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“Emerging PSA-based tests to improve screening”

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Introduction

Prostate cancer is the most commonly diagnosed malignancy in men in the United States. It is estimated that during 2013 in the United States alone some 238,590 new prostate cancer cases will be diagnosed, and 29,720 men will die from this disease [1]. Both early detection and treatment of clinically localised prostate cancer represent the most likely strategies that will enable clinicians to reduce the high rate of prostate cancer-related deaths. PSA-based testing has been widely used to aid prostate cancer detection since the late 1980s and prostate cancer-specific mortality has been shown to be decreased as a result of PSA-based screening programmes in recent years. However, there is a concern that this decline has come at the expense of over-diagnosis and over-treatment. This chapter provides an overview of emerging PSA-based biomarkers with the potential to improve the performance of prostate cancer screening programmes.

PSA-based screening for prostate cancer

The term “screening” describes the diagnosis of pre-clinical cases of a disease at an early stage in order to improve the outcomes for that condition. PSA-based screening for prostate

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cancer has to justify a number of important requirements in order to be acceptable and thereby become widely adopted by health care providers. A number of recent studies have demonstrated that clear survival benefits may occur as a result of population-based prostate cancer screening programmes using PSA [2, 3]. Moreover there is recent evidence that measuring a man's PSA level in early mid-life identifies a smaller subset of men who are at risk of developing metastatic prostate cancer several decades later. For example men with a PSA level in the highest decile at age 45 to 55 years have a ten-fold or higher risk of metastatic cancer 15-25 years later compared to men with a PSA level below the age-median [4]. Similarly a single PSA measurement at age 60 years may be very informative in predicting which men are at significant future risk of prostate cancer-specific death [5]. However, despite the clear survival benefits which arise as a result of PSA-based prostate cancer screening, there are a number of disadvantages to the widespread adoption of this practice, including the potential for over-diagnosis and subsequent over-treatment of men [2, 3, 6].

Over-diagnosis and over-treatment of insignificant prostate cancers unlikely to become clinically apparent during a man's lifetime are significant problems [7]. The European Randomised Study of Screening for Prostate Cancer (ERSPC) showed that PSA-based screening can reduce prostate cancer-specific mortality by 20% within nine years, but large numbers of men need to be screened for each life saved [6]. Further follow-up to eleven years demonstrated a greater reduction in prostate cancer-specific mortality and an improvement in the numbers needed to screen and treat in order to save each life [2].

Unfortunately PSA has a number of performance limitations when it is used for prostate cancer screening including a lack of specificity. As the specificity of PSA alone is limited, a prostate biopsy is positive in only around 25% of men with a PSA between 2-10ng/mL [8]. Data reported using the control arm of participants in the Prostate Cancer Prevention Trial (PCPT) clearly demonstrated that there is no absolute lower limit of PSA below which there is no risk of detecting prostate cancer at biopsy among men aged 62 to 91 [9]. It is worthwhile noting however that there is a distinct difference between using the prostate biopsy endpoint of PCPT versus the prostate cancer metastasis or cancer death endpoints used in recent reports describing PSA-based testing [4]. A further downside of using the PSA level alone in prostate cancer screening is that no single PSA cut-off threshold has particularly good test performance characteristics [6, 10-12]. Because PSA is an organ-specific rather than prostate cancer-specific biomarker, most men with an elevated level of PSA do not have prostate cancer. Men with a raised PSA may therefore be subjected to an unnecessary prostate biopsy and be exposed to the potential complications of this procedure along with considerable associated anxiety. Moreover, as explained above, some men with a low PSA result may actually have prostate cancer [9]. Many screen-detected prostate cancers may not actually require treatment, hence there is a need for a marker which can help to identify high-risk prostate cancer at an early stage, thereby enabling clinicians to undertake radical treatment enabling cure of those patients with the greatest risk of developing morbidity or mortality as a consequence of this malignancy.

