
Screening for prostate cancer: an update

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The introduction of total prostate-specific antigen (tPSA) testing in serum has revolutionized the detection and management of men with prostate cancer. This review will highlight some of the exciting new developments in the field of prostate cancer screening in general and from our SPORE research program at Memorial-Sloan Kettering Cancer Center. First, it is important to understand that the inherent variability of tPSA levels affects the interpretation of any single results. Total variation in tPSA includes both analytical (i.e., pre-analytical sample handling, laboratory processing, assay performance, and standardization) and biological variation (i.e., metabolism, renal elimination, medication, physical and sexual activity, size and integrity of the prostate). Second, recent evidence demonstrates that no single tPSA cut-off separates men at high risk for prostate cancer from men at low risk or men with "significant" (high grade, high volume) cancer from those with low grade, indolent cancer. Taken together with a man's age, family history, ethnicity, and digital rectal exam results, tPSA levels add to the overall estimate of the risk of cancer, allowing men to share in the decision about a biopsy. Third, men who will eventually develop prostate cancer have

increased tPSA levels years or decades before the cancer is diagnosed. These tPSA levels may reflect the long duration of prostate carcinogenesis and raise the question about a causal role for tPSA in prostate cancer development and progression. Total prostate-specific antigen measurements before age 50 could help risk stratify men for intensity of prostate cancer screening. Fourth, enhancing the diagnostic accuracy of tPSA, especially its specificity, is of particular importance, since higher specificity translates into fewer biopsies in men not affected by prostate cancer. While tPSA velocity has been shown to improve the specificity of tPSA, its sensitivity is too low to avoid prostate biopsy in a patient with an elevated tPSA level. Moreover, prospective screening studies have reported that tPSA velocity does not add diagnostic value beyond tPSA level. At this time, tPSA velocity appears most useful after diagnosis and after treatment, but its value in screening and prognostication remains to be shown. Finally, while free PSA molecular isoforms and human kallikrein-related peptidase 2 (hK2) hold the promise for detection, staging, prognosis, and monitoring of prostate cancer, evidence from large prospective clinical trials remain to be reported.

Key Words: prostate-specific antigen, human glandular kallikrein, prostate cancer, prognosis, detection

Introduction

Prostate cancer is the most commonly diagnosed cancer in American men and the second leading cause of cancer-related deaths. The wide availability of total prostate-specific antigen (tPSA) revolutionized prostate cancer

screening and ushered in the tPSA era. This has resulted in earlier prostate cancer detection and an increase in incidence. However, it remains unclear whether screening for prostate cancer results in lower prostate cancer mortality. Indirect evidence from observational and case control studies is not consistent but does suggest the highly prevalent screening in this country has played a substantial role in the decrease in prostate cancer mortality in the United States.¹ Advocates of screening point to an increased rate of discovery of lower stage cancer, a decline in the incidence of metastatic disease, and a reduction in cancer related mortality after

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widespread tPSA screening. Critics of tPSA screening, on the other hand, point to high rates of over detection: the lifetime risk of diagnosis is currently ~18%, whereas that of death from prostate cancer is ~3%. A major problem with tPSA is its lack of cancer specificity. An elevated tPSA level can reflect the presence of cancer but can also be caused by benign prostatic hyperplasia (BPH), infection, and/or chronic inflammation. All prostate epithelial cells, whether normal, hyperplastic or cancerous, synthesize PSA. Neoplastic cells produce somewhat lower tissue levels of tPSA compared to BPH cells although both conditions cause tPSA elevation in the blood. Therefore, it has been suggested that tPSA should be considered as a marker of BPH related prostate volume, growth, and outcome rather than a reliable marker of prostate cancer.²⁻⁴ In addition, there is continuing disagreement over the threshold level of tPSA that should indicate biopsy. Finally, tPSA levels do not directly correlate with the biological behavior of prostate cancer. This can lead to over detection and over treatment, resulting in increased cost, side effects, complications, and patient anxiety.

In addition to its use for early detection, use of tPSA testing has been found useful as an aid to predict prostate cancer risk and of treatment outcome. Indeed, tPSA is one of the key variables in pre and post treatment prognostic models for clinically localized prostate cancer.⁵⁻⁷ However, Stamey et al have reported that for patients with a tPSA level of < 9 ng/ml, tPSA poorly reflected the risk of biochemical recurrence (BCR) after radical prostatectomy but was significantly correlated with the volume of the radical prostatectomy specimen, a direct reflection of the degree of BPH present.⁸⁻¹⁰

The purpose of this review is to discuss: 1) the inherent variability of serum tPSA levels, 2) the need to replace tPSA cut-offs with prediction tools that incorporate established risk factors, 3) the predictive value of tPSA in young man and the impact it could have on the age of onset and intensity of prostate cancer screening, 4) the controversies of tPSA velocity (tPSAV), and 5) the association of free PSA and its isoforms as well as human kallikrein-related peptidase 2 (hK2) with prostate cancer risk and outcomes.

