# Prostate-Specific Antigen: Any Successor in Sight?

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Prostate cancer (PCa) is the most frequently diagnosed malignancy and the second leading cause of cancer death in men in the United States and other parts of the world. The lifetime risk of being diagnosed with PCa is approximately 16%. At present, the only widely accepted screening tools for PCa are prostate-specific antigen (PSA) and digital rectal examination. PSA is known to be prostate specific, but not PCa specific, and hence lacks the sensitivity to detect a large number of tumors, especially during the early stages. The PSA level is also known to be affected by many factors, such as medication, inflammation (benign prostatic hyperplasia and prostatitis), and urologic manipulation; hence, the controversy regarding the appropriate level of serum PSA that should trigger a biopsy or have clinical relevance to prostate metastases. Attempts to determine the level of prostate cells in peripheral blood by reverse transcriptase polymerase chain reaction did not significantly improve cancer diagnosis or predict postoperative failure. Therefore, the search continues for a novel biomarker or a panel of markers as well as other possible interventions to improve the use of PSA. This article reviews several possibilities.

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**KEY WORDS**

Prostate carcinoma • Prostate-specific antigen • Prostate diagnostic or screening test

Prostate cancer (PCa), an adenocarcinoma, is the most common cancer diagnosed in men today. The lifetime risk of being diagnosed with PCa is approximately 16%.[1] It affects one in nine men aged ≥ 65 years and is a leading cause of cancer-related death in men, second only to lung cancer.[2,3] The incidence of the disease presents a remarkable racial and national difference. The highest incidence of PCa is seen in North America and Scandinavia, especially among black men in the United States (137 per 100,000 per year).[4] Each year in the United States, approximately 220,000 new PCa cases are diagnosed, and 30,000 men die of the disease.[5] The lowest incidence is among Asian men (Japanese,

39/100,000; Chinese, 28/100,000) and men who are vegetarians.[6,7] Emerging data from Africa report an upsurge in the incidence of PCa, probably due to the increasing availability of screening facilities in recent years. PCa has been reported to be the most common cancer in Nigerian men and constitutes 11% of all cancers in men.[8,9] In South Africa, the incidence of invasive prostatic malignancy has risen in rural black Africans.[10] Also, a study from Yaounde, Cameroon, has indicated a high age-adjusted incidence rate for PCa.[11] PCa is increasingly common and becoming a global menace.

## Prostate-Specific Antigen

At present, the only widely accepted screening tools for PCa are prostate-specific antigen (PSA) and digital rectal examination (DRE). Since the PSA test was introduced into clinical practice in 1986, the early diagnosis and management of PCa has been revolutionized and much has been learned about the strengths and weaknesses of this assay. In fact, metastases and their comorbidities have decreased more than 75% since the early 1990s, resulting in a higher incidence of early organ-confined disease.[12] PSA testing not only helps with early diagnosis, but also assists in assessing the response to therapy, determining tumor progression, and, in its most controversial role, screening for PCa.

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Detection of PCa using a combination of PSA and DRE has been evaluated by a number of investigators. The positive predictive value of a PSA > 4.0 ng/mL is only 25% from a pooled meta-analysis of PSA studies.[13] Consequently, a value of > 4.0 ng/mL became the established level for recommending biopsy, although it was known that men could have cancer with PSA values < 4.0 ng/mL and a value > 4.0 ng/mL could be due to many other factors not related to prostate metastasis. Thompson and colleagues[14] showed that many cancers are missed with this cutoff, and that earlier medical intervention may lead to improved patient outcome. In men with PCa whose PSA level was < 4 ng/mL, normal DRE findings were present in 4% to 9%, whereas DRE findings were positive in 10% to 20%. When the PSA level was > 4 ng/mL, negative DRE results were found in 12% to 32% of patients, whereas positive DRE results were present in 42% to 72%. It was also discovered that PSA lacks the sensitivity to detect a large number of early-stage tumors, because > 15% of men with a normal serum PSA level have biopsy-proven PCa.[14] However, the level of PSA is known to correlate with the detection rate of PCa, especially in relation to age. Men aged > 50 years have a 20% to 30% possibility of having PCa if their PSA level is > 4.0 ng/mL. If the PSA level is 2.5 to 4.0 ng/mL, a biopsy is likely to detect cancer in 27% of men. For PSA levels > 10 ng/mL, the possibility of positive biopsy findings then increases to 42% to 64%.[15]

To achieve early diagnosis of PCa, the upper limit of normal PSA (4.0 ng/mL) has been recommended to be lowered. Catalona and colleagues observed that 20% to 30% of tumors will be missed if the only method of detection is serum PSA with a cutoff of 4.0 ng/mL.[16] A strategy for the early detection of PCa by Schröder and colleagues using a PSA cutoff of ≥ 3.0 ng/mL as the only indication for a biopsy, and excluding the DRE, was compared with one in which a PSA of ≥ 4.0 ng/mL, or the presence of a positive DRE, was the indication for a biopsy.[17] They identified 430 men with PCa out of the 8612 men who were screened who had a PSA level of ≥ 4.0 ng/mL or those with positive findings on DRE.

