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REVIEW ARTICLE

From prostate-specific antigen (PSA) to precursor PSA (proPSA) isoforms: a review of the emerging role of proPSAs in the detection and management of early prostate cancer

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What's known on the subject? and What does the study add?
Despite the popularity of PSA blood testing for prostate cancer, there are a number of important limitations of this popular serum marker including the limited ability to accurately distinguish patients with and without prostate cancer and those who harbour an aggressive form of the disease. This is especially true when the total PSA is <10 ng/mL. Thus, significant efforts have been placed to find new serum markers that can help overcome these limitations.

In this review article, we discuss the emerging role of the various precursor forms of PSA (proPSAs), with a special emphasis on [-2]proPSA in the detecion and management of early prostate cancer. The clinical utility of Prostate Health Index (*phi*) is also discussed.

- Despite the overall success of prostate-specific antigen (PSA) blood test, its use as a serum marker for prostate cancer has been limited due to the lack of specificity, especially in men presenting with a total PSA (tPSA) level of <10 ng/mL. PSA testing has also resulted in an increase in the number of patients being diagnosed with low-grade, potentially clinically insignificant prostate cancer.
- There is therefore an urgent need for new markers that can accurately detect as well as differentiate patients with aggressive vs unaggressive prostate cancer.
- In this review, we discuss the emerging role of precursor forms of PSA (proPSAs) and the Prostate Health Index (*phi*) measurement in the detection and management of early stage prostate cancer.
- A literature search was conducted using PubMed® to identify key studies.
- Studies to date suggest that [-2]proPSA, a truncated form of proPSA is the most cancer-specific form of all, being preferentially expressed in cancerous prostatic epithelium and being significantly elevated in serum of men with prostate cancer.
- There is evidence to suggest that %[-2]proPSA measurement ([-2]proPSA/free PSA [fPSA] × 100) improves the specificity of both tPSA and fPSA in detecting prostate cancer.
- *phi* incorporating [-2]proPSA, fPSA and tPSA measurements has also yielded promising results and appears superior to tPSA and fPSA in predicting those patients with prostate cancer. Increased *phi* levels also seem to preferentially detect patients harbouring more aggressive disease.
- Further studies in the form of large, multicentre, prospective trials with detailed health economic analyses are required to evaluate the true clinical applicability of these novel markers.

KEYWORDS PSA, proPSA, prostate, prostatic neoplasm, diagnosis, management

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INTRODUCTION

PSA (also known as human kallikrein 3 [hk3]) belongs to a family of proteases known as kallikreins that are encoded by a cluster of genes located within a 300 kb region on human chromosome 19q13.4 [1,2]. PSA is an androgen-regulated chymotrypsin-like serine protease that is produced in high levels within the prostatic ductal and acinar epithelium. PSA is initially produced with a 17 amino acid leader sequence that is cleaved to produce an inactive precursor enzyme (proPSA) containing a seven amino acid pro-leader peptide in addition to the 237 amino acids of mature PSA [3–5]. Once secreted into the prostatic ducts, the pro-leader peptide is removed and the proPSA becomes rapidly activated to PSA by hk2 and hk4 [3,6]. In the seminal fluid, PSA functions to cleave the gel proteins seminogelin I and II, leading to the liquefaction of the semen [7]. In healthy men, the normally tight and orderly prostatic glandular architecture functions to confine the enzymatically active PSA to within the prostate gland. Only a minute amount of PSA ever leaks in to the circulation, resulting in a concentration a million-fold lower in serum (<4 ug/L) than that in the seminal plasma (0.5–5 g/L) [8]. This dramatic disparity in concentration between the serum and seminal fluid may be one reason why PSA has evolved to become a useful serum marker for prostatic disease.

Despite the overall success of PSA, its use as a serum marker for prostate cancer (especially early stage prostate cancer) is far from ideal. The primary limitation of PSA has been its inability to accurately distinguish between a benign and malignant pathology [9]. This is especially true in the total PSA (tPSA) range of 2–10 ng/mL where benign and malignant prostatic conditions frequently co-exist (the so called diagnostic 'grey zone'). It is now clear that even at PSA levels previously considered normal (<4 ng/mL), a small but significant proportion of men will be found to have cancer. In the PSA ranges of <0.5, 0.6–1.0, 1.1–2.0, 2.1–3.0 and 3.1–4.0 ng/mL, ≈6.6%, 10.1%, 17%, 23.9% and 26.9% of patients will be found to have cancer on prostatic biopsies, respectively [10]. Of greater concern is that clinically significant prostate cancer (Gleason ≥7) may occur in ≈15% of patients who present with a low PSA level of <4 ng/mL [9]. Thus, considerable efforts have been made to find new PSA markers that can not only accurately detect prostate cancer but also, more crucially, differentiate between clinically significant and insignificant prostate cancers. In this regard, our increased understanding of the composition of PSA in serum has been pivotal in helping to identify new potential PSA biomarkers.

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In serum, most tPSA (80–95%) exists as a complexed form (cPSA) with a number of different endogenous protease inhibitors (e.g. α_1 anti-chymotrypsin, α_2 macroglobulin) [11,12]. These protease inhibitors function to prevent potentially damaging protease activity of PSA. However, a small proportion of PSA exists in an uncomplexed or 'free' form, which was assumed to be enzymatically inactive. It is now known that this free PSA (fPSA) fraction is composed of at least three different types of enzymatically inactive PSA: benign PSA (BPSA), intact inactive PSA and proPSA, of which BPSA and proPSA are the best characterised (Fig. 1) [13]. BPSA is a degraded form of PSA that is identical to the native, mature PSA with 237 amino acids but contains two internal peptide bond cleavages at Lys182 and Lys145. Immunohistochemical studies have shown that BPSA is expressed preferentially in the transitional zone of the prostate and is associated with pathological BPH [14,15]. In contrast, proPSAs are expressed almost exclusively in the peripheral zone of the prostate where most prostate cancers are known to emerge [16].

Truncated forms of proPSAs were first identified in serum of patients with prostate cancer in 1997 [17]. These truncated proPSAs have their normal seven amino acids pro-leader peptides removed by post-translational proteolytic cleavage resulting in a shorter pro-leader peptide of varying amino acid numbers (Fig. 2). By subjecting the pooled serum of patients with prostate cancer (containing 63 ng/mL tPSA) to hydrophobic interaction chromatography-HPLC techniques, Mikolajczyk *et al.* [17] were able to resolve proPSAs into two different peaks containing different truncated versions of proPSA: namely [-5, -7] proPSA and [-4] proPSA. Subsequent studies on prostate tissues revealed that proPSAs were preferentially elevated in the peripheral zone of prostatic tissues containing cancer whilst remaining largely undetectable in the transitional

FIG. 1. Molecular forms of PSA. Most of the PSA in serum exists in a complexed form with several different endogenous protease inhibitors, e.g. α_1 anti-chymotrypsin. fPSA on the other hand exists in an uncomplexed form and is comprised of proPSAs, BPSA and intact PSA. proPSA is associated with cancer, BPSA with benign diseases whilst the association of intact PSA is currently unknown. Adapted from Mikolajczyk et al. *[13], with permission from Elsevier.*

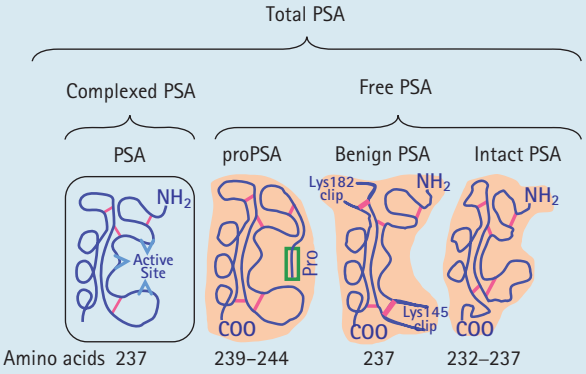
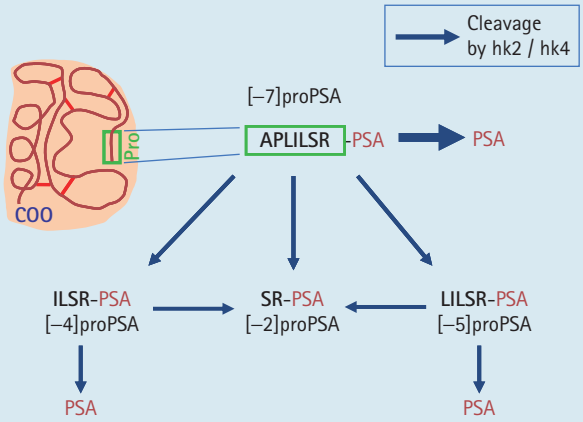