PSA variability

It is important to consider the variability of tPSA and its derivatives in screening and monitoring of individuals over time. Total variation in tPSA includes analytical and biological variation. Analytical variation depends on assay performance, sample handling, and laboratory processing.^{11,12} Biological variation relates

to individual factors such as tPSA metabolism, renal elimination, and physical and sexual activity.^{13,14}

First, transitory tPSA outliers, which may be due to infection, or following digital rectal examination (DRE) or prostate biopsies, may lead to non-cancer related higher tPSA value and result in a higher tPSA velocity (tPSAV). Oscillations up to 20%-30% in the tPSA range 0.1 ng/ml-20 ng/ml may be due to biological variation.^{15,16}

Second, the use of different detection assays may be another important cause of variation. Differences in assay standardization can give an artificially high or low estimate of tPSA and tPSAV.¹⁷⁻¹⁹ Assays are not interchangeable and caution should be exercised when comparing results from different commercial tPSA assays. Patients and physicians should be aware of which assay was used each time a tPSA measurement is performed, and an effort should be made to use the same assay at the next screening visit. In addition, studies of tPSA kinetics over time using different assays should be interpreted with caution.

Third, the effect of previous BPH treatment on tPSA remains mostly unpredictable. For example, the effect of commonly used 5- α -reductase inhibitors on the predictive value of tPSA kinetics for tumor progression is uncertain. Because 5- α -reductase inhibitors are known to decrease the PSA level with ~50% and mostly suppress the benign components of PSA secretion, they may enhance the utility of tPSA and tPSAV.²⁰ In addition, by shrinking the prostate gland, finasteride may increase the likelihood of detecting a small cancer on needle biopsy. It was initially implicated but recently refuted that finasteride also may induce the regression of low grade but not high grade prostate cancer.²¹

This large normal variability of tPSA requires larger changes between two consecutive measurements to distinguish pathological changes from changes resulting from analytical and biological variations. Nixon et al calculated the coefficient of variation (CV) over 2 weeks and demonstrated that a change between two tPSA measurements of approximately 25% indicated a significant change.^{22,23} Bunting et al reported a critical difference, defined as the minimum percent change between two consecutive measurements that suggests a significant change beyond the normal variation, close to 60% over a time period of 1 year.²⁴ Bruun et al recently assessed the long term variability of the different forms of tPSA at several different tPSA levels in a randomly selected population of asymptomatic and apparently healthy men whose tPSA levels were < 2.0 ng/ml at the end of the 8 year observation period.²⁵ They found that the total intra-individual variation of tPSA was much less than that reported by Bunting et al²⁴ and somewhat

higher than the intra-individual variation for either free PSA or percent free PSA. This suggests that free PSA concentration in blood may vary less than complexed PSA concentration, which is the major contributor to tPSA. One explanation is that free PSA and complexed PSA may have different elimination pathways, and hence different elimination rates.^{13,26-28}

Recently, Eastham et al evaluated the year-to-year fluctuations in tPSA levels over a period of 4 years in a cohort of men selected from a polyp-prevention trial study group.²⁹ Several cut-off points for tPSA were studied; 30% and 26% of the men with a tPSA level > 4 ng/ml and > 2.5 ng/ml, respectively, had a tPSA value below these cut-offs at the next tPSA-testing.

Optimal tPSA cut-off values

No single tPSA cut-off separates men at high risk for prostate cancer from men at low risk, nor men affected with high grade disease from those with low grade disease.

At a tPSA cut-off of ≥ 4 ng/ml, a significant cancers remain undetected³⁰ and intervention at lower tPSA levels has been proposed to improve patient outcomes.^{31,32} Catalona et al found that 22% of men with a normal digital rectal examination and a serum tPSA level between 2.6 ng/ml and 4.0 ng/ml have prostate cancer, and 81% of them have organ confined disease.³³ Data from the Prostate Cancer Prevention Trial (PCPT) revealed that as many as 15% of men with normal digital rectal examination and a serum tPSA less than 4.0 ng/ml have prostate cancer.³⁰ Among men with tPSA levels ≤ 0.5 , 0.6-1.0, 1.1-2.0, 2.1-3.0, and 3.1-4.0 ng/ml, prostate cancer was detected in 6.6%, 10.1%, 17.0%, 23.9%, and 26.9%, respectively. Moreover, approximately 25% of these men had a tumor with Gleason score of 7 or higher. These and other investigators demonstrated that increasing levels of tPSA are associated with increasing probability of prostate cancer risk within the 0-4.0 ng/ml interval.^{30,34,35} There is no tPSA threshold at age 62-91 below which prostate cancer can be ruled out with high specificity.³⁰ No single tPSA cut-off separates men with "significant" (high grade, high volume) cancer from those with low grade, possibly insignificant cancer. Similar to the detection of prostate cancer, high grade cancer can also be found in men with low tPSA levels.

On the other hand, as of now, there is no evidence that lowering the tPSA threshold below 4 ng/ml improves the long term survival in men with prostate cancer while continuing to maintain the cost effectiveness of screening programs. Lowering the tPSA threshold combined with decreasing the age of tPSA screening may be beneficial for men who are at an increased risk for prostate cancer (i.e.,

strong family history of prostate cancer and/or African-American race). However, consideration must be given to the possibility that lowering the tPSA threshold could result in unnecessary biopsies and increased detection of indolent cancers. Finally, determination of the optimal, institution specific, and management guiding threshold involves not only clinical and epidemiologic features but should also consider the social and psychological implications of prostate biopsy and possible prostate cancer detection.

