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The role of biomarkers in undiagnosed men

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Abstract

Purpose of review—This article intends to review biomarkers derived from blood, urine, and tissue that can aid in the diagnosis of prostate cancer.

Recent findings—Prostate cancer (PCa) screening requires tools that complement prostate-specific antigen (PSA) with a higher specificity for clinically significant disease. Novel blood biomarkers, such as the prostate health index and 4Kscore, utilize isoforms of PSA to more accurately predict high-grade PCa than traditional tools such as PSA and the percentage free-to-total PSA. Several gene products associated with PCa can be detected in the urine through commercially available assays. Prostate cancer antigen 3 (PCA3), though approved for repeat biopsy decisions, appears inferior to other biomarkers such as phi for identifying aggressive disease. However, combinations of PCA3 with other urine assays have shown promising results. One tissue-based hypermethylation test, named ConfirmMDx, can also be used to determine the need for repeat biopsy in men with a prior negative biopsy.

Summary—Several biomarkers have been developed to aid in the screening and diagnosis of PCa. Such tests are often indicated in men with moderately elevated PSA or history of a prior negative biopsy. Their use facilitates reduction of unnecessary biopsies without sacrificing the early diagnosis of clinically significant PCa.

Keywords

prostate cancer; PSA; screening; biomarkers

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Conflicts of interest

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Introduction

Although most prostate cancer (PCa) is currently diagnosed through prostate-specific antigen (PSA) screening, this test is not specific for clinically significant PCa. False positives trigger unnecessary biopsies, and biopsy risks have increased. Furthermore, PCa represents a wide spectrum of disease, ranging from clinically indolent to aggressive, high-grade cancers. PSA-based screening leads to overdiagnosis, and better markers are needed for significant cancer. Several PCa marker tests are now available, derived from blood, urine and tissue. Our objective is to review the evidence on currently available biomarkers for prostate biopsy decisions.

Blood Biomarkers

Free PSA(fPSA)

Serum PSA exists in two forms, either bound to proteins (complexed PSA) or unbound (free PSA). The majority of total PSA (tPSA) is complexed to α_1 -antichymotrypsin, α_2 -macroglobulin, or α_1 -protease inhibitor.(1) Men with PCa have a lower fraction of fPSA (%fPSA) compared PCa-free men.(2)

The FDA approved the use of %fPSA for screening in men with PSA's of 4–10ng/mL. The test is also included in professional guidelines on early detection of PCa from the National Comprehensive Cancer Network (NCCN) and European Association of Urology (EAU). The NCCN suggests %fPSA in men with no prior biopsy and PSA >3ng/mL, or prior negative biopsy with continued suspicion of cancer.(3) The EAU recommends its use in men with PSA 2–10ng/mL prior to prostate biopsy.(4) There is currently no standardized threshold of %fPSA for clinical use.

The utility of %fPSA is predicated on improved specificity for PCa and clinically significant PCa in men with moderately elevated PSA.(5, 6) A prospective, multicenter study of 773 men with PSA 4–10ng/mL demonstrated that a 25% fPSA cutoff detects 95% of PCa and avoids 20% of unnecessary biopsies (6). Even men with PSA <4ng/mL can harbor significant disease (7), and %fPSA has further proven valuable in this group (8, 9).

Ankerst *et al* recently evaluated serial measurements of PSA and %fPSA in 2,183 men from a screening study (10). In men that developed PCa, %fPSA was an earlier indicator of cancer than PSA in 71% and 34% using %fPSA thresholds of 25% and 15%, respectively. The study also demonstrated that %fPSA values were prone to fluctuations, particularly in patients never diagnosed with PCa. Therefore, repeat measurements might be informative when initial values are near the cutoff.

Though fPSA has demonstrated significant value as a standalone test, it is also an important part of newer tests such as the Prostate Health Index and 4Kscore.

Prostate Health Index (phi)

Incomplete processing of precursor PSA yields an isoform of fPSA named [-2]proPSA. This marker is elevated in men with PCa and has higher specificity than tPSA (11). Phi combines

tPSA, fPSA, and [-2]proPSA as follows: $([-2]proPSA/fPSA) \times PSA$, to predict the probability of PCa and high-grade disease on biopsy.

In 2012, the FDA approved phi for men with PSA levels of 4–10ng/mL. The NCCN and EAU suggest phi before initial or repeat biopsy.(3, 4)

Numerous studies have demonstrated improved accuracy of phi compared with tPSA and fPSA (12–15). Catalona *et al* examined 892 men with PSA 2–10ng/mL and found that phi improved specificity of cancer detection and was significantly associated with higher Gleason score.(12) A multicenter, prospective study of 658 men with PSA between 4–10ng/mL investigated phi specifically for detection of clinically significant cancer.(13) Phi was most accurate for detection of all cancers (AUCs phi 0.708, %fPSA 0.648, [-2]proPSA 0.550, PSA 0.516), Gleason ≥ 7 (AUCs phi 0.707, %fPSA 0.661, [-2]proPSA 0.558, PSA 0.551), and Epstein significant cancer (AUCs phi 0.698, %fPSA 0.654, [-2]proPSA 0.550, PSA 0.549). Using a phi cutoff of 28.6, 10.1% of Epstein significant cancers, 4.8% of Gleason $\geq 3+4$, and 1.2% of Gleason $\geq 4+3$ would be missed. However, 30.1% of men with benign or insignificant disease could avoid an unnecessary biopsy using phi compared to only 21.7% avoided with %fPSA. By reducing unnecessary biopsies, phi as a reflex test after PSA may improve cost-effectiveness of screening (16).

