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Tumor markers in prostate cancer I: blood-based markers

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Abstract

INTRODUCTION—The introduction of total prostate specific antigen (total PSA) testing in blood has revolutionized the detection and management of men with prostate cancer (PCa). The objective of this review was to discuss the challenges of PCa biomarker research, definition of the type of PCa biomarkers, the statistical considerations for biomarker discovery and validation, and to review the literature regarding total PSA velocity and novel blood-based biomarkers.

METHODS—An English-language literature review of the Medline database (1990 to August 2010) of published data on blood-based biomarkers and PCa was undertaken.

RESULTS—The inherent biological variability of total PSA levels affects the interpretation of any single result. Men who will eventually develop PCa have increased total PSA levels years or decades before the cancer is diagnosed. Total PSA velocity improves predictiveness of total PSA only marginally, limiting its value for PCa screening and prognostication. The combination of PSA molecular forms and other biomarkers improve PCa detection substantially. Several novel blood-based biomarkers such as human glandular kallikrein 2 (hK2), urokinase plasminogen activator (uPA) and its receptor (uPAR), transforming growth factor-beta 1 (TGF-β1); interleukin-6 (IL-6) and its receptor (IL-6R) may help PCa diagnosis, staging, prognostication, and monitoring. Panels of biomarkers that capture the biologic potential of PCa are in the process of being validated for PCa prognostication.

CONCLUSIONS—PSA is a strong prognostic marker for long-term risk of clinically relevant cancer. However, there is a need for novel biomarkers that aid clinical decision making about biopsy and initial treatment. There is no doubt that progress will continue based on the integrated collaboration of researchers, clinicians and biomedical firms.

Keywords

Prostate neoplasms; molecular markers; prostate specific antigen

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INTRODUCTION

In Western societies, prostate cancer (PCa) is the most common solid malignancy and the second leading cause of cancer death in men [1]. The wide availability of total prostate-specific antigen (PSA, formal name human kallikrein 3, hK3) revolutionized PCa screening and ushered in the PSA era resulting in a decrease of PCa metastasis and death. However, the ubiquitous application of PSA screening has also led to over-detection and overtreatment. The lifetime risk of PCa diagnosis is estimated at ~18%, whereas that for death from PCa is ~3%. In addition, PSA is neither cancer specific nor a surrogate for the biologic behavior of PCa. An elevated PSA level can reflect the presence of cancer but can also be caused by benign prostatic hyperplasia (BPH), infection, and/or chronic inflammation. Virtually all prostate epithelial cells, whether normal, hyperplastic or cancerous, synthesize PSA. Therefore, there has been a concerted effort to discover and validate novel PCa biomarkers. In this review article, we first discuss the challenge of PCa biomarker research, types of PCa biomarkers, and the statistical considerations for biomarker discovery and validation. Then, we discuss the limitations of measuring total PSA and its derivatives such as total PSA velocity (total PSAV) and different molecular forms (i.e., free PSA, BPSA, pro-PSA, and intact PSA). Moreover, we briefly discuss several promising novel blood-based biomarkers for PCa diagnosis, staging, prognostication, and monitoring i.e., human glandular kallikrein 2 (hK2), urokinase plasminogen activator (uPA) and its receptor (uPAR), transforming growth factor-beta 1 (TGF-β1); interleukin-6 (IL-6) and its receptor (IL-6R).

BIOMARKER CHALLENGE IN PROSTATE CANCER

A PubMed Search on “prostate cancer” AND (“biomarker” OR “marker”) in English language yielded 3016 hits (accessed 8/8/2010). The number of articles published on PCa biomarkers has increased steadily over the years. Despite this plethora of biomarkers reported to be “promising”, only one biomarker, (i.e. total PSA in blood) is routinely used by urologists. Why are PCa biomarkers not living up to their promise? For one, there are remarkable analytical and regulatory barriers to the application of biomarkers in PCa care. [2, 3] These include but are not limited to the status of intellectual property protection, availability of standard reference materials for the assay, complexity of assay format, implementation of quality control to assure reproducibility and accuracy, sufficient market testing size to assess methods of commercialization, lack of clear guidelines for good manufacturing/laboratory practice and quality control requirements for all phases of biomarker development, cost and effort required to accumulate clinical data under appropriately designed and Institutional Review Board-approved prospective trials, and the interval required for resolution of patent issues, assay standardization, validation, testing, and regulatory approval.

Besides analytical and regulatory barriers, the lack of PCa biomarker use in daily clinical practice is also a result of poor application of statistics and study design. There has been a lack of effective and efficient strategies to determine which biomarker candidates justify the great investment of time and money required for assay development, optimization and demonstration of analytical robustness. There are now guidelines intended to ensure that biomarker studies conform to some basic standards of design and reporting.[2, 3] For a new biomarker to be clinically useful, it has to answer a clinically relevant question and provide information that is not available in a more simple and cost-effective way. Any new biomarker needs to provide a benefit over these standard criteria or at least improve their accuracy. Before a biomarker assay can be implemented in the community setting, it needs to address four concepts: “*better, easier, faster and cheaper*”.[2]

Conceptually, the development of new biomarkers should be a process that is similar to therapeutic drug evaluation. In 2002, the National Cancer Institute's Early Detection Research Network developed a five phase approach to systematic discovery and validation of biomarkers (Figure 1).[2-6] This schema is not only an intellectual process but also provides a clear scale by which researchers, patients, and investors can evaluate the status of the biomarker in the development process. The expected failure rate of biomarkers in development can be expected to be similar to the one of drugs. A large concerted effort is required to advance the field of PCa biomarker through systematic discovery, verification, and validation- each step coupled with adequate statistical analysis.