The difficulty in selecting a cut-off to define what constitutes an abnormal tPSA suggests that tPSA is most useful as a continuous variable, providing a spectrum of prostate cancer risk. Therefore, we prefer to include serum tPSA levels in an overall estimate of the risk of cancer, inform the patient of his particular risk, then make a shared decision about a biopsy.^{7,30,34,36-40} Nam et al, for example, developed a model that predicts an individual's risk for prostate cancer in a cohort of 3108 men who underwent a prostate biopsy for the first time.⁴⁰ The model comprises factors that can be easily determined at the time of screening such as age, ethnicity, family history of prostate cancer, the presence of urinary symptoms, tPSA, percent free PSA, and digital rectal examination, Figure 1. Addition of all

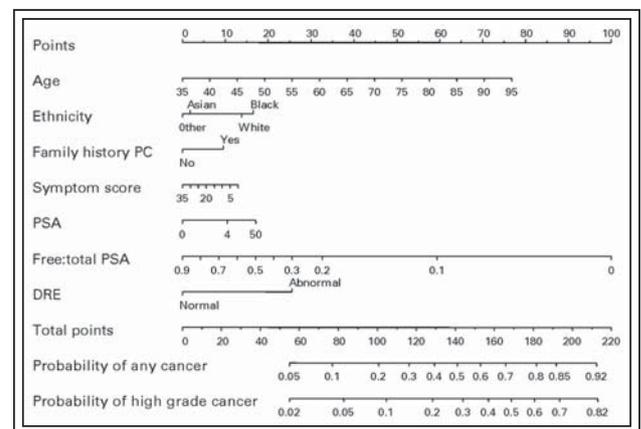


Figure 1. Nomogram prediction model for predicting prostate cancer at the time of biopsy. The nomogram is used by first locating a patient's position for each variable on its horizontal scale and then a point value is assigned according to the points scale (top axis) and summed for all variables. Total points correspond to a probability value for having prostate cancer or aggressive prostate cancer.

PSA = prostate-specific antigen; DRE = digital rectal examination.

Reproduced, with permission, from Nam RK et al. Assessing individual risk for prostate cancer. *J Clin Oncol* 2007;25(24):3582-3588.⁴⁰

these risk factors improved the predictive accuracy of a base model from 0.62 to 0.74. The main advantage of this and other predictive tools⁷ is that clinicians can assess prostate cancer risk on an individual basis and make management decisions. However, despite the reasonable accuracy, similar to all predictive tools, the exact probability cut-off for undergoing or foregoing a biopsy is left with the treating physician and patient and should be individualized.

Long term prediction of the future risk of prostate cancer using tPSA

Several studies have suggested that tPSA levels are associated with the risk of prostate cancer years, or even decades, before its diagnosis. The first long term prediction study, which reported that tPSA levels > 2.5 ng/ml predicted diagnosis of prostate cancer over the subsequent decade was limited by the small number of cancer cases (n = 44) and by the degradation of tPSA in archived serum samples.⁴¹ In a prospective study involving a large number of cases, the lead time between tPSA levels ≥ 4 ng/ml and the subsequent clinical diagnosis of PCa was estimated at 5.5 years.⁴² Similarly, Fang *et al* studied the risk of prostate cancer diagnosis in a cohort of 549 men following a baseline tPSA measurement at age 40-60 while providing a median follow-up of ~13 years.⁴³ They concluded a tPSA value above the age adjusted median carried a relative risk of subsequent cancer diagnosis of ~3.6.

Two larger studies extended prediction models to lower tPSA ranges and longer follow-up intervals. Loeb *et al* examined 1178 men in their 40s who had risk factors for prostate cancer.⁴⁴ The risk of subsequent prostate cancer diagnosis was 14.6-fold higher for men with a baseline tPSA level between 0.7 ng/ml and 2.5 ng/ml compared to men with tPSA < 0.7 ng/ml. Lilja *et al* assessed prostate cancer risk among 21,277 men younger than 50 years when they attended the Malmö Preventive Medicine study (MPM), a cardiovascular risk assessment study conducted between 1974 and 1986 in Malmö, Sweden.⁴⁵ The investigators measured tPSA levels in archived plasma obtained from 462 participants diagnosed with prostate cancer within a median of 18 years from start of the study and from 1222 matched controls. Of note, the attendance rate was high (74%) and the rate of tPSA testing in Sweden was low during most of the study period, leaving this study largely free of over detection or selection biases. The tPSA level at age 44-50 was very strongly associated with the likelihood of developing prostate cancer up to 25 years later, Figure 2. The odds ratio for a prostate cancer diagnosis at a tPSA value of 0.51 ng/ml-

