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PSA and beyond: Biomarkers in prostate cancer

A literature review suggests multiplexed models combining several biomarkers could help reduce the number of negative prostate biopsies and improve detection of clinically significant prostate cancers.

ABSTRACT: Prostate-specific antigen is one of the best-known biomarkers in medicine. Despite ongoing debate on the utility of this proteolytic enzyme in population screening, PSA continues to play a role in the diagnosis and management of prostate cancer. In an attempt to move beyond problems related to insufficient specificity, investigators are considering ways to enhance the positive predictive value of PSA and its isoforms, and are looking at tests based on several promising biomarkers, including human kallikrein-related peptidase 2 and prostate cancer antigen 3. Assays that have recently become available show promise in reducing the number of unnecessary prostate biopsies and improving detection of clinically significant prostate cancers while avoiding the overdiagnosis and overtreatment of biologically irrelevant tumors.

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Prostate-specific antigen (PSA) is a highly sensitive serum biomarker that has changed the management of prostate cancer over the past 20 years by allowing clinicians to detect prostate cancer earlier. However, PSA testing is not perfect, as indicated by the US Preventive Services Task Force's recent recommendation against using it for routine prostate cancer screening. This recommendation highlights possible limitations of PSA testing and the need for better biomarkers to facilitate diagnosis, prognosis, and stratification of treatment.

Investigators are continuing to explore the utility of PSA and its isoforms, as well as a few emerging biomarkers, and expect that multiplexed models based on more cancer-specific biomarkers will eventually pave the way for better prostate cancer diagnosis and care.

Biomarkers

Since the completion of the Human Genome Project in 2001, and with improving technological advances such as mass-spectrometry-based profiling and advanced bioinformatics, there has been an explosion in research and discovery in cancer biomarkers. Biomarkers can be divided into three categories:

- Diagnostic biomarkers used to detect cancer in an individual.
- Prognostic biomarkers used after disease status has been established to predict the probable clinical course, including recurrence.
- Stratification biomarkers used

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before treatment is selected to identify patients as likely responders or nonresponders.[1]

Prostate cancer biomarkers can be sourced from serum, urine, prostatic fluid, or from prostate tissue. They can be used to identify DNA, mRNA, protein, metabolites, or processes such as apoptosis, angiogenesis, or proliferation.[2] An ideal biomarker is measurable with a simple, inexpensive, minimally invasive test, and has high sensitivity (few false-negatives) and high specificity (few false-positives).

The development of biomarkers typically passes through five phases:[3]

1. Preclinical exploratory studies.
2. Clinical assay and validation.
3. Retrospective longitudinal studies.
4. Prospective screening studies.
5. Randomized control trials.

To date, PSA is the only biomarker that has been through all five phases of testing. Because prostate cancer is a very heterogeneous disease, finding newer biomarkers that can improve our ability to diagnose, prognosticate, or predict patient outcomes remains highly challenging.

Total PSA

One of the best-known biomarkers in cancer biology, prostate-specific antigen is a proteolytic enzyme belonging to the kallikrein family of serine proteases. Produced primarily by the human prostatic epithelium, PSA is normally secreted in high concentrations into seminal fluid and functions in the liquefaction of the seminal coagulum. It is organ-specific but not disease-specific, and can be detected in patients with normal prostate and benign prostatic hyperplasia (BPH), and in both primary and metastatic prostate cancer cells.

Total PSA production is determined by the number of PSA-producing cells, the level of PSA gene expression, the rate of PSA protein secretion per cell, and the degree of PSA leakage from glandular acini into the serum. Serum PSA levels may be elevated from inspissated secretions, distorted architecture, or disruption of basement membrane integrity occurring with prostatic infarction, prostatitis, ejaculation, digital rectal manipulation (DRE), or prostatic instrumentation. DRE has been shown to raise total PSA levels by 0.26 to 0.4 ng/mL, primarily as a result of elevated free PSA; complexed PSA appears to be more stable, with only modest elevations after DRE.[4] Ornstein and colleagues showed that at least 24 hours are needed for PSA to return to baseline levels after DRE.[5] Cystoscopy can increase serum PSA levels fourfold, while needle biopsies and transurethral resection of the prostate (TURP) can temporarily increase PSA levels up to fiftyfold, all as a result of increased PSA leakage into the serum.[6] The relatively long half-life of PSA (2.2 ± 0.8 days), coupled with a slow resolution of inflammation, may lead to a delay of several months before serum PSA returns to a baseline level after TURP, biopsy, or infection. Ejaculation has been reported to cause an increase of more than 15% in serum PSA within 1 hour, and could thus lead to a false-positive test result.[7] After 48 hours, the PSA level would be expected to return to baseline levels in most men.