The standard PSA reference range of 0.0 to 4.0 ng/mL does not account for age-related volume changes in the prostate that are related to the development of benign prostatic hyperplasia (BPH). In a study of 411 black men with PCa, it was reported that 40% of these cancers would have been missed using the standard PSA values.[18] Oesterling and associates presented the concept that age-related reference ranges would improve cancer detection rates in younger men and would increase the specificity of PSA testing in older men.[19] Using reference ranges of 0 to 2.5 ng/mL for men aged 40 to 49 years, 0 to 3.5 for men aged 50 to 59 years, 0 to 4.5 for men aged 60 to 69 years, and 0 to 6.5 for men aged 70 to 79 years, they reported an overall specificity of 95%. These researchers used a different reference range for black men. With a PSA range of 0 to 2 ng/mL for men aged 40 to 49 years, specificity was 93%. A PSA range of 0 to 4 ng/mL produced a specificity of 88% for men aged 50 to 59 years, a PSA range of 0 to 4.5 ng/mL produced a specificity of 81% for men aged 60 to 69 years, and a PSA range of 0 to 5.5 ng/mL produced a specificity of 78% for men aged 70 to 79 years. Using these reference ranges, Partin and colleagues[20] detected 74 additional cancers in men aged ≤ 60 years in a study of 4600 men with clinically localized PCa. Pathology results were favorable in men undergoing radical prostatectomy (RP); 80% of these men had organ-confined

disease with a Gleason score of ≤ 7. Using the same ranges for men aged > 60 years, < 3% of the cancers missed were nonpalpable, of which 95% had favorable histology results. The potential detection of PCa increased 18% in younger men and decreased 22% in older men. Reissigl and Bartsch studied the effect of biopsy rates and PCa detection using age-specific ranges and a PSA cutoff of 4 ng/mL.[21] The data came from an Austrian screening study of more than 21,000 men aged 45 to 75 years. They reported an 8% increase in cancer diagnosis of organ-confined disease in men aged < 59 years. In men aged > 60 years who had normal DRE findings, 21% fewer biopsies were performed, and 4% of organ-confined cancers were missed.

Controversy exists regarding the advantage of age-specific PSA reference ranges compared with the standard PSA cutoff of 4.0 ng/mL. In an early detection study of 6600 men, Catalona and colleagues reported that the standard PSA cutoff was optimal for all age groups.[22] Lee and Littrup concluded that the standard reference range was the most effective and least costly means for screening. These investigators argued that a lower PSA cutoff in younger men could result in additional unnecessary biopsies and greater health care costs, whereas raising the cutoff level for older men could result in fewer cancers being detected.[23]

PSA can be used to identify metastasis, even at PSA levels of 4 to 10 ng/mL. Partin and colleagues found that 50% of patients treated with RP had extraprostatic extension. When the PSA level is > 10 ng/mL, the risk of extraprostatic cancer is increased greatly. In the same study, it was noted that 80% of men with PSA levels > 20.0 ng/mL had extraprostatic disease.[20]

PSA is prostate specific, but not PCa specific.[1] The serum PSA level can be altered by various medications, BPH, prostatitis, and urologic manipulations. It can also increase for 24 hours after ejaculation. Finasteride and dutasteride, 5-α-reductase inhibitors that are commonly prescribed for the treatment of BPH, can produce a 50% decrease in total prostate levels within 6 months of therapy.[24] There is controversy regarding the appropriate level of serum PSA that should trigger a biopsy. It has been known for many years that cancer will not be found on an initial biopsy in as many as 65% of men with PSA > 4.0 ng/mL; even in early studies it was shown that men can have cancer and have normal PSA levels.

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### *Free PSA*

PSA exists in serum predominantly as a complex with the protease inhibitor α-1-antichymotrypsin, whereas only approximately 10% to 30% is present as uncomplexed or free PSA (fPSA). fPSA is generally lower in PCa than in benign prostate enlargement. A high ratio of fPSA to total PSA (tPSA; eg, > 25%) greatly reduces the probability of cancer. On the other hand, a low percentage of fPSA (%fPSA; eg, < 10%) greatly increases the probability of cancer.[25,26] Minardi and colleagues observed that, although tPSA and fPSA values appeared to be correlated with patient age and prostatic volume, %fPSA did not show a relationship with these parameters. The specificity, sensitivity, and overall diagnostic accuracy were better assuming a 16% cutoff value for %fPSA than with other cutoff values.[27] A study investigated 113 men with PSA levels of 4 to 10 ng/mL and included 63 men with BPH, 30 men with PCa (prostate size > 40 $cm^3$), and 20 men with small prostates. The median f:tPSA ratio was 0.188 (in BPH), 0.159 (in PCa [prostate size > 40 $cm^3$]), and 0.092 (in small prostates).[28] This implies that prostate size is an important variable in selecting a cutoff value for fPSA. For men whose prostates are < 40 $cm^3$, a %fPSA of 0.137 or lower are used to detect 90% of the cancers eliminating 76% of negative biopsy findings. For men with prostates > 40 $cm^3$, a cutoff of 0.205 allows detection of 90% of the cancers, and 38% of the negative biopsy findings can be eliminated. If the patient has a normal-sized prostate on DRE, a value of 0.234 is necessary to detect 90% of the cancers, sparing 31.3% of the patients an unnecessary biopsy.[28] In another study, Brawer and colleagues compared the specificity of tPSA and f:tPSA at various sensitivities. At a sensitivity of 80% and a tPSA of 4.11, the specificity was 35.6% compared with 46.2% for f:tPSA with a cutoff point of 19%. At a sensitivity of 90% with a cutoff for tPSA of 3.4, the specificity was 25.3%, whereas the f:tPSA