FIG. 2. ProPSA molecules. The native proPSA (otherwise known as [-7]proPSA) exists in a truncated form containing a seven amino acid N-terminal pro-leader peptide (APLILSR). Post-translational proteolytic cleavage by hk2 or hk4 results in the release of the active form of PSA. Partial cleavage of [-7]proPSA results in the shortening of the seven amino acid pro-leader peptide to form [-5]proPSA (LILSR), [-4]proPSA (ILSR) and [-2]proPSA (SR) with release of mature PSA from [-5] and [-4]proPSAs. As [-2]proPSA cannot be cleaved further, this isoform accumulates preferentially in the serum of men with prostate cancer.



zone of the prostate [16]. Furthermore, amino acid sequencing showed that the proPSA comprised primarily of a truncated form of proPSA that contains a pro-leader peptide consisting of only two ([-2]proPSA) rather than the usual seven amino acids ([-7]proPSA). Thus, serum proPSAs gained attention as a potentially more cancer-specific form of PSA that may help overcome the current limitations of PSA, that is, to be able to help reliably differentiate between prostate cancer and benign prostatic disease.

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In this review, we discuss the emerging role of proPSA isoforms, with a special emphasis on [–2]proPSA in the detection and management of early stage prostate cancer. The clinical utility of the Beckman Coulter Prostate Health Index (*phi*) will also be discussed.

METHODS

A literature search was performed on PubMed® until March 2012 to identify key studies looking at the association between proPSA, *phi* and prostate cancer. The search terms included: 'proprostate specific antigen', 'pro-prostate specific antigen', 'pPSA', 'proPSA', 'precursor PSA', 'proenzyme prostate specific antigen', 'proenzyme PSA', '[-2]proPSA', '[-4]proPSA', '[-5]proPSA', '[-7]proPSA' and 'Prostate Health Index'. The search was restricted to publications in the English language and publications were selected according to relevance to the subject and quality.

IMPROVING PSA SPECIFICITY: THE ROLE OF proPSAS IN EARLY PROSTATE CANCER DIAGNOSIS

To evaluate the potential clinical utility of proPSAs in early prostate cancer detection, Sokoll *et al.* [18] conducted a small, retrospective, preliminary analysis of archival prostatic biopsy specimens from 119 men (88 with cancer, 31 without cancer) who presented with a tPSA level of 2.5–4 ng/mL. Serum from these men was assayed for tPSA, fPSA and proPSAs (where proPSAs were the sum of [-2], [-4] and [-7] proPSA isoforms). Although no difference in PSA and %fPSA (fPSA/tPSA × 100) were noted between the two groups, %proPSAs (proPSAs/fPSA × 100) tended to be higher in the cancer group than in the group without cancer (50.1% vs 35.5%). Furthermore, receiver operating characteristic (ROC) analysis showed that the area under the curve (AUC) for %proPSAs was higher (0.688) than with %fPSA (0.567) and that at a fixed sensitivity of 75%, %proPSAs had a higher specificity than %fPSA (59% vs 33%). In a follow-up study [19], the same group evaluated further the potential of proPSAs in men presenting with a PSA level of 4–10 ng/mL. On multivariate logistic regression analyses, when the sum of proPSAs ([-2], [-4] and [-7]) was analysed in conjunction with tPSA and %fPSA, the specificity of these combined variables for early prostate cancer detection was significantly higher than those of the individual variables (44% combined vs 23%, 33% and 13% for tPSA, %fPSA and proPSAs, respectively) at a fixed sensitivity of 90%. These results were later confirmed in a larger, two-centre study by Catalona *et al.* [20] who analysed serum specimens obtained from 1091 (635 benign, 456 cancer) men who underwent prostatic biopsies. These samples were retrospectively assayed for tPSA, fPSA and the different forms of proPSAs ([-2], [-4], [-5, -7]). Although this study was somewhat limited by the lack of standardisation in PSA assays used across the two institutions, %proPSAs was found to be superior to tPSA or %fPSA across the PSA level range of 2–10 ng/mL at either institutions. However, at a lower PSA level range of 2–4 ng/mL, %[-2]proPSA ([-2]proPSA/fPSA × 100) had the highest specificity amongst all other variables. In a follow-up study using the same patient group [21], sub-group analysis of men with a low PSA level range of 2–4 ng/mL revealed that %proPSAs at a threshold of 1.8% detected 90% of cancers including 100% (16 of 16) of extracapsular tumours and 96.6% (28 of 29) of tumours with a Gleason score of ≥7, while still avoiding 19% of unnecessary biopsies. Thus for the first time, it was shown that %proPSAs not only appeared to increase the specificity for early prostate cancer detection but might also be helpful in preferentially identifying those patients who might harbour a more aggressive form of the disease.

Previously, the ratio of fPSA to tPSA (f/tPSA) has been shown to significantly improve the discrimination between prostate cancer and BPH, where an increase in %fPSA correlates with a lower risk of prostate cancer (and vice versa) [22,23]. Furthermore, components of fPSA have also been noted to be preferentially associated with prostate cancer or BPH (proPSAs and BPSA, respectively). It was therefore only a matter of time before studies evaluating the clinical utility of proPSAs and BPSA in men with low (<15%) or high (>25%) %fPSA emerged. In 2004, Mikolajczyk *et al.* [24] retrospectively evaluated the serum samples of 380 (238 with cancer, 142 without cancer) men enrolled in prostate cancer screening studies at Washington University with a tPSA level of 4–10 ng/mL. In agreement with previous studies, %proPSAs ([-2], [-4], [-5, -7]) had a higher AUC than did %[-2]proPSA, fPSA and complexed PSA (AUC 0.69, 0.64, 0.63 and 0.57, respectively). In men with a %fPSA of >25%, %[-2]proPSA had the highest discriminatory ability with an AUC of 0.77. Using a threshold level of 2.5%, the sensitivity of %[-2]proPSA for prostate cancer detection in men with a tPSA level of 4–10 ng/mL and %fPSA of >25% was 90%, with 36% of men potentially being spared from having to undergo prostatic biopsies. However, in those with a %fPSA of <15%, %proPSAs had a higher predictive ability (AUC 0.703) and specificity (36%) at 90% sensitivity than with %[-2]proPSA (AUC 0.669, specificity 21%). In a separate study, Khan *et al.* [25] analysed the pre-biopsy serum of men who were enrolled in the National Cancer Institute Early Detection Research Network (NCI EDRN) prostate cancer early detection biomarker programme at Johns Hopkins, for tPSA, fPSA, proPSAs ([-2], [-4], [-5, -7]) and BPSA. All patients in this study had a %fPSA of <15%. In this select group of men, BPSA and proPSAs/BPSA ratio had identical predictive ability (AUC of 0.72) and was superior to tPSA (AUC 0.51) and %fPSA (AUC 0.54). Although the AUC for BPSA and proPSAs/BPSA ratio were identical, BPSA alone had a statistically significantly lower specificity of 20% when the sensitivity was maintained at 90%. In contrast, the specificity of proPSAs/BPSA ratio at this sensitivity level was 46%. Thus, %[-2]proPSA or proPSAs/BPSA ratio may be useful adjunct markers to consider when considering %fPSA of patients.

