Published in final edited form as:

Nat Rev Urol. 2009 July ; 6(7): 384–391. doi:10.1038/nrurol.2009.123.

Prostate kallikrein markers in diagnosis, risk stratification and prognosis

David Ulmert¹, M. Frank O'Brien³, Anders S. Bjartell², and Hans Lilja^{1,3,4,5}

¹Department of Laboratory Medicine and Clinical Sciences, University Hospital UMAS, SE 205 02 Malmö, Sweden

²Department of Urology, Lund University, University Hospital UMAS, SE 205 02 Malmö, Sweden

³Department of Surgery (Urology), Memorial Sloan-Kettering Cancer Center, New York, NY 10065, USA

⁴Clinical Laboratories, Memorial Sloan-Kettering Cancer Center, New York, NY 10065, USA

⁵Department of Medicine (GU-Oncology), Memorial Sloan-Kettering Cancer Center, New York, NY 10065, USA

Summary

The kallikrein, prostate-specific antigen (PSA), is one of the world's most frequently used disease biomarkers. After almost two decades of research and clinical experience, we are now starting to comprehend its diagnostic and monitoring limitations. Most physicians are aware of PSA's low cancer-specificity among older men with benign prostatic conditions, but fewer are aware of recent data showing that a prior negative biopsy or a prior PSA below cut-off may have even stronger negative impact. Furthermore, a subtle PSA rise at early middle age strongly correlates to prostate cancer of unquestionable significance. We thoroughly review current and past reports on prostate kallikreins in relation to pathology and epidemiology.

Keywords

diagnosis; human kallikrein 2; kallikreins; prostate-specific antigen; prostate cancer

Introduction

Human kallikrein-related peptidase 3, more commonly referred to as prostate-specific antigen (PSA), and kallikrein-related peptidase 2 (hK2) are abundantly produced by and released from the prostate in a manner highly specific to this gland, which is key to their recognized clinical significance in man. They share 80% identity in primary structure, are both proteases but manifest distinctly different substrate specificity. Only dogs and some

Corresponding Author, Hans Lilja, MD, PhD, Department of Clinical Laboratories, Surgery (Urology), and Medicine (GU-Oncology), 1275 York Ave, Box 213, Memorial Sloan-Kettering Cancer Center, New York, NY, Tel: +1-212-639-6982, Fax: +1-646-422-2379, liljah@mskcc.org.

Competing Interests

Hans Lilja holds patents for free PSA and hK2 assays.

old-world primates such as humans possess functional orthologs to genes coding for either hK2, PSA, or both, and interestingly man and dogs are among the few animal kingdom species known to suffer from prostate cancer (PCa). This review will evaluate the literature pertaining to current risk stratification, diagnostic, and prognostic applications and limitations of these kallikreins in prostate disease and discuss potential future usefulness. Many other kallikreins (KLK4-KLK15) have been investigated as candidate biomarkers, but will not be discussed in this review.

History of prostate-specific antigen

PSA, initially designated as gamma-seminoprotein, was identified in 1966 and characterized in 1971 by Hara et al.¹. The authors anticipated that it could be utilized as a marker for seminal fluid in forensic medicine. In 1979, a protein designated prostate-specific antigen was purified from prostatic tissue by Wang and associates, and later demonstrated to be identical to gamma-seminoprotein and to the P30 antigen^{2,3,4}. Several subsequent studies recognized PSA as a potential marker for PCa⁵⁻⁷. The first report on the detection of PSA in serum was contributed in 1991 by Papsidero et al.⁶.

Normal expression of prostate kallikreins

The genes that encode hK2 and PSA, *KLK2* and *KLK3*, are clustered with other kallikrein genes on chromosome 19⁸. Expression of both *KLK2* and *KLK3* is induced by androgens. Both proteins are synthesized in a pre-pro precursor form, and activation is a multistep enzymatic process. Low levels of expression of both PSA and hK2 have been detected in several other tissues, both neoplastic and normal, such as mammary, salivary, pituitary and thyroid glands⁹.

Free PSA and Complex PSA

Based on two independent studies, Lilja et al. and Stenman et al.^{10,11} presented solid evidence in 1991 that the major immunodetected fraction of PSA in serum occurs in complex with α 1-antichymotrypsin. In addition, Lilja et al. identified a distinct PSA-epitope only available on the free, non-complexed minor fraction, which together with the major fraction of PSA complexed to antichymotrypsin made up most of PSA detected in serum. The report by Stenman et al. also suggested that the proportion of PSA complexed with α 1-antichymotrypsin is higher in men with PCa than in men with benign prostatic hyperplasia (BPH), which was consistent with the independent findings reported by Christensson et al.¹² and that conversely, the proportion of free-to-total PSA was lower in men with PCa than in those with BPH. Since then, numerous investigators have reported improved discrimination of PCa from BPH by using the ratio of free to total PSA. For example, Prestigiacomo et al. and Björk et al.^{13,14} showed improved identification of men with BPH and those with PCa by using the ratio of free to total PSA (%fPSA) while a report by Leinonen et al.¹⁵ implicated that a ratio of PSA in complex with antichymotrypsin to total PSA could be similarly useful. However since the ratio of complexed PSA to total PSA added no new information over %fPSA, future investigation centered on free and total rather than complexed PSA. Catalona and coworkers¹⁶, in a large multicenter trial, found that prostate biopsies were positive in 56% of the subjects with %fPSA <10% but in only 8% of those

with %fPSA >25%. Moreover, %fPSA has been reported to increase the diagnostic accuracy at both low and high levels of total PSA (tPSA). With respect to low tPSA levels, a prospective study revealed that a significant number of clinically relevant tumors are found in men who have PSA levels in the range 1–3 ng/mL and %fPSA <20%¹⁷. Furthermore, data collected in a large randomized population study indicated that low %fPSA in men with PSA levels <3 ng/mL is associated with a 5–10-fold greater risk of a PCa diagnosis¹⁸. With respect to higher tPSA levels, Morote et al.¹⁹ demonstrated that determining %fPSA benefited the accuracy of predicting biopsy results in subjects with tPSA in the range 10–20 ng/mL. A 2005 meta-analysis of 66 studies²⁰ clearly verified that %fPSA offers better diagnostic performance (i.e., a higher PCa detection rate) compared to tPSA. Data from the Finnish arm of the European Randomized Screening for Prostate Cancer (ERSPC) have also been used to evaluate %fPSA as a possible risk predictor among men with a PSA <3 ng/mL²¹. In this study, the predictive accuracy declined with decreased tPSA level; with the cut-off set at %fPSA <15%, the positive predictive value of %fPSA was 21% for those with PSA level of 2–2.9 ng/mL and 10% for those with PSA <2 ng/mL.