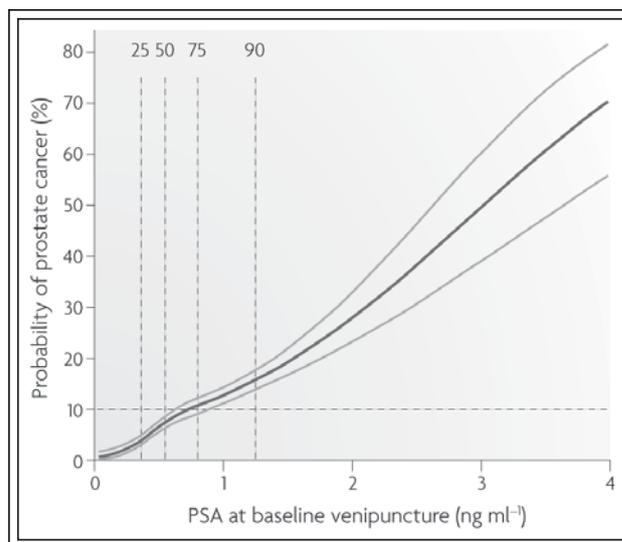


Figure 2. Early prediction of prostate cancer risk. The predicted probability of a prostate cancer diagnosis before the age of 75 years by total prostate-specific antigen (tPSA) measured at age 44-50 years, with 95% confidence intervals. The vertical lines represent the 25th, 50th, 75th and 90th percentiles of baseline tPSA, and the horizontal line represents the average lifetime risk (10%) of a prostate cancer diagnosis before the age of 75 years. Note that the tPSA levels reported from this study are approximately 13% lower than values derived from assays calibrated against the World Health Organization standard.

Reproduced, with permission, from Lilja H *et al*. Long-term prediction of prostate cancer up to 25 years before diagnosis of prostate cancer using prostate kallikreins measured at age 44 to 50 years. *J Clin Oncol* 2007;25:431-436.⁴⁵

1.0 ng/ml was 2.51 compared to tPSA ≤ 0.50 ng/ml, which roughly corresponded to the population average. The odds ratio increased to 7.02 for a tPSA of 1.0 ng/ml-1.5 ng/ml, and further up to 19.01 for a tPSA of 2.01 ng/ml-3.0 ng/ml compared to a tPSA ≤ 0.50 ng/ml. In a follow-up study, the authors have further shown that tPSA level at age 44-50 predicts the likelihood of developing advanced prostate cancer, defined as either locally advanced (clinical T3 or higher) or metastatic disease at the time of diagnosis.⁴⁶ In another analysis of the MPM study cohort, the value of PSA assessments in these younger men were compared with the blood taken from 1167 men of ages 59-61 years.⁴⁷ In this study, the prognostic accuracy of PSA (both tPSA and complexed PSA, described below) decreased with age. The authors hypothesized that these findings result from a greater prevalence of BPH

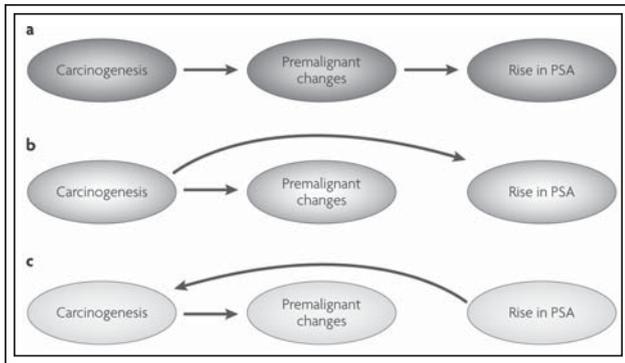


Figure 3. Three non-exclusive hypotheses to explain the association between total prostate-specific antigen (tPSA) level in younger men and prostate cancer diagnosed up to 25 years subsequently.

a) A carcinogenic process causes premalignant changes in prostate cells, which in turn increases leaking of PSA into the bloodstream.

b) A carcinogenic process causes premalignant changes in prostate cells. These changes are not sufficient to cause increased levels of serum PSA; however, carcinogenesis independently causes increased serum PSA by a separate process.

c) An unknown process causes an increase in serum PSA; extracellular PSA is causally influencing the carcinogenic process, which leads to premalignant changes in the prostate.

Reproduced, with permission, from Lilja H et al. Prostate-specific antigen and prostate cancer: prediction, detection and monitoring. *Nature Reviews Cancer* 2008;8:268-278.⁴⁸

(and therefore of non cancer related tPSA increase) among older men.

In summary, these studies indicate that men who will eventually develop prostate cancer have increased tPSA levels years or decades before the cancer is diagnosed. These tPSA levels may reflect the long duration of prostate carcinogenesis or could reflect a causal role of tPSA in prostate cancer development and/or progression, Figure 3. A tPSA measurement before age 50 could help risk stratify men for frequency and/or type of later prostate cancer screening.

Approaches to enhance the diagnostic accuracy of tPSA for detecting prostate cancer

Enhancing the diagnostic accuracy of tPSA, particularly specificity, is critical, since higher specificity would reduce the number of biopsies performed in men not affected by prostate cancer. Several different strategies have been investigated, including the use of

age-specific tPSA cut-offs, tPSA density, tPSA density of the transition zone, tPSA velocity (tPSAV), and the measurement of various molecular forms of PSA.^{7,48-50} We will focus on tPSAV and the measurement of various molecular forms of PSA since they have the highest potential for improving our predictive accuracy.

