The Prostate Health Index (*phi*)

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GeneNews

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Test Description

The Prostate Health Index (*phi*) is a simple blood test that improves the accuracy of prostate cancer detection. Prostate cancer is a leading cause of cancer mortality in men; it is estimated that 238,590 US men were newly diagnosed and 29,720 died of prostate cancer in 2013.[1] Prostate-specific antigen (PSA), a serine protease produced by prostate epithelial cells, is a commonly-used serum marker for prostate cancer, as cancer-induced changes to prostate gland architecture can lead to increased "leakage" of PSA into the bloodstream (Figure 1).[2] However, total PSA (tPSA) tests alone lack the specificity for accurate prostate cancer detection, because PSA leakage and resultant increases in serum PSA can also be caused by benign conditions such as prostatitis, nonmalignant enlargement of the prostate (known as benign prostatic hyperplasia or BPH), and prostate biopsy.[3] Overtreatment of prostate cancer due to misdiagnosis or overdiagnosis (which is defined as the detection of cancer that would not otherwise cause symptoms or death) often causes lasting damage, including urinary incontinence, problems with bowel function, erectile dysfunction, and infection.[4]

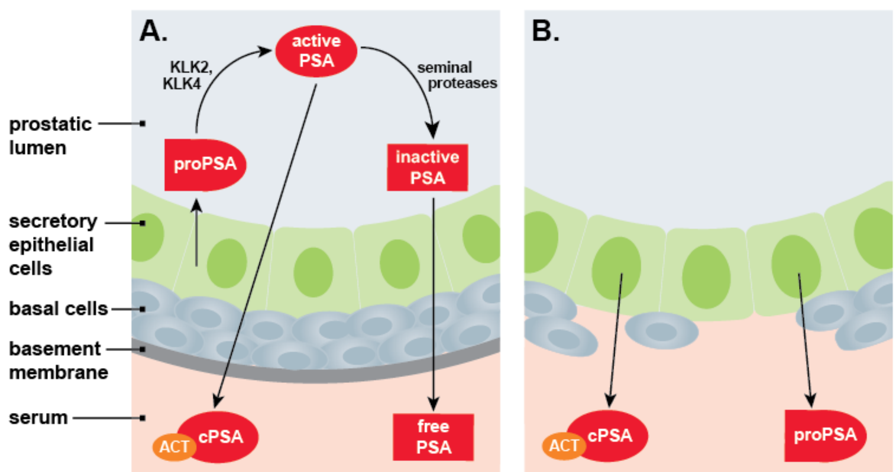

Figure 1. PSA biosynthesis in normal vs. cancerous prostate epithelium. Normal secretory epithelium **(A)** is surrounded by basal cells and a basement membrane and secretes proPSA into the prostatic lumen, where the proteases KLK2 and KLK4 remove the propeptide to generate active PSA. A small fraction of this active PSA diffuses to the circulation and is bound by protease inhibitors such as alpha-1 antichymotrypsin (ACT) to form cPSA. Active PSA also undergoes proteolysis by seminal proteases to generate inactive PSA, which enters the bloodstream and circulates as free PSA. In prostate cancer **(B)**, loss of basal cells and degradation of the basement membrane results in decreased luminal processing of proPSA to active PSA, and increased levels of cPSA and proPSA in the serum.[2]

PSA is first synthesized as preproPSA, which includes a 17–amino acid leader sequence that is cotranslationally cleaved to generate an inactive 244–amino acid precursor protein called proPSA; the mature PSA enzyme (237 amino acids) is then generated via cleavage of the N-terminal 7 amino acids of proPSA by the proteases KLK2 and KLK4 (Figure 2). ProPSA may also undergo cleavage at various positions within the propeptide; the most stable of these truncated forms is pro2PSA, which has two extra amino acids relative to mature PSA, is the primary form found in prostate tumor tissue, and has been associated with more aggressive disease.[2,5,6] The majority of PSA that enters the bloodstream (70-90%) is bound by various protease inhibitors—primarily alpha-1 antichymotrypsin (ACT)—to inactivate its catalytic activity, forming complexed PSA (cPSA); the remaining 10-30% is inactivated via cleavage by seminal proteases while still in the prostatic lumen, and circulates in the bloodstream as free PSA (fPSA).[2] Total PSA (tPSA) includes both complex and free forms of the protein, which comprises a mixture of mature PSA (active and inactive), full-length proPSA, and truncated proPSA.[7]