As %fPSA is the only marker that became clinically implemented as a supplementary decision-guiding tool for when to biopsy, it is not unlikely that the clinical impact of testing for %fPSA may at times be somewhat underestimated. Although there is no current data; the number of negative biopsies would probably be even higher without access to %fPSA.

Free PSA subforms

α 1-Antichymotrypsin and other specific inhibitors of PSA (e.g. α 2-macroglobulin) are present in blood at concentrations 10^4 – 10^5 -fold higher than the concentration of PSA. The PSA that remains unbound in blood (i.e. free PSA) therefore consists of various noncatalytic forms that are incapable of binding the inhibitors; these forms include various precursor forms and nicked PSA. Increased levels of various precursor forms have been found in men with PCa^{22–24}. However, increased levels of pro-forms have also been found in men with prostatitis²⁵. In addition to the precursor forms, several different internally nicked forms of PSA have been described. A consistent result of putative clinical value is that PSA nicked between lysine 145 and 146 is elevated in patients with benign prostatic hyperplasia (BPH)^{26–28}. The potential of the described PSA subform-assays are currently under investigation and could, in a future perspective, become clinically useful by enhancing the predictive value of currently established markers.

BPH and PSA

BPH incidence increases linearly with age, from 3 cases per 1000 man-years at ages 45–49 (prevalence 2.7%) to 38 cases per 1000 man-years at ages 75–79 (prevalence 24%)²⁹. BPH is characterized by hyperplasia of both stromal and epithelial cells in various proportions, resulting in large microscopic and macroscopic nodules.

Among men with BPH, the amount of PSA in blood is linearly associated with the size of the prostate, and this correlation appears to be stronger when enlargement is restricted to the transition zone and easily detectable by transrectal ultrasonography (TRUS)³⁰. Stamey and collaborators reported that BPH tissue produces more PSA per gram tissue than normal

prostate tissue³¹, but the accuracy of their data may be questioned. Cross-sectional data from autopsy studies³², population-based studies^{33,34}, and clinical trials in men with BPH demonstrate an increase in prostate size with advancing age that is reflected by a longitudinal rise in PSA.

Prostatitis and PSA

Prostatitis is a common, though ill-defined, benign condition without clear diagnostic criteria³⁵ or pathological mechanisms³⁶. To limit confusion, a four-category classification system for prostatitis was developed under the auspices of the National Institutes of Health (NIH)³⁷. The lifetime prevalence for symptomatic persistent prostatitis is around 10–15%, and the incidence increases with age³⁸. Analogous to BPH and PCa, the incidence is highest among black men and lowest among Asian men^{38–41}.

Prostatitis is correlated with a rise in PSA, irrespective of the NIH category, underlying cause, or chronicity of the prostatitis^{42–46}. Although the putative mechanism for the PSA increase is that inflammation increases vascular permeability, which permits PSA to seep into the circulation^{47,48}, no correlation was found between serum PSA or PSA density and the degree and pattern of inflammation or presence of bacteria⁴⁹. Bozeman et al retrospectively analyzed 95 men who presented with serum PSA >4 ng/mL and were subsequently diagnosed with NIH category IV prostatitis. After 4 weeks on antimicrobial and/or anti-inflammatory treatment, mean PSA decreased 36%, and it decreased to levels below the biopsy cut-off in 46% of the patients⁴⁴. Other studies on chronic prostatitis have shown similar results⁴³. In a prospective study, PSA was measured over 12 months in 54 men with febrile urinary bacterial infection⁵⁰. Total PSA levels decreased slowly, remaining elevated for up to 6 months, while fPSA decreased rapidly and reached stable levels within 1 month. The leakage of enzymes during inflammation and the increased cell division that occur during healing and reconstruction processes would, theoretically, be a very tempting explanation for initiation of malignant growth. However, no correlation between prostatitis and cancer has been shown.

PSA and obesity

Obesity appears to influence PSA levels somewhat. In a prospectively screened cohort of 4458 men with a median age of 60 years⁵¹, obese men (BMI >30 kg/m²) were 1.8 times as likely to have a lower PSA level compared to non-obese men (median PSA 1.78ng/mL vs 1.48ng/mL, $p<0.01$). Since PSA levels are influenced by androgens⁵², speculatively, these findings could result from the correlation of BMI with higher estrogen levels and lower serum androgen levels, due to an altered hormonal environment created by the excess body fat⁵³. In a study by Banez et al, men with a BMI>35kg/m² had a 10–21% lower PSA concentration compared with normal-weight men. Furthermore in that same study obesity was also associated with larger prostate size⁵⁴. Given these factors, relating PSA levels to the risk of PCa is somewhat problematic among obese men. In a recent report based on 28,380 men from the Prostate, Lung, Colorectal, Ovarian (PLCO) Cancer Screening Trial the authors proposed that a larger plasma volume in men with higher BMI resulted in the apparent reduction in PSA concentration in men with higher BMI, hence leading to the

inverse relationship between PSA concentration and BMI⁵⁵. However, more research is required to quantify if an adjustment is required in the cut-off values for PSA based on BMI for clinical practice or for screening trials.

Furthermore, obesity could both be due to, and caused by, a spectrum of hormonal imbalances. Medical treatment targeted at obesity related disease complicates the research situation even further. Hamilton and colleagues recently reported that recent initiation of a statin medication treatment in men was associated with a significant drop in serum PSA. For men with serum PSA >2.5 ng/ml and a drop of >41% in low density lipoprotein, they found a median 17% decline in PSA⁵⁶.