The value of phi is evident in patient populations from the US, Europe and Asia. It has demonstrated utility for both initial and repeat biopsy (17, 18). Although phi is most commonly utilized in men with a “borderline” PSA of 4–10ng/ml, Lazzeri *et al* also showed its utility in men with PSA >10ng/mL.(19) Furthermore, phi has been validated in men with a family history of PCa, African-American ethnicity, age <60, and obesity (20–23).

Multiple guidelines now recommend a multivariable, risk-adapted approach to biopsy decisions, and biomarkers like phi can be considered in the context of other risk factors. Several multivariable tools incorporating phi have been developed to predict an individual patient’s risk of PCa. Lughezzani *et al* developed a nomogram using age, prostate volume, digital rectal examination (DRE), biopsy history, and phi that outperformed the base model with tPSA.(24, 25) The European Randomized Study of Screening for Prostate Cancer Risk Calculator (ERSPC-RC) developed another tool based on age, family history, DRE, prior biopsy results, tPSA, and prostate volume. Addition of phi improved prediction of clinically significant PCa (AUC 0.78 vs 0.72, $p=0.04$). (26) This calculator is available as a smartphone application for clinical use. More recently, Loeb *et al* developed a new nomogram using phi with other variables to predict clinically significant PCa.(27)

Phi also appears to provide prognostic information. Several studies have demonstrated that preoperative phi can predict pathological stage, grade, tumor volume, and biochemical recurrence following radical prostatectomy (RP) (28–31). In patients on active surveillance (AS), baseline and longitudinal phi values are predictive of biopsy reclassification (32). Thus, phi can be useful for both selecting and monitoring patients for AS.

4Kscore

The 4Kscore incorporates both serum biomarkers (tPSA, fPSA, intact PSA, and human kallikrein 2) and clinical variables (age, DRE, and prior biopsy results) to predict risk of high-grade PCa on biopsy. The NCCN and EAU recommend its use as a reflex test in men with moderately elevated PSA (3, 4). The 4Kscore is commercially available, but unlike %fPSA and phi, it is not currently FDA-approved.

Many studies have established that the 4-kallikrein panel improves accuracy of PCa detection compared with other multivariable tools, including the Prostate Cancer Prevention Trial Risk Calculator (PCPTRC)(33–35). It has been validated for both initial and repeat biopsy (36, 37). The largest of these trials examined 6,129 men from the ProtecT study with elevated PSA who underwent prostate biopsy (34). The base model for PCa detection incorporating age and tPSA was compared to the base model plus %fPSA or the 4-kallikrein panel. The 4-kallikrein model most accurately predicted high-grade cancer (AUCs 4-kallikrein 0.820, %fPSA 0.799, tPSA 0.738; $p < 0.001$). Using one particular threshold for the 4-kallikrein panel, 43% of unnecessary biopsies would be avoided while missing 11% of high-grade cancers. In a comparative study, Nordström *et al* demonstrated that the 4-kallikrein panel and phi had similar accuracy for detecting any PCa and high-grade PCa (38).

The 4Kscore was recently evaluated in the Canary AS cohort. In this setting, longitudinal monitoring of the 4K score improved prediction of reclassification on the initial AS biopsy, but was no better than PSA for monitoring patients subsequent to that (39).

The aforementioned studies validate the 4Kscore using biopsy outcomes. However, prostate biopsy, particularly non-targeted biopsy, is imperfect and thus may misrepresent true disease burden. Using a different approach for validation, one study demonstrated that the 4-kallikrein panel improved prediction of aggressive RP pathology.(40) Stattin *et al* reported that men with moderately elevated PSA but low-risk 4Kscore had a very low likelihood of subsequent metastatic disease and thus may safely avoid biopsy (41). For example, men aged 60 with PSA 3ng/mL and 4Kscore <5% had a 20-year risk of metastasis <0.5%.

Urine Biomarkers

Prostate Cancer Antigen 3(PCA3)

PCA3 is a noncoding mRNA that is overexpressed in PCa tissue and detectable in urine after vigorous DRE (42). The FDA approved the use of PCA3 utilizing a cutoff of 25 in men age 50 with 1 prior negative biopsy. The NCCN recommends using a cutoff of 35 in men with moderately elevated PSA for whom repeat biopsy is being considered, but the EAU makes no distinction regarding biopsy history (3, 4).

Numerous studies indicate that PCA3 has greater accuracy for overall PCa detection in the repeat biopsy setting compared with tPSA and %fPSA (43–46). Additionally, some evidence suggests that PCA3 improves cancer detection prior to initial biopsy (46–48). Wei *et al* studied both indications in 859 men undergoing prostate biopsy (49). A threshold of PCA3 <20 in the repeat setting would avoid 46% of biopsies while missing 3% of high-grade

cancer. However, that same threshold would miss 13% of high-grade cancer on initial biopsy.