Total PSA velocity

Total prostate-specific antigen velocity refers to the serial evaluation of serum tPSA concentration over time.^{51,52} Different methods of calculating tPSAV are available (e.g., based on the first and the last measured values only or on a regression line through all available measurements, based on normal or logarithmic values), but only small differences in predictive value have been found among these derivatives. Connolly et al found that using all available PSA measurements in a linear regression analysis should be the method of choice for calculating tPSAV.⁵³ When using the first and last measurements only, these should at least be separated by a sufficiently long time period.

Carter et al showed that patients with BPH demonstrated a linear increase in tPSA levels over time, whereas patients with prostate cancer had an initial linear increase with a subsequent exponential rise that occurred approximately 5 years before cancer detection.⁵¹ In men with an initial tPSA level between 4 ng/ml and 10 ng/ml, a tPSAV cut-off value of 0.75 ng/ml/year provided a sensitivity and specificity for prostate cancer of 79% and > 90%, respectively. If the initial tPSA concentration was less than 4 ng/ml, the specificity of remained > 90%, but the sensitivity dropped to an abysmal 11%. These results were questioned for using relatively short tPSA intervals of 1 and 2 years.⁵⁴ Subsequently, Carter et al showed that tPSAV values are useful if a minimum of three consecutive measurements are taken over a 2 year period.⁵⁵ While the specificity of tPSAV is high, its sensitivity is too low to advise against prostate biopsy in a patient with an elevated tPSA level who is otherwise healthy and a good candidate for curative therapy. Other limitations of tPSAV include imprecision due to biological and analytical intra-individual variability (see section on PSA variability) and tPSA stability. Moreover, to date, appropriate tPSAV cut-offs have not been determined for men with tPSA levels below 4 ng/ml. Finally, tPSAV may be of most use in patients whose serum tPSA concentration at initial screening is below 4 ng/ml to help predict who should be biopsied when they reach the 4 ng/ml threshold.^{49,56-58}

Prospective screening studies have reported that tPSAV does not appear to add diagnostic value for prostate cancer detection beyond that of a single tPSA level. In an analysis of PCPT data, Thompson et al found that when tPSAV was used alone, it was an independent predictor of prostate cancer presence and aggressiveness.³⁶ However, when tPSAV was adjusted for the effect of tPSA and other standard variables, it lost independent predictive value. Similarly, the first two screening rounds of the Rotterdam section of the ERSPC found that tPSAV did not improve accuracy when combined with tPSA in the prospective setting.^{57,58} Finally, a recent analysis from the Prostate, Lung, Colon, and Ovarian (PLCO) cancer screening trial showed that although tPSAV was an independent predictor of high grade disease, addition of tPSAV to tPSA only slightly increased its performance for prediction of high grade tumors.⁵⁹ Finally, using a large population based cohort of men in early middle age who were likely to have a low incidence of BPH, Ulmert et al found no benefit to calculating tPSAV or the velocity of any other PSA form over tPSA for long term prostate cancer prediction.⁶⁰ Of note, the predictive value of tPSAV alone was 0.712, while the predictive value of a single tPSA was higher (concordance index: 0.771) and the combined model including both PSA velocity and tPSA did not alter the predictive accuracy. The observed lack of additional predictive value for tPSAV indicates that tPSA levels do not increase sharply before prostate cancer diagnosis but rise gradually and slowly over many years, also in those men who later present with advanced cancer.

The most compelling support for the role of tPSAV in prostate cancer comes from prognostic studies. Several studies have shown that a high pretreatment tPSAV is strongly associated with a poor disease specific survival following diagnosis and could help identify men with low tPSA values who are at increased risk of harboring a potentially lethal tumor.⁶¹⁻⁶⁴ Carter et al found a strong association between survival and higher tPSAV as early as 10-15 years before diagnosis in the Baltimore Longitudinal Study of Aging project.⁶⁴ Based on these findings, they proposed that a tPSAV threshold of 0.35 ng/ml/year be used in screening men with low tPSA levels to increase the detection of potentially lethal tumors still in the window of curability. These data have prompted debate as to whether this would suffice as evidence to warrant the National Comprehensive Cancer Network to recommend a prostate biopsy if the tPSAV is greater than 0.5 ng/ml/year.⁶⁵

D'Amico et al reported that men with a preoperative tPSAV greater than 2.0 ng/ml/year had a 9.8-fold

increased relative risk of death from prostate cancer than men with a lower tPSAV.⁶¹ In a more recent study, these investigators reported that tPSAV was also significantly associated with the risk of cancer specific mortality following external beam radiation therapy.⁶² Conversely, using data from 267 Scandinavian men with localized prostate cancer and baseline tPSA levels < 50 ng/ml, Fall and colleagues found that, although prognostically relevant, baseline tPSA levels and relative tPSAV in the first 2 years following diagnosis were not able to predict accurately which patients would have a lethal PCa outcome.⁶⁶ Nevertheless, there exists substantial evidence that tPSAV before treatment is associated with outcome, albeit, there is lack of evidence as to whether the predictive accuracy is improved by the combination of tPSAV and tPSA compared to a single tPSA alone.