REFINING THE DEFINITION OF *PROSTATE CANCER BIOMARKER*

According to the National Institute of Health (NIH) in the US, a biomarker is a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmaceutical responses to a therapeutic intervention [7]. Cancer biomarkers are either produced by the tumor or by the body in response to the tumor. Six different types of biomarker can be differentiated in PCa:

1. Detection/screening: this biomarker is used for evaluating patients with either risk factors for or symptoms of PCa.
2. Diagnostic: this biomarker can help classical histopathological characteristics in assessing presence or absence of cancer.
3. Prognostic: this biomarker is used to predict the outcome of patients based on different risk of recurrence or progression thereby allowing individualized management.
4. Predictive: this biomarker is used to predict whether the treatment (drug or other therapy) will be effective, and/or to monitor the effectiveness of the treatment. It can help identify the best treatment modality.
5. Therapeutic target: this biomarker can help identify the patients who will benefit from a particular treatment regimen. It identifies the molecular targets of novel therapies and is affected by therapy. As of now there is no such blood-based biomarker in clinical use for prostate PCa.
6. Surrogate endpoint: this biomarker is used to substitute for a clinical endpoint and/or to measure clinical benefit, harm or lack of benefit or harm. Surrogates could replace traditional endpoints, such as mortality due to disease or the recurrence or relapse of disease. Biomarkers can reduce time factors and costs for Phase I and II clinical trials by replacing clinical endpoints.

For the purposes of the current review, we will focus on blood-based biomarkers that are the end result of a bioassay, for processing biological material from humans, expressed quantitatively or categorically. Tissue-based and urinebased PCa biomarkers are discussed in separate reports.

STATISTICAL CONSIDERATIONS FOR BIOMARKER DISCOVERY AND VALIDATION

An issue that has received less attention is the degree to which research on biomarkers has made sufficient use of clinically relevant statistics, such as the assessment of predictive accuracy, decision analysis, and/or experimental methodology. Most biomarkers do not provide sufficient information to be used independent of other information. The optimal use of biomarkers lies in incorporating it in a model that also includes standard clinical data.[3,

8-12] To determine the value of a new biomarker, it is not sufficient to show that it is significantly related to the outcome, statistically significant in a multivariable model including the standard clinical and pathologic factors, or more significant than the standard clinical and pathologic factors. A variable that is statistically significant in a multivariable model might not improve the model's predictive accuracy. P-value and odds/hazard ratio do not meaningfully describe a biomarkers' ability to classify patients. For a biomarker to be potentially clinically useful, it is necessary to show that adding the biomarker to an existing model based on the most important clinical and pathologic factors improves the predictive accuracy (discrimination and calibration) of the model.[3, 11, 13-16]

One major issue with model development is the need for appropriate validation. There are two general types of validation: internal validation on original dataset and external validation on independent dataset.[2, 3] External validation on a different data set allows for evaluation of the generalizability of the risk prediction tool to wider populations than originally reported. Finally, methods that incorporate clinical consequences such as decision curve analysis are crucial to the evaluation of biomarkers. Several methods are available including decision curve analysis which combines simplicity with efficient computations. [17-20]

TOTAL PSA AND ITS LIMITATIONS

Neoplastic cells produce somewhat lower and varying tissue levels of PSA compared to benign epithelial cells although both conditions cause total PSA elevation in the blood.[3] Therefore, it has been suggested that total PSA should be considered as a marker of BPH-related prostate volume, growth, and outcome rather than a reliable marker of PCa.[3] Moreover, some aggressive PCa 's do not produce PSA.

PSA levels are inherently variable thereby affecting the interpretation of any single result. [21] Variation in total PSA 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). Oscillations up to 20–30% in the total PSA range 0.1–20 ng/mL may be due to biologic variation.[22, 23] Furthermore, the use of different detection assays may be another important cause of variation. Differences in assay standardization can give an artifactually high or low estimate of total PSA and total PSAV.[24-26] Assays are not interchangeable and caution should be exercised when comparing results from different commercial total PSA assays. Patients and physicians should be aware of which assay was used each time a total PSA measurement is performed, and an effort should be made to use the same assay at the next visit. In addition, studies of total PSA kinetics over time using different assays should be interpreted with caution. Of note, there is an expected 20% lower value when the World Health Organization standard is adopted and laboratories should be obliged to mention the name of the PSA assay used on the lab report as well as stating the assay specific reference range and the type of master-calibration (i.e. WHO standardized or traditional calibration).[27-29]

The effect of previous BPH treatment on total PSA remains mostly unpredictable. For example, the effect of commonly used 5-α-reductase inhibitors on the predictive value of total PSA 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 total PSA.[30] In addition, by shrinking the prostate gland, finasteride has been suggested to increase the likelihood of detecting a small cancer on needle biopsy.[30]

This large normal variability of total PSA 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 over 2 weeks and demonstrated that a change between two total PSA measurements of approximately 25% indicated a significant change.[31, 32] 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.[33] Bruun et al. recently assessed the long-term variability of the different forms of PSA at several different total PSA levels in a randomly selected population of asymptomatic and apparently healthy men whose total PSA levels were <2.0 ng/mL at the end of the 8-year observation period.[34] They found that the total intra-individual variation of total PSA was much less than that reported by Bunting et al.[33] 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 total PSA. One explanation is that free PSA and complexed PSA may have different elimination pathways, and hence different elimination rates.[35-38]