Data are conflicting regarding the association of PCA3 score with high-grade or clinically-significant cancer detected on biopsy (43, 44, 48, 50). Furthermore, studies suggest that PCA3 score is not associated with progression on AS or RP pathology.(51–54) Comparative studies have demonstrated that phi outperforms PCA3 for prediction of clinically significant PCa at biopsy and RP.(55, 56) In 120 men eligible for AS who opted for RP, pre-operative MRI was best predictor of clinically significant PCa (57); phi was also a significant predictor but PCA3 was not. As the current paradigm emphasizes detection of aggressive PCa, such evidence diminishes the value of PCA3.

Perhaps the most appropriate application of PCA3 is in conjunction with other clinical tools. PCA3 combined with either MRI or real-time elastography was shown to improve the detection of significant PCa (58, 59). Several PCA3-based nomograms have been developed and validated, including one incorporating PCA3 into the PCPTRC (49, 60–63). One such study estimated that its nomogram would avoid up to 55% of unnecessary biopsies while missing 2% of high-grade PCa (60).

TMPRSS2:ERG

TMPRSS2:ERG (T2:ERG) is a gene fusion product that is highly specific for PCa and detectable in urine after DRE (64, 65). In a multi-institutional study of 1312 men, T2:ERG demonstrated greater diagnostic accuracy than tPSA (66). Furthermore, T2:ERG levels were associated with clinically significant PCa detected on biopsy and RP specimens in a subset of patients undergoing surgery.

PCA3 and T2:ERG

Although T2:ERG has high specificity for PCa, its low sensitivity reduces its value as a standalone test. Several studies have shown that combining PCA3 with T2:ERG can improve the prediction of PCa on biopsy (66–68).

A commercially available test, termed the Mi-Prostate Score (MiPS), incorporates serum tPSA and urinary PCA3 and T2:ERG to predict risk of PCa and high-grade PCa. In 1244 men undergoing biopsy, Tomlins *et al* examined the added value of PCA3, T2:ERG, or MiPS to a base model of either tPSA or the PCPTRC (69). For the prediction of high-grade PCa, MiPS (AUC 0.772) outperformed PSA+PCA3 (AUC 0.747, $p<0.001$), PSA+T2:ERG (AUC 0.729, $p<0.01$), and tPSA alone (AUC 0.651, $p<0.001$). Similarly, the addition of MiPS to the PCPTRC was superior to the base models. These results held true in the setting of either initial biopsy or repeat biopsy. Using various cutoffs, the MiPS-PCPTRC model would avoid 35–47% of unnecessary biopsies while missing 1.0–2.3% of high-grade PCa. MiPS is another promising reflex test following PSA screening, but it has not yet been directly compared with other biomarkers such as phi and 4Kscore.

SelectMDx

SelectMDx is another commercially available test that measures urinary RNA levels of two genes (DLX1 and HOXC6) following DRE. Using an algorithm including tPSA, PSA density, DRE, age, and family history, it provides an individual patient's likelihood of low- and high-grade PCa. Van Neste *et al*/developed this tool in 519 men undergoing prostate biopsy and subsequently validated it in a cohort of 386 men (70). SelectMDx demonstrated an AUC of 0.86 (95% CI 0.80–0.92) for high-grade PCa and outperformed the base model without RNA markers and the PCPTRC. One model, which excluded DRE, was superior to the PCPTRC combined with PCA3. Decision curve analysis suggested that 42% of biopsies could be avoided while missing 2% of high-grade PCa.

Urine Exosomes

ExoDx Prostate IntelliScore is a novel test analyzing exosomal RNA in urine without a prior DRE. It examines three PCa-associated genes (ERG, PCA3, and SPDEF) to predict the risk of high-grade PCa in men undergoing initial biopsy. It is intended to be used alongside standard of care (SOC) clinical factors such as tPSA, age, race, and family history. A validation study compared SOC with the urine exosome gene expression assay plus SOC for the detection of high-grade PCa (71). In 519 men, it demonstrated greater diagnostic accuracy than SOC. Setting a predefined cutoff would avoid 27% of unnecessary biopsies while missing 5% of dominant Gleason pattern 4 disease. It is unknown how this test compares with other available urine biomarkers.

Tissue Biomarkers

ConfirmMDx

Since prostate biopsy represents only a sampling of prostatic tissue, it may miss some significant PCa, particularly when performed in a non-targeted fashion. ConfirmMDx is a commercially available test that examines the tissue from a negative prostate biopsy to predict the presence of missed cancer in adjacent tissue. This test capitalizes on the presence a field effect around tumors. Epigenetic changes that alter gene expression are found not only in prostate tumors, but also in benign tissue adjacent the tumor. ConfirmMDx examines prostatic tissue for epigenetic changes, specifically the hypermethylation of three markers previously associated with prostate cancer (GSTP1, APC, and RASSF1).

Unlike other commercially available markers that evaluate tumor tissue to guide prognosis and decisions about treatment (e.g. Prolaris, OncotypeDx, and Decipher), ConfirmMDx is the only tissue marker that examines benign tissue to predict the presence of occult cancer. The NCCN guidelines suggest ConfirmMDx as another optional test to aid in decisions about repeat prostate biopsy (3).