PSA testing was approved in 1986 by the FDA and since then has been used commonly for the diagnosis and management of prostate cancer. In 1991, Catalona and colleagues concluded that PSA testing was useful in addition to rectal examination and transrectal ultrasound (TRUS) when screening for prostate cancer.[8] These methods each have the ability to detect prostate cancer, but PSA has the greatest predictive value.[8]

Most clinicians who deal with prostate cancer on a regular basis agree that PSA testing needs to be used in a smart manner, and that the main concerns involve overtreatment rather than overdiagnosis.

When used together, PSA testing and DRE detect 27% more cancers than would be detected by PSA testing alone, and 34% more than by DRE alone.[8,9] Used alone as a screening tool, transrectal ultrasound will miss up to 40% of prostate cancers.[10] Although TRUS does not reliably identify tumors, it does offer an exceptional way to perform systematic biopsy sampling of the entire gland.

Role in screening

Major cancer organizations around the world express various opinions on the role of PSA in prostate cancer

Table 1. Relationship of PSA to prevalence of prostate cancer and high-grade prostate cancer (Gleason score ≥ 7) in a study of 2950 men with PSA levels of 4.0 ng/mL or less.[24]

PSA level (ng/mL)	Number of men	Men with prostate cancer n (%)	Men with Gleason score ≥ 7 prostate cancer n (%)
0.0–0.5	486	32 (6.6)	4 (0.8)
0.6–1.0	791	80 (10.1)	8 (1.0)
1.1–2.0	998	170 (17.0)	20 (2.0)
2.1–3.0	482	115 (23.9)	22 (4.6)
3.1–4.0	193	52 (26.9)	13 (6.7)

Table 2. PSA level by age.

Age (years)	Total PSA, normal reference range (ng/mL)
Younger than 50	0.0–2.5
50–59	0.0–3.5
60–69	0.0–4.5
70 and older	0.0–6.5

screening.[11-14] While there is no universal agreement on the relationship between early detection and survival, most clinicians who deal with prostate cancer on a regular basis agree that PSA testing needs to be used in a smart manner, and that the main concerns involve overtreatment rather than overdiagnosis.

All experts recommend shared decision making that includes discussion of the pros and cons of treatment with patients, and then individualized screening practices. Validated prostate risk calculators developed from population-based cohort data are available online (e.g., prostaterisk.ca), and can provide individualized risk assessment of prostate cancer prior to a prostate biopsy. A total lack of screening may result in men with highly aggressive tumors missing their window of opportunity for a cure.

Role in predicting outcomes

PSA levels can be used to determine the long-term risk of a particular prostate cancer, especially high-grade, lethal disease.[15-17] An initial PSA level above the age-adjusted median of 0.7 to 0.9 ng/mL in men younger than 60 predicts an increased risk of prostate cancer during the man's remaining lifetime.[15] It can also predict more aggressive tumor features and a greater risk of biochemical progression after treatment.[17] Other investigators have found that a PSA level less than 1.0 ng/mL before age 60 predicts a low lifetime risk of metastasis and death from prostate cancer.[18] Men in this category may harbor prostate cancer, but the disease is unlikely to become life-threatening. These men could be screened on a biennial or triennial basis, allowing for more cost-effective population screening.

PSA levels can be used before radical prostatectomy to predict outcomes, including tumor volume, grade of disease, and biochemical progression.[19,20] The ability of PSA to indicate biomedical progression also makes it useful as a surveillance marker for recurrent disease before a tumor is detectable by DRE or bone scan. Postoperatively, PSA doubling time has been shown to stratify the risk of clinical progression to metastases and death in those with PSA recurrence.[21]

Role in monitoring

PSA also plays a role in the monitoring of patients on hormone therapy for advanced/metastatic prostate cancer or those with PSA recurrence following definitive therapy. A good example of the utility of PSA as a disease biomarker is in men on hormone-withdrawal therapy, which today is given either continuously or intermittently. Recent large trials have shown that intermittent androgen deprivation is noninferior oncologically when compared with continuous therapy, and has the potential advantage of improved tolerability and quality of life.[22] During a treatment cycle of intermittent hormone therapy, PSA levels usually fall toward a nadir level. The PSA nadir in these circumstances is believed to be an important prognostic parameter, associated with time-to-androgen-independent progression, clinical progression, and death.[23] During the off-treatment phase, PSA measurements can guide the timing of future cycles.