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at a cutoff point of 24% was 26.2%. Among men with PSA levels of 4 to 10 ng/mL with a cutoff point of ≤ 25%, 95% of the cancers would be detected, and 20% of the patients would be spared a biopsy.[29] fPSA is most useful in men with persistently elevated PSA levels who have had a previous biopsy with negative findings. As the %fPSA declines, the probability of a cancer being present increases. Conversely, higher %fPSA indicates a lower probability that cancer exists.[29]

### *PSA Density*

In 1992, Benson and colleagues introduced the concept of PSA density (PSAD) to correlate PSA and prostate volume. This was based on the knowledge that most PSA is produced in the transition zone (TZ) of the prostate; cancer cells produce more PSA per unit volume than benign cells. PSAD is defined as the total serum PSA divided by prostate volume, as determined by transrectal ultrasound measurement. Theoretically, PSAD could help distinguish between PCa and BPH in men whose PSA levels are between 4 and 10 ng/mL. The value of PSAD is limited because of its dependency on the individual performing the prostate volume measurement. In addition, the BPH volume does not always correlate with serum PSA values because of the variation that exists between individuals in their epithelial-to-stromal ratios. PSA is made only by the epithelial cells, which produces a lower PSA level, even though the total volume of the prostate is high.[30] Using a cutoff of 0.15, different investigators had different outcomes—Seaman and colleagues reported that the value of PSAD could improve the detection rate of cancer at that cutoff value.[31] Catalona and colleagues reported that nearly 50% of cancers would be missed using the cutoff of 0.15.[32] Brawer and colleagues studied 107 men with PSA levels in the 4 to 10 ng/mL range and found no statistical difference between those with positive and negative biopsy findings using the 0.15 cutoff.[29]

### *PSA Isoforms*

Some isoforms of fPSA have been identified from detailed examination of the fPSA fraction, among which a mixture of precursor isoforms of PSA (pPSA or proPSA) and a form designated "benign" PSA [ie, BPA-associated PSA (BPHA)].[33-35] When specifically concentrating on the precursor isoform of PSA containing two amino acids in the propeptide leader, it confirmed the presence of [-2]proPSA in serum of men with PCa, in which [-2]proPSA formed 25% to 95% of the fPSA fraction, in contrast with 6% to 19% in biopsy-negative men.[34] Other investigations of [-2]proPSA showed that [-2]proPSA serum concentrations were, in general, higher in men with PCa compared with men without cancer, and is able to significantly improve PCa detection.[36,37] In evaluating the (−5, −7) proPSA isoform against fPSA and tPSA in men with low PSAs (2.0-4.0 ng/mL), the area under the receiver operating characteristic curve (ROC) was not significantly better for this proPSA isoform or the ratio of proPSA:PSA compared with tPSA or the fPSA:tPSA ratio.[37] In the PSA range of 4.0 to 10.0 ng/mL, the proPSA:fPSA ratio had a better area under the ROC compared with tPSA {0.67 [95% confidence interval (CI), 0.65-0.68] vs 0.53 [95% CI, 0.52-0.55]}, but added no diagnostic information over the fPSA:tPSA ratio (0.69; 95% CI, 0.67-0.70).[38] In a study of 376 men with PCa undergoing prostatectomy, proPSA was tested for its ability to distinguish between aggressive and nonaggressive PCa. The ratio of (−5, −7) proPSA:fpsa was associated with higher Gleason grade ($P = .001$) and non–organ-confined disease ($P < .0001$).[39] In another study, %p2PSA outperformed PSA and %fPSA for differentiating between PCa and benign disease. Setting the sensitivity at 88.5%, p2PSA led to a substantial improvement in specificity as well as positive and negative predictive values.[38] When considered together, however, a model including proPSA, PSA, and fPSA was superior to any of the individual tests. At a sensitivity of 95%, the combined model had greater specificity (37%) than PSA (15%) or fPSA (27%) alone, in a study of men undergoing prostate biopsy with PSA levels between 4.0 and 10.0 ng/mL.[40]