In an attempt to investigate whether a low BPSA and a high [-2]proPSA (or vice versa) reflect poor prostate cancer prognosis, patients with prostate cancer on a watchful-waiting policy from the screen arm of the European Randomised Study of Screening for Prostate Cancer (ERSPC), section Rotterdam [26], were arbitrarily divided into those with favourable disease characteristics (clinical T1c disease, one biopsy core with Gleason 3 + 3 or less) or aggressive disease characteristics (clinical T3c, more than four biopsy cores invaded with Gleason 4 + 4 or greater). To adjust for possible tPSA to prostatic volume bias, a sub-group analysis of men with a PSA level of 4–10 ng/mL with a prostatic volume of <60 mL was also

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TABLE 1 Summary of studies evaluating the role of proPSA isoforms in prostate cancer

Reference	Year	PSA range, ng/mL	Number of patients	Methods	Main findings
Sokoll *et al.* [18]	2003	2.5–4	119	Retrospective analysis of sera from men undergoing TRUS-guided biopsy	75% of cancers could be detected with 59% of unnecessary biopsies being spared using %proPSAs ([-2], [-4], [-7]).
Khan *et al.* [19]	2003	4–10	93	Retrospective analysis of sera from men undergoing TRUS-guided biopsy	Sum of proPSA ([-2], [-4], [-7]), tPSA and %fPSA in combination improved the specificity of early prostate cancer detection.
Catalona *et al.* [20]	2003	2–10	1091	Retrospective, two centre analysis of sera from men undergoing TRUS-guided biopsy	%proPSAs ([-2], [-4], [-5, -7]) improved specificity for prostate cancer detection whilst decreasing unnecessary biopsies.
Catalona *et al.* [21]	2004	2–10	1091	Retrospective, two centre analysis of sera from men undergoing TRUS-guided biopsy	%proPSAs ([-2], [-4], [-5, -7]) was superior to %fPSA and complexed PSA for prostate cancer detection and had selectivity for detecting more aggressive cancers
Mikolajczyk *et al.* [24]	2004	4–10	380	Retrospective analysis of sera from men undergoing TRUS-guided biopsy	%proPSAs ([-2], [-4], [-5, -7]) had higher prostate cancer detection than fPSA and complexed PSA.[-2]proPSA significantly discriminated cancer in men with fPSA of >25%.
Khan *et al.* [25]	2004	1.8–24	161	Retrospective analysis of sera from men prospectively enrolled in the EDRN programme	Ratio of proPSAs ([-2], [-4], [-5, -7]) and BPSA can distinguish prostate cancer with greater accuracy when fPSA is <15%.
de Vries *et al.* [26]	2005	1.4–14.8	61	Retrospective analysis of sera from men with prostate cancer from the screen arm of the ERSPC (section Rotterdam)	Combination of proPSAs ([-2], [-4], [-5, -7]) and %fPSA could accurately distinguish patients with favourable or aggressive prostate cancer.

performed. On univariate analysis, a decrease in the [-2]proPSA/BPSA ratio was noted amongst patients with favourable prostate cancer characteristics ($P = 0.043$). When tPSA was adjusted to prostatic volume, the preferential decrease in the [-2]proPSA/BPSA ratio seen in the favourable disease characteristic group remained ($P = 0.033$). On multivariate logistic regression analysis, proPSAs ([-2], [-4], [-5, -7]) and %fPSA remained significant predictors of unfavourable disease characteristics. In combination, proPSAs and %fPSA were able to accurately predict 95.5% (42 of 44) of men with arbitrarily defined favourable prostate cancer (sensitivity of 95.5%) and 82.4% (14 of 17) of patients with aggressive prostate cancer characteristics (specificity of 82.4%). However, subgroup analysis of patients with a PSA level of 4–10 ng/mL with prostatic volume of <60 mL, revealed that the sensitivity and specificity of combined proPSAs and %fPSA was lower in this group of patients, albeit still of statistical significance. Thus, prostatic volume appears to have some influence on the sensitivity and specificity of proPSAs and fPSAs. This study suggests that proPSAs may be able to differentiate between those patients with favourable or unfavourable disease characteristics. It must be noted that the small sample size and the arbitrarily defined disease characteristics at the extreme ends of the spectrum limits the applicability of their findings.

> ‘proPSAs may be able to differentiate between those patients with favourable or unfavourable disease characteristics’

Thus, initial studies suggest that proPSA isoforms combined with fPSA or BPSA, may improve cancer detection in men presenting with a tPSA level of 2–10 ng/mL (Table 1) [18–21,24–26]. Further studies are required to study the potential predictive role of proPSAs in identifying those men with low PSA levels who might be harbouring aggressive disease.

CLINICAL UTILITY OF [-5, -7]proPSA ISOFORMS IN PROSTATE CANCER

Following the development of immunoassays specific to [-5, -7]proPSA in serum, the clinical utility in prostate cancer detection was explored. In a pilot study, Bangma *et al.* [27] examined sera from men enrolled in the Rotterdam section of the ERSPC presenting with a tPSA level of 1–10 ng/mL. In all, 146 men with biopsy confirmed prostate cancer, 143 without prostate cancer and 142 with BPH were examined. The authors found that [-5, -7]proPSA measurements did not improve the specificity of fPSA for discriminating patients with and without prostate cancer. Additionally, there was no association between tumour aggressiveness and [-5, -7]proPSA levels. In a subsequent multicentre study involving 2055 White men (1046 with cancer, 1009 without cancer), the ROC curve for serum [-5,-7]proPSA levels was found not to significantly differ from those of tPSA or f/tPSA ratio [28]. In the tPSA level range of 2–4 ng/mL for example, the AUC for [-5, -7]proPSA (0.53) and proPSA/fPSA (0.59) was not significantly

Table 2 Summary of studies evaluating the role of [-5, -7]proPSA in prostate cancer

Reference	Year	PSA range, ng/mL	Number of patients	Methods	Main findings
Bangma *et al.* [27]	2004	1–10	146	Retrospective analysis of sera from subset of men in the ERSPC (section Rotterdam)	[-5, -7]proPSAs did not improve the specificity of fPSA for discriminating patients with and without prostate cancer.
Lein *et al.* [28]	2005	0.28–81	2055	Retrospective analysis of sera from men undergoing TRUS-guided biopsy	No improvement in diagnostic accuracy was noted when comparing [-5, -7]proPSA and the corresponding ratios with tPSA or f/t PSA.
Stephan *et al.* [29]	2006	1–10	898	Retrospective, two centre ANN analysis of sera from men undergoing TRUS-guided biopsy	[-5, -7]proPSA measurements did not improve specificity over %fPSA.
Stephan *et al.* [31]	2007	1–25	376	Retrospective analysis of sera from men undergoing TRUS-guided biopsy who subsequently had laparoscopic radical prostatectomy	[-5, -7]proPSA alone did not improve the detection of non-organ confined or aggressive prostate cancer in men with prostate cancer. [-5, -7]proPSA / %fPSA; however, was able to predict more aggressive/ advanced prostate cancer.
Miyakubo *et al.* [30]	2009	4.1–20	257	Retrospective analysis of sera obtained from men undergoing age and volume-adjusted TRUS-guided biopsy	In both tPSA ranges of <10 and 10–20 ng/mL, [-5, -7]proPSA measurements did not improve the specificity over other PSA-based parameters, e.g. PSA density or PSA transitional zone density.

larger than that for tPSA (0.60) and f/tPSA ratio (0.64). In the tPSA level range of 4–10 ng/mL, [-5, -7] proPSA levels once again did not improve the discriminatory power of f/tPSA ratio. The role of [-5, -7]proPSA in prostate cancer detection was also evaluated in an artificial neural network (ANN) analysis conducted by Stephan *et al.* [29]. Serum measurements of 898 men with and without cancer showed that [-5, -7]proPSA could not improve the specificity of %fPSA in predicting biopsy outcome when sensitivity was set at 95%. A Japanese study in 2009 by Miyakubo *et al.* [30] reported that although [-5, -7]proPSA/fPSA (AUC 0.699) had a modestly higher discriminatory power than f/tPSA ratio (AUC 0.675) in predicting men with prostate cancer, it remained inferior to PSA density measurement (AUC 0.715) in men with a PSA level of <10 ng/mL who underwent age- and volume-adjusted prostatic biopsies. In men with a PSA level of 10–20 ng/mL, the AUC of [-5, -7]proPSA/fPSA remained higher than that of f/tPSA, but lower than those of PSA density and PSA transitional zone density (AUC 0.742, 0.708, 0.788 and 0.842, respectively).