OPTIMAL TOTAL PSA CUT-OFF VALUES

No single total PSA cut-off separates men at high risk for PCa from men at low risk, nor men affected with high-grade disease from those with low-grade disease. At a total PSA cut-off of ≥4 ng/mL, a significant number of PCas remain undetected.[39-42] In addition, intervention at lower total PSA levels has been proposed to improve patient outcomes.[43, 44] Catalona et al. found that 22% of men with a normal digital rectal examination (DRE) and a serum total PSA level between 2.6 and 4.0 ng/mL have PCa, and 81% of them have organ-confined disease.[45] Data from the Prostate Cancer Prevention Trial (PCPT) revealed that as many as 15% of men with normal DRE and a serum total PSA less than 4.0 ng/mL have PCa.[39] Among men with total PSA levels ≤0.5, 0.6–1.0, 1.1–2.0, 2.1–3.0, and 3.1–4.0 ng/mL, PCa 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 total PSA are associated with increasing probability of PCa risk within the 0-4.0 ng/mL interval.[39, 41, 46] There is no total PSA threshold at age 62-91 below which PCa can be ruled out with high specificty.[39] No single total PSA cut-off separates men with “significant” (high grade, high volume) cancer from those with low-grade, possibly insignificant cancer. Similar to PCa presence, high-grade cancer can be found in men with low total PSA levels.

On the other hand, as of now, there is no evidence that lowering the total PSA threshold below 4 ng/mL improves the long-term survival in men with PCa Lowering the total PSA threshold combined with decreasing the age of total PSA screening may be beneficial for men who are at an increased risk for PCa (i.e., strong family history of PCa and/or African-American race). However, consideration must be given to the possibility that lowering the total PSA threshold could result in unnecessary biopsies and an increased detection of insignificant 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 PCa detection.

The difficulty in selecting a cut-off to define what constitutes an abnormal total PSA suggests that total PSA is most useful as a continuous variable, providing a spectrum of prostate cancer risk. Therefore, we prefer to include serum total PSA 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.[39-41, 47-51] Nam et al., for example, developed a model that

predicts an individual's risk for PCa in a cohort of 3,108 men who underwent a prostate biopsy for the first time.[51] This model comprises factors that can be easily determined at the time of screening such as age, ethnicity, family history of PCa, the presence of urinary symptoms, total PSA, percent free PSA, and DRE. Addition of all 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 tools[47] is that clinicians can assess PCa 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 patient and his treating physician and should be individualized.

LONG-TERM PREDICTION OF THE FUTURE RISK OF PROSTATE CANCER USING TOTAL PSA

Several studies have suggested that total PSA levels are associated with the risk of PCa years, or even decades, before its diagnosis. The first long-term prediction study, which reported that total PSA levels >2.5 ng/mL predicted diagnosis of PCa over the subsequent decade was limited by the small number of cancer cases (n=44) and by the degradation of total PSA in archived serum samples.[52] In a prospective study involving a large number of cases, the lead time between total PSA levels ≥4 ng/mL and the subsequent clinical diagnosis of PCa was estimated at 5.5 years.[53] Similarly, Fang *et al.* studied the risk of PCa diagnosis in a cohort of 549 men following a baseline total PSA measurement at age 40–60 while providing a median follow-up of ~13 years.[54] They concluded a total PSA 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 total PSA ranges and longer follow-up intervals. Loeb *et al.* examined 1,178 men in their 40s who had risk factors for PCa.[55] The risk of subsequent PCa diagnosis was 14.6-fold higher for men with a baseline total PSA level between 0.7 and 2.5 ng/mL compared to men with total PSA <0.7 ng/mL. Lilja *et al.* assessed PCa risk among 21,277 men younger than 50 years when they attended the Malmö Preventive Medicine study (MPM), a cardiovascular risk assessment study.[56] The investigators measured total PSA levels in archived plasma obtained from 462 participants diagnosed with PCa within a median of 18 years from start of the study and from 1,222 matched controls. Total PSA level at age 44–50 was very strongly associated with the likelihood of developing PCa up to 25 years later. The odds ratio for a PCa diagnosis at a total PSA value of 0.51–1.0 ng/mL was 2.51 compared to total PSA ≤0.50 ng/mL, which roughly corresponded to the population average. The odds ratio increased to 7.02 for a total PSA of 1.0-1.5 ng/mL, and further up to 19.01 for a total PSA of 2.01-3.0 ng/ml compared to a total PSA ≤0.50 ng/mL. In a follow-up study, the authors have further shown that total PSA level at age 44–50 predicts the likelihood of developing advanced PCa, defined as either locally advanced (clinical T3 or higher) or metastatic disease at the time of diagnosis.[57] In another analysis of the MPM-study cohort, the prognostic accuracy of PSA (both total PSA and complexed PSA, described below) decreased with age.[58] The authors hypothesized that these findings result from a greater prevalence of BPH (and therefore of non-cancer-related total PSA increase) among older men.

An analysis of the same cohort demonstrated that PSA at 60 is an extremely strong predictor of the risk of prostate cancer metastasis (AUC 0.86) and death (AUC 0.90) by age 85. Almost all deaths (90%) occurred in men in the top quartile of PSA levels (> 2 ng / ml); men with PSA below the median (<1 ng / ml), had an extremely low risk of clinically relevant prostate cancer by age of 85 (0.5% risk of metastasis, 0.2% risk of death from prostate cancer).This suggests that at least half of men can be exempted from prostate cancer

screening at age 60, with early detection efforts focusing on a sub-group of men at elevated risk.[59]

In summary, these studies indicate that men who will eventually develop PCa have increased total PSA levels years or decades before the cancer is diagnosed. These total PSA levels may reflect the long duration of prostate carcinogenesis or could reflect a causal role of total PSA in PCa development and/or progression. A total PSA measurement before age 50 could help risk-stratify men for frequency and/or type of later PCa screening; a PSA at 60 could determine which men need to continue with screening.