Given the clear limitations of PSA-based testing for prostate cancer there are a number of concerns regarding the routine use of this test within the context of a national prostate cancer

screening programme. Currently around half of all American men undergo regular PSA testing [13-15] and over 1 million prostate biopsies are performed per annum in the United States alone [16]. The U.S. Preventative Services Task Force has recently recommended against PSA-based screening for prostate cancer [17]. This announcement has caused considerable controversy in the United States and concerns have been raised regarding the methodology used to review the evidence base for screening, together with the interpretation of this evidence. One approach to improve the benefit:cost ratio of a prostate cancer screening programme would be to focus more on those men with the highest risk of developing clinically significant malignancy [5]. Another strategy would be to improve methods of detection, perhaps by identifying novel diagnostic tests with a greater positive predictive value than the currently used PSA test.

Prostate specific antigen

Prostate cancer is an example of a urological malignancy that has benefitted from the discovery and application of a tumour marker. Prostate specific antigen (PSA), or human kallikrein 3 (hK3, KLK3), was discovered in 1979 and first applied to clinical use in the late 1980s and early 1990s [12, 18-28]. The PSA-era has led to a greater detection of non-palpable clinically localised disease, and this has resulted in a subsequent reduction in prostate cancer-specific mortality [29-32].

PSA is a 33-kD glycoprotein and serine protease produced under androgen-regulation [33, 34] by the luminal cells of the prostate epithelium [35-37]. PSA acts to liquefy semen after ejaculation [38-42], and it is normally found in serum at low concentrations compared with the amount within the ejaculate. Several different molecular forms of PSA can be found within serum, where around 70% of PSA is bound as complexed PSA (cPSA) in association with molecules such as α_1 -antichymotrypsin (ACT, *SERPINA3*) or macroglobulin (A2M) [41, 43-48]. PSA bound to ACT, but not A2M, represents the largest proportion of bound PSA that remains detectable with immunological assays [49, 50], and in addition free inactive PSA (fPSA) is also detectable [46]. The development of antibodies specific for fPSA and cPSA has enabled accurate assays for the particular forms of PSA to be developed [46, 48] so that the percentage of free PSA (%fPSA) can be measured and utilised to try and improve the accuracy of prostate cancer detection [8, 43, 51-56].

PSA also exists as several different isoforms. Luminal prostate epithelial cells produce proPSA, an inactive precursor of PSA which has a 7-amino-acid leader sequence, and this is cleaved by the protease action of human kallikrein 2 (hK2) and other kallikreins resulting in the 237-amino-acid mature form of PSA [57-63]. Numerous truncated or clipped forms of proPSA may arise due to the incomplete removal of the 7-amino-acid leader sequence, and these and other forms of PSA may be released more freely within the circulation of men with prostate cancer [61-73]. It has recently been demonstrated that the levels of [-2] proPSA are higher in prostate cancer than the benign setting and that the use of this biomarker significantly improves the rate of cancer detection compared to using tPSA and the free-to-total PSA ratio [66, 74-87]. Intact PSA describes an intact and inactive form of proPSA that does not form a complex and which is released from prostate cancer cells [69, 73, 88]. In fact, it is now known that prostate cancer cells actually produce less PSA than

benign epithelial cells, however changes in the architecture of the prostate epithelium arising from malignant progression [23, 26, 89] result in the serum PSA level becoming elevated in many, though not all, of these men.

As PSA is organ-specific and not prostate cancer-specific there is a considerable degree of overlap in PSA levels between patients with benign pathologies [21, 26, 90-93] such as prostatitis, benign prostate hyperplasia (BPH) or urinary retention, and patients with prostate cancer [28, 94]. A “normal” level of PSA was previously described as being below 4.0ng/mL for men aged 50-80 years without prostate disease [95] however in more recent years it has become clear that there is no such thing as a “normal” PSA in terms of prostate cancer risk. Moreover there is no PSA cut-off threshold below which the risk of detecting a prostate cancer on biopsy is zero [9] and the choice of PSA threshold at which a clinician might recommend a patient biopsy is controversial [96-98]. If the PSA threshold was to be set too high then clinically significant prostate cancers might be missed – conversely, if it was to be set too low then an unacceptably high number of men without prostate cancer would be subjected to an unnecessary biopsy and thereby be exposed to the inherent risks and anxieties associated with this invasive procedure. The use of a number of PSA derivatives, such as PSA density, PSA velocity, age-adjusted PSA, free-to-total PSA ratio, and different molecular derivatives of PSA has led to various refinements in the performance of the PSA test [53]. Despite this the limited sensitivity and specificity of PSA means that there remains an urgent need to identify prostate cancer biomarkers with better performance characteristics than PSA alone.