Enhancing the positive predictive value of PSA testing

Urologists recognized early on that the substantial overlap in serum PSA levels in men with early prostate cancer and those with BPH resulted in a lack of sufficient specificity for PSA to be considered ideal for use in a screening test. Consequently, research efforts have focused on developing methods that improve the ability of PSA to predict for the presence of clinically important early prostate cancers, while minimizing the number of false-positive results.

PSA reference ranges and density

Generally, the higher the PSA level the greater the chance of prostate cancer. However, low PSA levels do not necessarily exclude a diagnosis of cancer. **Table 1** shows the rates of prostate cancer in those with a PSA level lower than 4.0 ng/mL, a level once considered "normal."[24] Rather than the previously held view that a particular level can be used to categorize the risk of prostate cancer as an "all or nothing" phenomenon, the patient's PSA level is now seen to reflect a continuum of prostate cancer risk.

PSA level is also influenced by age, race, and prostate volume. An age-specific range is now commonly used, as outlined in **Table 2**. PSA increases with age and is higher in people of certain races, such as African Americans.

Determining PSA density, which is equal to serum PSA divided by prostate gland volume, has been proposed as a way to improve PSA specificity. On a gram-for-gram basis, cancer will increase serum PSA levels more than BPH or normal prostate tissue will do. Early studies suggested that PSA density could help differentiate between BPH and early nonpalpable cancer, especially at serum levels of 4.0 to 10.0 ng/mL.[25] However, PSA density has since been found to provide no more information than PSA level alone in many men. This is due to difficulty in obtaining accurate and reproducible TRUS volume determinations of the prostate gland, the heterogeneous stromal and epithelial composition between prostate glands that lead to marked variation in the amount of PSA produced per gram of prostate tissue, and biopsy sampling error (larger prostates may hide small cancers). It may have a prognostic role in men on active surveillance.

PSA velocity and doubling time

Serial PSA measurements, termed PSA velocity (ng/mL/year) and PSA doubling time (the relative time for the PSA level to double), can reflect biological changes within the prostate over time. A PSA velocity greater than 0.75 ng/mL/year has been shown to increase both the positive predictive value of PSA testing and the likelihood of diagnosing cancers while they are organ-confined.[26]

Unfortunately, despite optimism about past longitudinal study results, PSA velocity and doubling time are not totally reliable when differentiating between benign and malignant disease because of variations in PSA measurement intervals, variations in PSA lab values, and variations in PSA from BPH. These measurements do, however, have an important role to play in managing men with known prostate cancer.

Free/total PSA ratio

While PSA is organ-specific, it is not cancer-specific. This limited specificity results in a large number of negative prostate biopsies, and their associated complications. Approximately 25% of patients will have a positive biopsy when their PSA level is in the range of 2.0 to 10.0 ng/mL.[27] Various fractions of prostate-specific antigen, such as free or unbound PSA and complexed PSA, have been reviewed to increase testing accuracy. When enzymatically active PSA leaks or is secreted into the serum (as occurs in patients with Gleason score 4 or 5 cancers) its proteolytic activity is immediately neutralized by binding to serine antiproteases such as alpha1-protease inhibitor, alphal-antichymotrypsin, or alpha2-macroglobulin. Thus, the ratio of free PSA to total PSA is lower in men with prostate cancer than in those without,[28] and when the PSA level is between 4.0 and 10.0 ng/mL with a negative digital rectal examination, the ratio can help determine the need for a prostate biopsy (**Figure**).

The risk of cancer is 55% to 56% when the free/total PSA ratio is 0% to 10%, and only 8% when the ratio is greater than 25%. Although there is a higher risk of prostate cancer with increasing age, BPH is also more common, meaning that the free/total PSA ratio may be inaccurate with larger prostates. Because more recent studies have not been as conclusive as