PSA and age

PSA levels tend to increase with age. The increasing levels can most likely be explained by age-related benign prostatic diseases. In a large Swedish cohort, median PSA among men aged 40–50, 51–55 and 60 was 0.63 ng/mL, 0.81 ng/mL, and 1.1 ng/mL^{57–59}. In 1993, based on an evaluation of PSA in 471 non-symptomatic “healthy” men of ages 40–79⁶⁰, Oesterling et al proposed age-related reference ranges for PSA: 40–49 years, 0.0–2.5 ng/mL; 50–59 years, 0.0–3.5 ng/mL; 60–69 years, 0.0–4.5 ng/mL; 70–79 years, 0.0–6.5 ng/mL. The upper limits of these ranges are much higher than those in more contemporary series, which might be due to the fact that PCa was ruled out using DRE and TRUS, not biopsy. Hence the indication for biopsy was mostly criticized due to the smaller number of older men getting a biopsy due to the higher PSA threshold.

PSA and prostate cancer

The increased PSA level associated with PCa is believed to be due to perturbation of the normal tissue anatomy. PCa is characterized by loss of the basal cell layer, derangement of the basal lamina, diminished epithelial cell polarity, and lack of connection of the glandular acini formed by the prostate epithelial cells. All these changes could allow some of the PSA from cancerous cells to leak into intercellular compartments drained by lymphatics and then into the bloodstream. In advanced cases of PCa these architectural changes may eventually cause a thousand-fold increased release of PSA into the blood. Prostate intraepithelial neoplasia (PIN), characterized as either low-grade or high-grade PIN (HGPIN), is defined by cytologically atypical cells that line architecturally normal ducts. Similarly to PCa, these lesions display nuclear and nucleolar abnormalities⁶¹, but due to an intact basement membrane^{62,63} serum PSA levels appear to remain unaltered.

The first prospectively performed evaluation of the usefulness of PSA as a screening test for PCa was conducted in 1991 by Catalona et al⁶⁴. They reported that 4 ng/mL was selected as the optimal cut-off value to achieve both the best rate of detection of curable disease and minimizing the number of unnecessary biopsies. Subsequent studies have repeatedly confirmed the association between PSA and prostate cancer, but the evidence for selecting the 4 ng/mL cut-off appears arbitrary and has been questioned again recently⁶⁵. This was highlighted in 1996 by Smith and colleagues who investigated 10,248 study cohort and observed that 48% of the men whose PSA levels were initially <4 ng/mL had concentrations >4 ng/mL within four years. Furthermore 13% of those men were diagnosed with PCa

during that time period⁶⁶ suggesting that lower cutoff values may allow earlier diagnosis. A further study later indicated that a PSA level of 2.6–4 ng/mL carried a 26% positive predictive value for a positive sextant biopsy⁶⁷. In the same investigation, tumors from patients with PSA 2.6–4 ng/mL were found on pathologic analysis to be smaller and more often localized to the gland than those removed from the patients with PSA 4.1–10 ng/mL. Following these two studies many urologists adjusted their cut-off down to 2.5ng/mL for performing a prostate biopsy as can be seen in widespread publications in the literature. The clinical accuracy of this new cut-off point was confirmed by the findings of the control arm of the Prostate Cancer Prevention Trial (PCPT), in which 5,112 men underwent annual PSA monitoring for seven years followed by an end-of-study biopsy. A cut-off of 2.5 ng/mL showed a PCa sensitivity of 80%⁶⁸. However, the risk of PCa diagnosis below standard cut-off points never reaches zero, thus demonstrating the limited ability of a single PSA as predictor of PCa risk on biopsy of older men. Furthermore, the accuracy of PSA testing (assessed by area under the receiver operating characteristic curve [AUC]) was 0.681 for detection of any PCa and 0.781 for detection of aggressive PCa (Gleason ≥ 7), demonstrating that PSA level in men aged 62–91 years was associated with clinically significant PCa.

Many subsequent studies have focused on how to increase sensitivity and specificity of PSA as a test for PCa. Strategies such as PSA dynamics, and PSA density have been proposed, with varying degrees of acceptance. Studies on these strategies are summarized below. A caveat is that concise summary is difficult, since the cohorts show a great diversity in ethnicity, disease demographics, socioeconomic status, and BMI and have some limitation due to selection or verification bias. These factors are seldom taken into consideration even in the primary studies.

PSA dynamics: velocity and doubling time

In 1992, Carter et al.⁶⁹ introduced the concept of PSA velocity (PSAv), defined as the annual rate of increase in serum PSA value. Their initial study included 54 participants in the Baltimore Longitudinal Study of Aging (BLSA). Five years before diagnosis of PCa, they found no difference in PSA levels between participants with PCa and those with BPH, but PSAv was significantly greater in those who were subsequently diagnosed with PCa. The threshold indicating malignancy, set at 0.75 ng/mL/yr, was based on analysis of 18 men with PCa, 20 with BPH, and 16 with no diagnosis of prostate disease. In a later investigation based on the Baltimore cohort, Fang et al.⁷⁰ studied PSAv in 89 men with PSA levels between 2.0 and 4.0 ng/mL (21 men with PCa and 68 controls). The probability of a PCa diagnosis within 10 years was 65% for men with a PSAv >0.1 ng/mL/yr, compared to 3% for the men with a PSAv <0.1 ng/ml/year.

A recent analysis of data from the BLSA focused on 20 patients who died of PCa. At 10–15 years before diagnosis, when most participants' PSA values were <4 ng/ml, PSAv was associated with risk of dying from PCa⁷¹. A cut-off of PSAv >0.35 ng/ml was able to identify those at risk of death from prostate cancer. However, it is concerning that these observations were based on no more than 20 men who had died of PCa compared to 334 control subjects. In addition, PSAv was highly correlated with the PSA value (Pearson correlation coefficient, $r=0.70$), but these authors did not report whether the combination of

PSAv plus the PSA value actually improved the predictive accuracy beyond that of PSA alone. Ulmert et al. used the Malmö Preventive Project cohort with 4907 participants, of whom 443 were later diagnosed with PCa, to demonstrate that a single PSA measurement up to 15 years prior to diagnosis was highly predictive of a subsequent diagnosis of PCa. PSAv was also strongly associated with a later diagnosis of PCa, but since the single PSA value and PSAv were highly correlated ($r=0.93$), PSAv combined with PSA did not increase the accuracy beyond that of the single PSA. Restricting the analysis to those patients with advanced cancer at diagnosis did not alter the results⁵⁸. Other groups investigating the use of PSA dynamics closer to the time of diagnosis have reported similar results^{72,73}. In the PCPT study, PSAv did not add any predictive value beyond that of PSA alone as a predictor of biopsy outcome⁷². Similarly, in the Rotterdam arm of the ERSPC, PSAv did not improve the prediction of PCa diagnosis in men with PSA >4.0 ng/mL⁷³.