When BPHA concentrations were measured in serum, it was demonstrated that BPHA represented 25% of the fPSA in biopsy-negative men and was significantly higher in benign compared with PCa serum.[33] In another study, BPHA outperformed fPSA and tPSA in the prediction of TZ enlargement.[41] When the use of BPHA in discriminating PCa patients from patients without evidence of PCa was evaluated, it showed that BPHA might improve PCa detection.[42]

## Early PCa Antigens

Early PCa antigens (EPCA) and EPCA-2 are nuclear structural proteins that have been identified as expressed in PCa, but not in other normal tissues or cancer types.[43,44] Changes in nuclear matrix proteins are associated with carcinogenesis in a variety of tissues. The nuclear matrix proteins of the Dunning rat model of PCa were identified as different from those of the normal rat prostate.[44] In an analysis of the nuclear matrix proteins in human prostate tissues, 1 protein (designated PC-1) later renamed EPCA,

was identified in 14 of 14 of the PCa nuclear matrix preparations, but was not detected in similar preparations of any of 13 benign prostate specimens or 13 BPH specimens.[45]

In a small study of 12 cancer patients, using a cutoff of 1.7, EPCA identified 92% (11/12) of patients with cancer. None of the 16 healthy donors had EPCA levels above the cutoff, but 2 of the 6 bladder cancer control subjects did have EPCA levels above 1.7 for an overall specificity of 94%.[46] In another study, Getzenberg and colleagues established assay cutoffs in an initial pilot set of 10 men, each with negative PSA, organ-confined PCa, and non–organ-confined PCa. None of the samples from patients without evidence of prostate disease or the other control subjects had EPCA-2 levels above the positive cutoff. However, 8 of 35 patients (23%) with BPH had a serum EPCA-2 greater than the cutoff. Interestingly, in patients with serum PSA $<$ 2.5 ng/mL and with biopsy-documented PCa, the EPCA-2 enzyme-linked immunosorbent assay (ELISA) was positive in 14 of 18 men (78%). The EPCA-2 ELISA test was positive in 36 of 40 men (90%) with organ-confined PCa and 39 of 40 men (97.5%) with non–organ-confined PCa. The assay equally separated those men with organ-confined PCa from those with non–organ-confined PCa.[44]

## α-Methylacyl-CoA racemase

α-Methylacyl-CoA racemase (*AMACR*) is a well-characterized enzyme that plays a key role in peroxisomal β-oxidation of dietary branched-chain fatty acids and C27-bile acid intermediates.[47] Low levels of *AMACR* expression were detected in 9 of 9 (100%) BPH specimens, but *AMACR* was overexpressed relative to a common reference an average of 5.7-fold in 13 of 16 (81%) PCa samples.[48] In subsequent complementary DNA microarray analyses, *AMACR* messenger RNA (mRNA) was increased in 20 of 23 (87%) PCa specimens.[49] In a more direct comparison, *AMACR* mRNA was increased in 9 of 12 PCa samples (75%) versus matched normal prostate from the same patient.[48] By quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR), in the same research, *AMACR* mRNA levels were an average of 8.8-fold higher in 8 samples of PCa versus 8 samples of benign prostate. *AMACR* increased by immunohistochemistry (IHC) in the vast majority of 168 primary PCa cases and was also variably increased in high-grade prostatic intraepithelial neoplasia (HGPIN). *AMACR* epithelial IHC score cutoffs were established by which 95.6% of PCa versus only 3.5% of benign prostates were immunopositive.[48] On tissue microarrays including 108 benign prostates, 75 prostatic intraepithelial neoplasia, 116 clinically localized PCa, and 17 metastatic PCa samples, along with IHC scoring from 0 to 4, *AMACR* was significantly increased in clinically localized PCa versus benign prostate, with mean scores of 3.2 versus 1.3, respectively.[49]

*AMACR* is not a prostate-specific gene, and increased expression of *AMACR* in human neoplasia is not limited to PCa, which could influence specificity for PCa detection using either blood or urine. *AMACR* is also increased in papillary renal cell carcinomas, including approximately 75% of hepatocellular carcinomas, 31% urothelial carcinomas and colon adenocarcinomas,[50-52] BPH, prostatitis, and other urologic disorders, such as kidney stones or nephritis.[53]

qRT-PCR for *AMACR* mRNA was performed on total cellular RNA extracted from postprostatic massage urine specimens from 21 patients, including 10 with PCa, 2 with HGPIN, and 9 cancer-free individuals. Similar methodology quantitated PSA mRNA in order to verify prostate cell-derived RNA recovery, and normalized *AMACR* mRNA in urine to PSA mRNA for a relative *AMACR* score. Using cutoffs defined by the cancer-free control group, 7 of 10 (70%) with PCa had scores above the cutoff. The two patients with HGPIN were also above the positive cutoff for *AMACR* score.[54] Zehentner and colleagues analyzed postprostatic massage urine samples using the qRT-PCR *AMACR* assay in samples from seven patients with PCa, three with BPH, and one with prostatitis. Urine sediment samples demonstrated elevated normalized *AMACR* mRNA in four of six stage T1 PCa patients and in the one patient with stage T2 PCa only.[53] When examined by Western blot in urine specimens obtained after prostate biopsy for suspected PCa, *AMACR* protein was detected in the urine of 18 of 26 patients (69%), including 12 of 12 (100%) patients with biopsy-confirmed PCa, 1 of 2 with atypia on biopsy, and 5 of 12 patients (42%) with negative concurrent biopsies.[55]

