for predicting response to radiotherapy [132] and circulating tumour DNA for predicting resistance to enzalutamide in castrate-resistant patients [133]. In 2016, the US Food and Drug Administration (FDA) granted Breakthrough Therapy designation (BTD) for olaparib for the treatment of *BRCA1/2* (or ATM) gene mutated metastatic castrate-resistant prostate cancer patients who have received a prior taxane-based chemotherapy and abiraterone or enzalutamide (<https://www.fda.gov/Drugs/InformationOnDrugs/ApprovedDrugs/ucm592357.htm>).

Conclusions

Despite its limitations, PSA is likely to remain the only biomarker available for prostate cancer screening for the foreseeable future. Although traditionally, expert panels differed in their recommendations on whether or not to screen for prostate cancer, most currently recommend limited screening following a process of education and informed consent [33–37]. Although for the present, PSA is the best validated and most widely used prostate cancer biomarker, several other tests are likely to be increasingly used in the future. These will include PHI, the 4K score or multi-parametric MRI for enhancing the specificity of PSA for prostate cancer and reducing the number of men undergoing unnecessary biopsy. For determining tumour aggressiveness and predicting patient outcome, it is likely that multiple factors will be used such as blood biomarkers (e.g. PSA, PHI, 4K score, *PCA3*), gene expression profiles (in selected cases), histological and clinical criteria. With the increasing number of biomarkers becoming

available and validated, we will finally be on the road to personalised management for men with suspected or diagnosed prostate cancer.

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