Similarly, studies of pre-treatment PSAv for predicting disease aggressiveness have yielded mixed results. Thiel et al.⁷⁴, in a retrospective study of PSAv before radical prostatectomy in 82 men with clinically organ-confined PCa, found no statistically significant relationship between PSAv and the extent of the cancer. However, in a more recent study based on 1,095 men who underwent radical prostatectomy, D'Amico et al.⁷⁵ noted that a PSAv >2.0 ng/mL/yr during the year before diagnosis was significantly associated with PCa-specific mortality. Similar results were observed in a subsequent investigation of 358 patients treated with external radiation therapy⁷⁷. However, neither of these studies demonstrated that PSAv improved the ability of PSA alone to predict outcome after treatment. This type of analysis was undertaken for 22 published definitions of PSA dynamics, which were retrospectively applied for pretreatment assessment of 2938 patients who underwent radical prostatectomy. Even though PSA doubling time and PSAv did associate with outcome, no PSA dynamic definition when included in a model containing PSA, improved the ability of a single pretreatment PSA value alone to predict outcome⁷⁸. Although some PSA dynamic definitions do associate with outcome, there may be little if any justification to include PSA dynamics in models predicting outcome in patients treated with radical prostatectomy, and clinicians were advised to use the most recent PSA value rather than relying on any current definitions of PSA dynamics.

Despite PSA dynamics being included in some guidelines and suggestions that PSA dynamics should be inclusion criteria for clinical trials, the published evidence on the utility of PSA dynamics is insufficient. A recent systematic review found that the majority of articles presenting such evidence did not include PSA dynamics combined with PSA in a prediction model⁷⁹. Only one article demonstrated that PSAv could improve the predictive accuracy beyond that of PSA alone, and this study suffered from verification bias⁸⁰.

PSA density

PSA density was proposed as a diagnostic tool based on the hypothesis that the diagnostic inaccuracy of tPSA is due to excess PSA production and release by benign nodules in men with BPH^{81,82}. PSA density is defined as the tPSA level divided by the volume of the entire gland as measured by TRUS. Theoretically, PSA density should be higher in subjects with a prostatic malignancy, because leakage of PSA into the bloodstream per gram of cancerous

tissue is up to ten times greater than such leakage from benign or healthy tissue⁸². Several studies show that PSA density is more reliable than tPSA alone as an indication for biopsy^{83,84}, but the method is unfortunately hampered by several factors. Primarily, measurements of prostate volume vary considerably because TRUS is largely an operator-dependent method. Furthermore, PSA density may give false positive results due to increased PSA leakage caused by subclinical prostatitis and infarction. Finally, increased blood concentrations of PSA derived from a tumor may be masked, or diluted, by release of PSA from excessive BPH tissue

Long-term PCa prediction based on the Malmö Preventive Project cohort

Several recent reports on the long-term prognostic capacity of kallikreins have been based on the Malmö Preventive Project (MPP), a large, representative, population-based study on cardiovascular risk factors that took place in Malmö, Sweden between 1974 and 1992⁸⁵. This study enrolled 22,444 men (participation rate 71.2%), of whom 21,277 were 50 years at the initial blood draw at baseline. Certain age groups were invited for a re-screening six years from baseline (participation rate: 72%). By the end of 1999, 498 of the 21,277 participants who were <50 at baseline had been diagnosed with PCa. As the rate of PSA screening in Sweden during this study period was very low, the study constitutes a “natural experiment” to test hypotheses about PSA and prostate cancer.

In a nested case-control study of MPP-participants 50 years old at baseline, a single baseline-measurement of tPSA was highly predictive (AUC=0.762) for later diagnosis of PCa. The predictive value of tPSA extended up to 20 years prior to diagnosis⁵⁷. Even a small increase above population-based median levels – i.e. ≈ 0.6 ng/ml – translated into greatly increased PCa-risk; compared to PSA 0.5 ng/mL, a PSA of 1–2 ng/mL was associated with 7-fold higher risk, and PSA of 2–3 ng/mL with 19-fold higher risk. Although PSA subforms and hK2 were associated with later PCa-diagnosis, the predictive accuracy did not increase beyond that of a PSA alone upon adding PSA-subforms or hK2. However, in a study on the influence of age and delay to diagnosis using a nested case-control design based on 1167 MPP participants aged 60 years at baseline, the predictive value of tPSA was found to decrease with age while the predictive effect of adding PSA subforms and hK2 increases with age⁵⁹. In a more recent study⁸⁶, inclusion criteria were narrowed to advanced or metastatic cancers only. The majority (66%) of these cancers occurred in men whose tPSA levels were in the top 20%. This analysis demonstrated that as much as 25 years prior to diagnosis, tPSA was a strong and statistically significant predictor of these advanced cancers (AUC, 0.791; $p < 0.0001$).

European Randomized Study of Screening for Prostate Cancer

The European Randomized Study of Screening for Prostate Cancer recruited 182,000 men aged 50–74 from seven European countries with a tPSA cut-off at 3 ng/ml as the principal screening method. In the screening group, 82% of men accepted to be screened at least once. Based on a median follow-up of 9 years of 162,000 men in the core age group (55–69), PSA-based screening was found to reduce death of prostate cancer by 20%, but also associated with a high risk of over-diagnosis: 1410 men would need to be screened and 48

additional cases of prostate cancer would need to be treated to prevent one death from prostate cancer⁸⁷. In an analysis adjusted for noncompliance among the men actually screened, screening reduced death from prostate cancer by 27%. Interim results were also reported from the randomized screening trial in US, the PLCO Cancer Screening Trial (initiated around the same time as ERSPC) and which found no reduction of death from prostate cancer by screening⁸⁸. This may, in part, be attributed to smaller sample size ($\approx 76,600$ men), high rate of PSA-testing among controls (52%), higher rate of prior PSA-testing than ERSPC, and 41–64% compliance (vs. 86% in ERSPC) to get a prostate biopsy among men with elevated PSA^{88,89}.