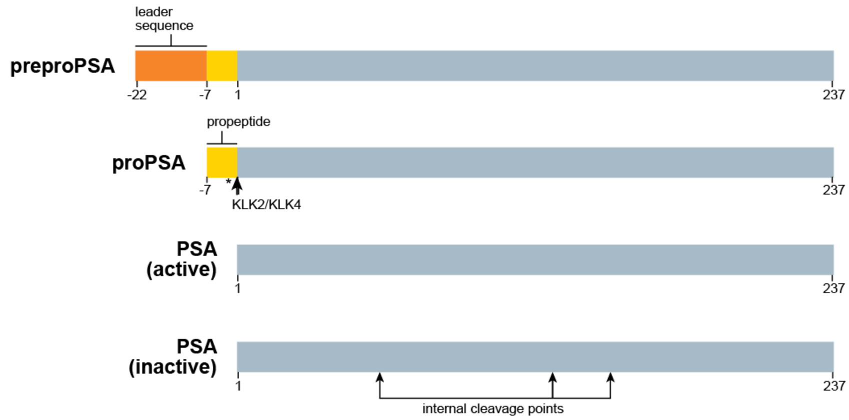

Figure 2. PSA protein structure. The leader sequence of preproPSA (amino acids -22 to -7) is removed to generate proPSA. Cleavage of the propeptide (-7 to 1) by KLK2 and KLK4 then generates active PSA. (ProPSA is sometimes cleaved at various positions within the propeptide to generate truncated forms; pro2PSA is produced by cleavage at the asterisk.) Active PSA may be further cleaved at the indicated internal points to generate inactive PSA.[2]

Measurement of alternate forms of PSA and its precursors has been explored as a means of increasing prostate cancer testing accuracy. In prostate cancer, loss of the prostatic basement membrane results in increased serum cPSA (Figure 1), reducing the fPSA/tPSA ratio.[2] Accordingly, the percentage of fPSA in serum (fPSA/tPSA x 100%; %fPSA) is inversely associated with prostate cancer risk and has been demonstrated to significantly improve the discrimination of prostate cancer from benign conditions, especially in patients with PSA levels in the 4-10 ng/mL range.[8,9] Nevertheless, %fPSA-based screening still results in a high number of unnecessary prostate biopsies and needless treatment of slow-growing tumors that otherwise may persist for many years with no ill effects (sometimes referred to as indolent tumors).

The *phi* test is designed to improve upon the specificity of PSA and %fPSA for prostate cancer detection. Developed by Beckman Coulter and widely used in Europe under CE mark approval, it was granted approval by the US Food and Drug Administration (FDA) in June 2012 for determining the probability that prostate cancer is present. *phi* is calculated as follows:

$$phi = (\text{pro2PSA} / \text{fPSA})(\text{tPSA}^{1/2})$$

This risk score, along with factors such as overall health and life expectancy, can help clinicians and patients determine whether a man would benefit from prostate biopsy.

Clinical Interpretation

In 2011, a multi-center pivotal clinical trial sponsored by Beckman Coulter demonstrated that *phi* significantly enhanced specificity for prostate cancer detection compared to PSA and %fPSA for men over age 50 with PSA in the 2-10 ng/mL range; in a receiving operator characteristic (ROC) analysis, the diagnostic accuracy of *phi* (~70%) was significantly greater than those for PSA, fPSA, and %fPSA (~53%, 62%, and 65%, respectively).[10] Higher *phi* values were significantly associated with increased probability of prostate cancer being present, and with more aggressive disease; for example, men with *phi* > 55 had a greater than 52% probability of prostate cancer (Figure 3) and a 4.7-fold increased risk of positive biopsy, while *phi* > 21.3 conveyed a 1.61-fold increased risk of moderately- or highly-aggressive cancer.[10] Moreover, *phi*—unlike PSA and fPSA—was not found to be associated with age or prostate volume. All study participants were between 50 and 84 years of age, had digital rectal examination (DRE) findings that were not suspicious for cancer, and had PSA levels in the diagnostic "gray zone" of 2-10 ng/mL; in this range, biopsy confirms the presence of cancer in only about 25% of patients.[10]