PROSTATE-SPECIFIC ANTIGEN DERIVATIVES

Enhancing the diagnostic accuracy of total PSA, particularly specificity, is critical, since higher specificity would reduce the number of biopsies performed in men not affected by PCa. Several different strategies have been investigated, including the use of age-specific total PSA cut-offs, total PSA density, total PSA density of the transition zone, total PSA velocity (total PSAV), and the measurement of various molecular forms of PSA.[47, 60-62] In this section, we will focus on total PSAV and the measurement of various molecular forms of PSA.

Total PSA Velocity

Total PSAV refers to the serial evaluation of serum total PSA concentration over time.[63, 64] Different methods of calculating total PSAV are available (eg, 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 total PSA measurements in a linear regression analysis should be the method of choice for calculating total PSAV.[65] 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 total PSA levels over time, whereas patients with PCa had an initial linear increase with a subsequent exponential rise that occurred approximately 5 years before cancer detection.[63] In men with an initial total PSA level between 4 and 10 ng/mL, a total PSAV cut-off value of 0.75 ng/mL per year provided a sensitivity and specificity for PCa of 79% and >90%, respectively. If the initial total PSA concentration was less than 4 ng/mL, the specificity of total PSAV remained >90%, but the sensitivity dropped to 11%. These results were questioned using relatively short total PSA intervals of 1 and 2 years.[66] Subsequently, Carter et al. showed that total PSAV values are only useful if a minimum of three consecutive measurements are taken over a two year period.[67] While the specificity of total PSAV is high, its sensitivity is too low to advise against prostate biopsy in a patient with an elevated total PSA level who is otherwise healthy and a good candidate for curative therapy. Other limitations of total PSAV include imprecision due to biological and analytical intra-individual variability (see section entitled “Total PSA and its limitations”) and total PSA stability.

Prospective screening studies have reported that total PSAV does not appear to add diagnostic value for PCa detection beyond that of a single total PSA level. In an analysis of PCPT data, Thompson et al. found that when total PSAV was used alone, it was an independent predictor of PCa presence and aggressiveness.[40] However, when total PSAV was adjusted for the effect of total PSA and other standard variables, it lost independent predictive value. Similarly, the first two screening rounds of the Rotterdam section of the ERSPC found that total PSAV did not improve accuracy when combined with total PSA in

the prospective setting.[68, 69] A recent analysis from the Prostate, Lung, Colon, and Ovarian (PLCO) cancer screening trial showed that although total PSAV was an independent predictor of high-grade disease, addition of total PSAV to total PSA only slightly increased its performance for prediction of high-grade tumors.[70] 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 calculate total PSAV or the velocity of any other PSA form over total PSA for long-term PCa prediction.[71] Of note, the predictive value of total PSAV alone was 71.2%, while the predictive value of a single total PSA was higher (77.1%) and the combined model including both total PSAV and total PSA did not alter the predictive accuracy. The observed lack of additional predictive value for total PSAV indicates that total PSA levels do not increase sharply before PCa diagnosis but rise gradually and slowly over many years, also in those men who later present with advanced cancer.

Several studies have shown that that a high pre-treatment total PSAV is strongly associated with a poor disease-specific survival following diagnosis and could help identify men with low total PSA values who are at increased risk of harboring a potentially lethal tumor. [72-75] Carter et al. found a strong association between survival and higher total PSAV as early as 10-15 years before diagnosis in the Baltimore Longitudinal Study of Aging project. [75] Based on these findings, they proposed that a total PSAV threshold of 0.35 ng/mL per year be used in screening men with low total PSA 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 total PSAV is greater than 0.5 ng/mL per year.[76] However, in analysis of the PCPT data, Vickers and colleagues showed that biopsying men with low PSA but elevated PSAV, led to a large increase in unnecessary biopsies without detecting an important number of clinically significant cancers.[77]

D'Amico et al. reported that men with a pre-operative total PSAV greater than 2.0 ng/mL per year had a 9.8-fold increased relative risk of death from PCa than men with a lower total PSAV.[72] This analysis is compromised by a low number of events; accordingly, it is impossible to tell whether PSAV adds predictive value to standard predictors such as stage, grade and absolute level of PSA. In a more recent study, these investigators reported that total PSAV was also significantly associated with the risk of cancer-specific mortality following external beam radiation therapy.[73] Conversely, using data from 267 Scandinavian men with localized PCa and baseline total PSA levels <50 ng/mL, Fall and colleagues found that, although prognostically relevant, baseline total PSA levels and relative total PSAV in the first 2 years following diagnosis were not able to predict accurately which patients would have a lethal PCa outcome.[78] Several other studies have found that PSAV does not aid prediction in men treated by radical prostatectomy [79, 80]or conservatively[81].

It may be that the observation period necessary for obtaining a valid calculation of total PSAV that is not disturbed by considerable short-term fluctuations is be too long, or that number of total PSA measurements is too high for use in clinical practice. In addition, total PSAV 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 total PSA is more common in men with a high starting total PSA level.[82] This proportion of men is expected to be much smaller in a screened cohort than in a clinically diagnosed cohort. PSAV is a practical parameter after treatment, when PSA is a sensitive measure of cancer; its value in men with a prostate, in whom rises in PSA may be cause by benign disease, remains to be established.