ConfirmMDx has been validated in clinical studies from multiple countries. The MATLOC study included 483 men from the UK and Belgium with prior negative biopsy (72). ConfirmMDx was a significant predictor of PCa on repeat biopsy (OR 3.17, 95% CI 1.81–5.53), with a negative predictive value (NPV) of 90%. A subsequent US validation study of 320 men similarly reported a 2.5-fold increased risk of PCa on repeat biopsy with positive

methylation status and a NPV of 88% (73). These two previously published cohorts were combined in a subsequent study, which confirmed that ConfirmMDx was a significant predictor of high-grade PCa, with a NPV of 96% (74). These results affirm a low risk of detecting significant PCa in men with negative findings on ConfirmMDx, suggesting that these men can safely defer a repeat biopsy.

In contrast to serum and urine markers, ConfirmMDx offers a potential advantage by localizing where additional sampling is warranted on repeat biopsy. However, the cost-effectiveness of ConfirmMDx within the context of an experienced multiparametric MRI program is unknown.

Conclusion

PSA remains the only marker demonstrated to reduce prostate cancer mortality in randomized trials. Nevertheless, there are an abundance of novel biomarkers which are commercially available and being incorporated into guidelines to assist with prostate biopsy decisions. Though some are intended specifically for either initial or repeat biopsy, there is much overlap in their application. Studies comparing phi with other biomarkers have been published, but there remains a need for further studies that compare these biomarkers head-to-head. As more data on MRI continues to emerge and its use in prostate cancer detection expands, more studies are also needed examining the cost-effectiveness of integrating biomarkers with MRI in this setting. With that, we might elucidate how to best incorporate all of these new tools into a risk-adapted screening protocol that accurately detects clinically significant PCa while minimizing diagnosis and treatment of insignificant PCa.

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References

1. Christensson A, Laurell CB, Lilja H. Enzymatic activity of prostate-specific antigen and its reactions with extracellular serine proteinase inhibitors. *Eur J Biochem.* 1990; 194(3):755–63. [PubMed: 1702714]
2. Christensson A, Bjork T, Nilsson O, Dahlen U, Matikainen MT, Cockett AT, et al. Serum prostate specific antigen complexed to alpha 1-antichymotrypsin as an indicator of prostate cancer. *J Urol.* 1993; 150(1):100–5. [PubMed: 7685416]
- 3**. NCCN Clinical Practice Guidelines in Oncology. Prostate Cancer Early Detection. 2016. Available from: https://www.nccn.org/professionals/physician_gls/pdf/prostate_detection.pdf These guidelines provide useful guidance regarding the use of biomarkers in prostate cancer detection
- 4**. European Association of Urology. Prostate Cancer. 2016. Available from: <https://uroweb.org/guideline/prostate-cancer/> The EAU guidelines provide evidence-based guidance on prostate cancer
5. Partin AW, Brawer MK, Subong EN, Kelley CA, Cox JL, Bruzek DJ, et al. Prospective evaluation of percent free-PSA and complexed-PSA for early detection of prostate cancer. *Prostate Cancer Prostatic Dis.* 1998; 1(4):197–203. [PubMed: 12496895]