Multiple clinical trials have since corroborated the findings of the original Beckman Coulter-sponsored study. A recent systematic review and meta-analysis of eight studies totaling nearly 3,000 patients concluded that *phi* significantly improves the accuracy of prostate cancer detection in comparison with PSA or %fPSA, particularly in patients with PSA between 2-10 ng/mL.[11] The marked improvement in specificity of *phi*(Figure 4) represents a substantial advance in testing to distinguish prostate cancer from benign conditions.

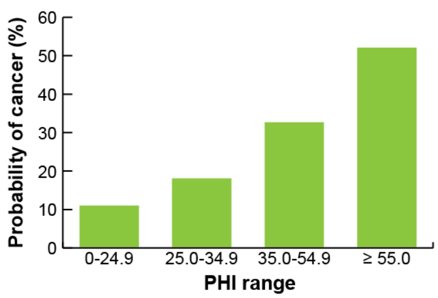

Figure 3. Probability of prostate cancer on biopsy, by *phi*. For PSA from 2-10 ng/mL.[10]

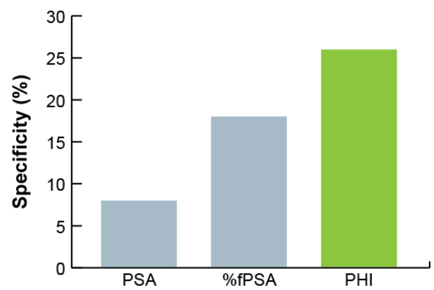

Figure 4. Specificity of PSA, %fPSA, and *phi* at 90% sensitivity, for PSA from 2-10 ng/mL.[10]

Treatment Considerations

Total PSA and %fPSA have limited utility for specifically detecting clinically significant prostate cancer. Reliance on these tests alone for prostate cancer diagnosis can lead to unnecessary biopsies and treatment of indolent tumors. To limit overtreatment, clinicians should consider screening male patients over the age of 50 with PSA and/or fPSA (%fPSA), and reflexing to *phi*/pro2PSA for those whose results indicate increased prostate cancer risk (i.e., PSA ≥ 2 ng/mL or %fPSA ≤ 25).[9,12] *Phi* is currently FDA approved as a reflex for PSA of 4-10 ng/mL; however, a growing body of evidence suggests that this test is also significantly more specific in patients with PSA from 2-4 ng/mL.[11,13] These findings suggest that clinicians may consider using *phi* as a reflex test for patients who have PSA from 2-4 ng/mL when other prostate cancer risk factors are present.

Prostate cancer risk factors include the following:[14]

- Age (risk rises rapidly after age 50; about 60% of cases are found in men over the age of 65)
- Race/ethnicity (prostate cancer occurs more often in men of African ancestry)

- Family history of prostate cancer (risk is more than doubled for men who have a father or brother with prostate cancer, and is much higher for men with several affected relatives)
- Diet high in red meat or high-fat dairy products, and low in fruits and vegetables
- Obesity (linked to risk of more aggressive prostate cancer)
- Smoking (linked to risk of more aggressive prostate cancer)
- Excessive alcohol intake
- Genetic mutations (e.g., *BRCA1* or *BRCA2*)
- Exposure to Agent Orange

Selection of a *phi* cutoff for referral to biopsy

Higher *phi* scores are associated with an increased probability of prostate cancer on biopsy. However, prostate biopsy is not without risk, and may cause complications such as pain, bleeding, and infection.[14] Furthermore, prostate biopsy carries a high risk of overdiagnosis; modeling analysis of a randomized controlled trial of PSA screening revealed rates of overdiagnosis ranging from 27% for 55-year-old individuals to 56% for 75-year-olds.[15] Rampant overdiagnosis of prostate cancer is problematic because ~90% of patients elect to undergo treatment, which may cause serious complications and side effects.[16] Prostate cancer diagnosis has also been shown to contribute to anxiety and depression, and is associated with significantly increased risk of cardiovascular events and suicide.[17,18] The decision of when to refer a patient for biopsy must therefore balance the potential benefits and harms of prostate cancer treatment, and may vary for each individual, depending upon factors such as age, overall health, family history of disease, and patient preference.