Additional human kallikreins as tumour markers

In addition to PSA, fourteen other human kallikrein-related peptidases have now been identified and structurally characterized. Human kallikrein-related peptidase 2 (hK2, KLK2) is a protease with a number of similarities to PSA. For example there is 80% identity in primary structure between hK2 and PSA, the expression of both the *KLK2* and *KLK3* genes is regulated by the signalling activity of the androgen receptor (*AR*) stimulated by the supply of androgens, and both PSA and hK2 display a similar and highly extensive degree of prostate-specific expression pattern. hK2 is a trypsin-like serine protease responsible for cleaving pro-prostate-specific antigen into its enzymatically active form during a cascade reaction which regulates seminal clot liquefaction. Although the concentration of hK2 in serum is about two orders of magnitude lower than that of PSA, the development of reliable assays for hK2 suggests that this kallikrein may potentially be used as a prostate cancer biomarker [58, 72, 99-110]. Interestingly whereas PSA is strongly expressed in benign epithelium, hK2 becomes strongly expressed in malignant tissue, particularly in high-grade prostate cancer cases [99, 111-113]. Compared with individuals without prostate cancer, men with this malignancy have higher levels of hK2 in their serum, and there is reasonable correlation between the serum level of hK2 and the aggressiveness of prostate cancer present in an individual [101, 112].

Many of the kallikreins interact with each other within both normal physiological pathways and during the development of prostate pathology. Several of the recently described kallikreins, including *KLK4*, *KLK5*, *KLK6*, *KLK8*, *KLK10*, *KLK11* and *KLK14*, have been

tested as potential prostate cancer biomarkers in relatively small numbers of patients [114-120] however to date these additional kallikreins have not been proven to be clinically useful..

The kallikreins can be subject to degradation if samples undergo repeated freeze-thaw cycles, and the values of both free and intact PSA are lowered if samples are repeatedly frozen and thawed [73, 121]. In general the plasma or serum should be separated from the cellular component of blood within around one hour of blood draw, and free PSA in serum should be assayed within the same working day, whilst plasma-based assays should be undertaken within a day or two [105] given that there is evidence to suggest that kallikreins are more stable in plasma than in serum samples. After this the samples should be stored at -80°C and repeated freeze/thaw cycles should be avoided. In general the performance characteristics of different PSA isoforms and novel kallikreins as prostate cancer biomarkers may be optimised by the correct handling and utilisation of clinical samples [122].

The use of combinatorial panels of kallikrein biomarkers

In recent years there has been interest in developing a prostate cancer screening test based on the use of assays for a number of kallikreins in combination, based on the principle that the use of a panel of biomarkers might outperform the use of PSA alone. As an example of such an approach a panel of four kallikrein markers measured in blood – specifically comprising free PSA (fPSA), single-chain “intact” PSA (iPSA), total PSA (tPSA), and (hK2) – has been demonstrated to outperform the use of PSA alone in predicting the outcome of a prostate biopsy in several cohorts of men enrolled in randomised studies of screening [121, 123-127]. Importantly it has become apparent that predicting biopsy outcome based on this panel of four kallikrein markers() may be better than using total PSA alone in both previously screened [125-127] and hitherto unscreened [121, 123, 124] men. Crucially in terms of developing a test which might be applicable to a population-based screening programme for prostate cancer, the four kallikrein marker panel has the potential to dramatically reduce the number of unnecessary biopsies conducted as part of the screening programme. If the four kallikrein marker panel was used in a screening programme along with a man’s age, regardless of whether a digital rectal examination (DRE) is incorporated, evidence suggests that unnecessary biopsies can be greatly reduced in number without missing many high grade cancers [121, 123, 128].