Despite the limited overall evidence of [-5, -7]proPSA for use in early prostate cancer detection, Stephan *et al.* [31] reported a potential role for this proPSA isoform in discriminating between aggressive and unaggressive prostate cancer. In examining the sera of 376 patients with prostate cancer undergoing laparoscopic radical prostatectomy, the authors noted that the ratio of [-5, -7]proPSA/%fPSA differed significantly between patients with G2 and G3 disease ($P = 0.004$), Gleason < 7 and >7 ($P = 0.001$) and pT2 and pT3 tumours ($P < 0.001$) at tPSA levels of 1–25 ng/mL. Thus, [-5, -7]proPSA measurements might play some role in predicting tumour aggressiveness in patients with an established diagnosis of prostate cancer.

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Overall, most studies to date have found [-5, -7]proPSA no better than fPSA or other current PSA-based measurements in improving prostate cancer detection rates particularly in men with a tPSA level of <10 ng/mL (Table 2) [27–31].

CLINICAL UTILITY OF [-2]proPSA, A CANCER-SPECIFIC proPSA

Enthusiasm for [-2]proPSA as a prostate cancer-specific marker emerged from the observation that this truncated form of proPSA was differentially elevated in the peripheral zone of the prostate where most cancers occur [16]. Also, [-2]proPSA was largely devoid in the transition zone of the prostate, an area often associated with the development of BPH. Furthermore, serum levels of [-2]proPSA were found to be high in men with prostate cancer whilst it was low in men without prostate cancer [32]. Thus, [-2]proPSA appears to be a more cancer specific form of PSA.

The NCI EDRN is an American National Institute of Health-funded programme that aims to facilitate the development of biomarkers capable of detecting cancers at an early stage. Based on the promising results to date, [-2]proPSA was chosen by the NCI EDRN to undergo further validation using a common, archival, referenced set of serum samples collected from men who underwent prostatic biopsies at three different EDRN recruiting sites (Beth Israel Deaconess Medical Center, Johns Hopkins

University and the University of Texas Health Science Centre at San Antonio). By using serum samples of 123 men (51% with cancer, 49% without cancer, and PSA levels of 0.48–33.18 ng/mL) enrolled in the EDRN study, Sokoll *et al.* [33] evaluated the relationship between various PSA forms (including [-2]proPSA) and prostate cancer. Overall, the %fPSA was significantly lower in patients with prostate cancer, whilst [-2]proPSA and %[-2]proPSA levels were higher in patients with prostate cancer. When the more clinically relevant PSA range of 2–10 ng/mL was examined, [-2]proPSA as well as %[-2]proPSA continued to be significantly associated with prostate cancer. In this PSA range, the AUC for %[-2]proPSA was 0.73 compared with 0.53 for %fPSA. When PSA, BPSA, %fPSA, %[-2]proPSA, [-2]proPSA / BSA and testosterone were evaluated together using multivariate logistic regression models, %[-2]proPSA remained a significant discriminator for prostate cancer (AUC 0.73).

In a follow-up study to investigate the potential correlation between [-2]proPSA and prostate cancer aggressiveness, Sokoll *et al.* [34] analysed the serum samples of 566 men (43% cancer, 57% without cancer) who were prospectively enrolled in the NCI EDRN study who met their inclusion criteria. In all, %[-2]proPSA was found to have equivalent predictive ability compared with PSA and %fPSA in detecting prostate cancer across the overall study population (PSA levels of 0.29–310.6 ng/mL). At lower, more diagnostically challenging PSA levels, %[-2]proPSA performed significantly better than %fPSA at a PSA range of 2–4 ng/mL. However, for a higher PSA level range of 4–10 ng/mL, the AUC for %[-2]proPSA was only slightly larger than %fPSA and this was not statistically significant. Despite this discrepancy (which may be due to differences in study designs), multivariate regression models incorporating clinical, demographic and individual PSA forms showed a significant improvement in AUC over the individual PSA forms in agreement with previous studies. In addition, increasing [-2]proPSA as well as %[-2]proPSA was noted to be associated with increasing Gleason score and worsening disease characteristics.

Evaluating [-2]proPSA as part of mathematical models (e.g. multivariate logistic regression models), with or without other proPSAs, have consistently been shown to be better than considering individual PSA forms on their own. To evaluate this observation further, Stephan *et al.* [35] created a [-2]proPSA based ANN alongside conventional logistic regression models incorporating various PSA parameters, e.g. tPSA, %fPSA and %[-2]proPSA, age and prostatic volumes. In all, 586 patients were included in the analysis of which 475 patients (264 with cancer, 211 without cancer) were in the clinically relevant PSA range of 2–10 ng/mL. The authors found that the ANN and logistic regression models using %[-2]proPSA, %fPSA, tPSA and age but without prostatic volume reached the highest AUCs (ANN 0.85 and logistic regression 0.84) and had superior specificities (ANN 62.1%, logistic regression 53.1%) compared with tPSA (22.7%), %fPSA (45.5%) and %[-2]proPSA (41.7%) alone at a fixed sensitivity of 90%. Interestingly, both the ANN and logistic regression models that included the additional input parameter of prostatic volume did not enhance the AUC on either model. This important observation presents an obvious advantage, whereby an ANN model based entirely on laboratory parameters alone spares the patient from having to undergo an invasive procedure for prostatic volume measurement.

More recently, changes in [-2]proPSA levels over time have been suggested as a potentially useful predictor of future prostate cancer development. By using frozen sera from 420 White men randomly selected from the Olmsted County Study of Urinary Symptoms and Health Status among Men (OCS), Rhodes *et al.* [36] investigated changes in [-2]proPSA levels with the longitudinal changes of common clinical urological measures including age, prostatic volume and subsequent detection of prostate cancer. The authors reported that the levels of [-2]proPSA increased with advancing age and prostatic volume; however, the greatest change in [-2]proPSA levels were seen amongst men who subsequently developed prostate cancer (annualised percentage change of 8.1%) compared with those who did not (annualised percentage change of 3.5%) after a median follow-up of 7 years. In a subsequent study by the same group [37], the authors investigated the baseline and cross-sectional associations between [-2]proPSA and urological measures in a

Table 3 Summary of studies evaluating the role of [-2]proPSA in prostate cancer

Reference	Year	PSA range, ng/mL	Number of patients	Methods	Main findings
Sokoll *et al.* [33]	2008	0.48–33.18	123	Retrospective, multicentre analysis of sera obtained from men prospectively enrolled in the EDRN study who underwent TRUS-guided biopsy	%[-2]proPSA was the best predictor of prostate cancer detection compared with %fPSA.
Stephan *et al.* [35]	2009	0.26–28.4	586	Retrospective, ANN analysis of sera obtained from men undergoing TRUS-guided biopsy	Incorporation of %[-2]proPSA into an ANN and logistic regression model enhanced the diagnostic accuracy to differentiate between malignant and non-malignant prostatic diseases.
Sokoll *et al.* [34]	2010	0.29–310.6	566	Retrospective, multicentre analysis of sera obtained from men prospectively enrolled in the EDRN study who underwent TRUS-guided biopsy	%[-2]proPSA performed significantly better than %fPSA in PSA level range of 2 to 4 ng/mL. %[-2]proPSA was also noted to increase with increasing Gleason score and in aggressive cancers.
Rhodes *et al.* [36]	2012	<8.8	443	Retrospective analysis of sera obtained from men enrolled in a population based study (OCS)	Men subsequently diagnosed with prostate cancer had more than twice the rate of increase in [-2]proPSA levels compared with those men without prostate cancer.
Rhodes *et al.* [37]	2012	0.5–1.8*	748	Retrospective analysis of sera obtained from men enrolled in two population based studies (OCS and FMHS)	Baseline [-2]proPSA levels were slightly higher in Black men compared with White men (median 6.3 vs 5.6 pg/mL). Men with higher baseline [-2]proPSA were at an almost eight-fold increased risk of developing subsequent prostate cancer.