6. Catalona WJ, Partin AW, Slawin KM, Brawer MK, Flanigan RC, Patel A, et al. Use of the percentage of free prostate-specific antigen to enhance differentiation of prostate cancer from benign prostatic disease: a prospective multicenter clinical trial. *JAMA*. 1998; 279(19):1542–7. [PubMed: 9605898]
7. Thompson IM, Pauler DK, Goodman PJ, Tangen CM, Lucia MS, Parnes HL, et al. Prevalence of prostate cancer among men with a prostate-specific antigen level < or =4.0 ng per milliliter. *N Engl J Med*. 2004; 350(22):2239–46. [PubMed: 15163773]
8. Catalona WJ, Smith DS, Ornstein DK. Prostate cancer detection in men with serum PSA concentrations of 2.6 to 4.0 ng/mL and benign prostate examination. Enhancement of specificity with free PSA measurements. *JAMA*. 1997; 277(18):1452–5. [PubMed: 9145717]
9. Haese A, Dworschack RT, Partin AW. Percent free prostate specific antigen in the total prostate specific antigen 2 to 4 ng./ml. range does not substantially increase the number of biopsies needed to detect clinically significant prostate cancer compared to the 4 to 10 ng./ml. range. *J Urol*. 2002; 168(2):504–8. [PubMed: 12131298]
10. Ankerst DP, Gelfond J, Goros M, Herrera J, Strobl A, Thompson IM Jr, et al. Serial Percent Free Prostate Specific Antigen in Combination with Prostate Specific Antigen for Population Based Early Detection of Prostate Cancer. *J Urol*. 2016; 196(2):355–60. [PubMed: 26979652]
11. Mikolajczyk SD, Millar LS, Wang TJ, Rittenhouse HG, Marks LS, Song W, et al. A precursor form of prostate-specific antigen is more highly elevated in prostate cancer compared with benign transition zone prostate tissue. *Cancer Res*. 2000; 60(3):756–9. [PubMed: 10676664]
12. Catalona WJ, Partin AW, Sanda MG, Wei JT, Klee GG, Bangma CH, et al. A multicenter study of [-2]pro-prostate specific antigen combined with prostate specific antigen and free prostate specific antigen for prostate cancer detection in the 2.0 to 10.0 ng/ml prostate specific antigen range. *J Urol*. 2011; 185(5):1650–5. [PubMed: 21419439]
13. Loeb S, Sanda MG, Broyles DL, Shin SS, Bangma CH, Wei JT, et al. The prostate health index selectively identifies clinically significant prostate cancer. *J Urol*. 2015; 193(4):1163–9. [PubMed: 25463993]
14. de la Calle C, Patil D, Wei JT, Scherr DS, Sokoll L, Chan DW, et al. Multicenter Evaluation of the Prostate Health Index to Detect Aggressive Prostate Cancer in Biopsy Naive Men. *J Urol*. 2015; 194(1):65–72. [PubMed: 25636659]
15. Guazzoni G, Nava L, Lazzeri M, Scattoni V, Lughezzani G, Maccagnano C, et al. Prostate-specific antigen (PSA) isoform p2PSA significantly improves the prediction of prostate cancer at initial extended prostate biopsies in patients with total PSA between 2.0 and 10 ng/ml: results of a prospective study in a clinical setting. *Eur Urol*. 2011; 60(2):214–22. [PubMed: 21482022]
16. Heijnsdijk EA, Denham D, de Koning HJ. The Cost-Effectiveness of Prostate Cancer Detection with the Use of Prostate Health Index. *Value Health*. 2016; 19(2):153–7. [PubMed: 27021748]
17. Lazzeri M, Briganti A, Scattoni V, Lughezzani G, Larcher A, Gadda GM, et al. Serum index test % [-2]proPSA and Prostate Health Index are more accurate than prostate specific antigen and %fPSA in predicting a positive repeat prostate biopsy. *J Urol*. 2012; 188(4):1137–43. [PubMed: 22901578]
18. Boegemann M, Stephan C, Cammann H, Vincendeau S, Houlgatte A, Jung K, et al. The percentage of prostate-specific antigen (PSA) isoform [-2]proPSA and the Prostate Health Index improve the diagnostic accuracy for clinically relevant prostate cancer at initial and repeat biopsy compared with total PSA and percentage free PSA in men aged <=65 years. *BJU Int*. 2016; 117(1):72–9. [PubMed: 25818705]
19. Lazzeri M, Lughezzani G, Haese A, McNicholas T, de la Taille A, Buffi NM, et al. Clinical performance of prostate health index in men with tPSA>10ng/ml: Results from a multicentric European study. *Urol Oncol*. 2016; 34(9):415.e13–9.
20. Lazzeri M, Haese A, Abrate A, de la Taille A, Redorta JP, McNicholas T, et al. Clinical performance of serum prostate-specific antigen isoform [-2]proPSA (p2PSA) and its derivatives, %p2PSA and the prostate health index (PHI), in men with a family history of prostate cancer: results from a multicentre European study, the PROMetheuS project. *BJU Int*. 2013; 112(3):313–21. [PubMed: 23826841]
21. Schwen ZR, Tosoian JJ, Sokoll LJ, Mangold L, Humphreys E, Schaeffer EM, et al. Prostate Health Index (PHI) Predicts High-stage Pathology in African American Men. *Urology*. 2016; 90:136–40. [PubMed: 26688190]