Studies of the four kallikrein markers have facilitated the development of mathematical “laboratory models” (based on assays of the four kallikrein-markers and a man’s age) and “clinical models” (using the four kallikreins, a man’s age, and the findings at DRE). A “laboratory model” is potentially very useful as it obviates the need for all men within a screening programme to undergo a DRE, and raises the concept of the use of a “finger-prick” blood test, which may be performed at home or in the office, rather than a visit to a particular screening centre and a clinical examination. The adoption of a simpler and less intrusive test may raise compliance with the screening programme. Fortunately assays for tPSA and fPSA are now widely available and assays for iPSA and hK2 have now been developed therefore all four assays could be readily and conveniently incorporated into a single test with relatively low cost.

The performance of fewer unnecessary prostate biopsies clearly has potential benefits such as a reduction in costs, a reduced risk of biopsy-associated complications such as bleeding and sepsis, and a reduction in the anxiety experienced by men who undergo screening. An additional potential benefit to undertaking fewer biopsies within a screening programme might be that fewer clinically insignificant prostate cancers are detected, thereby reducing the associated problems of over-diagnosis and over-treatment of low-grade cancers which are otherwise considered to be “over-diagnosed” using current PSA-based detection methods [121, 123]. However, undertaking fewer prostate biopsies within a fixed population of screened men, and thereby detecting fewer overall cancers, might be expected to result in the under-detection of clinically significant cancer cases. In fact, the available evidence from the studies performed thus far suggests that very few high grade prostate cancers appear to be missed using the four kallikrein marker panel during an initial round of screening [121, 123]. In addition it might be reasonable to assume that any high grade cancers potentially missed by a round of screening using the four kallikrein panel could subsequently be detected during a further round of screening, and still be within a “window of curability”, provided the screening rounds were sufficiently frequent to pick up any progressing malignancy before it had become incurable. All of the studies performed to date to evaluate the four kallikrein panel of biomarkers have been performed in European population cohorts using serum samples however plasma-EDTA anti-coagulated samples may be more beneficial as they can be shipped to other centres in order for novel biomarkers to be evaluated in other laboratories. Moreover validation studies performed using cohorts of men from the United States are needed in order to verify the applicability of this test in these populations. The four kallikrein marker assay is not yet readily available in the United States however it is likely to be introduced in the near future.

The use of different PSA isoforms and additional kallikreins as newer biomarkers within the context of a screening programme has the potential to improve upon the diagnostic performance of using PSA alone, resulting in a fewer number of men needing to undergo a prostate biopsy procedure. Although the four kallikrein marker panel for example has shown particular promise in retrospective studies, many of the cohorts in the studies reported to date comprise serum, rather than plasma, samples, and many of the protocols within these screening studies included the use of sextant rather than extended core biopsies. It is now recognised that the use of extended core biopsies to detect prostate cancer is superior to sextant samples due to improved sampling of the gland [129]. Moreover, many of the studies of PSA-based screening for prostate cancer have included men in the eighth decade of life, whereas it is now recognised that the men who are likely to benefit the most from prostate cancer screening are those in earlier mid-life, perhaps between the ages of 40 and 60 years. A prospective study evaluating the contemporaneous use of a panel of PSA variants and additional kallikreins, focussing on early middle-aged men with the most to gain from a well-performing screening test, would be extremely helpful in clarifying the clinical utility of such a panel of kallikrein biomarkers.

A novel approach to improve the clinical performance of PSA is to combine the results of three automated blood tests (tPSA, fPSA and [−2] proPSA) using a mathematical formula termed the Prostate Health Index (phi). The phi test specifically uses the formula $([-2] \text{ proPSA/fPSA}) \times \text{tPSA}$ to calculate and report a phi result which can improve the rate of

prostate cancer detection compared with either tPSA or f/tPSA alone [74-77, 81, 83, 84, 87]. Levels of phi have been demonstrated to differ significantly between men with and without prostate cancer in European cohorts of men [84]. The phi had clinical specificities of 23% and 31% at a sensitivity of 95% and 90%, compared with specificities of 10% and 8% for tPSA alone [84]. The phi has also been prospectively evaluated in a large cohort of men in the United States with PSA levels of 2-10 ng/mL [74]. The performance of phi was compared with tPSA, fPSA and [-2] proPSA, and at a sensitivity of 80% to 95% the specificity of phi to detect prostate cancer was higher than for tPSA and fPSA [74]. The phi has also been shown to be a stronger predictor of prostate cancer at biopsy in men with PSA levels of 2-10 ng/mL in an Italian study [75].