**25–75th percentiles.*

subset of patients from two population based studies of White (OCS) and Black men (Flint Men's Health Study, FHMS). In all, frozen serum samples from 420 and 328 men were measured for [-2]proPSA from the OCS and FHMS cohorts, respectively. This study reported that the baseline [-2]proPSA levels in Black men were slightly higher than those in White men (median of 6.3 vs 5.6 pg/mL, respectively). Interestingly, men with higher baseline [-2]proPSA levels in the OCS cohort had an almost eight-fold increase in risk of subsequent prostate cancer diagnosis (hazard ratio 7.8, 95% CI 2.2–27.8) than men who had low baseline levels of [-2]proPSA. Thus, measurements of baseline [-2]proPSA, as well as changes in [-2]proPSA levels over time, might be useful predictors of prostate cancer development and warrants further investigation.

Clinical studies to date evaluating the cancer-specific form of [-2]proPSA have yielded very promising results (Table 3) [33–37] and appear to confirm the growing observation that [-2]proPSA could be the most clinically relevant proPSA isoform in serum for the detection of prostate cancer. The predictive ability of [-2]proPSA in detecting prostate cancer within the diagnostic grey zone of 2–10 ng/mL PSA appears to be optimal when it is evaluated as part of a mathematical model.

'measurements of baseline [-2]proPSA, as well as changes in [-2]proPSA levels over time, might be useful predictors of prostate cancer development'

THE *PHI* IN EARLY PROSTATE CANCER DETECTION

With increasing positive evidence for the role of [-2]proPSA in early prostate cancer detection, Beckman Coulter Inc. developed a mathematical algorithm incorporating [-2]proPSA, tPSA and fPSA for use in patients with PSA levels of 2–10 ng/mL with a non-suspicious prostate on DRE.

***Phi* has been defined as:**
([-2]proPSA/FPSA) X √ TPSA

phi currently has approval for use in early prostate cancer detection in Europe but it is not yet available in the USA. Le *et al.* [38] were the first to evaluate the predictive ability of [-2]proPSA and *phi* in a prospective prostate cancer screening setting. Of the 2034 men undergoing prostate cancer screening, 322 patients were advised to undergo prostatic biopsies due to an elevated PSA level (>2.5 ng/mL) and/or an abnormal DRE finding. Of the 74 patients who eventually underwent prostatic biopsies, 63 had a tPSA level of 4–10 ng/mL and a non-suspicious DRE. Of these 63 men, the authors found no statistically significant

difference in tPSA between men with negative prostatic biopsies and those who had positive biopsies (median 5.37 vs 5.14 ng/mL, $P = 0.88$). However, men with prostate cancer were noted to have a significantly higher median %[-2]proPSA ($P < 0.001$). ROC analysis showed that *phi* had the highest predictive ability with an AUC of 0.77, followed by %[-2]proPSA (AUC 0.76) and %fPSA (AUC 0.68). The tPSA level lacked both sensitivity and specificity in the 2.5–10 ng/mL range (AUC 0.50). In a separate two-centre retrospective study, serum samples of 405 patients enrolled in the Rotterdam arm of the ERSPC study and 351 samples from Innbruck Medical University were evaluated to investigate the use of [-2]proPSA, BPSA and *phi* [39]. Although the authors found no improvement in the predictive accuracy of tPSA and %fPSA with BPSA, a significant improvement in the predictive accuracy of PSA was reported with the additional measurement of %[-2]proPSA and *phi*. At 90% sensitivity, *phi* had the highest specificity of 31% compared with only 8% for tPSA.

More recently, a multicentre, double-blind, case-control clinical trial led by Catalona *et al.* [40] was conducted to validate and select *phi* thresholds that could be used in clinical practice. In all, 892 men (out of 1372 men enrolled) who met the eligibility criteria of a normal DRE and a PSA level of 1.5–11 ng/mL were included for analysis. The AUC of prostate cancer detection was found to be significantly greater for *phi* (AUC 0.703) than for fPSA/tPSA (AUC 0.648), fPSA (AUC 0.615), [-2]proPSA (AUC 0.557) or tPSA (AUC 0.525). Higher *phi* values were also associated with an increased probability of prostate cancer detection. For *phi* < 25, the percentage probability of prostate cancer detection was 11% whilst for *phi* values of 25–34.9, 35–54.9 and ≥55, the probability of prostate cancer detection was 18.1%, 32.7% and 52.1%, respectively. Furthermore, it was noted that increasing *phi* values were associated with an increased probability of detecting Gleason ≥ 7 prostate cancer. Interestingly, as *phi* values did not differ with age and race, this suggests that the use of *phi* may be applicable to all men, irrespective of race, age and background.

> 'increasing *phi* values were associated with an increased probability of detecting Gleason ≥ 7 prostate cancer'

In 2011, Guazzoni *et al.* [41] conducted an observational prospective study of 268 consecutive men who were scheduled to undergo prostatic biopsies for investigation of a mildly elevated PSA value (2–10 ng/mL) and a normal DRE. Consistent with previous studies, %[-2]proPSA and *phi* were the strongest predictors of a positive prostatic biopsy outcome. Multivariate accuracy analyses showed that both *phi* and %[-2]proPSA improved the accuracy of established predictors in determining the presence of prostate cancer at biopsy by 11% and 10%, respectively. Furthermore, the same group have recently conducted an observational, prospective study of 350 consecutive men diagnosed with clinically localised prostate cancer who underwent radical prostatectomy [42]. By performing tPSA, fPSA, %fPSA, [-2]proPSA, %[-2]proPSA and *phi* measurements on preoperative serum samples of these patients, the authors were able to determine that %[-2]proPSA and *phi* were significantly higher in patients with pT3 disease, pathological Gleason ≥ 7 score and those that had Gleason sum upgrading (all $P <$ 0.001). Thus, preoperative serum measurement of %[-2]proPSA and *phi* appear to predict patients with more aggressive disease on final pathology and might therefore be useful in the preoperative counselling of patients with newly diagnosed, clinically localised prostate cancer.

Overall, studies to date suggest that both *phi* and %[-2]proPSA substantially improve the predictive value of PSA in the detection of early stage prostate cancer (Table 4) [38–42]. Early evidence suggests that *phi* and [-2]proPSA may also be able to predict prostate cancer aggressiveness on prostatic biopsies, as well as on final histology after radical prostatectomy. More studies are required to investigate these promising results.

ROLE OF [-2]proPSA AND *PHI* IN MEN WITH PROSTATE CANCER ON ACTIVE SURVEILLANCE REGIMES

One of the recognised complications of PSA-based screening is the over-detection of low-risk, indolent prostate cancers and their subsequent overtreatment. It has been previously estimated that a single screening test at the age of 55 years could lead to an

Table 4 Summary of studies evaluating the role of phi *in prostate cancer*

Reference	Year	PSA range, ng/mL	Number of patients	Methods	Main findings
Le *et al.* [38]	2010	2.5–10	2034	Sera from men with PSA levels of 2.5–10 ng/mlL with normal DRE recruited in a prospective screening study conducted over a week in 2007	In 63 men with PSA levels of 2.5–10 ng/mL with normal DRE, %[-2]proPSA outperformed tPSA and %fPSA in differentiating between negative and positive biopsy results. Additionally, *phi* measurement had the best overall performance characteristic.
Jansen *et al.* [39]	2010	2–10	756	Retrospective, two centre analysis of sera from men undergoing TRUS-guided biopsy	*phi* and %[-2]proPSA was superior to tPSA and %fPSA in predicting negative biopsy results.
Catalona *et al.* [40]	2011	1.5–11	892	Prospective, multi-centre analysis of sera from men enrolled in a case-control trial to determine *phi* thresholds in clinical practice	Higher *phi* values were associated with an increased probability of prostate cancer detection as well as increased probability of detecting Gleason sum ≥7 tumours.
Guazzoni *et al.* [41]	2011	2–10	268	Sera from 268 consecutive men with PSA levels of 2–10 ng/mL with normal DRE undergoing TRUS-guided biopsy	%[-2]proPSA and *phi* were more accurate than tPSA, %fPSA and PSA density in predicting the presence of cancer on biopsy.
Guazzoni *et al.* [42]	2012	0.64–19.79	350	Sera from 350 consecutive men diagnosed with clinically localised prostate cancer who underwent radical prostatectomy	%[-2]proPSA and *phi* levels were significantly higher in patients with pT3 disease, Gleason sum ≥7 and Gleason sum upgrading after radical prostatectomy.