PSA molecular forms

Improvements in measuring PSA isoforms have allowed the measurement of free PSA and its ratio to total PSA.[83-85] Other forms include complexed PSA, which is a measure of how much PSA in serum is bound to α2-macroglobulin, α1-protease inhibitor, or α1-antichymotrypsin. Currently there is no assay commercially available which specifically measures the complex of α2-macroglobulin with PSA.

The FDA has approved the use of percent free PSA testing [i.e., (fPSA/tPSA) × 100] as an adjunct to total PSA in men with a serum total PSA concentration between 4 and 10 ng/mL. A higher percent free PSA value indicates a lower probability of finding PCa on biopsy and raises the likelihood that the elevation in total PSA is due to the presence of BPH.[86, 87] In a multicenter, prospective trial, Catalona et al. reported that when a percent free PSA of <25% is used for triggering a sextant prostate biopsy, it yielded a 95% sensitivity for PCa detection and increased the specificity by 20% over PSA alone.[86] The area under the curve for percent free PSA was significantly higher than that for total PSA (AUC=0.72 versus AUC=0.53). However, in response to the realization that sextant biopsies misclassify up to one third of patients who have PCa as without cancer, a more recent evaluation of the utility of percent free PSA in patients undergoing extended 10- or 12-core biopsy has suggested a lower diagnostic efficiency of percent free PSA.[88] While most investigators agree that percent free PSA can improve the diagnostic performance of total PSA between 4 and 10 ng/mL, the most appropriate percent free PSA cutoff value remains debatable. Catalona et al. determined that with a percent free PSA cutoff of less or equal to 27%, they were able to obtain a sensitivity of 90% and avoid 18% of unnecessary biopsies in men over the age of 50 with a total PSA of 2.6 to 4.0 ng/mL.[45] In addition, 83% of these cancers were clinically significant. Finally, data on the utility of percent free PSA for the prediction of pathologic grade and stage of PCa is inconclusive. Catalona et al. determined that with a percent free PSA cutoff of less or equal to 27%, they were able to obtain a sensitivity of 90% and avoid 18% of unnecessary biopsies in men over the age of 50 with a total PSA of 2.6 to 4.0 ng/mL.[45] In addition, 83% of these cancers were clinically significant.

In response to the realization that sextant biopsies misclassify up to one third of patients who have CaP as being without cancer, a more recent evaluation of the usefulness of free PSA values in patients undergoing extended 10- or 12-core biopsy has suggested a lower diagnostic efficiency of free PSA.[88] While most investigators agree that free PSA can improve the diagnostic performance of total PSA between 4 and 10 ng/mL, the most appropriate free PSA cutoff value remains debated.

Data on the usefulness of free PSA to predict clinical outcomes is inconclusive. Graefen and coworkers[89] failed to detect an independent association of preoperative free PSA with biochemical failure in 581 unscreened patients who underwent radical prostatectomy for clinically localized CaP.[90] In contrast, Shariat and colleagues found that lower preoperative serum free PSA is an independent predictor of advanced pathologic features, biochemical progression, and patterns of aggressive disease progression in 402 consecutive men treated with radical prostatectomy for clinically localized CaP who had total PSA levels less than 10 ng/mL.[84]

There are three distinct cleavage isoforms of free PSA in the serum: pro-PSA, BPH-associated PSA (BPSA), and intact free PSA.[91] The precursor of PSA is a 261 amino acid pre-pro-protein. Subsequent processing by human glandular kallikrein 2 (hK2) and other proteases produces the active 237 amino acid mature PSA.[91] Studies have shown that higher levels of pro-PSA are associated with PCa. In men with PSA levels between 6.0-24.0 ng/ml, the [−2]proPSA fraction was found to be significantly higher in men with PCa. [91, 92] Moreover, authors demonstrated the utility of the pro-PSA to free PSA ratio for

screening patients with PSA levels between 2.5-4.0 ng/ml and between 4.0-10.0 ng/ml.[93] Elevated pro-PSA to free PSA ratios have also been associated with aggressive pathological features and decreased biochemical disease free survival after radical prostatectomy[94, 95]. A new automated tool using the [−2]proPSA assay with a percent free PSA based artificial neural network was capable of detecting PCa and more aggressive disease with higher accuracy than total PSA or percent free PSA alone.[96] In a recent prospective cohort of men enrolled into active surveillance for PCa, serum and tissue levels of pro-PSA at diagnosis were associated with need for subsequent treatment.[97] The authors hypothesized that the increase in the ratio of serum pro-PSA to percent free PSA might be driven by increased pro-PSA production from “premalignant” cells.

Molecular forms of PSA may differ in their in-vitro stability properties and information about the preanalytical conditions is therefore essential for proper clinical interpretation. For proper measurement of [−2]proPSA, blood samples should be centrifuged within 3 hours of blood draw. Serum may be stored at room temperature or refrigerated (+4°C) for a maximum of 48 hours and should be frozen if stored for a longer period. Two freeze-thaw cycles have no effect on [−2]proPSA stability.[98]

BPH-associated PSA (BPSA) is formed by the internal cleavage of free PSA between Lys182 and Ser183. BPSA is expressed in nodular hyperplasia limited to the transitional zone of men with BPH. BPSA can be detected in semen, blood and prostate, and its levels correlate with transitional zone volume and obstructive voiding symptoms.[91, 99] BPSA seems to be a promising marker of BPH since it has been shown a direct association between its secretion and the volume of the transition zone.[99] As such, BPSA is a better predictor of prostate enlargement than total and free PSA.[100] In addition, BPSA is not affected by age and is significantly higher in the presence of BPH symptoms. Adjusting the level of free PSA for BPSA resulted in 13-17% improvement in specificity compared to free PSA alone while maintaining a sensitivity of 90-95%.[101]