Selection of an appropriate *phi* score to guide clinical patient management should take into account both the percentage of actual cancers detected (sensitivity) and the percentage of healthy men who are accurately identified as cancer-free, or “true negatives" (specificity; see Table). For example, a *phi* value of 22.1 corresponds to 95% sensitivity and 14.1% specificity; therefore, choosing to refer patients with *phi* < 22.1 for biopsy will detect 95% of cancers while identifying 14% of true negatives (i.e., 1 in 7 cancer-free individuals would avoid biopsy). Similarly, using a *phi* cutoff of 27.0 (90% sensitivity, 31.1% specificity) would detect 90% of cancers while allowing nearly 1 in 3 cancer-free men to avoid biopsy. Raising the *phi* cutoff value to 31.3 (80%, sensitivity, 46.1% specificity) results in detection of 80% of cancers, while avoiding nearly half of unnecessary biopsies.[19]

It should also be noted that the intermediate-timeframe mortality rate for prostate cancer is extremely low; 5-, 10-, and 15-year survival rates are >99%, >98%, and 93%, respectively.[14] Clinical trials of active surveillance, in which men with a positive screening test for low-risk prostate cancer are closely monitored rather than receiving therapeutic treatment, consistently demonstrate high survival and low rates of cancer progression.[20] In one such study of 450 patients, the 10-year overall and prostate cancer-specific survival rates were 79%

Table. Sensitivity and specificity of *phi* cutoffs for men over age 50 with non-suspicious DRE. The percentage of cancers detected (sensitivity) and the percentage of cancer-free individuals spared from biopsy (specificity) must be considered, along with other factors, when selecting an appropriate *phi* cutoff.[19]

Sensitivity (%)	*phi* cutoff	Specificity (%)
99	**17.2**	4.2
98	**19.4**	8.4
95	**22.1**	14.1
90	**27.0**	31.1
85	**28.9**	37.7
80	**31.3**	46.1
75	**34.0**	55.7
70	**36.2**	63.2
65	**38.1**	65.9
60	**40.9**	73.4
55	**42.8**	76.3
50	**44.4**	80.5
45	**47.6**	83.8
40	**49.3**	85.3
35	**51.7**	88.9
30	**54.8**	89.8
25	**58.2**	91.0
20	**62.7**	92.5
15	**68.1**	94.3
10	**77.1**	96.7
5	**99.9**	100

and 97%, respectively, and only 30% of participants exhibited signs of disease progression over a 7-year follow-up period.[21] Even more strikingly, study participants were nearly 20 times more likely to die of unrelated causes than of prostate cancer.[21] Clinicians and patients may thus wish to consider the patient's expected lifespan, and whether prostate cancer treatment would significantly increase quality life-years, when determining whether biopsy is appropriate.

Using *phi* for clinical patient management

Patients whose test results indicate elevated prostate cancer risk may choose to undergo prostate biopsy or, instead, to be closely monitored for signs of disease progression ("active surveillance"). To minimize overtreatment, it is important to consider reflex testing prior to biopsy.

Prostate cancer prevention

Although the exact causes of prostate cancer are unknown, the following lifestyle and dietary modifications may reduce men's risk of developing the disease:[22,23]

- Weight loss (as appropriate)
- Exercise
- Smoking cessation
- Decreased alcohol consumption
- Increased consumption of green tea
- Increased intake of foods that have been shown to significantly reduce inflammation and cancer risk, including fresh fruits, carotenoid-rich foods, non-starchy vegetables, raw nuts and seeds, and omega-3 fatty acid-containing foods such as oily fish[24]
- Decreased intake of foods that may increase inflammation and cancer risk, such as red/processed meat, refined grains and sugars, highly heated or oxidized oils, and *trans* fats[24,25]
- Replacement of calories from carbohydrates and animal fats with calories from vegetable fats[26]
- Increased dietary intake of folate, lycopene, and soy
- Vitamin D supplementation