22. Fossati N, Lazzeri M, Haese A, McNicholas T, de la Taille A, Buffi NM, et al. Clinical performance of serum isoform [-2]proPSA (p2PSA), and its derivatives %p2PSA and the Prostate Health Index, in men aged <60 years: results from a multicentric European study. *BJU Int.* 2015; 115(6):913–20. [PubMed: 24589357]
23. Abrate A, Lazzeri M, Lughezzani G, Buffi N, Bini V, Haese A, et al. Clinical performance of the Prostate Health Index (PHI) for the prediction of prostate cancer in obese men: data from the PROMetheuS project, a multicentre European prospective study. *BJU Int.* 2015; 115(4):537–45. [PubMed: 25130593]
24. Lughezzani G, Lazzeri M, Larcher A, Lista G, Scattoni V, Cestari A, et al. Development and internal validation of a Prostate Health Index based nomogram for predicting prostate cancer at extended biopsy. *J Urol.* 2012; 188(4):1144–50. [PubMed: 22901589]
25. Lughezzani G, Lazzeri M, Haese A, McNicholas T, de la Taille A, Buffi NM, et al. Multicenter European external validation of a prostate health index-based nomogram for predicting prostate cancer at extended biopsy. *Eur Urol.* 2014; 66(5):906–12. [PubMed: 24361258]
26. Foley RW, Maweni RM, Gorman L, Murphy K, Lundon DJ, Durkan G, et al. The ERSPC Risk Calculators Significantly Outperform The PCPT 2.0 In The Prediction Of Prostate Cancer; A Multi-Institutional Study. *BJU Int.* 2016
27. Loeb S, Shin SS, Broyles DL, Wei JT, Sanda M, Klee G, et al. Prostate Health Index improves multivariable risk prediction of aggressive prostate cancer. *BJU Int.* 2016
28. Fossati N, Buffi NM, Haese A, Stephan C, Larcher A, McNicholas T, et al. Preoperative Prostate-specific Antigen Isoform p2PSA and Its Derivatives, %p2PSA and Prostate Health Index, Predict Pathologic Outcomes in Patients Undergoing Radical Prostatectomy for Prostate Cancer: Results from a Multicentric European Prospective Study. *Eur Urol.* 2015; 68(1):132–8. [PubMed: 25139197]
29. Cantiello F, Russo GI, Cicione A, Ferro M, Cimino S, Favilla V, et al. PHI and PCA3 improve the prognostic performance of PRIAS and Epstein criteria in predicting insignificant prostate cancer in men eligible for active surveillance. *World J Urol.* 2016; 34(4):485–93. [PubMed: 26194612]
30. Cantiello F, Russo GI, Ferro M, Cicione A, Cimino S, Favilla V, et al. Prognostic accuracy of Prostate Health Index and urinary Prostate Cancer Antigen 3 in predicting pathologic features after radical prostatectomy. *Urol Oncol.* 2015; 33(4):163.e15–23.
31. Lughezzani G, Lazzeri M, Buffi NM, Abrate A, Mistretta FA, Hurle R, et al. Preoperative prostate health index is an independent predictor of early biochemical recurrence after radical prostatectomy: Results from a prospective single-center study. *Urol Oncol.* 2015; 33(8):337.e7–14.
32. Tosoian JJ, Loeb S, Feng Z, Isharwal S, Landis P, Elliot DJ, et al. Association of [-2]proPSA with biopsy reclassification during active surveillance for prostate cancer. *J Urol.* 2012; 188(4):1131–6. [PubMed: 22901577]
33. Vickers AJ, Cronin AM, Aus G, Pihl CG, Becker C, Pettersson K, et al. A panel of kallikrein markers can reduce unnecessary biopsy for prostate cancer: data from the European Randomized Study of Prostate Cancer Screening in Goteborg, Sweden. *BMC Med.* 2008; 6:19. [PubMed: 18611265]
34. Bryant RJ, Sjoberg DD, Vickers AJ, Robinson MC, Kumar R, Marsden L, et al. Predicting high-grade cancer at ten-core prostate biopsy using four kallikrein markers measured in blood in the ProtecT study. *J Natl Cancer Inst.* 2015; 107(7)
35. Parekh DJ, Punnen S, Sjoberg DD, Asroff SW, Bailen JL, Cochran JS, et al. A multi-institutional prospective trial in the USA confirms that the 4Kscore accurately identifies men with high-grade prostate cancer. *Eur Urol.* 2015; 68(3):464–70. [PubMed: 25454615]
36. Vickers A, Cronin A, Roobol M, Savage C, Peltola M, Pettersson K, et al. Reducing unnecessary biopsy during prostate cancer screening using a four-kallikrein panel: an independent replication. *J Clin Oncol.* 2010; 28(15):2493–8. [PubMed: 20421547]
37. Gupta A, Roobol MJ, Savage CJ, Peltola M, Pettersson K, Scardino PT, et al. A four-kallikrein panel for the prediction of repeat prostate biopsy: data from the European Randomized Study of Prostate Cancer screening in Rotterdam, Netherlands. *Br J Cancer.* 2010; 103(5):708–14. [PubMed: 20664589]