The Swedish arm of the ERSPC randomized 20,000 men (aged 50–65 yrs) in Göteborg, half of them for screening. Men with a PSA <3.0 ng/mL or a benign biopsy have been invited for biennial testing up to age 70 since 1995. Results from the 10-year follow-up of this study showed that screening increases the chance of PCa diagnosis by 1.8, which substantiates a common caveat about screening: increased diagnosis of indolent cancers. On the other hand, the risk of being diagnosed with metastatic disease was reduced by 49%. Individualized frequency of PSA-retesting was addressed by Aus and co-workers who evaluated the cumulative PCa risk in men with different PSA levels, based on the Swedish arm of ERSPC⁹⁰. During a follow up of 7.6 years, 539 cancer cases were detected in the screening arm. No men with a baseline PSA <0.5 ng/mL at baseline had been diagnosed with PCa. Participants with levels of 0.50–0.99 ng/mL also showed low risk: 0.9%⁹⁰. The implication is that men with very low PSA levels could safely undergo screening at longer time intervals. Aus et al specifically proposed repeat tests at every three years for men with PSA 1 ng/mL.

Several studies based on ERSPC cohorts have shown that PSA levels are strongly predictive of a positive biopsy during initial but not at subsequent screening rounds. Although the influence of prior PSA-testing to the predictive value of the PSA-test may be well-known to many clinicians, there is little if any evidence from prior investigations which has focused on this important aspect of the predictive value of the PSA test.

Conclusions

It is common clinical knowledge that an increased level of serum PSA could, aside from malignancy, be due to several benign conditions. In older men, these benign prostatic conditions have higher prevalence than the malignancies. Studies based on the MPP cohort in Sweden have shown that a single PSA measurement performed at early middle age provides a very powerful predictive value⁵⁷, at a time when benign conditions are less prevalent. For older men, adding PSA subforms and other kallikreins to the laboratory panel could enhance precision of risk prognostication among older men^{21,59}. In contrast, PSA dynamics have not been shown to enhance diagnostic or prognostic precision and do not aid clinical decision-making⁷⁸. The predictive value of a PSA test decreases dramatically if prior tests have been negative. However, adding PSA subforms and hK2 increases the precision of PSA-based prediction in patients with a history of a PSA below standard cut-off levels for biopsy or an elevated PSA and a negative biopsy⁹¹.

The clinical dilemma is that there is no diagnostic method to prognosticate if, and when, a well-differentiated tumor is going to progress into life-threatening disease. Future research to identify new and better uses of PSA will need to be focused on not only diagnostics but also to identify and prognosticate which prostate cancers will be clinically significant.

Acknowledgments

The work on this research was funded by a P50-CA92629 SPORE and a R21-CA127768 phased innovation grant in cancer prognosis and prediction from the National Cancer Institute, Swedish Cancer Society project no. 3555 and 07-0458, European Union 6th Framework contract LSHC-CT-2004-503011 (P-Mark), and Swedish Research Council (Medicine) project no. **20095 and** 20760.

MF O'Brien was funded by the Boxer Family Fellowship.

Supported by: The Sidney Kimmel Center for Prostate and Urologic Cancers

We thank Janet Novak, PhD, of Helix Editing for substantive editing of the manuscript; this work was paid for by Memorial Sloan-Kettering Cancer Center.

References

1. Hara M, Koyanagi Y, Inoue T, Fukuyama T. [Some physico-chemical characteristics of "gamma-seminoprotein", an antigenic component specific for human seminal plasma. Forensic immunological study of body fluids and secretion. VII]. *Nihon Hoigaku Zasshi*. 1971; 25:322–324. [PubMed: 5106556]
2. Graves HC, Kamarei M, Stamey TA. Identity of prostate specific antigen and the semen protein P30 purified by a rapid chromatography technique. *J Urol*. 1990; 144:1510–1515. [PubMed: 1700160]
3. Schaller J, et al. Isolation, characterization and amino-acid sequence of gamma-seminoprotein, a glycoprotein from human seminal plasma. *Eur J Biochem*. 1987; 170:111–120. [PubMed: 3691515]
4. Lilja H, Abrahamsson PA. Three predominant proteins secreted by the human prostate gland. *Prostate*. 1988; 12:29–38. [PubMed: 3347596]
5. Kuriyama M, et al. Use of human prostate-specific antigen in monitoring prostate cancer. *Cancer Res*. 1981; 41:3874–3876. [PubMed: 7284995]
6. Papsidero LD, Wang MC, Valenzuela LA, Murphy GP, Chu TM. A prostate antigen in sera of prostatic cancer patients. *Cancer Res*. 1980; 40:2428–2432. [PubMed: 7388802]
7. Wang MC, et al. Prostate antigen: a new potential marker for prostatic cancer. *Prostate*. 1981; 2:89–96. [PubMed: 6169079]
8. Riegman PH, Vlietstra RJ, Suurmeijer L, Cleutjens CB, Trapman J. Characterization of the human kallikrein locus. *Genomics*. 1992; 14:6–11. [PubMed: 1385301]
9. Lundwall A, Clauss A, Olsson AY. Evolution of kallikrein-related peptidases in mammals and identification of a genetic locus encoding potential regulatory inhibitors. *Biol Chem*. 2006; 387:243–249. [PubMed: 16542144]
10. Lilja H, et al. Prostate-specific antigen in serum occurs predominantly in complex with alpha 1-antichymotrypsin. *Clin Chem*. 1991; 37:1618–1625. [PubMed: 1716536]
11. Stenman UH, et al. 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; 51:222–226. [PubMed: 1703033]
12. Christensson A, et al. Serum prostate specific antigen complexed to alpha 1-antichymotrypsin as an indicator of prostate cancer. *J Urol*. 1993; 150:100–105. [PubMed: 7685416]
13. Prestigiacomo AF, Lilja H, Pettersson K, Wolfert RL, Stamey TA. A comparison of the free fraction of serum prostate specific antigen in men with benign and cancerous prostates: the best case scenario. *J Urol*. 1996; 156:350–354. [PubMed: 8683677]