REFERENCES

1. National Cancer Institute. *SEER Cancer Statistics Factsheets: Prostate Cancer*. http://seer.cancer.gov/statfacts/html/prost.html. Accessed 12/20/2013.
2. Balk SP, Ko YJ, Bubley GJ. Biology of prostate-specific antigen. *J Clin Oncol* 2003;21(2):383-91.
3. Fauci AS, Braunwald E, Kasper DL, *et al.*Harrison's Principles of Internal Medicine. McGraw-Hill Professional. 2004.
4. National Cancer Institute. National Cancer Institute. *Prostate-Specific Antigen (PSA) Test*. http://www.cancer.gov/cancertopics/factsheet/detection/PSA. Accessed 12/19/2013.
5. Mikolajczyk SD, Catalona WJ, Evans CL, *et al.* Proenzyme forms of prostate-specific antigen in serum improve the detection of prostate cancer. *Clin Chem* 2004;50(6):1017-25.
6. Sokoll LJ, Sanda MG, Feng Z, *et al.* A prospective, multicenter, National Cancer Institute Early Detection Research Network study of [-2]proPSA: improving prostate cancer detection and correlating with cancer aggressiveness. *Cancer Epidemiol Biomarkers Prev* 2010;19(5):1193-200.
7. Lilja H, Ulmert D, Vickers AJ. Prostate-specific antigen and prostate cancer: prediction, detection and monitoring. *Nat Rev Cancer* 2008;8(4):268-78.
8. Woodrum DL, Brawer MK, Partin AW, *et al.* Interpretation of free prostate specific antigen clinical research studies for the detection of prostate cancer. *J Urol* 1998;159(1):5-12.
9. Catalona WJ, Partin AW, Slawin KM, *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.
10. Catalona WJ, Partin AW, Sanda MG, *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.
11. Filella X, Gimenez N. Evaluation of [-2] proPSA and Prostate Health Index (phi) for the detection of prostate cancer: a systematic review and meta-analysis. *Clin Chem Lab Med* 2013;51(4):729-39.
12. Catalona WJ, Richie JP, Ahmann FR, *et al.* Comparison of digital rectal examination and serum prostate specific antigen in the early detection of prostate cancer: results of a multicenter clinical trial of 6,630 men. *J Urol* 1994;151(5):1283-90.
13. Stephan C, Vincendeau S, Houlgatte A, *et al.* Multicenter evaluation of [-2]proprostate-specific antigen and the prostate health index for detecting prostate cancer. *Clin Chem* 2013;59(1):306-14.
14. American Cancer Society. American Cancer Society. *Prostate Cancer*. http://www.cancer.org/cancer/prostatecancer/detailedguide/. Accessed 12/20/2013.

15. Draisma G, Boer R, Otto SJ, *et al.* Lead times and overdetection due to prostate-specific antigen screening: estimates from the European Randomized Study of Screening for Prostate Cancer. *J Natl Cancer Inst* 2003;95(12):868-78.
16. Borza T, Konijeti R, Kibel AS. Early detection, PSA screening, and management of overdiagnosis. *Hematol Oncol Clin North Am* 2013;27(6):1091-110, vii.
17. Klotz L. Active surveillance for prostate cancer: patient selection and management. *Curr Oncol* 2010;17 Suppl 2:S11-7.
18. Fall K, Fang F, Mucci LA, *et al.* Immediate risk for cardiovascular events and suicide following a prostate cancer diagnosis: prospective cohort study. *PLoS Med* 2009;6(12):e1000197.
19. Hybritech p2PSA [package insert]. Beckman Coulter, Brea, CA: February 2012.
20. Dall'Era MA, Albertsen PC, Bangma C, *et al.* Active surveillance for prostate cancer: a systematic review of the literature. *Eur Urol* 2012;62(6):976-83.
21. Klotz L, Zhang L, Lam A, *et al.* Clinical results of long-term follow-up of a large, active surveillance cohort with localized prostate cancer. *J Clin Oncol* 2010;28(1):126-31.
22. National Cancer Institute. National Cancer Institute. *PDQ® Prostate Cancer Prevention*. http://www.cancer.gov/cancertopics/pdq/prevention/prostate/healthprofessional. Accessed 1/3/2014.
23. Hori S, Butler E, McLoughlin J. Prostate cancer and diet: food for thought? *BJU Int* 2011;107(9):1348-59.
24. World Cancer Research Fund / American Institute for Cancer Research. *Food, nutrition, physical activity, and the prevention of cancer: a global perspective.* Washington, DC: 2007.
25. Hu J, La Vecchia C, de Groh M, *et al.* Dietary transfatty acids and cancer risk. *Eur J Cancer Prev* 2011;20(6):530-8.
26. Richman EL, Kenfield SA, Chavarro JE, *et al.* Fat intake after diagnosis and risk of lethal prostate cancer and all-cause mortality. *JAMA Intern Med* 2013;173(14):1318-26.