- 38*. Nordstrom T, Vickers A, Assel M, Lilja H, Gronberg H, Eklund M. Comparison Between the Four-kallikrein Panel and Prostate Health Index for Predicting Prostate Cancer. *Eur Urol.* 2015; 68(1):139–46. This head-to-head study compares the 4 kallikrein panel versus the prostate health index. [PubMed: 25151013]
39. Lin D, Brown M, Newcomb L, Sjoberg D, Brooks J, Carroll P, et al. PD08-02 EVALUATING THE FOUR KALLIKREIN PANEL OF THE 4KSCORE FOR PREDICTION OF HIGH-GRADE PROSTATE CANCER IN MEN IN THE CANARY PROSTATE ACTIVE SURVEILLANCE STUDY (PASS). *The Journal of Urology.* 195(4):e229.
40. Carlsson S, Maschino A, Schroder F, Bangma C, Steyerberg EW, van der Kwast T, et al. Predictive value of four kallikrein markers for pathologically insignificant compared with aggressive prostate cancer in radical prostatectomy specimens: results from the European Randomized Study of Screening for Prostate Cancer section Rotterdam. *Eur Urol.* 2013; 64(5):693–9. [PubMed: 23683475]
41. Stattin P, Vickers AJ, Sjoberg DD, Johansson R, Granfors T, Johansson M, et al. Improving the Specificity of Screening for Lethal Prostate Cancer Using Prostate-specific Antigen and a Panel of Kallikrein Markers: A Nested Case-Control Study. *Eur Urol.* 2015; 68(2):207–13. [PubMed: 25682340]
42. Bussemakers MJ, van Bokhoven A, Verhaegh GW, Smit FP, Karthaus HF, Schalken JA, et al. DD3: a new prostate-specific gene, highly overexpressed in prostate cancer. *Cancer Res.* 1999; 59(23): 5975–9. [PubMed: 10606244]
43. Marks LS, Fradet Y, Deras IL, Blase A, Mathis J, Aubin SM, et al. PCA3 molecular urine assay for prostate cancer in men undergoing repeat biopsy. *Urology.* 2007; 69(3):532–5. [PubMed: 17382159]
44. Haese A, de la Taille A, van Poppel H, Marberger M, Stenzl A, Mulders PF, et al. Clinical utility of the PCA3 urine assay in European men scheduled for repeat biopsy. *Eur Urol.* 2008; 54(5):1081–8. [PubMed: 18602209]
45. Gittelman MC, Hertzman B, Bailen J, Williams T, Koziol I, Henderson RJ, et al. PCA3 molecular urine test as a predictor of repeat prostate biopsy outcome in men with previous negative biopsies: a prospective multicenter clinical study. *J Urol.* 2013; 190(1):64–9. [PubMed: 23416644]
46. Bradley LA, Palomaki GE, Gutman S, Samson D, Aronson N. Comparative effectiveness review: prostate cancer antigen 3 testing for the diagnosis and management of prostate cancer. *J Urol.* 2013; 190(2):389–98. [PubMed: 23545099]
47. Deras IL, Aubin SM, Blase A, Day JR, Koo S, Partin AW, et al. PCA3: a molecular urine assay for predicting prostate biopsy outcome. *J Urol.* 2008; 179(4):1587–92. [PubMed: 18295257]
48. Chevli KK, Duff M, Walter P, Yu C, Capuder B, Elshafei A, et al. Urinary PCA3 as a predictor of prostate cancer in a cohort of 3,073 men undergoing initial prostate biopsy. *J Urol.* 2014; 191(6): 1743–8. [PubMed: 24333241]
49. Wei JT, Feng Z, Partin AW, Brown E, Thompson I, Sokoll L, et al. Can urinary PCA3 supplement PSA in the early detection of prostate cancer? *J Clin Oncol.* 2014; 32(36):4066–72. [PubMed: 25385735]
50. Aubin SM, Reid J, Sarno MJ, Blase A, Aussie J, Rittenhouse H, et al. PCA3 molecular urine test for predicting repeat prostate biopsy outcome in populations at risk: validation in the placebo arm of the dutasteride REDUCE trial. *J Urol.* 2010; 184(5):1947–52. [PubMed: 20850153]
51. Tosoian JJ, Loeb S, Kettermann A, Landis P, Elliot DJ, Epstein JI, et al. Accuracy of PCA3 measurement in predicting short-term biopsy progression in an active surveillance program. *J Urol.* 2010; 183(2):534–8. [PubMed: 20006883]
52. Hessels D, van Gils MP, van Hooij O, Jannink SA, Witjes JA, Verhaegh GW, et al. Predictive value of PCA3 in urinary sediments in determining clinico-pathological characteristics of prostate cancer. *Prostate.* 2010; 70(1):10–6. [PubMed: 19708043]
53. Liss MA, Santos R, Osann K, Lau A, Ahlering TE, Ornstein DK. PCA3 molecular urine assay for prostate cancer: association with pathologic features and impact of collection protocols. *World J Urol.* 2011; 29(5):683–8. [PubMed: 21152924]