14. Björk T, et al. Comparison of analysis of the different prostate-specific antigen forms in serum for detection of clinically localized prostate cancer. *Urology*. 1996; 48:882–888. [PubMed: 8973671]
15. Leinonen J, Lövgren T, Vornanen T, Stenman UH. Double-label time-resolved immunofluorometric assay of prostate-specific antigen and of its complex with alpha 1-antichymotrypsin. *Clin Chem*. 1993; 39:2098–2103. [PubMed: 7691441]
16. Catalona WJ, 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; 279:1542–1547. [PubMed: 9605898]
17. Recker F, et al. Prospective detection of clinically relevant prostate cancer in the prostate specific antigen range 1 to 3 ng./ml. combined with free-to-total ratio 20% or less: the Aarau experience. *J Urol*. 2001; 166:851–855. [PubMed: 11490232]
18. Hugosson J, Aus G, Lilja H, Lodding P, Pihl CG. Results of a randomized, population-based study of biennial screening using serum prostate-specific antigen measurement to detect prostate carcinoma. *Cancer*. 2004; 100:1397–1405. [PubMed: 15042673]
19. Morote J, et al. The percentage of free prostatic-specific antigen is also useful in men with normal digital rectal examination and serum prostatic-specific antigen between 10.1 and 20 ng/ml. *Eur Urol*. 2002; 42:333–337. [PubMed: 12361897]
20. Roddam AW, et al. Use of prostate-specific antigen (PSA) isoforms for the detection of prostate cancer in men with a PSA level of 2–10 ng/ml: systematic review and meta-analysis. *Eur Urol*. 2005; 48:386–399. discussion 398–389. [PubMed: 15982797]
21. Finne P, et al. Diagnostic value of free prostate-specific antigen among men with a prostate-specific antigen level of <3.0 microg per liter. *Eur Urol*. 2008; 54:362–370. [PubMed: 18006214]
22. Mikolajczyk SD, et al. A precursor form of PSA (pPSA) is a component of the free PSA in prostate cancer serum. *Urology*. 1997; 50:710–714. [PubMed: 9372880]
23. Peter J, Unverzagt C, Krogh TN, Vorm O, Hoesel W. Identification of precursor forms of free prostate-specific antigen in serum of prostate cancer patients by immunosorption and mass spectrometry. *Cancer Res*. 2001; 61:957–962. [PubMed: 11221890]
24. Stephan C, et al. A [-2]proPSA-based artificial neural network significantly improves differentiation between prostate cancer and benign prostatic diseases. *Prostate*. 2009; 69:198–207. [PubMed: 18942119]
25. Nadler RB, et al. Prostate-specific antigen test in diagnostic evaluation of chronic prostatitis/chronic pelvic pain syndrome. *Urology*. 2006; 67:337–342. [PubMed: 16442595]
26. Chen Z, Chen H, Stamey TA. Prostate specific antigen in benign prostatic hyperplasia: purification and characterization. *J Urol*. 1997; 157:2166–2170. [PubMed: 9146608]
27. Hilz H, et al. Molecular heterogeneity of free PSA in sera of patients with benign and malignant prostate tumors. *Eur Urol*. 1999; 36:286–292. [PubMed: 10473986]
28. Noldus J, Chen Z, Stamey TA. Isolation and characterization of free form prostate specific antigen (f-PSA) in sera of men with prostate cancer. *J Urol*. 1997; 158:1606–1609. [PubMed: 9302183]
29. Verhamme KM, et al. Incidence and prevalence of lower urinary tract symptoms suggestive of benign prostatic hyperplasia in primary care--the Triumph project. *Eur Urol*. 2002; 42:323–328. [PubMed: 12361895]
30. Perrin P, et al. [Circulating prostatic-specific antigen in benign hypertrophy and localized prostate cancer: can PSA be considered a screening examination for localized cancer?]. *Prog Urol*. 1991; 1:419–431. [PubMed: 1726943]
31. Hammerer PG, McNeal JE, Stamey TA. Correlation between serum prostate specific antigen levels and the volume of the individual glandular zones of the human prostate. *J Urol*. 1995; 153:111–114. [PubMed: 7526003]
32. Berry SJ, Coffey DS, Walsh PC, Ewing LL. The development of human benign prostatic hyperplasia with age. *J Urol*. 1984; 132:474–479. [PubMed: 6206240]
33. Bosch JL, et al. Parameters of prostate volume and shape in a community based population of men 55 to 74 years old. *J Urol*. 1994; 152:1501–1505. [PubMed: 7523706]
34. Oesterling JE, et al. Serum prostate-specific antigen in a community-based population of healthy Japanese men: lower values than for similarly aged white men. *Br J Urol*. 1995; 75:347–353. [PubMed: 7537604]