Prostate Health Index (*phi*)

Scientific Papers

Scientific Papers

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. Journal of Urology, 2011

Evaluation of pro2PSA and Prostate HealthIndex (*phi*) for the detection of prostate cancer:a systematic review and meta-analysis; DOI 10.1515/cclm-2012-0410 Clin Chem Lab Med 2012; aop Filella phi Review Meta analysis CCLM 2012

The Prostate Health Index: a new test for the detection of prostate cancer, Stacy Loeb and William J. Catalona, PHI a New PCa Test Catalona-Loeb 2014

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 PHI PCA3 Sarcosine 2015 Path Outcomes in RP

Prognostic accuracy of Prostate Health Index and urinary Prostate CancerAntigen 3 in predicting pathologic features after radical prostatectomy. Urol Oncol 2015 Jan 6. pii: S1078-1439(14)00448-7.doi: 10.1016/j.urolonc.2014.12.002.PHI vs PCA3 Urol Oncol 2015 Jan 6

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A Multi-Center Study of [−2]Pro-Prostate-Specific Antigen (PSA) in Combination with PSA and Free PSA for Prostate Cancer Detection in the 2.0 to 10.0 ng/mL PSA Range

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*Not intended as off-label promotion of any Beckman Coulter, Inc. product.

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Mizrahi, Broyles, Shin and Cruz had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.
Study concept and design: Catalona, Mizrahi, Broyles, Shin.
Acquisition of the data: Catalona, Partin, Sanda, Wei, Klee, Bangma, Slawin, Marks, Broyles, Chan, Sokoll, Roberts, van Schaik, Mizrahi.
Analysis and interpretation of the data: Catalona, Partin, Sanda, Klee, Slawin, Marks, Chan, Sokoll, Roberts, van Schaik, Wei, Bangma, Broyles, Shin, Cruz, Loeb, Mizrahi. **An independent statistical analysis was performed by Edward F. Vonesh, PhD of the Department of Preventive Medicine, Northwestern University**.
Drafting of the manuscript: Catalona, Mizrahi, Broyles, Loeb.
Critical revision of the manuscript for important intellectual content: Catalona, Mizrahi, Broyles, Partin, Sanda, Wei, Loeb, Bangma, van Schaik, Vonesh.
Statistical analysis: Shin. *Obtained funding*: Catalona, Partin, Sanda, Wei, Klee, Bangma, Slawin, Marks, Chan, Sokoll, Roberts, van Schaik.
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Abstract

Purpose—PSA and free PSA (fPSA) have limited specificity for detecting clinically significant, curable prostate cancer (PCa), leading to unnecessary biopsies and detection and treatment of some indolent tumors. [−2]proPSA (p2PSA) may improve specificity for detecting clinically significant PCa. Our objective was to evaluate p2PSA, fPSA, and PSA in a mathematical formula (prostate health index [*phi*] = [−2]proPSA / fPSA) × $PSA^{1/2}$) to enhance specificity for detecting overall and high-grade PCa.

Materials and Methods—We enrolled 892 men in a prospective multi-institutional trial with no history of PCa, normal rectal examination, a PSA of 2–10 ng/mL, and ≥6- core prostate biopsy. We examined the relationship of serum PSA, %fPSA and *phi* with biopsy results. The primary endpoints were the specificity and AUC using *phi* to detect overall and Gleason ≥7 prostate cancer on biopsy compared with %fPSA.