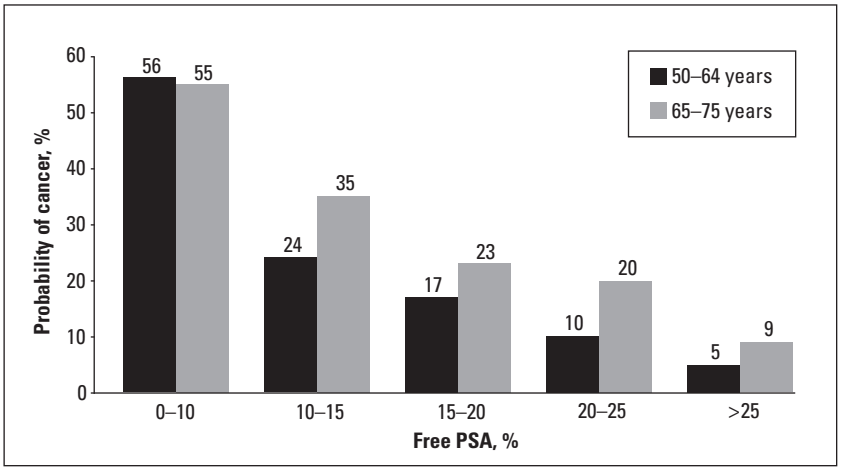

Figure. Probability of cancer by patient age and percentage of free PSA for patients with PSA levels between 4.0 and 10.0 ng/mL.[27]

earlier studies, free/total PSA ratio has not become standard for determining the need for a prostate biopsy. It can, however, help to decide on next steps if the ratio is very high or very low in men with smaller glands, negative biopsies, and high total PSA levels.

[-2] proPSA

There are a number of components of free PSA. One of these, proPSA, is highly expressed by all grades of prostate cancer. The most stable form of proPSA is [-2] proPSA, and in 2012 testing for this biomarker was approved by the FDA to aid in the initial biopsy decision for men with a PSA between 4.0 and 10.0 ng/mL and negative DRE. The biomarker has also been found to correlate significantly with higher Gleason scores and may help select patients for active surveillance.[29]

Prostate Health Index

The Prostate Health Index (PHI) is a mathematical regression model that relies on total PSA, free PSA, and [-2] proPSA values. The ratio of [-2] proPSA to free PSA is multiplied by the square root of the total PSA at diagnosis to obtain a PHI score. A meta-analysis of the evidence for the usefulness of PHI scores compared with PSA and free PSA measurements found the pooled sensitivity was 90%, while the pooled specificity was 31.6%.[30-32] While not yet in common use, PHI testing shows potential as a tool for detecting prostate cancer and has been approved in some jurisdictions.

Human kallikrein-related peptidase 2

Human kallikrein-related peptidase 2 (KLK2) belongs to the human kallikrein family, a group of 15 serine proteases that also includes prostate-specific antigen. KLK2 shares 80% of sequence homology with PSA, is expressed primarily by the prostate gland, and is androgen-regulated. Several studies have reported overexpression of human glandular kallikrein 2 in prostate cancer tissues.[33,34]

A prediction model for prostate cancer, using serum levels of four kallikrein markers (total PSA, free PSA, intact PSA, and KLK2) has been developed. In a study of 740 previously unscreened men, use of the kallikrein panel reduced the number of biopsies by 57%, and missed 31 of 152 low-grade cancers and 3 of 40 high-grade cancers.[35] The results were replicated in another cohort of 2914 previously unscreened men, reducing biopsies by 51% while missing 54 of 177 low-grade cancers and 12 of 100 high-grade cancers.[36] Similarly, the kallikrein panel has been validated in previously screened cohorts and found to reduce biopsy rates by 36% to 71%.[37,38]

The 4Kscore test (OPKO Health) combines these four kallikrein markers with patient age and digital rectal exam status to determine the likelihood of aggressive prostate cancer prior to a biopsy. A clinical validation study is currently underway in 21 urology centres in the United States. The first part of the study, a calibration confirmation phase, has successfully validated the algorithm, paving the way for a commercial launch in the US in 2014.

Prostate cancer antigen 3

Prostate cancer antigen 3 (PCA3) is a noncoding mRNA protein that is elevated in more than 95% of prostate cancer tissues, with a median sixty-six-fold upregulation compared with adjacent nonmalignant prostate tissues.[39,40] In the US, a commercially available assay, Progensa PCA3 (Gen-Probe), was approved in 2012 by the FDA as a diagnostic test to help clinicians determine the need for repeat prostate biopsies in the setting of a previous negative biopsy. In Canada, the PCA3 commercial assay is currently available only through a private laboratory in Laval, Quebec.