35. Pontari MA, Joyce GF, Wise M, McNaughton-Collins M. Prostatitis. *J Urol.* 2007; 177:2050–2057. [PubMed: 17509285]
36. Weidner W, et al. Chronic prostatitis: a thorough search for etiologically involved microorganisms in 1,461 patients. *Infection.* 1991; 19(Suppl 3):S119–S125. [PubMed: 2055646]
37. Krieger JN, Nyberg L Jr, Nickel JC. NIH consensus definition and classification of prostatitis. *Jama.* 1999; 282:236–237. [PubMed: 10422990]
38. Mehik A, Hellstrom P, Lukkarinen O, Sarpola A, Jarvelin M. Epidemiology of prostatitis in Finnish men: a population-based cross-sectional study. *BJU Int.* 2000; 86:443–448. [PubMed: 10971269]
39. Nickel JC, Downey J, Hunter D, Clark J. Prevalence of prostatitis-like symptoms in a population based study using the National Institutes of Health chronic prostatitis symptom index. *J Urol.* 2001; 165:842–845. [PubMed: 11176483]
40. Tan JK, Png DJ, Liew LC, Li MK, Wong ML. Prevalence of prostatitis-like symptoms in Singapore: a population-based study. *Singapore Med J.* 2002; 43:189–193. [PubMed: 12188063]
41. Ejike CE, Ezeanyika LU. Prevalence of chronic prostatitis symptoms in a randomly surveyed adult population of urban-community-dwelling Nigerian males. *Int J Urol.* 2008; 15:340–343. [PubMed: 18380824]
42. Dalton DL. Elevated serum prostate-specific antigen due to acute bacterial prostatitis. *Urology.* 1989; 33:465. [PubMed: 2471345]
43. Potts JM. Prospective identification of National Institutes of Health category IV prostatitis in men with elevated prostate specific antigen. *J Urol.* 2000; 164:1550–1553. [PubMed: 11025702]
44. Bozeman CB, Carver BS, Eastham JA, Venable DD. Treatment of chronic prostatitis lowers serum prostate specific antigen. *J Urol.* 2002; 167:1723–1726. [PubMed: 11912396]
45. Kwak C, et al. Effect of subclinical prostatic inflammation on serum PSA levels in men with clinically undetectable prostate cancer. *Urology.* 2003; 62:854–859. [PubMed: 14624908]
46. Stancik I, Luftenegger W, Klimpfinger M, Muller MM, Hoeltl W. Effect of NIH-IV prostatitis on free and free-to-total PSA. *Eur Urol.* 2004; 46:760–764. [PubMed: 15548444]
47. Ulleryd P, et al. Prostatic involvement in men with febrile urinary tract infection as measured by serum prostate-specific antigen and transrectal ultrasonography. *BJU Int.* 1999; 84:470–474. [PubMed: 10468764]
48. Neal DE Jr, Clejan S, Sarma D, Moon TD. Prostate specific antigen and prostatitis. I. Effect of prostatitis on serum PSA in the human and nonhuman primate. *Prostate.* 1992; 20:105–111. [PubMed: 1372426]
49. Nickel JC, Downey J, Young I, Boag S. Asymptomatic inflammation and/or infection in benign prostatic hyperplasia. *BJU Int.* 1999; 84:976–981. [PubMed: 10571623]
50. Zackrisson B, et al. Evolution of free, complexed, and total serum prostate-specific antigen and their ratios during 1 year of follow-up of men with febrile urinary tract infection. *Urology.* 2003; 62:278–281. [PubMed: 12893335]
51. Barqawi AB, Golden BK, O'Donnell C, Brawer MK, Crawford ED. Observed effect of age and body mass index on total and complexed PSA: analysis from a national screening program. *Urology.* 2005; 65:708–712. [PubMed: 15833513]
52. Kim MR, et al. Serum prostate specific antigen, sex hormone binding globulin and free androgen index as markers of pubertal development in boys. *Clin Endocrinol (Oxf).* 1999; 50:203–210. [PubMed: 10396363]
53. Tchernof A, et al. Reduced testosterone and adrenal C19 steroid levels in obese men. *Metabolism.* 1995; 44:513–519. [PubMed: 7723675]
54. Bañez LL, et al. Obesity-related plasma hemodilution and PSA concentration among men with prostate cancer. *JAMA.* 2007; 298:2275–2280. [PubMed: 18029831]
55. Grubb RL 3rd, et al. Serum prostate-specific antigen hemodilution among obese men undergoing screening in the prostate, lung, colorectal and ovarian cancer screening trial. *Cancer Epidemiol Biomarkers Prev.* 2009; 18:748–51. [PubMed: 19258472]
56. Hamilton RJ, Goldberg KC, Platz EA, Freedland SJ. The influence of statin medications on prostate-specific antigen levels. *J Natl Cancer Inst.* 2008; 100:1511–1518. [PubMed: 18957682]

57. 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. [PubMed: 17264339]
58. Ulmert D, et al. Long-term prediction of prostate cancer: prostate-specific antigen (PSA) velocity is predictive but does not improve the predictive accuracy of a single PSA measurement 15 years or more before cancer diagnosis in a large, representative, unscreened population. *J Clin Oncol.* 2008; 26:835–841. [PubMed: 18281654]
59. Vickers AJ, et al. The predictive value of prostate cancer biomarkers depends on age and time to diagnosis: Towards a biologically-based screening strategy. *Int J Cancer.* 2007; 121:2212–2217. [PubMed: 17657743]
60. Oesterling JE, et al. Serum prostate-specific antigen in a community-based population of healthy men. Establishment of age-specific reference ranges. *Jama.* 1993; 270:860–864. [PubMed: 7688054]
61. Bostwick DG, Pacelli A, Lopez-Beltran A. Ultrastructure of prostatic intraepithelial neoplasia. *Prostate.* 1997; 33:32–37. [PubMed: 9294624]
62. Morote J, Encabo G, Lopez M, de Torres IM. Influence of high-grade prostatic intra-epithelial neoplasia on total and percentage free serum prostatic specific antigen. *BJU Int.* 1999; 84:657–660. [PubMed: 10510111]
63. Morote J, Raventos CX, Encabo G, Lopez M, de Torres IM. Effect of high-grade prostatic intraepithelial neoplasia on total and percent free serum prostatic-specific antigen. *Eur Urol.* 2000; 37:456–459. [PubMed: 10765077]
64. Catalona WJ, et al. Measurement of prostate-specific antigen in serum as a screening test for prostate cancer. *N Engl J Med.* 1991; 324:1156–1161. [PubMed: 1707140]
65. Punglia RS, D'Amico AV, Catalona WJ, Roehl KA, Kuntz KM. Impact of age, benign prostatic hyperplasia, and cancer on prostate-specific antigen level. *Cancer.* 2006; 106:1507–1513. [PubMed: 16518812]
66. Smith DS, Catalona WJ, Herschman JD. Longitudinal screening for prostate cancer with prostate-specific antigen. *Jama.* 1996; 276:1309–1315. [PubMed: 8861989]
67. Krumholtz JS, et al. Prostate-specific antigen cutoff of 2.6 ng/mL for prostate cancer screening is associated with favorable pathologic tumor features. *Urology.* 2002; 60:469–473. discussion 473–464. [PubMed: 12350486]
68. Thompson IM, et al. Effect of finasteride on the sensitivity of PSA for detecting prostate cancer. *J Natl Cancer Inst.* 2006; 98:1128–1133. [PubMed: 16912265]
69. Carter HB, et al. Longitudinal evaluation of prostate-specific antigen levels in men with and without prostate disease. *Jama.* 1992; 267:2215–2220. [PubMed: 1372942]
70. Fang J, et al. Low levels of prostate-specific antigen predict long-term risk of prostate cancer: results from the Baltimore Longitudinal Study of Aging. *Urology.* 2001; 58:411–416. [PubMed: 11549490]
71. Carter HB, et al. Detection of life-threatening prostate cancer with prostate-specific antigen velocity during a window of curability. *J Natl Cancer Inst.* 2006; 98:1521–1527. [PubMed: 17077354]
72. Thompson IM, et al. Assessing prostate cancer risk: results from the Prostate Cancer Prevention Trial. *J Natl Cancer Inst.* 2006; 98:529–534. [PubMed: 16622122]
73. Schroder FH, Roobol MJ, van der Kwast TH, Kranse R, Bangma CH. Does PSA velocity predict prostate cancer in pre-screened populations? *Eur Urol.* 2006; 49:460–465. discussion 465. [PubMed: 16442212]
74. Thiel R, Pearson JD, Epstein JI, Walsh PC, Carter HB. Role of prostate-specific antigen velocity in prediction of final pathologic stage in men with localized prostate cancer. *Urology.* 1997; 49:716–720. [PubMed: 9145976]
75. D'Amico AV, Chen MH, Roehl KA, Catalona WJ. Preoperative PSA velocity and the risk of death from prostate cancer after radical prostatectomy. *N Engl J Med.* 2004; 351:125–135. [PubMed: 15247353]