This discrepancy between the prognostic and screening setting can be partially explained by the mode of detection, the lead time bias, and how tPSAV was measured. Due to the retrospective nature of these articles, there is no proof that the prospective use of tPSAV thresholds can identify men with an unfavorable prognosis at the time when curative treatment is still possible. The observation period necessary for obtaining a valid calculation of tPSAV that is not disturbed by considerable short term fluctuations may be too long, or the number of tPSA measurements may be too high for use in clinical practice. In addition, tPSAV may not correlate with early tumor progression, but could be a mere indicator of aggressive disease for which the window of curability has already closed. Furthermore, a quickly rising tPSA is more common in men with a high starting tPSA level.⁶⁷ This proportion of men is expected to be much smaller in a screened cohort than in a clinically diagnosed cohort. In the absence of better alternatives, tPSAV is an important and very practical parameter after diagnosis and/or treatment, but its value in screening and prognostication remains to be proven.

Free PSA

The serine protease, PSA, circulates in the serum in multiple molecular forms consisting of both free (unbound to other proteins) and complexed PSA (i.e. mainly bound to the protease inhibitor alpha-1-antichymotrypsin, ACT), Figures 4 and 5. The FDA has approved the use of percent free PSA testing [i.e., (free PSA/tPSA) x 100] as an adjunct to tPSA in men with a serum tPSA concentration between 4 and 10 ng/ml. While several studies have shown that

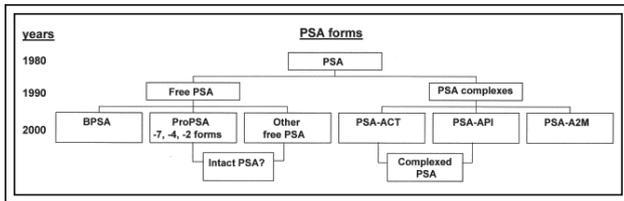


Figure 4. Time line for the discovery of various forms of PSA.

BPSA = benign or BPH-associated free PSA (fPSA); proPSA = precursor propeptide form of fPSA with an intact 7 amino acid-containing peptide leader sequence (-7proPSA) or with either a 3 amino acid peptide [leaving a 4 amino acid peptide remaining (-4proPSA)] or a 5 amino acid peptide (-2proPSA) clipped from the leader sequence of the parent pPSA molecule; other fPSA, refers to other truncated, enzymatically inactive forms of fPSA; intact PSA?, refers to other, as yet unidentified, intact enzymatically inactive forms of free PSA; PSA-ACT, PSA bound to α_1 -antichymotrypsin; PSA-API, PSA bound to α_1 -protease inhibitor; PSA-A2M, PSA bound to α_2 -macroglobulin.

Reproduced, with permission, from Stephan C, Jung K, Diamandis EP, Rittenhouse HG, Lein M, Loening SA. Prostate-specific antigen, its molecular forms, and other kallikrein markers for detection of prostate cancer. *Urology* 2002;59:2-8.⁹⁵

percent free PSA helps discriminate men with BPH from those with prostate cancer, the magnitude of this effect varies across populations.^{68,69} Explanations for these inconsistencies may lie in the limited stability of free PSA in blood, particularly in stored sera.^{12,70} In addition, prostate cancer patients with larger prostate volumes have higher percent free PSA thereby resulting in lower specificity due to the dilution effect.⁷¹ Finally, the most appropriate percent free PSA cut-off value for clinical decision making remains controversial and percent free PSA may be more valuable as a continuous risk variable. Despite all these limitations, in a recent meta analysis of 66 studies, percent free PSA has been shown to outperform tPSA and complexed PSA as a predictor for biopsy outcome.⁷²

Overall, percent free PSA might be less useful as a long term predictor of prostate cancer presence in younger men. Ulmert et al investigated the value of PSA isoforms in a retrospective study comprising a highly representative subset with over 4900 men aged ≤ 50 at the baseline blood draw from the MPM study cohort.⁶⁰ They found that among all men aged 44 to 50 years, the combination of tPSA, free PSA, percent free