Combination of PSA molecular forms for improved cancer detection

It is most unlikely that a single biomarker will have the single decision as to a diagnosis and/or a prognosis of a particular pathology. The future of cancer profiling relies on the combination of a panel of complimentary biomarkers that can give accurate molecular staging and indicate the likelihood of aggressive behavior.[10, 11, 102, 103]

The group of Vickers and Lilja has developed a statistical model that predicts prostate biopsy outcomes based on age, DRE and a panel of four kallikrein markers – total PSA, free PSA, intact PSA and hK2. Using data from the randomized prostate cancer screening trial in Göteborg, Sweden [one centre of the European Randomized Study of Prostate Cancer screening (ERSPC)], they estimated that for every 1000 previously unscreened men with elevated total PSA, use of the model to determine biopsy would reduce biopsy rates by 573, while missing only a small number of cancers (31 out of 152 low-grade cancers and 3 out of 40 high-grade cancers).[15] These findings were subsequently replicated in an independent cohort (reduction in biopsy by 513 per 1000 men with elevated total PSA, missing 54 out of 177 low-grade cancer and 12 out of 100 high-grade cancers).[104] These findings have also been verified in men who recently have undergone previous screening, with resultant improvements in predictive accuracy.[105, 106] Recently, Gupta et al. demonstrated that the panel of four kallikrein markers can predict the outcome of prostate biopsy in men who had previously undergone prostate biopsy during previous screening.[107] This model in addition to age and DRE substantially improved the predictive accuracy of a base model (comprising of total PSA, age and DRE), for both low- and high-grade cancers.

EMERGING BLOOD-BASED BIOMARKERS FOR BOTH PROSTATE CANCER DETECTION AND PROGNOSTICATION

Human Kallikrein 2 (hK2)

Human kallikrein related peptidase 2 is a secreted serine protease from the same gene family as PSA.[108] They share an 80% sequence homology and are both primarily expressed in the prostate gland.[108] Despite these structural similarities, hK2 and total PSA differ in their enzymatic activity. The levels of hK2 in prostate tissue, plasma, semen, and serum are less than 2% that of total PSA, although hK2 mRNA transcript expression represents half that of total PSA. Similar to total PSA, serum hK2 is present in two forms in the blood: one bound to various protease inhibitors, and the other (preponderant) free in the circulation. Several studies have shown that, when used in conjunction with free and total PSA, serum hK2 could improve the discrimination of men with PCa from men without cancer.[109, 110] [111] It has also been suggested that hK2 could predict poor differentiation, extra-capsular extension and biochemical recurrence in patients treated with radical prostatectomy. [112-114] However, this finding has not been validated by other authors.[115] The usefulness of hK2 for the preoperative staging of localized PCa therefore remains controversial. As mentioned above (see “Combination of PSA molecular forms for improved cancer detection” section), the addition of hK2 to three other kallikreins (total, free, and intact PSA) improved the prediction of prostate biopsy results in men with elevated total PSA (increase of predictive accuracy from 68-72% to ~83%).[15] Considering the risk of PCa at 20%, the number of biopsies would have reduced by half, missing only 3 out of 40 high-grade tumors.[15]

EMERGING BLOOD-BASED BIOMARKERS FOR PROSTATE CANCER PROGNOSTICATION

Urokinase Plasminogen Activation (uPA)

The urokinase plasminogen activation (uPA) axis represents a potential target for PCa markers by being involved in various phases of tumor development and progression though degradation of the extra-cellular matrix. The serum protease uPA may play a role in cancer progression by binding to the uPA receptor (uPAR) and consequently converting plasminogen to plasmin, which activates proteases related to the degradation of extracellular matrix proteins.[116] Immunohistochemical staining of radical prostatectomy specimens revealed that overexpression of both uPA and its inhibitor (PAI-1) were associated with aggressive PCa recurrence.[117] In patients with a total PSA level above 2 ng/mL, soluble uPAR and free PSA measured in serum before prostate biopsy improved the regression model accuracy for prediction of PCa.[118] Steuber et al recently showed that uPAR fragments were significant predictors of PCa on biopsy specimens of patients with an elevated PSA.[118]

Both uPA and uPAR might also have a prognostic value. Elevated circulating levels of uPA and uPAR have been linked to PCa stage and bone metastases.[117, 119-122] In a study of 429 patients treated with radical prostatectomy, preoperative plasma uPA was a strong predictor of biochemical recurrence after surgery. Both preoperative uPA and uPAR were associated with features of aggressive biochemical recurrence such as development of distant metastasis suggesting an association with occult metastatic disease at time of local therapy. Moreover, elevation of plasma uPA and uPAR levels in PCa patients seemed to be partly caused by local release from the prostate. Larger multi-institutional studies are under way to validate the potential role of uPA and uPAR as markers of metastatic PCa.