over detection rate of 27%, whilst at age 75 years, this would be as high as 56% [43]. In other words, an increasing number of patients are being diagnosed with potentially low-risk, clinically insignificant cancers that would have not been diagnosed without screening. In response to this growing trend, active surveillance has been proposed as an alternative management strategy with the aim of reducing the risk of overtreatment in patients with clinically confined, low-risk prostate cancer. Although most Urologists would agree that an effective active surveillance programme should include a model incorporating regular periodic DRE, PSA testing and possibly further prostatic biopsies, there is currently no consensus on what constitutes an ideal definition of indolent prostate cancer and what an optimal active surveillance regime entails. In this regard, two recent studies have evaluated the predictive role of [-2]proPSA and *phi* in identifying those men with prostate cancer, enrolled in an active surveillance programme who might be at an increased risk of disease progression. Makarov *et al.* [44] using serum and prostatic biopsy samples from 71 men under active surveillance programme at Johns Hopkins, evaluated the potential association of serum and tissue proPSA levels in predicting those patients who will develop unfavourable biopsy conversion on annual surveillance examination. The authors found that the tissue-staining intensity or extent of the benign adjacent areas for [-5] and [-7]proPSA were significantly associated with future unfavourable biopsy profile. Similar results were seen with the ratio of [-2]proPSA/%fPSA defined using the serum levels of the PSA isoforms. Therefore, the measurement of serum levels of [-2]proPSA at diagnosis, can help identify those men at risk of future unfavourable biopsy conversion during active surveillance and aid with the appropriate counselling and management of this group of patients.

A couple of years later, the same group extended their investigation to incorporate *phi* in this same cohort of patients [45]. The *phi* levels from serum in men diagnosed with prostate cancer who ultimately developed an unfavourable biopsy finding was significantly higher than those who did not (*phi* 37.23 vs 30.6, $P = 0.03$). Multivariate logistic regression also showed that *phi* and %[-2]proPSA were significant predictors of an unfavourable biopsy conversion. In addition, the inclusion of biopsy tissue DNA content to *phi* and %[-2]proPSA in the multivariate model further enhanced the predictive accuracy of determining the likelihood of an unfavourable biopsy finding (c-index 0.6908 and 0.6884, respectively).

> ‘measurement of serum levels of [-2]proPSA at diagnosis, can help identify those men at risk of future unfavourable biopsy conversion during active surveillance’

Thus, although studies evaluating the potential role of *phi* and %[-2]proPSA in active surveillance regime is currently sparse, the preliminary results are encouraging (Table 5) [44,45]. Further studies are required to define how the *phi* index or %[-2]proPSA could be used to select men that would most benefit from an active surveillance programme and how these markers could be incorporated into the follow-up schedule of patients.

TABLE 5 Summary of studies evaluating the role of [-2]proPSA and the phi *in men with prostate cancer on active surveillance regimes*

Reference	Year	PSA range, ng/mL	Number of patients	Methods	Main findings
Makarov *et al.* [44]	2009	N/R*	71	Sera was retrospectively analysed for 71 patients with prostate cancer on active surveillance regime	[-2]proPSA/%fPSA was significantly higher at diagnosis in men with prostate cancer who subsequently developed unfavourable biopsy characteristics on follow-up surveillance examination.
Isharwal *et al.* [45]	2011	N/R*	71	Sera was retrospectively analysed for 71 patients with prostate cancer on active surveillance regime	The *phi* and [-2]proPSA/%fPSA combined with biopsy tissue DNA content improved the accuracy of predicting unfavourable biopsy conversion at the annual surveillance biopsy examination to 70%.

*N/R, not reported in the study. *tPSA in men with favourable and unfavourable biopsy on follow-up surveillance examination was 4.61 and 5.35 ng/mL, respectively.*

COST-EFFECTIVENESS OF THE BECKMAN COULTER *PHI*

Considering that the *phi* index showed an improved specificity over PSA for prostate cancer detection, the use of the *phi* in prostate cancer detection could potentially reduce the number of patients with negative prostatic biopsies. This reduction in unnecessary procedures was estimated to be 20–25% and could lead to limit the direct cost of prostate cancer detection [40]. To evaluate the budgetary impact of *phi* on prostate cancer detection, Nichol *et al.* [46] constructed two budget impact models using a PSA threshold of ≥2 and ≥4 ng/mL for recommending a prostatic biopsy in a hypothetical health plan with 100 000 men aged 50–74 years. When *phi* was added to the current PSA screening strategies (using tPSA and %fPSA), it increased the total cost of blood tests by $51 524 and $13 611 for models with a PSA threshold of ≥2 and ≥4 ng/mL, respectively. Despite the additional costs of the blood tests, these were offset by a reduction in the number of required office visits, laboratory tests and prostatic biopsies, resulting in a 1 year net saving of $356 647 (for PSA threshold of ≥2 ng/mL) and $94 219 (for PSA threshold of ≥4 ng/mL).

> 'health economic studies to date suggest that the addition of *phi* to PSA-based screening may be a more cost-effective solution than PSA-based screening alone'

In a separate study by the same group, Nichol *et al.* [47] estimated the costs and utilities of prostate cancer detection and treatment for men aged 50–75 years who were enrolled in an annual prostate cancer screening programme from a USA societal perspective. Over 25 annual screening cycles, it was estimated that the screening strategy using the combined PSA and *phi* measurement resulted in a saving of $1199 or $443 with an expected gain of 0.08 or 0.03 quality adjusted life years per person for PSA thresholds of ≥2 and ≥4 ng/mL, respectively. As the combination of tPSA and *phi* is expected to increase the true positive and reduce false positive results, the additional costs of fPSA and [-2]proPSA assay added to the tPSA assay cost was postulated to be offset by a reduction in the number of patients undergoing unnecessary prostatic biopsies. In agreement with these results, a recent study presented at the 2012 European Association of Urology congress in Paris by a group from the Erasmus Medical Centre in Rotterdam showed that by incorporating the *phi* test into a PSA-based screening model from ERSPC, resulted in a predicted reduction in negative biopsies by 29%, a reduction in prostate cancers detected by 2% and a total cost reduction for prostate cancer care by 2% [48].

In summary, health economic studies to date suggest that the addition of *phi* to PSA-based screening may be a more cost-effective solution than PSA-based screening alone.

CONCLUSION

It has now been over a decade since the discovery that truncated forms of proPSA are differentially expressed in the tissue and serum of men with prostate cancer [16,17,32]. Since then, a growing body of evidence suggests that proPSA and its derivatives (in particular [-2]proPSA and the *phi*) are more accurately able to differentiate those patients with and without prostate cancer in the diagnostically challenging tPSA range of 2–10 ng/mL. Studies also suggest that %[-2]proPSA and *phi* might be able to distinguish patients who harbour a more aggressive form of the disease. The full clinical potential of %[-2]proPSA and in particular, the threshold for the *phi* index for preferential identification of aggressive disease have yet to be determined. If the maximum number of aggressive prostate cancers were to be detected, it might be acceptable to not detect some of the less aggressive, clinically insignificant prostate cancer especially if the *phi* index could also predict disease progression as indicated by some preliminary reports [44,45]. In this context, one important aspect that needs addressing is the clinical relevance and predictive value of the *phi* kinetic, in determining whether a change in the *phi* value over a period could predict disease progression.