76. D'Amico AV, Renshaw AA, Sussman B, Chen MH. Pretreatment PSA velocity and risk of death from prostate cancer following external beam radiation therapy. *Jama*. 2005; 294:440–447. [PubMed: 16046650]
77. O'Brien MF, et al. Both pretreatment PSA velocity and doubling time associate with outcome but neither improves prediction of outcome beyond pretreatment PSA alone in patients treated with radical prostatectomy. *J Clin Oncol*. 2009 [in press].
78. Vickers AJ, Savage C, O'Brien MF, Lilja H. Systematic review of pretreatment prostate-specific antigen velocity and doubling time as predictors for prostate cancer. *J Clin Oncol*. 2009; 27:398–403. [PubMed: 19064972]
79. Loeb S, Roehl KA, Catalona WJ, Nadler RB. Prostate specific antigen velocity threshold for predicting prostate cancer in young men. *J Urol*. 2007; 177:899–902. [PubMed: 17296371]
80. Babaian RJ, Fritsche HA, Evans RB. Prostate-specific antigen and prostate gland volume: correlation and clinical application. *J Clin Lab Anal*. 1990; 4:135–137. [PubMed: 1690280]
81. Veneziano S, et al. Correlation between prostate-specific antigen and prostate volume, evaluated by transrectal ultrasonography: usefulness in diagnosis of prostate cancer. *Eur Urol*. 1990; 18:112–116. [PubMed: 1699766]
82. Stamey TA, et al. Prostate specific antigen in the diagnosis and treatment of adenocarcinoma of the prostate. II. Radical prostatectomy treated patients. *J Urol*. 1989; 141:1076–1083. [PubMed: 2468795]
83. Bazinet M, et al. Prospective evaluation of prostate-specific antigen density and systematic biopsies for early detection of prostatic carcinoma. *Urology*. 1994; 43:44–51. discussion 51–42. [PubMed: 7506853]
84. Rommel FM, et al. The use of prostate specific antigen and prostate specific antigen density in the diagnosis of prostate cancer in a community based urology practice. *J Urol*. 1994; 151:88–93. [PubMed: 7504748]
85. Nilsson PM, Nilsson JA, Ostergren PO, Berglund G. Social mobility, marital status, and mortality risk in an adult life course perspective: the Malmo Preventive Project. *Scand J Public Health*. 2005; 33:412–423. [PubMed: 16332606]
86. Ulmert D, et al. Prostate-specific antigen at or before age 50 as a predictor of advanced prostate cancer up to 25 years later: a case-control study. *BMC Med*. 2008; 6:6. [PubMed: 18279502]
87. Schröder FH, et al. Screening and prostate-cancer mortality in a randomized European study. *N Engl J Med*. 2009; 360:1320–1328. [PubMed: 19297566]
88. Andriole GL, et al. Mortality results from a randomized prostate-cancer screening trial. *N Engl J Med*. 2009; 360:1310–1319. [PubMed: 19297565]
89. Pinsky PF, et al. Prostate biopsy following a positive screen in the prostate, lung, colorectal and ovarian cancer screening trial. *J Urol*. 2005; 173:746–750. [PubMed: 15711261]
90. Aus G, et al. Individualized screening interval for prostate cancer based on prostate-specific antigen level: results of a prospective, randomized, population-based study. *Arch Intern Med*. 2005; 165:1857–1861. [PubMed: 16157829]
91. Vickers AJ, et al. A panel of kallikrein markers can reduce unnecessary biopsy for prostate cancer: data from the European Randomized Study of Prostate Cancer Screening (ERSPC) in Goteborg, Sweden. *J Urol*. 2008; 179:719. [abstract].
92. Sakr WA, et al. High grade prostatic intraepithelial neoplasia (HGPIN) and prostatic adenocarcinoma between the ages of 20–69: an autopsy study of 249 cases. *In Vivo*. 1994; 8:439–443. [PubMed: 7803731]
93. Sanchez-Chapado M, Olmedilla G, Cabeza M, Donat E, Ruiz A. Prevalence of prostate cancer and prostatic intraepithelial neoplasia in Caucasian Mediterranean males: an autopsy study. *Prostate*. 2003; 54:238–247. [PubMed: 12518329]
94. Soos G, et al. The prevalence of prostate carcinoma and its precursor in Hungary: an autopsy study. *Eur Urol*. 2005; 48:739–744. [PubMed: 16203079]
95. Stamatiou K, Alevizos A, Agapitos E, Sofras F. Incidence of impalpable carcinoma of the prostate and of non-malignant and precarcinomatous lesions in Greek male population: an autopsy study. *Prostate*. 2006; 66:1319–1328. [PubMed: 16688747]

Review Criteria

PubMed was searched for papers published from 1970 through to December 2008 using the terms “PSA”, “hK2”, “kallikreins”, “prostate cancer”. For selected sections “BMI” and “prostatitis” was also included. From the citations identified by the searches, publications were selected on the basis of relevance to the subject matter, as well as scientific and clinical value. Only papers published in English have been included in this Review.