Transforming Growth Factor-Beta 1 (TGF-β1) and Interleukin-6 (IL-6)

TGF-β1 is a growth factor involved in the regulation of several cellular mechanisms including proliferation, immune response, differentiation and angiogenesis.[123] TGF-β1 has been shown to promote cell progression in PCa models and its local expression has been associated with higher tumor grade, tumor invasion and metastasis in PCa patients.[124, 125] Several studies have shown that increased levels of circulating TGF-β1 were associated with cancer progression, occult and documented metastasis and biochemical progression in PCa patients.[125-127]

IL-6 is a cytokine with variable effects on immune and hematopoietic mechanisms. In vitro and in vivo studies have shown that both IL-6 and its receptors (IL-6R) were expressed in PCa.[128, 129] Several authors reported that elevated serum levels of IL-6 and IL-6R were associated with metastatic and hormone refractory disease, and suggested that IL-6 could predict progression and survival of PCa patients.[130, 131]

Based on these findings, Kattan and associates developed and internally validated a prognostic model that incorporates plasma TGF-β1 and IL-6R into a standard nomogram for prediction of biochemical recurrence following radical prostatectomy.[14] This combination of serum markers and classical clinical parameters improved the predictive accuracy by a statistically and prognostically substantial margin (increase in predictive accuracy from 75 to 84%). However, before a biomarker can become useful in daily clinical management, it needs to be externally validated in an independent cohort of patients (figure 1).[2, 3] Therefore, in a multi-institutional dataset of 423 patients treated with radical prostatectomy, Shariat et al. confirmed that plasma levels of TGF-beta1 and IL6-SR considerably enhance the accuracy of the standard preoperative nomogram for the prediction of biochemical recurrence (accuracy of clinical features plus biomarkers 87.9% versus 71.1% for clinical features alone; $p<0.001$). Such prognostic models refine our ability to identify patients at a high risk of biochemical recurrence after radical prostatectomy who may benefit from inclusion into perioperative clinical trials and/other intensified follow-up protocols.

Endoglin

Endoglin, or CD 105, is a transmembrane glycoprotein that is typically expressed by human vascular endothelial cells. Functionally, it is a cell-surface coreceptor for transforming growth factor β1 (TGF-β1) and β3 (TGF-β3) [132] that modulates cellular responses to TGF-β in the early steps of endothelial cell proliferation. Its critical role in angiogenesis has prompted investigators to evaluate the role of Endoglin in cancer progression and metastasis. In PCa, Endoglin is preferentially found on new, immature blood vessels and immunohistochemical analysis supports an association between Endoglin expression and disease progression [133]. Urine levels of Endoglin may distinguish patients with PCa and may help in the staging of the disease.[134] In addition, pre-operative plasma Endoglin were found to be associated with metastasis to regional lymph nodes,[135] established features of biologically aggressive PCa such as higher pathologic Gleason sum, and biochemical recurrence following radical prostatectomy.[136] Use of pre-operative plasma Endoglin could help decide whether and how extensively to perform a lymphadenectomy as well as preoperative identification of patients at risk for disease progression. This would help select patients for neo-adjuvant and/or adjuvant therapy or enrollment into clinical trials. Moreover, Endoglin may be valuable as a surrogate biomarker for occult metastatic disease in patients with presumed organ-confined disease. Further investigation is needed to validate Endoglin as a useful biomarker in men with PCa and to elucidate the mechanistic role of this biomarker in the progression of PCa.

Combination of blood-based biomarkers for prostate cancer prognostication

A biomarker may reflect disruption of a biochemical pathway by a particular mechanism. Given the complexity of the molecular abnormalities associated with PCa, it is improbable that a single marker can accurately segregate tumors of similar clinico-pathologic phenotypes into distinct prognostic categories. Therefore, combinations of independent, yet complementary markers, may provide a more accurate prediction of outcome compared to a single marker.[62]

Shariat et al. found that the addition of a panel comprising pre-operative plasma levels of TGF-β1, soluble IL-6R, IL-6, Endoglin, vascular endothelial growth factor (VEGF), and vascular cell adhesion molecule-1 (VCAM-1 or CD 106)[121, 123, 125, 135-140] improved the predictive accuracy the Kattan pre-operative nomogram[9] by 15.0% (i.e., 71.6 to 86.6%).[11, 103] This increase substantially exceeds accuracy gains obtained from the consideration of detailed pathologic descriptors of prostate cancer at radical prostatectomy. Svatek et al. confirmed the strong predictive value of *pre*-operative levels of the candidate biomarkers after adjusting for the effect of postoperative features.[102] Addition of *pre*-operative levels of the candidate biomarkers improved the accuracy of the base model (i.e., total PSA, surgical margin status, extracapsular extension, seminal vesicles invasion, lymph node involvement, and pathologic Gleason sum) for prediction of biochemical recurrence by a statistically and prognostically significant margin (79% to 86%, $p<0.001$). Predictive tools integrating biomarker levels could constitute the new standard for counseling patients regarding their risk of recurrence following curative therapy and for designing clinical trials to test neo-adjuvant and/or adjuvant treatment strategies in high-risk patients. However, while prediction of biochemical recurrence is important, prediction of response to therapy as well as metastasis and survival is more important for the management of PCa patients.[141]

BLOOD-BASED BIOMARKERS FOR MONITORING OF PROSTATE CANCER TREATMENT

TOTAL PSA

Treatment monitoring is the most accepted clinical application for total PSA. Total PSA is used for monitoring response to local treatments such as radical prostatectomy, various methods of radiation therapy, and other local therapies including cryosurgery, as well as systemic treatments such as androgen deprivation therapy and chemotherapy. Post-treatment total PSA levels can provide invaluable information about the effectiveness of the therapy given and the existence of residual cancer in men treated with local therapy with curative intent. In such patients, rising total PSA levels can signal cancer activity well before any clinical signs of recurrence appear. This lead-time can be further increased by months and even years.