To perform the PCA3 test, first-void urine (20 to 30 mL) is collected immediately after a digital rectal exam, which causes shedding of prostate epithelial cells into the prostatic urethra.[41] The presence of PCA3 in the urine is evaluated using reverse transcriptase polymerase chain reaction (RT-PCR). A score is calculated as the ratio of PCA3 RNA copies to PSA RNA copies, multiplied by 1000. If the reported PCA3 score is below 25, the result is interpreted as negative. Unlike a PSA level, a PCA3 score is not influenced by prostate volume.[42]

The main feature of the PCA3 test is a direct correlation between the PCA3 score and the risk of finding prostate cancer on a subsequent biopsy.[43] Results from both the PCA3 and PSA test are continuous variables. While the commercial assay product has set the threshold level to trigger intervention at 25, there is clearly a trade-off in maintaining a balance between specificity and sensitivity. In a study of 466 men who had at least one previous negative biopsy and were scheduled for a repeat biopsy, a PCA3 score of 25 was associated with 77% sensitivity, 57% specificity, a negative predictive value of 90%, and a positive predictive value of 33%. Men with a PCA3 score of less than 25 were 4.56 times more likely to have a negative repeat biopsy than men with a score of greater than 25.[44] The PCA3 test serves to complement PSA testing, and can aid clinicians in making more informed decisions about a repeat biopsy.

Evidence on the role of PCA3 in the initial biopsy setting is also

becoming available. In a study of 3073 men undergoing their first biopsy, PCA3 testing outperformed PSA testing in predicting prostate cancer.[45] Research results regarding the correlation between PCA3 scores and Gleason scores upon subsequent prostatectomy have, however, been conflicting, so the role of PCA3 testing in prognostication and stratification remains undetermined.[45,46]

Gene fusion

The *TMPRSS2-ERG* gene fusion (T2E) is one of the most common genetic events underlying prostate cancer, occurring in 50% of all cases.[47] A urine test can be used to detect an mRNA transcript arising when the *TMPRSS2* gene fuses to the *ERG* oncogene.

Like the PCA3 test, the T2E assay begins with a prostate massage, then measures the amount of T2E RNA in a man's urine relative to PSA mRNA. The PSA mRNA level is used to determine the yield of prostate cells in the urine sediment. If the PSA level is too low, the test is uninformative.[48] As the *TMPRSS2-ERG* gene fusion occurs in only half of prostate cancers, the T2E test, when performed on a urine specimen, has a specificity of 86% and sensitivity of 45%.[49] This high specificity may be useful in reducing the number of unnecessary prostate biopsies.

A prospective study of T2E scores at the University of Michigan among men who underwent radical prostatectomy found that the score may be prognostic as well. There was a statistically significant association between T2E scores and characteristics common in clinically important disease, including tumor volume, Gleason score, and Epstein criteria.[50]

The eventual role of the T2E test will likely involve multiplexed panels to improve overall diagnostic accuracy, such as those combining T2E and PCA3 urine assays.[48,51] The MiProstate Score (MiPS) test, an assay commercially available to Canadians through MLabs at the University of Michigan, relies on blood PSA levels and urinary PCA3 and T2E test results to predict a patient's risk of having prostate cancer detected by standard assay. For example, an MiPS of 10% indicates that the patient has a 10% chance of having prostate cancer detected on biopsy. The model is able to improve cancer prediction and increase the overall specificity to 90% and sensitivity to 80%.[51] Such multiplexed panels provide an individualized assessment of prostate cancer risk beyond those of traditional population-based nomograms.

Current research efforts are directed at understanding the role of gene fusion in men on active surveillance[52] or in those found to have high-grade prostatic intraepithelial neoplasia on needle biopsies.[53]

Conclusions

Despite testing controversies, PSA remains one of the most useful biomarkers in cancer biology and is here to stay. In today's complex world of RCTs and global debate, serum PSA test results require experienced contextual interpretation and judgment when applied to individual men. The heterogeneity of prostate cancer means multiplexed models combining several biomarkers can be expected to improve the accuracy of prostate cancer diagnosis as we strive to reduce the number of unnecessary (and anxiety-provoking) prostate biopsies and improve detection of clinically significant prostate cancers while avoiding overdiagnosis and overtreatment of biologically irrelevant tumors.

Serum PSA test results require experienced contextual interpretation and judgment when applied to individual men.

We remain very optimistic that we can achieve these goals, especially with parallel advances taking place in the realm of multiparametric prostate MRI and image-guided fusion biopsies.

Competing interests

None declared.

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