Results—For the 2–10 ng/mL PSA range, at 80–95% sensitivity, the specificity and AUC (0.703) of *phi* exceeded those of PSA and %fPSA. Increasing *phi* was associated with a 4.7-fold increased risk of PCa and 1.61-fold increased risk of Gleason ≥7 disease on biopsy. The AUC for *phi* (0.724) exceeded that of %fPSA (0.670) in discriminating between PCa with Gleason ≥ 4+3 vs. lower grade disease or negative biopsies. *Phi* results were not associated with age and prostate volume.

Conclusions—*Phi* may be useful in PCa screening to reduce unnecessary biopsies in men age ≥50 years with PSA 2–10 ng/mL and negative DRE, with minimal loss in sensitivity.

INTRODUCTION

PSA testing was approved by the FDA using a 4.0 ng/mL cutoff for recommending prostate biopsy. Lower cutoffs further enhance early prostate cancer (PCa) detection,[1] since PSA correlates with the risk of overall and high-grade PCa at PSA concentrations <4 ng/mL.[2] However, PSA testing may be confounded by benign conditions.

The low specificity at PSA <10.0 ng/mL has created a diagnostic gray zone in which PCa is found on biopsy in ~25% of patients. This is important, since most PCa is curable at PSA <10.0 ng/mL; whereas, PSA >10 ng/mL often portends advanced disease.[3]

PSA in serum is either complexed with proteins or in an unbound form called free PSA (fPSA).[4] At PSA levels of 4.0–10.0 ng/mL, the ratio of fPSA to PSA (%fPSA) significantly improves discrimination between PCa and benign conditions.[5]

Different regions of the prostate contain varying proportions of fPSA isoforms, including proPSA that is associated with PCa. [−2]proPSA (p2PSA) is the primary form in PCa tissue.[6–8] At PSA of 2.0–10.0 ng/mL, p2PSA further improves specificity for PCa detection relative to %fPSA.[9–13]

The utility of p2PSA at PSA <4.0 ng/mL and its relationship to PCa aggressiveness are relevant to the PCa screening debate, including concerns about overdiagnosis and overtreatment.[13–19] Preliminary evidence suggests that a higher percentage of p2PSA may be associated with more aggressive PCa.[10, 12, 13, 19]

Selecting thresholds for clinical use of p2PSA has received limited study. We evaluated the relationship of p2PSA** combined with fPSA and PSA in a mathematical formula called Prostate Health Index (*phi*) with prostate cancer detection and tumor features.

METHODS

Study Design

We conducted a multi-center, double-blind, case-control clinical trial to validate *phi* in the 2.0–10.0 ng/mL PSA range. This formula was developed from an independent dataset,[20] and is calculated as (p2PSA pg/mL / fPSA ng/mL) × (PSA ng/mL) ^{½}. Intuitively, higher [−2] proPSA and PSA with a lower fPSA has greater likelihood of PCa. The study protocol was approved by the IRB of each participating institution, and all participants provided informed consent.

Study population

We evaluated 1372 men from October 2003 through June 2009 from 8 medical centers. The study cohort included men age ≥50 years of all ethnic backgrounds who met the following criteria: (1) no history of PCa, (2) non-suspicious digital rectal examination (DRE) findings, (3) pre-study PSA of 1.5–11.0 ng/mL (all PSA concentrations were re-tested in the Access Hybritech assay, and only those 2–10 ng/mL were included), (4) ≥6 core biopsy within 6 months of blood draw, and (5) a histologic diagnosis from prostate biopsy.

Exclusion criteria were: (1) treatment with medications that alter PSA levels or interventions such as transurethral resection of the prostate prior to blood draw, (2) acute prostatitis or urinary infection at blood draw, (3) a final Access Hybritech PSA value outside the 2.0–10.0 ng/mL range, (4) no blood draw or biopsy at the appropriate time interval, or (5) prior androgen-replacement therapy.