*AMACR* mRNA levels were determined in blood using quantitative RT-PCR and were normalized to a non–prostate-specific housekeeping gene. Normalized AMACR mRNA levels were above the cutoff values in the blood of 28 of 58 patients (48%) with known metastatic PCa who were undergoing treatment. In 39 of 88 patients (44%) with presumed organ-confined PCa, *AMACR* mRNA was detectable in blood. *AMACR* mRNA transcripts in blood were detected in 3 of 9 patients (33%) with BPH, 10 of 20 patients (50%) with prostatitis, and 3 of 12 patients (25%) with other urologic disorders, such as kidney stones or nephritis.[53]

## Methylated Glutathione S-transferase π 1

Glutathione S-transferase-π 1 (*GSTP1*), the gene for glutathione S-transferase-π, which functions in the metabolic detoxification of potentially carcinogenic reactive oxygen metabolites, is the most extensively characterized gene that is methylated in PCa.[56] Hypermethylation of *GSTP1* is the most common (> 90%) reported epigenetic alteration in PCa. It occurs early in cancer progression and is a promising marker for detecting organ-confined disease. The quantitation of *GSTP1* hypermethylation can accurately detect the presence of cancer even in small, limited tissue samples.[57]

Yegnasubramanian and colleagues found that CpG islands were hypermethylated in > 85% of PCa and cancer cell lines but not in normal prostate cells and tissues. CpG island hypermethylation patterns in PCa metastases were very similar to the primary PCas and tended to show greater differences between cases than between anatomic sites of metastasis.[58] *GSTP1* promoter methylation is not present in benign prostate epithelium, but was detected in 6% of proliferative inflammatory atrophy (PIA) lesions.[59] In a study using conventional (nonquantitative) methylation-specific PCR, *GSTP1* promoter methylation was detected by Hoque and colleagues in only 27% of urine samples from patients with *GSTP1* methylation in the corresponding PCa tumor tissue.[60] In yet another study, ethanol-fixed paraffin-embedded prostate tumor specimens were obtained from eight different patients with clinically localized PCa (organ-confined) who were treated by curative RP. When analyzed using quantitative methylation-sensitive (QMS)-PCR, all samples demonstrated *GSTP1* methylation in both the tumor-associated endothelium and tumor epithelium. None of the normal epithelium samples demonstrated methylation at *GSTP1* and only two cases demonstrated methylation in the normal endothelium.[61]

## TMPRSS2:ERG Fusion

TMPRSS2 is an androgen-regulated transmembrane serine protease that is expressed in normal prostate epithelium, with increased expression reported in PCa.[62] ERG is a member of the ETS family of transcription factors, which contribute to the regulation of expression of genes that could be involved in carcinogenesis or tumor progression, and which are known to be involved in oncogenic transformations in Ewing sarcoma and myeloid leukemias.[63] These gene fusions presumably result in the increased expression of ETS transcription factors under the control of the androgen-response elements present in the 5′ region of TMPRSS2.[64] RNA isolated from sedimented urine and subjected to quantitative PCR, revealed the presence of TMPRSS2:ERG fusions in 8 of 19 patients (42%) with PCa.[65] In a study by Perner and colleagues, TMPRSS2:ERG fusions were detected in 5 of 26 (19%) of HGPIN foci studied. Positive HGPIN foci in close association with invasive PCa showed the same ERG fusion as the corresponding invasive PCa, neither BPH nor atrophy/PIA samples, showed ERG rearrangements.[66] Hessels and colleagues used RT-PCR followed by Southern blot hybridization to detect possible TMPSS2:ERG mRNAs in urine sediments following DRE in 78 patients with PCa on prostate biopsy versus 30 men with negative biopsy findings. The urinary sediments of 29 of the 78 (37%) PCa patients and 2 of the 30 men with negative biopsies (7%) harbored TMPRSS2:ERG fusion transcripts.[67]