Another interesting aspect for future consideration would be to investigate the

potential synergistic association between [-2]proPSA, *phi* and the recently described multivariate model incorporating a panel of hk markers. In evaluating the sera from men with elevated PSA levels who underwent prostatic biopsies during the first round of ERSPC (section Göteborg), Vickers *et al.* [49] reported that the use of four hk markers (tPSA, fPSA, iPSA and hk2) significantly enhanced the predictive ability of age and tPSA in identifying those patients who have prostate cancer (AUC improved from 0.68 to 0.83). In a separate study, the authors managed to replicate their initial findings in men with high PSA levels enrolled in the ERSPC (section Rotterdam) who underwent prostatic biopsies [50]. In this validation study, the AUC of the multivariate model incorporating the various hk markers was 0.76 whilst the baseline model incorporating age and tPSA alone was 0.64. As similar enhancements in AUC were also noted with [-2]proPSA and *phi*, it would be useful to determine whether [-2]proPSA, *phi* and the above hk markers in combination can further enhance the ability to predict a positive prostate cancer diagnosis.

Although most studies to date have focussed on the role of [-2]proPSA and *phi* as a potential diagnostic markers, the clinical utility of both markers are expected to expand considerably in the future. A recent study for example, reported the potential association of [-2]proPSA and the *phi* index with probability of metastatic progression in men with biochemical recurrence after radical prostatectomy [51]. This, together with studies reporting the predictive value of [-2]proPSA/%fPSA and *phi* in men with localised prostate cancer on expectant treatment [44,45] is paving the way for exploring other novel uses of these biomarkers.

It must be noted that most of the reviewed articles were retrospective studies, where the study population was assembled once the final diagnosis and tPSA values were already known (thus prone to selection bias). Even in studies where serum for analysis was obtained prospectively, the final decision on whether to biopsy or not was still based on elevated tPSA values and not based on the novel markers themselves. In addition, as most studies were performed in the context of a restricted range of tPSA (for example, PSA level of <10 ng/mL), this may further exaggerate the predictive accuracy of these novel markers in prostate cancer diagnosis compared with tPSA if it was left unrestricted. Lastly, the differences in the PSA assays used, especially between the older and more recent studies, makes the direct comparison of results difficult and the sample size in most of the studies was relatively small.

Despite these limitations, proPSAs and in particular [-2]proPSA and *phi* have yielded encouraging results and appear to improve the specificity of PSA and %fPSA in detecting men with and without prostate cancer and preferentially detects aggressive disease. However, the true clinical role of these novel markers needs to be carefully evaluated in the context of several large, multicentre, prospective trials with full health economic analyses before any advice can be made about its use in routine clinical practice.

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CONFLICT OF INTEREST

Jean-Sebastien Blanchet works for the Department of Scientific Affairs at Beckman Coulter Inc. John McLoughlin has acted as a consultant for Beckman Coulter Inc. Satoshi Hori has no conflict of interest to declare.

REFERENCES

1 **Yousef GM, Luo LY, Diamandis EP.** Identification of novel human kallikrein-like genes on chromosome 19q13.3-q13.4. *Anticancer Res* 1999; **19**: 2843–52
2 **Clements J, Hooper J, Dong Y, Harvey T.** The expanded human kallikrein (KLK) gene family: genomic organisation, tissue-specific expression and potential functions. *Biol Chem* 2001; **382**: 5–14
3 **Kumar A, Mikolajczyk SD, Goel AS, Millar LS, Saedi MS.** Expression of pro form of prostate-specific antigen by mammalian cells and its conversion to mature, active form by human kallikrein 2. *Cancer Res* 1997; **57**: 3111–4
4 **Lovgren J, Rajakoski K, Karp M.** Lundwall Å, Lilja H. Activation of the zymogen form of prostate-specific antigen by human glandular kallikrein 2. *Biochem Biophys Res Commun* 1997; **238**: 549–55
5 **Takayama TK, Fujikawa K, Davie EW.** Characterization of the precursor of prostate-specific antigen. Activation by trypsin and by human glandular kallikrein. *J Biol Chem* 1997; **272**: 21582–8
6 **Takayama TK, McMullen BA, Nelson PS, Matsumura M, Fujikawa K.** Characterization of hK4 (prostase), a prostate-specific serine protease: activation of the precursor of prostate specific antigen (pro-PSA) and single-chain urokinase-type plasminogen activator and degradation of prostatic acid phosphatase. *Biochemistry* 2001; **40**: 15341–8
7 **Lilja H, Oldbring J, Rannevik G, Laurell CB.** Seminal vesicle-secreted proteins and their reactions during gelation and liquefaction of human semen. *J Clin Invest* 1987; **80**: 281–5
8 **Stephan C, Jung K, Lein M, Diamandis EP.** PSA and other tissue kallikreins for prostate cancer detection. *Eur J Cancer* 2007; **43**: 1918–26
9 **Stamey TA, Yang N, Hay AR, McNeal JE, Freiha FS, Redwine E.** Prostate-specific antigen as a serum marker for adenocarcinoma of the prostate. *N Engl J Med* 1987; **317**: 909–16
10 **Thompson IM, Pauler DK, Goodman PJ *et al*.** Prevalence of prostate cancer among men with a prostate-specific antigen level < or =4.0 ng per milliliter. *N Engl J Med* 2004; **350**: 2239–46
11 **Lilja H, Christensson A, Dahlen U *et al*.** Prostate-specific antigen in serum occurs predominantly in complex with alpha 1-antichymotrypsin. *Clin Chem* 1991; **37**: 1618–25
12 **Stenman UH, Leinonen J, Alfthan H, Rannikko S, Tuhkanen K, Alfthan O.** A complex between prostate-specific antigen and alpha 1-antichymotrypsin is the major form of prostate-specific antigen in serum of patients with prostatic cancer: assay of the complex improves clinical sensitivity for cancer. *Cancer Res* 1991; **51**: 222–6
13 **Mikolajczyk SD, Marks LS, Partin AW, Rittenhouse HG.** Free prostate-specific