In patients treated with systemic therapy, post-therapy total PSA changes are a seemingly perfect outcome measure because they are easily assessable, quantitative, reproducible, and inexpensive; this is true regardless of whether this outcome measure is applied to evaluate drugs in the clinical trial setting or in clinical practice. Critical to the successful application of total PSA measurement as an endpoint are the therapeutic objectives of the trial and the mechanisms of action of the treatment administered. Criteria were proposed to screen for treatment effects in prostate cancer clinical trials on the basis of the hypothesis that total PSA declines reflect significant cell kill in response to agents that cause reduction in overall tumor burden. With the recognition that short-term declines in total PSA levels might simply reflect the effect of the drug on the marker, and not on cell growth or survival, it is recommended that the declines be documented on more than one occasion and, equally importantly, over time. Indeed, different total PSA-based outcomes would be required for

different classes of drug. For example, drugs that are not anticipated to kill cells would not be expected to produce declines in total PSA. Similarly, 'differentiating effect' might produce an initial rise in total PSA that might be an indication that the drug is actually working. A single set of outcomes would not only be inapplicable to agents that act via diverse mechanisms, but could also be misleading.

Unfortunately, the significance of post-therapy changes in total PSA have been misinterpreted as indicators of tumor response, and/or as a measure of clinical benefit. An additional misconception is that the demonstration of a particular degree of decline in total PSA, in a proportion of patients, is an indication that the treatment prolongs life, and as such, should be used as an endpoint for accelerated drug approval.

The use of a post-therapy decline in total PSA as an outcome measure has been justified in part by statistical analyses exploring the relationship between the defined outcome measure and survival.[142] Most of these reports include multivariate techniques, and in some, the resultant models were validated on independent data sets. These early reports did not assess randomized comparative trials showing a survival benefit, a necessary but not sufficient condition in which to explore measures of surrogacy.[142, 143]

There is no clear demonstration that a post-therapy total PSA change can account for all of the treatment effects seen is not yet available. Large-scale prospective studies incorporating different post-therapy total PSA change definitions, as well as other potential biomarkers, are ongoing. At this point, one can conclude that a cytotoxic drug that does not produce any total PSA declines is unlikely to be effective, but that the corollary is not always true. In addition, it is important to recognize that there is a range of clinical benefits to patients that can improve the quality, and more than likely, the duration of survival, which are independent of total PSA levels. This includes differentiating agents that might produce a rise in total PSA before a decline is observed, or act through immunization strategies where the effect on total PSA levels might be delayed or not occur at all, and angiogenesis and growth factor inhibitors. It is also important to note that there remains significant variation in the methodologies and assays used by different laboratories, which makes it difficult to compare results between groups. All of these factors must be considered carefully to ensure that a drug is not discontinued prematurely on the basis of a total PSA rise that is not relevant to the drug under study.

CONCLUSIONS

The introduction of total PSA in clinical practice has resulted in early detection and reduced mortality from PCa.[144] However, PCa screening remains controversial, because of the risk of overdiagnosis reduced mortality and overtreatment and the inability to detect a significant proportion of dangerous tumors. A large concerted effort has been made to improve and/or monitoring the activity of PCa and to guide molecular targeted therapy and/or assess therapeutic response. An integrated approach with blood-based measurement of different molecular forms of PSA in combination with genetic and urine biomarkers hold the promise of improving screening for and diagnosis of PCa. Panels of blood-based biomarkers will allow a fingerprinting of the tumors biologic behavior resulting in individualized therapy and monitoring. In addition, the emergence of new therapeutic approaches for PCa cannot flourish without a set of markers to serve as prognosticators, predictors, therapeutic targets, and/or surrogate end points.

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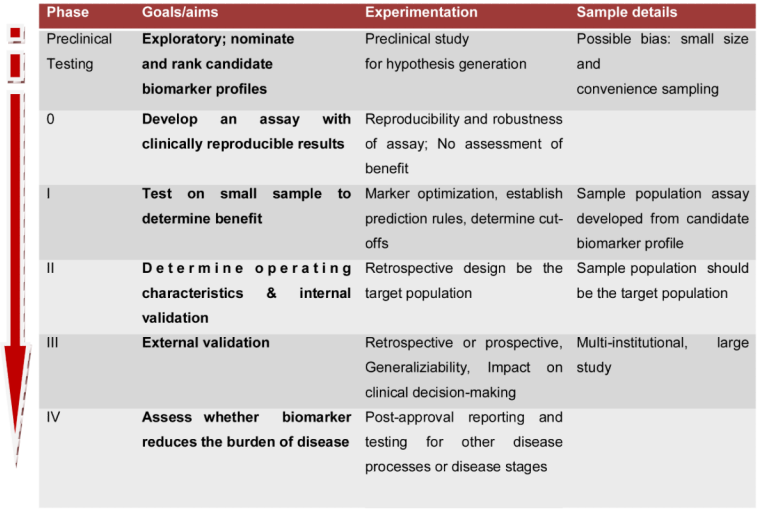

Phase	Goals/aims	Experimentation	Sample details
Preclinical Testing	**Exploratory; nominate and rank candidate biomarker profiles**	Preclinical study for hypothesis generation	Possible bias: small size and convenience sampling
0	**Develop an assay with clinically reproducible results**	Reproducibility and robustness of assay; No assessment of benefit	
I	**Test on small sample to determine benefit**	Marker optimization, establish prediction rules, determine cut-offs	Sample population assay developed from candidate biomarker profile
II	**Determine operating characteristics & internal validation**	Retrospective design be the target population	Sample population should be the target population
III	**External validation**	Retrospective or prospective, Generaliziability, Impact on clinical decision-making	Multi-institutional, large study
IV	**Assess whether biomarker reduces the burden of disease**	Post-approval reporting and testing for other disease processes or disease stages	

Figure 1.
Modification of the structured phase-approach to the systematic discovery, evaluation, and validation of biomarkers[2, 3]