## Golgi Membrane Protein 1

Golgi membrane protein 1 (GOLM1, NM_016548) is a resident *cis*-Golgi membrane protein of unknown function. The first evidence of its upregulation was shown in the hepatocytes of patients with acute and chronic forms of hepatitis and hepatocellular cancer.[68] In a study by Varambally and colleagues, the mean score for urine-associated GOLM1 reactivity in PCa patients (mean = 2.77) was significantly greater ($P < .0001$) than in the control subjects (mean = 0.96). Significantly greater percentage of PCa urine samples (75%) had GOLM1 reactivity score in the range of 2 to 4 in contrast to control subjects (28%). A ROC curve was generated for GOLM1 reactivity, and an optimum cutoff point was selected at the region where the slope of the curve had the highest value. At this cutoff point, GOLM1 had the best discriminatory power in distinguishing between urine from PCa patients and control populations (area under the curve [AUC] = 0.785; 95% CI, 0.693-0.876; $P < .0001$) representing a sensitivity and specificity of 75% and 72%, respectively. Overall, 39 of 52 urine samples from patients with clinically localized PCa and 14 of 50 control samples were considered positive for GOLM1 reactivity.[69] On testing for the ability to detect PCa based on the ROC curves, GOLM1 (AUC = 0.622, $P = .0009$) outperformed serum PSA (AUC = 0.495, $P = .902$) suggesting the use of urine-based GOLM1 mRNA measurements for the noninvasive detection of PCa. The sensitivity, specificity, and positive and negative predictive values

for the detection of GOLM1 in urine were 0.594, 0.709, 0.732, and 0.490, respectively.[69]

## *PCA3* Mutation

PCa antigen 3 (*PCA3*) is a prostate-specific noncoding gene that is highly upregulated in the vast majority of PCas.[70] The differential display gene 3 (*DD3*) subsequently renamed *PCA3* to reflect its association with PCa, was identified as overexpressed in PCa versus benign prostate by differential display. By Northern blot analysis, *DD3* (*PCA3*) mRNA was upregulated 10- to 100-fold in PCa versus benign in 53 of 56 RP specimens, with only low or no expression detected in benign prostate or BPH tissue.[71] Using RT-PCR, *DD3* (*PCA3*) mRNA was detected in only PCa tissues or tissues of benign prostate or BPH. *PCA3* mRNA was not detected in other benign tissues, including normal bladder, seminal vesicles, or testis. *PCA3* mRNA was not detected in tumors or tumor cell lines of other tissues, including testis, bladder, or kidney.[71]

By qRT-PCR, similarly low levels of *PCA3* were detected in benign prostate as well as in BPH tissues. In contrast, there was a median 34-fold increase in PCA versus benign/BPH specimens.[72] In situ hybridization studies demonstrated that *PCA3* is overexpressed in the vast majority of HGPIN lesions, at least in cases associated with invasive PCa. To verify prostate cell recovery and to normalize expression of *PCA3*, Hessels and colleagues also quantitated mRNA for PSA. Following prostatic massage, voided urine was collected and total RNA was extracted from urine sediments from 108 patients scheduled for prostate biopsy for PSA $>$ 3 ng/mL. Based on correlating mRNA ratios with biopsy results, the area under the ROC curve for *DD3* (*PCA3*)/PSA was 0.72. At the optimal *DD3* (*PCA3*)/PSA $\times$ $10^{-3}$ cutoff of 200, the sensitivity was 67%, and the specificity was 83%, which represents a substantial improvement over the specificity for serum PSA.[73] In 233 men enrolled at three different North American institutions, the PCA3 test informative rate was 97%. For the PCA3 score, the area under the ROC curve was 0.678 compared with only 0.524 for serum PSA. A PCA3 score cutoff of 35 achieved an optimal combination of sensitivity and specificity. With 35 as a cutoff, the sensitivity for PCa diagnosis in the repeat biopsy was 58% and the specificity was 72%. Importantly, the risk of a positive biopsy increased in a continuous fashion with increasing PCA3 score ranges. Patients with a PCA3 score $<$ 5 had PCa on biopsy in only 12%, whereas in patients with PCA3 scores $>$ 100, the risk of a positive biopsy was 50%.[74] A particularly important role of *PCA3* appears to be in men with persistently elevated serum PSA levels, but a negative initial biopsy. In such men who constitute a large problematic group, the odds ratio for the PCA3 test to predict cancer upon re-biopsy is 3.6, compared with only 1.2 for serum PSA testing.[75] Nakanishi and associates reported that the PCA3 score was also significantly associated with prostatectomy Gleason score (6 vs 7 or greater, $P = .005$) and "significant" cancer ($P = .007$), a classification based on dominant tumor volume and Gleason score (dominant tumor volume $<$ 0.5 cc and Gleason score 6).[76]

One well-recognized problem with the application of serum PSA for PCa screening is the relationship of total serum PSA to prostate volume.[25] In contrast, urine PCA3 score is not related to prostate volume. In 529 men scheduled for prostate biopsy, urine PCA3 score was correlated with prostate volume determined by transrectal ultrasound at the time of biopsy. Prostate volume was divided into three categories: $<$ 30 cc, 30 to 50 cc, and $>$ 50 cc. In contrast to serum PSA, PCA3 scores did not increase with prostate volume. The mean PCA3 scores for the three groups were 45, 38, and 43, respectively. These encouraging results suggest that age- and volume-related effects that complicate application of serum PSA in PCa screening, particularly affecting specificity of mild PSA elevations, will not similarly be encountered with PCA3 testing.[77] In a study of 463 men, the PCA3 score (cutoff of 35) had a greater diagnostic accuracy than %fPSA (cutoff of 25%). The PCA3 score was independent of the number of previous biopsies, age, prostate volume, and tPSA level. Moreover, the PCA3 score was significantly higher in men with HGPIN versus those without HGPIN, clinical stage T2 versus T1, Gleason score $\geq$7 versus $<$7, and "significant" versus "indolent" (clinical stage T1c, PSAD $<$ 0.15 ng/mL, Gleason score in biopsy $\leq$ 6, and percentage positive cores $\leq$ 33%) PCa.[78]