antigen in serum is becoming more complex. *Urology* 2002; **59**: 797–802
14 **Mikolajczyk SD, Millar LS, Marker KM *et al*.** Seminal plasma contains 'BPSA,' a molecular form of prostate-specific antigen that is associated with benign prostatic hyperplasia. *Prostate* 2000; **45**: 271–6
15 **Mikolajczyk SD, Millar LS, Wang TJ *et al*.** 'BPSA,' a specific molecular form of free prostate-specific antigen, is found predominantly in the transition zone of patients with nodular benign prostatic hyperplasia. *Urology* 2000; **55**: 41–5
16 **Mikolajczyk SD, Millar LS, Wang TJ *et al*.** A precursor form of prostate-specific antigen is more highly elevated in prostate cancer compared with benign transition zone prostate tissue. *Cancer Res* 2000; **60**: 756–9
17 **Mikolajczyk SD, Grauer LS, Millar LS *et al*.** A precursor form of PSA (pPSA) is a component of the free PSA in prostate cancer serum. *Urology* 1997; **50**: 710–4
18 **Sokoll LJ, Chan DW, Mikolajczyk SD *et al*.** Proenzyme PSA for the early detection of prostate cancer in the 2.5–4.0 ng/ml total PSA range: preliminary analysis. *Urology* 2003; **61**: 274–6
19 **Khan MA, Partin AW, Rittenhouse HG *et al*.** Evaluation of proprostate specific antigen for early detection of prostate cancer in men with a total prostate specific antigen range of 4.0 to 10.0 ng/ml. *J Urol* 2003; **170**: 723–6
20 **Catalona WJ, Bartsch G, Rittenhouse HG *et al*.** Serum pro prostate specific antigen improves cancer detection compared to free and complexed prostate specific antigen in men with prostate specific antigen 2 to 4 ng/ml. *J Urol* 2003; **170**: 2181–5
21 **Catalona WJ, Bartsch G, Rittenhouse HG *et al*.** Serum pro-prostate specific antigen preferentially detects aggressive prostate cancers in men with 2 to 4 ng/ml prostate specific antigen. *J Urol* 2004; **171**: 2239–44
22 **Catalona WJ, Partin AW, Slawin KM *et al*.** Use of the percentage of free prostate-specific antigen to enhance differentiation of prostate cancer from benign prostatic disease: a prospective multicenter clinical trial. *JAMA* 1998; **279**: 1542–7
23 **Woodrum DL, Brawer MK, Partin AW, Catalona WJ, Southwick PC.** Interpretation of free prostate specific antigen clinical research studies for the detection of prostate cancer. *J Urol* 1998; **159**: 5–12
24 **Mikolajczyk SD, Catalona WJ, Evans CL *et al*.** Proenzyme forms of prostate-specific antigen in serum improve the detection of prostate cancer. *Clin Chem* 2004; **50**: 1017–25
25 **Khan MA, Sokoll LJ, Chan DW *et al*.** Clinical utility of proPSA and 'benign' PSA when percent free PSA is less than 15%. *Urology* 2004; **64**: 1160–4
26 **de Vries SH, Raaijmakers R, Blijenberg BG, Mikolajczyk SD, Rittenhouse HG, Schroder FH.** Additional use of [-2] precursor prostate-specific antigen and 'benign' PSA at diagnosis in screen-detected prostate cancer. *Urology* 2005; **65**: 926–30
27 **Bangma CH, Wildhagen MF, Yurdakul G, Schroder FH, Blijenberg BG.** The value of (-7, -5)pro-prostate-specific antigen and human kallikrein-2 as serum markers for grading prostate cancer. *BJU Int* 2004; **93**: 720–4
28 **Lein M, Semjonow A, Graefen M *et al*.** A multicenter clinical trial on the use of (-5, -7) pro prostate specific antigen. *J Urol* 2005; **174**: 2150–3
29 **Stephan C, Meyer HA, Kwiatkowski M *et al*.** A (-5, -7) proPSA based artificial neural network to detect prostate cancer. *Eur Urol* 2006; **50**: 1014–20
30 **Miyakubo M, Ito K, Yamamoto T, Takechi H, Ohi M, Suzuki K.** Proprostate-specific antigen: its usefulness in the era of multiple-core prostate biopsy. *Int J Urol* 2009; **16**: 561–5
31 **Stephan C, Meyer HA, Paul EM *et al*.** Serum (-5, -7) proPSA for distinguishing stage and grade of prostate cancer. *Anticancer Res* 2007; **27**: 1833–6
32 **Mikolajczyk SD, Marker KM, Millar LS *et al*.** A truncated precursor form of prostate-specific antigen is a more specific serum marker of prostate cancer. *Cancer Res* 2001; **61**: 6958–63
33 **Sokoll LJ, Wang Y, Feng Z *et al*.** [-2]proenzyme prostate specific antigen for prostate cancer detection: a national cancer institute early detection research network validation study. *J Urol* 2008; **180**: 539–43
34 **Sokoll LJ, Sanda MG, Feng Z *et al*.** A prospective, multicenter, National Cancer Institute Early Detection Research Network study of [-2]proPSA: improving prostate cancer detection and correlating with cancer aggressiveness. *Cancer Epidemiol Biomarkers Prev* 2010 **19**: 1193–200
35 **Stephan C, Kahrs AM, Cammann H *et al*.** A [-2]proPSA-based artificial neural network significantly improves differentiation between prostate cancer and benign prostatic diseases. *Prostate* 2009; **69**: 198–207
36 **Rhodes T, Jacobson DJ, McGree ME *et al*.** Longitudinal changes of benign prostate-specific antigen and [-2]proprostate-specific antigen in seven years in a community-based sample of men. *Urology* 2012; **79**: 655–61
37 **Rhodes T, Jacobson DJ, McGree ME *et al*.** Distribution and associations of [-2]proenzyme-prostate specific antigen in community dwelling black and white men. *J Urol* 2012; **187**: 92–6
38 **Le BV, Griffin CR, Loeb S *et al*.** [-2]Proenzyme prostate specific antigen is more accurate than total and free prostate specific antigen in differentiating prostate cancer from benign disease in a prospective prostate cancer screening study. *J Urol* 2010; **183**: 1355–9
39 **Jansen FH, van Schaik RH, Kurstjens J *et al*.** Prostate-specific antigen (PSA) isoform p2PSA in combination with total PSA and free PSA improves diagnostic accuracy in prostate cancer detection. *Eur Urol* 2010; **57**: 921–7
40 **Catalona WJ, Partin AW, Sanda MG *et al*.** A multicenter study of [-2]pro-prostate specific antigen combined with prostate specific antigen and free prostate specific antigen for prostate cancer detection in the 2.0 to 10.0 ng/ml prostate specific antigen range. *J Urol* 2011; **185**: 1650–5
41 **Guazzoni G, Nava L, Lazzeri M *et al*.** Prostate-specific antigen (PSA) isoform p2PSA significantly improves the prediction of prostate cancer at initial extended prostate biopsies in patients with total PSA between 2.0 and 10 ng/ml: results of a prospective study in a clinical setting. *Eur Urol* 2011; **60**: 214–22
42 **Guazzoni G, Lazzeri M, Nava L *et al*.** Preoperative prostate-specific antigen Isoform p2PSA and its derivatives, %p2PSA and prostate health index, predict pathologic outcomes in patients undergoing radical prostatectomy for

prostate cancer. *Eur Urol* 2012; **61**: 455–66

43 **Draisma G, Boer R, Otto SJ *et al*.** Lead times and overdetection due to prostate-specific antigen screening: estimates from the European Randomized Study of Screening for Prostate Cancer. *J Natl Cancer Inst* 2003; **95**: 868–78

44 **Makarov DV, Isharwal S, Sokoll LJ *et al*.** Pro-prostate-specific antigen measurements in serum and tissue are associated with treatment necessity among men enrolled in expectant management for prostate cancer. *Clin Cancer Res* 2009; **15**: 7316–21

45 **Isharwal S, Makarov DV, Sokoll LJ *et al*.** ProPSA and diagnostic biopsy tissue DNA content combination improves accuracy to predict need for prostate cancer treatment among men enrolled in an active surveillance program. *Urology* 2011; **77**: 763. e1–6

46 **Nichol MB, Wu J, An JJ *et al*.** Budget impact analysis of a new prostate cancer risk index for prostate cancer detection. *Prostate Cancer Prostatic Dis* 2011; **14**: 253–61

47 **Nichol MB, Wu J, Huang J, Denham D, Frencher SK, Jacobsen SJ.** Cost-effectiveness of prostate health index for prostate cancer detection. *BJU Int* 2011. [Epub ahead of print]. DOI: 10.1111/j.1464-410X.2011.10751.x

48 **Heijnsdijk E, Huang J, Denham D, De Koning H.** The cost-effectiveness of prostate cancer detection using Beckman Coulter Prostate Health Index. 2012. Proceeding of the 27th Annual European Association of Urology Congress in Paris, France. Available at: http://www.uroweb.org/index.php?id=108&tAID=36840. Accessed May 2012

49 **Vickers AJ, Cronin AM, Aus G *et al*.** A panel of kallikrein markers can reduce unnecessary biopsy for prostate cancer: data from the European Randomized Study of Prostate Cancer Screening in Goteborg, Sweden. *BMC Med* 2008; **6**: 19

50 **Vickers A, Cronin A, Roobol M *et al*.** Reducing unnecessary biopsy during prostate cancer screening using a four-kallikrein panel: an independent replication. *J Clin Oncol* 2010; **28**: 2493–8

51 **Sottile A, Ortega C, Berruti A *et al*.** A pilot study evaluating serum pro-prostate-specific antigen in patients with rising PSA following radical prostatectomy. *Oncol Lett* 2012 **3**: 819–24

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Abbreviations: **ANN**, artificial neural network; **AUC**, area under the curve; **BPSA**, benign PSA; **ERSPC**, European Randomised Study of Screening for Prostate Cancer; **FHMS**, Flint Men's Health Study; **fPSA**, free PSA; **hk**, human kallikrein; **NCI EDRN**, National Cancer Institute Early Detection Research Network; **OCS**, Olmsted County Study of Urinary Symptoms and Health Status among Men; ***phi***, Beckman Coulter Prostate Health Index; **proPSA**, precursor of PSA; **ROC**, receiver operating characteristic; **tPSA**, total PSA; **TRUS**, transrectal ultrasound guided.