54. van Gils MP, Hessels D, Hulsbergen-van de Kaa CA, Witjes JA, Jansen CF, Mulders PF, et al. Detailed analysis of histopathological parameters in radical prostatectomy specimens and PCA3 urine test results. *Prostate*. 2008; 68(11):1215–22. [PubMed: 18500693]
- 55*. Seisen T, Roupert M, Brault D, Leon P, Cancel-Tassin G, Comperat E, et al. Accuracy of the prostate health index versus the urinary prostate cancer antigen 3 score to predict overall and significant prostate cancer at initial biopsy. *Prostate*. 2015; 75(1):103–11. This study compares the performance of phi versus PCA3. [PubMed: 25327361]
56. Ferro M, Lucarelli G, Bruzzese D, Perdona S, Mazzarella C, Perruolo G, et al. Improving the prediction of pathologic outcomes in patients undergoing radical prostatectomy: the value of prostate cancer antigen 3 (PCA3), prostate health index (phi) and sarcosine. *Anticancer Res*. 2015; 35(2):1017–23. [PubMed: 25667489]
57. Porpiglia F, Cantiello F, De Luca S, Manfredi M, Veltri A, Russo F, et al. In-parallel comparative evaluation between multiparametric magnetic resonance imaging, prostate cancer antigen 3 and the prostate health index in predicting pathologically confirmed significant prostate cancer in men eligible for active surveillance. *BJU Int*. 2016; 118(4):527–34. [PubMed: 26350955]
58. Fenstermaker M, Mendhiratta N, Bjurlin MA, Meng X, Rosenkrantz AB, Huang R, et al. Risk Stratification by Urinary Prostate Cancer Gene 3 Testing Before Magnetic Resonance Imaging-Ultrasound Fusion-targeted Prostate Biopsy Among Men With No History of Biopsy. *Urology*. 2016
59. Nygard Y, Haukaas SA, Halvorsen OJ, Gravdal K, Frugard J, Akslen LA, et al. A positive Real-Time Elastography (RTE) combined with a Prostate Cancer Gene 3 (PCA3) score above 35 convey a high probability of intermediate- or high-risk prostate cancer in patient admitted for primary prostate biopsy. *BMC Urol*. 2016; 16(1):39. [PubMed: 27391229]
60. Hansen J, Auprich M, Ahyai SA, de la Taille A, van Poppel H, Marberger M, et al. Initial prostate biopsy: development and internal validation of a biopsy-specific nomogram based on the prostate cancer antigen 3 assay. *Eur Urol*. 2013; 63(2):201–9. [PubMed: 22854248]
61. Rubio-Briones J, Borque A, Esteban LM, Casanova J, Fernandez-Serra A, Rubio L, et al. Optimizing the clinical utility of PCA3 to diagnose prostate cancer in initial prostate biopsy. *BMC Cancer*. 2015; 15:633. [PubMed: 26362197]
62. Elshafei A, Chevli KK, Moussa AS, Kara O, Chueh SC, Walter P, et al. PCA3-based nomogram for predicting prostate cancer and high grade cancer on initial transrectal guided biopsy. *Prostate*. 2015; 75(16):1951–7. [PubMed: 26384170]
63. Greene DJ, Elshafei A, Nyame YA, Kara O, Malkoc E, Gao T, et al. External validation of a PCA-3-based nomogram for predicting prostate cancer and high-grade cancer on initial prostate biopsy. *Prostate*. 2016; 76(11):1019–23. [PubMed: 27197726]
64. Tomlins SA, Bjartell A, Chinnaiyan AM, Jenster G, Nam RK, Rubin MA, et al. ETS gene fusions in prostate cancer: from discovery to daily clinical practice. *Eur Urol*. 2009; 56(2):275–86. [PubMed: 19409690]
65. Tomlins SA, Rhodes DR, Perner S, Dhanasekaran SM, Mehra R, Sun XW, et al. Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer. *Science*. 2005; 310(5748):644–8. [PubMed: 16254181]
66. Tomlins SA, Aubin SM, Siddiqui J, Lonigro RJ, Sefton-Miller L, Miick S, et al. Urine TMPRSS2:ERG fusion transcript stratifies prostate cancer risk in men with elevated serum PSA. *Sci Transl Med*. 2011; 3(94):94ra72.
67. Hessels D, Smit FP, Verhaegh GW, Witjes JA, Cornel EB, Schalken JA. Detection of TMPRSS2-ERG fusion transcripts and prostate cancer antigen 3 in urinary sediments may improve diagnosis of prostate cancer. *Clin Cancer Res*. 2007; 13(17):5103–8. [PubMed: 17785564]
68. Leyten GH, Hessels D, Jannink SA, Smit FP, de Jong H, Cornel EB, et al. Prospective multicentre evaluation of PCA3 and TMPRSS2-ERG gene fusions as diagnostic and prognostic urinary biomarkers for prostate cancer. *Eur Urol*. 2014; 65(3):534–42. [PubMed: 23201468]
69. Tomlins SA, Day JR, Lonigro RJ, Hovelson DH, Siddiqui J, Kunju LP, et al. Urine TMPRSS2:ERG Plus PCA3 for Individualized Prostate Cancer Risk Assessment. *Eur Urol*. 2016; 70(1):45–53. [PubMed: 25985884]

70. Van Neste L, Hendriks RJ, Dijkstra S, Trooskens G, Cornel EB, Jannink SA, et al. Detection of High-grade Prostate Cancer Using a Urinary Molecular Biomarker-Based Risk Score. *Eur Urol*. 2016
71. McKiernan J, Donovan MJ, O'Neill V, Bentink S, Noerholm M, Belzer S, et al. A Novel Urine Exosome Gene Expression Assay to Predict High-grade Prostate Cancer at Initial Biopsy. *JAMA Oncol*. 2016; 2(7):882–9. [PubMed: 27032035]
72. Stewart GD, Van Neste L, Delvenne P, Delree P, Delga A, McNeill SA, et al. Clinical utility of an epigenetic assay to detect occult prostate cancer in histopathologically negative biopsies: results of the MATLOC study. *J Urol*. 2013; 189(3):1110–6. [PubMed: 22999998]
73. Partin AW, Van Neste L, Klein EA, Marks LS, Gee JR, Troyer DA, et al. Clinical validation of an epigenetic assay to predict negative histopathological results in repeat prostate biopsies. *J Urol*. 2014; 192(4):1081–7. [PubMed: 24747657]
74. Van Neste L, Partin AW, Stewart GD, Epstein JI, Harrison DJ, Van Criekinge W. Risk score predicts high-grade prostate cancer in DNA-methylation positive, histopathologically negative biopsies. *Prostate*. 2016; 76(12):1078–87. [PubMed: 27121847]

Key Points

- Several biomarkers, derived from blood, urine, and tissue, can aid in decisions regarding prostate biopsy.
- Phi and the 4Kscore more accurately detect high-grade PCa than do PSA and free PSA.
- PCA3 is another test for repeat biopsy but is inferior to phi for identifying high-grade disease
- ConfirmMDx is the only tissue biomarker to predict occult cancer in men with negative biopsies
- Such tests can selectively identify patients who should undergo prostate biopsy, thus reducing unnecessary biopsies and diagnosis of indolent PCa.