Use of the phi may help to reduce the number of unnecessary prostate biopsies undertaken in men with moderately elevated levels of PSA, and it may provide a useful tool in the discrimination of insignificant and aggressive prostate cancer. As an example of this, around three quarters of prostate cancers detected with a phi value less than 25 have been found to be clinically insignificant at prostate biopsy [74]. The phi has also been demonstrated to miss fewer prostate cancers with a Gleason score >7 than tPSA or free-to-total PSA [84], whilst the risk of detecting a clinically aggressive cancer has been shown to be increased in men with higher phi readings [74]. Studies have also shown an association between phi and [-2] proPSA levels and Gleason score suggesting that these biomarkers may be useful in the prediction of aggressive disease [75]. Whereas the level of PSA is known to correlate with prostate volume, the phi reading has been shown to correlate with tumour volume, providing further evidence that this tool may be helpful in the detection of clinically aggressive disease at prostate biopsy. By helping to reduce the number of unnecessary prostate biopsies, the use of phi not only provides a clinical benefit to men at lower levels of PSA, but it can also facilitate substantial cost savings for healthcare systems [130]. Taken together the available evidence suggests that phi may play an important role in the identification of prostate cancer at biopsy, especially in men aged over 50 years with a negative DRE and a tPSA in the range of 2-10 ng/mL. Following a prospective multicenter FDA registration study including men with contemporary and more extensive biopsy schemes the phi has now been approved by the FDA in the United States.

Conclusions

Whilst the use of PSA has revolutionised the detection and treatment of prostate cancer, there remains considerable scope for both improvements in the use of variations of the PSA test and the development of novel biomarkers. The use of PSA derivatives and additional kallikrein markers has the potential to improve upon the current performance characteristics of the PSA test alone. The use of PSA as part of a multivariable approach to early prostate cancer detection, including such tools as the phi test, has recently been supported by the Melbourne Consensus Statement on Prostate Cancer Testing [131]. An improved screening test may dramatically reduce the number of men undergoing an unnecessary biopsy whilst maintaining the ability to detect high risk cases of this common malignancy. Such an approach has the potential to reduce the over-diagnosis and over-treatment of prostate cancer whilst simultaneously enabling clinicians to focus on high-risk cases of localised prostate cancer in a population of men likely to benefit from radical intervention.

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Key points

1. Using PSA derivatives and additional kallikrein markers for prostate cancer diagnosis can improve upon the current performance characteristics of the PSA test alone.
2. An improved screening test may dramatically reduce the number of men undergoing an unnecessary biopsy whilst maintaining the ability to detect high risk prostate cancer.
3. The use of an improved screening test may reduce both over-diagnosis and over-treatment of prostate cancer.

Synopsis

The aim of this article is to provide an update regarding current advances in prostate cancer screening based on the use of prostate specific antigen (PSA) and its derivatives, along with the use of more novel human kallikrein markers. This update will hopefully be useful for urologists, general physicians and patients.

Prostate cancer is a heterogenous disease. Whilst it is the commonest male malignancy, and the second most common cause of male cancer-related death, many men are diagnosed with relatively indolent disease and may not need treatment. Prostate cancer screening based on PSA-testing is therefore a controversial issue. Whilst there is evidence of a survival benefit from screening, the numbers needed to screen and subsequently treat remain high, and there is a significant risk of over-detection and over-treatment, resulting in the exposure of a large number of men to the side effects of treatment when their disease may not actually become a threat in later life. That said prostate cancer remains a significant cause of cancer-associated morbidity and mortality. One of the key challenges in prostate cancer management is to develop a screening test with sufficiently good performance characteristics that it detects significant disease at a sufficiently early stage that treatment may be offered with curative intent, whilst simultaneously reducing the large number of negative or unnecessary biopsies currently being performed.