## PSA and CAG/GGN Repeat Polymorphisms

The first exon of the *AR* gene contains two polymorphic trinucleotide repeat segments that encode polyglutamine and polyglycine tracts localized in the NH2-terminal transactivation domain of the AR protein. The polyglutamine tract is encoded by a CAG trinucleotide repeat, and the polyglycine stretch is encoded by a GGN repeat. The number of CAG repeats ranges from approximately 8 to 35 repeats in normal individuals. Longer CAG repeat lengths appear to result in reduced AR transcriptional activity both in vivo and in vitro.[79]

**TABLE 1**

**Summary and Comparison of Possible Screening Tests**

| Screening Tests | Advantages | Disadvantages | Specifications and Comments |
|---|---|---|---|
| PSA | PSA is prostate specific, and therefore, a good prediction of metastasis of the prostate. Its throughput assay method is convenience for routine application. | PSA is not cancer specific and lacks the sensitivity to detect a large fraction of early-stage tumors. In addition, it does not account for age-related volume changes in the prostate. The serum PSA level can be altered by various factors other than PCa. | PSA and DRE are the most popular and widely accepted screening tools for screening of PCa. PSA test has revolutionized diagnosis and management of PCa and their comorbidities have decreased more than 75% since its introduction. |
| fPSA | %fPSA does not show a relationship to age. Provides better sensitivity than tPSA. | Prostate size is an important variable in selecting a cutoff value for %fPSA. | As the %fPSA declines, the probability of a cancer being present increases. Conversely, higher %fPSA indicates a lower probability that cancer exists. |
| PSAD | PSAD helps distinguish between PCa and BPH in men whose PSA levels are borderline (4-10 ng/mL) | The value of PSAD is dependent on the individual performing the prostate volume measurement. The BPH volume does not always correlate with serum PSA. | PSA is made only by the epithelial cells of the prostate; cancer cells produce more PSA per unit volume than benign cells. |
| PSA Isoforms | %p2PSA differentiates between PCa and benign disease better than PSA and %fPSA. The ratio of (–5, –7) propsa:fPSA distinguishes between aggressive and nonaggressive PCa. BPHA out performs fPSA as well as tPSA in the prediction of transition zone enlargement. | At a sensitivity of 95%, none of the isoforms has a good specificity among men with PSA levels between 4.0 and 10.0 ng/mL when compared with a model involving all the isoforms. | A detailed examination of the fPSA fraction of PSA yields a mixture of precursor isoforms of PSA (pPSA or proPSA) and a form designated "benign" PSA (ie, BPHA-associated PSA. |
| EPCA and EPCA-2 | EPCA-2.22 assay has a better specificity for PCa than PSA. It is also highly accurate in differentiating between localized and extra capsular disease. | EPCA-2 has been found in BPH samples thus question its ability to effectively differentiate between PCa and BPH. | EPCA and EPCA-2 are nuclear structural proteins that have been identified as expressed in PCa, but not in other normal tissues or cancer types. |
| *AMACR* | *AMACR* significantly differentiates clinically localized PCa from benign prostate tissues. | *AMACR* is not a prostate-specific gene, and its increased expression in human neoplasia is not limited to PCa, which could influence specificity for PCa. | *AMACR* is expressed in low levels in benign prostate tissues, over expressed in PCa tissues. |
| Methylated *GSTP1* | The quantitation of *GSTP1* hypermethylation can accurately detect the presence of cancer even in small tissue samples. | Routine and clinical diagnostic application of methylation experiment is still very limited. | Hypermethylation of *GSTP1* is the most common reported epigenetic alteration in PCa. It occurs early in cancer progression and is a promising marker for detecting organ-confined disease. |
| Androgen-Regulated Transmembrane Serine Protease - TMPRSS2:ERG Fusion | TMPRSS2:ERG fusions test has the potential of differentiating PCa tissues from BPH and atropy/PIA samples. | It has a low positive predictive value for PCa. | These gene fusions presumably result in the increased expression of ETS transcription factors under the control of the androgen-response elements present in the 5′ region of TMPRSS2. |