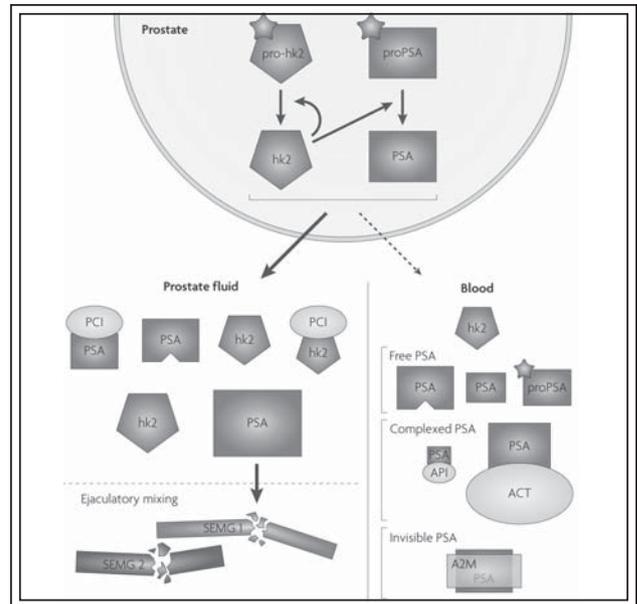


Figure 5. Prostate-specific antigen (PSA) subforms and interactions. Active forms of PSA and kallikrein-related peptidase 2 (hK2) and inactive forms are shown. In the prostate, propeptides are removed from proPSA and prohk2, leaving the mature, catalytic forms. hK2 might be one of the proteases responsible for these processing events. PSA and hK2 are released at high concentrations into prostatic fluid, then into seminal fluid, and at low concentrations into blood. PSA forms in prostatic fluid are active PSA, nicked PSA and PSA complexed with protein C inhibitor (PCI, encoded by SERPINA2), a protease inhibitor. The sizes in the figure indicate the relative abundances of the forms. In seminal fluid, active PSA is believed to be responsible for liquefaction of seminal fluid by proteolysing gel proteins (SEMG1 and SEMG2, which are secreted primarily by the seminal vesicles, though SEMG2 is also secreted in small amounts by the epididymis). Blood contains a variety of forms of PSA: free PSA forms (nicked, intact and proPSA) and complexed PSA. The most abundant form in blood is PSA complexed with α_1 -antichymotrypsin (ACT); complexes with α_2 -macroglobulin (A2M) or α_1 -protease inhibitor (API) are estimated to comprise only a 1-2% or lower proportion of PSA in blood. A2M envelopes PSA, masking the epitopes recognized by commercial PSA assays and thus rendering this form invisible to the assays. PSA levels in seminal fluid are $0.5^{\circ}V3.0$ mg/ml ($\sim 10^6$ -fold higher than in blood) and hK2 levels in seminal fluid are 2-12 microg/ml ($\sim 10^4$ -fold higher than in blood). (Reproduced, with permission, from Lilja, H. et al. Prostate-specific antigen and prostate cancer: prediction, detection and monitoring. *Nature reviews cancer*. 8, 268-278, 2008).⁴⁸

PSA, did not improve the predictive power of tPSA alone, albeit enhancements were found for men with tPSA ≥ 1.2 ng/ml, and more notably in men with tPSA ≥ 2.0 ng/ml. In addition, Vickers et al showed that in men aged 59 to 61 years, the combination of percent free PSA, hK2 and tPSA was significantly superior to tPSA alone (AUC 0.819 versus 0.794, respectively), whereas third combination of markers was unable to enhance cancer risk predictions among men aged ≤ 50 at baseline. A hypothesis for this finding is that a shorter time delay between tPSA measurement and cancer diagnosis and increased frequency of BPH enhances the predictive value of both percent free PSA and hK2 among older men, whereas increased frequency of BPH decreases the predictive value of tPSA in older men.⁴⁷

Measuring distinct subfractions of free PSA: proPSA, intact PSA, nicked PSA, and BPSA

The free PSA in the blood is (micro) heterogeneous and exists mainly as four distinctly defined subfractions, all of which are enzymatically inactive, Figure 5. Similar to most secreted peptide enzymes, PSA is initially produced as a 261 amino acid pre pro protein. Co-translational removal of an amino terminal leader generates a non catalytic zymogen (proPSA). Subsequent removal of the 7-residue propeptide generates the catalytically active mature form, a 237 residue single chain enzyme containing five intra chain disulphide bonds.

ProPSA

Compared to BPH associated transition zone epithelium, prostate cancer tissues have been found to contain higher levels of truncated versions of proPSA with either two (-2proPSA) or four (-4proPSA) extending N terminal of the mature 237 amino acid single chain sequence.⁷³ In a preliminary study of men with a tPSA value between 6 ng/ml and 24 ng/ml, the fraction of -2proPSA in the men with and without prostate cancer ranged from 25% to 95% and 6% to 19%, respectively.⁷³ In this study, -2proPSA was also reported to be a stable (i.e., not cleaved by either hK2 or trypsin), enzymatically inactive form of free PSA. In a follow-up study, Sokoll et al found that in men with tPSA levels between 2.5 ng/ml to 4.0 ng/ml, the percentage of proPSA to free PSA ratio was 50.1% in men with prostate cancer versus 35.5% in men with a negative prostate biopsy.⁷⁴ A higher percentage of proPSA to fPSA also has been associated with a higher risk for prostate cancer in men with tPSA levels between 4.0 ng/ml and

10 ng/ml.⁷⁵ Finally, a higher preoperative proPSA to free PSA ratio has been associated with higher Gleason grade, extracapsular tumor extension, and BCR after radical prostatectomy.⁷⁶⁻⁷⁸ After validation in large, prospective studies, addition of preoperative proPSA to free PSA ratio measurements to standard preoperative predictive models may improve prediction of prostate cancer features and outcomes.