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| GOLM1 | Urine-based GOLM1 messenger RNA measurements for the detection of PCa outperforms serum PSA. | GOLM1 is upregulated in other cancers apart from PCa. | The mean score for urine-associated GOLM1 reactivity in PCa is consistently greater than control subjects in available studies. |
| *PCA3* Mutation | *PCA3* is prostate specific. Urine PCA3 score is independent of the number of previous biopsies, age, prostate volume, and tPSA level. It exhibit better specificity than serum PSA levels. | Expression of *PCA3* is not PCa specific. It is also expressed in BPH, though in low quantities. | The risk of a positive biopsy increases in a continuous fashion with increasing PCA3 score ranges. The PCA3 score is significantly higher in men with HGPIN vs those without HGPIN, and clinical stage T2 vs T1. |
| AR-CAG/GGN Repeat Polymorphisms | Shorter AR polyglutamine tracts have been associated with increased risk of PCa. | Routine analysis of CAG and GGN repeats pose no preference advantage in diagnoses of PCa. | The studies on AR polymorphism have helped to understand differences in inherent risk to PCa especially in different ethnical groups. |

*AMACR*, α-Methylacyl-CoA racemase; AR, androgen receptor; BPH, benign prostatic hyperplasia; DRE, digital rectal examination; EPCA, early PCa antigens; fPSA, free PSA; GOLM1, Golgi membrane protein 1; *GSTP1*, glutathione S-transferase π 1; HGPIN, high-grade prostatic intraepithelial neoplasia; PCa, prostate cancer; PIA, proliferative inflammatory atrophy; PSA, prostate-specific antigen; PSAD, PSA density.

Shorter AR polyglutamine tracts, and thus a more transcriptionally active AR, has been associated with increased PCa risk,[79] higher cancer grade at diagnosis,[80] earlier age of cancer onset in white men,[81] and aggressive early-stage PCa (defined as clinically unsuspected metastatic disease in men undergoing radical prostatectomy).[82]

Striking differences in CAG repeat lengths have been observed between populations. Black men tend to have significantly shorter repeats than their white counterparts.[80] These genetic differences may be potentially important in understanding why populations of African descent are more susceptible to developing PCa. Specifically, in vitro studies[83] have demonstrated an inverse relationship between the length of both repeats and AR activity levels. Lange and colleagues found no significant evidence of an association between shorter alleles at *AR* CAG or GGN and increased risk of PCa in the black American population.[84] Furthermore, other studies have investigated the hypothesis that shorter CAG repeat length is associated with an increased risk of clinically significant PCa, this was found to be true in both the Nigerian population[85] and the Chinese population.[86] The studies on AR polymorphism had helped to understand differences in inherent risk to PCa especially in different ethnical groups. In addition, the shorter the CAG and GGN repeat the stronger the interaction of the receptor with the ligand. This has a direct implication in androgen replacement therapy and supports the concept of individualized medicine. Although our earlier results showed no direct relationship between PSA level and AR repeat polymorphisms, the results demonstrated some CAG instability as PSA level increases.[85] These results provide potential tools to assist prediction strategies in this important disease. However, routine analysis of CAG and GGN repeats pose no preference advantage in diagnoses of PCa.

## Conclusions

Despite the numerous limitations of PSA, it remains the most useful single test for routine screening of PCa. Its combined use with DRE also remains the most popular diagnostic regiment of PCa. Advances in molecular biology and increasing discovery of other possible potent tumor markers (Table 1) will revolutionize the diagnosis and management of PCa in the near future. Many have proposed the use of panels of markers including PSA to increase the specificity and sensitivity of diagnosis but this is still far from routine use. However, there is a common agreement of ethnical diversity in prostate metastasis and more emphasis on individualized diagnosis and management of this disease. This is a challenge of the future. However, increasing understanding and continued collaborative effort to improve the diagnosis of PCa is a global challenge that will soon be overcome. ■

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## MAIN POINTS

- Prostate cancer (PCa) is the second leading cause of death as a result of cancer in men around the world with the lifetime risk of diagnosis at 16%. The only widely accepted screening tools are prostate-specific antigen (PSA) and digital rectal examination.
- PSA testing not only helps with early diagnosis but also assists in assessing the response to therapy, determining tumor progression, and, in its most controversial role, screening for PCa.
- PSA is known to be prostate specific, but not PCa specific, so lacks the sensitivity to detect a large fraction of tumors especially during the early stages. PSA levels are also known to be affected by many factors such as medication, inflammation (benign prostatic hyperplasia and prostatitis), and urologic manipulation; therefore, controversy continues regarding the appropriate level of serum PSA that should trigger a biopsy or have clinical relevance to prostate metastases.
- Attempts to determine the level of prostate cells in peripheral blood by reverse transcriptase polymerase chain reaction do not significantly improve cancer diagnosis or predict postoperative failure; therefore, the search continues for a novel biomarker or a panel of markers as well as other possible interventions to improve the use of PSA.
- The PSA test has revolutionized diagnosis and management of PCa; comorbidities have decreased more than 75% since its introduction. Further understanding and continued collaborative effort to improve the diagnosis of PCa is a global challenge that will soon be overcome.

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