Intact PSA

Other assays recognizing distinctly different antigenic epitopes on free PSA have also been implicated to be useful tools to distinguish critical free PSA heterogeneity in blood as it measures only intact (i.e. both mature and proPSA single chain PSA), but does not recognize any nicked mutli chain PSA forms that are cleaved between Lys145 and Lys146. The level of intact PSA and the ratio of nicked to tPSA have shown potential for improving the discrimination of prostate cancer from BPH.^{79,80} Similarly to free PSA, intact PSA levels degrade with freezing, storage, and thawing.⁷⁹

Vickers et al evaluated whether a multivariable model including tPSA, free PSA, intact PSA, and hK2 predict the results of a prostate biopsy in previously unscreened men with elevated tPSA from the Göteborg cohort of the European Randomized study of Screening for Prostate Cancer screening (ERSPC).⁷⁰ They found that a statistical model including the four markers predicts the result of biopsy more accurately than a model incorporating tPSA and age alone. The area under the curve (AUC) for a predictive "base" model including age and tPSA was 0.680; incorporating the additional markers into the model significantly increased predictive accuracy to an AUC of 0.832. A similar significant increment in predictive accuracy was seen if the digital rectal exam was added to the base model. Moreover, decision analytic methods revealed that application of the model would lead to notably superior clinical outcomes than the current strategy of biopsying all men with elevated tPSA.

BPSA

Another distinct form of the multi chain cleaved free PSA, BPSA, forms through the clipping of intact single chain free PSA between amino acid residues Lys182 and Ser183 resulting in a neo-epitope. BPSA is present in prostatic tissue, blood, and seminal plasma.⁷³ BPSA expression has been localized to the nodular hyperplasia of the transition zone of men with BPH.⁸¹ Serum levels of BPSA are higher in men with symptomatic BPH compared to men without lower urinary tract symptoms suggestive of BPH.⁷³

Moreover, serum BPSA levels are almost undetectable in healthy men. Further studies have confirmed that serum levels of BPSA correlate with pathological nodular hyperplasia of the prostate.^{82,83} Therefore, measurements of BPSA may hold most promise as a serum marker for BPH.

In summary, while assays capable of distinguishing distinct free PSA subfractions in the blood hold the promise of providing new tools for detection, staging, prognosticating, and monitoring of prostate cancer, independent replication of data from large prospective clinical trials remain to be reported. In addition, BPSA or nicked PSA, either alone or (quite likely) in combination with free or total PSA, may be useful in studying the development, clinical progression, and response to therapy of BPH.

Human kallikrein-related peptidase 2

Human kallikrein-related peptidase 2 (hK2) is a serine protease that shares 78% and 80% identity at the amino acid and DNA level with PSA. Moreover, both enzymes are mainly expressed in the prostate and are under androgen regulation. hK2 mRNA amounts to 10%-50% of the PSA mRNA in the prostate tissue but in serum and seminal plasma, hK2 concentration is only 1%-3% that of PSA. The low levels in serum pose analytical challenges for hK2 measurements but reliable assays are available in several research laboratories.

Some studies have suggested that tissue expression of hK2 may be more strongly associated with prostate cancer presence and progression than tPSA.^{84,85} In addition, serum levels of hK2 and its ratios to free PSA and percent free PSA have been reported to outperform tPSA for prostate cancer detection.⁸⁵⁻⁸⁸ Furthermore, preoperative serum hK2 has been suggested to be a stronger predictor of prostate cancer grade, stage, and volume in the prostatectomy specimens than tPSA or free PSA.⁸⁹⁻⁹³ Recently, preoperative serum hK2 has also been shown to predict BCR with high accuracy.⁹³ In a cohort of 867 patients treated with radical prostatectomy for clinically localized disease, the predictive accuracy of hK2 for BCR after surgery was 0.721 (concordance index) versus 0.691 for tPSA. This difference in predictive accuracy was more pronounced in men with a tPSA < 10 ng/ml (0.739 for hK2 versus 0.599 for tPSA, $p < 0.0005$). Moreover, addition of hK2 significantly improved the predictive accuracy of a preoperative nomogram for prediction of BCR consisting of tPSA, clinical stage and biopsy Gleason grade.

In summary, hK2 seems to add statistically and clinically important information for prostate cancer detection and, more importantly, for prostate cancer prognostication, especially in the tPSA range below 10 ng/ml. This is particularly important, as most men diagnosed nowadays with prostate cancer have a tPSA below 10 ng/ml and this is the range where risk stratification using tPSA alone does not perform very well. Nevertheless, these findings need to be externally validated using independent large, well designed studies before hK2 can applied in clinical practice.

Decision analysis tools that integrate risk factors with markers to improve clinical decision making

In addition to tPSA and digital rectal examination findings, there are other risk factors of importance, such as age, family history of prostate cancer, ethnicity, other hereditary and environmental factors and attributes (e.g., diet, body mass index, supplement use), and a prior biopsy with negative results for cancer. Historically, physicians estimated a patient's risk based on clinical and anecdotal experience combined with an understanding of the medical literature, but such an approach is clearly biased.^{7,37,94} Formal predictive/prognostic tools based on statistical models provide more accurate estimates and are widely available.^{7,37,94} These models generally perform as well as or better than clinical judgment.^{7,37,94} The estimates of risk and their potential consequences, the advantages and disadvantages of this knowledge, and subsequent treatment options can be discussed with the patient prior to undergoing biopsy or repeat biopsy.^{7,37,94} Patients can then use their own priorities regarding disease, treatment and functional changes after treatment to decide whether to proceed with a biopsy. Ultimately, this counseling process will create a better-informed patient if a prostate biopsy is performed and cancer is detected.

Disclosure

Dr. Hans Lilja holds patents for free PSA and hK2 assays.

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