Seven men were excluded due to unevaluable tests from hemolyzed or lipemic samples or p2PSA duplicate results with >15% coefficient of variation at p2PSA concentrations ≤ 20 pg/mL, for which samples could not be retested. Finally, one site enrolled only men aged 55–75 years (our study enrolled men aged ≥ 50 years), and our study-specific sample storage limit (≤ 5 years) further limited the evaluable population to men aged 62–74. Because the age distribution from this site may not be representative of the target population, we performed separate analyses excluding and including these men.

The final study population of 892 men included: (1) 121 (13.6%) prospectively enrolled, (2) 743 (83.3%) prospectively enrolled under separate protocols, and (3) 28 (3.1%) retrospective samples. The study population included 706 (79.2%) initial biopsies, 159 (17.8%) repeat biopsies, and 27 (3%) with unknown history of prior biopsy. Each institution enrolled an approximately equal number of men with or without PCa, for a total of 430 (48.2%) men with PCa and 462 (51.8%) without. Participants and investigators were blinded to p2PSA results, and testing sites were blinded to individual clinical information.

Test Methods

Access Hybritech p2PSA, PSA, and fPSA assays were measured on the Beckman Coulter Access 2 Immunoassay Analyzer***. Serum samples were collected and processed within 8 hours, then stored frozen at ≤−70°C prior to testing (≤5 years from the date of blood draw), conditions that allowed accurate measurement of *phi*.[21] Samples were tested at one of 3

**Pending FDA approval.

***All trademarks are the property of their respective owners.

laboratories. PSA and fPSA assays were run using one-sample replicate. The p2PSA assay was run in duplicate (first replicate used for data analysis, consistent with the proposed product labeling) according to the testing protocol. Evaluation of the first replicate compared to the mean of duplicates using Passing-Bablock regression analyses showed no difference (Spearman R=0.9985). The p2PSA assay is a two-site immunoenzymatic sandwich assay using specific monoclonal antibodies and 6 calibrators from 0- 5000 pg/mL.

Statistical Methods

The minimum sample size was estimated as 295 patients without cancer to detect a 10% difference in specificity between *phi* and % fPSA at $\alpha = 0.05$ and $\beta = 0.10$. In addition, a minimum sample size of 350 cancer patients was determined to accurately estimate sensitivity at 95% with a 95% confidence interval of ± <3%. The target sample size was then increased to 400 participants in each group.

The primary null hypothesis was that *phi* has no greater specificity than %fPSA at 95% sensitivity. This hypothesis was tested using bootstrap-based receiver operating characteristic (ROC) analysis.[22] Briefly, 1000 datasets of benign and PCa patients were generated to repetitively sample the study population.[23–25] Differences in the specificity between phi and %fPSA at 95% sensitivity were calculated for the 1000 pairs of replicate datasets. The standard error of the difference in specificities was then estimated with adjustment for correlation between the results of the two tests. Finally, the bootstrap-estimated standard error was used to evaluate whether the difference in specificities is >0 assuming normal distribution of the differences. A one-sided statistical test was performed for this analysis. This method was also used to compare the specificities of *phi* and %fPSA at 90%, 85%, and 80% sensitivities.

The secondary null hypothesis was that the area under the ROC curve (AUC) for *phi* equals that of %fPSA. This hypothesis was tested by evaluating whether the difference between the estimated AUCs for the two tests equals 0 using empirical methods.[26, 27] The standard error of the difference was calculated accounting for the correlation in AUCs as appropriate for comparison of paired data. The difference between the two estimated AUCs has been shown to have a Chi-square distribution with one degree of freedom. The AUCs for *phi* and %fPSA were also estimated for each prostate volume tertile to determine whether the observed trend in AUCs differed by prostate volume.

The validity of pooling data across sites was evaluated by fitting a logistic regression model with cancer status as the dependent variable, with *phi* (dichotomized at the estimated cutoff for 95% sensitivity) and site as independent predictors including interaction terms for site and *phi*. A statistically significant parameter estimates for this interaction terms was considered evidence of heterogeneity in *phi* performance by site.

Comparisons between participant subgroups were performed using the Wilcoxon Rank-Sum test for continuous variables and the $\chi 2$ test for categorical variables. Two-sided statistical tests were used on all analyses except as noted above, and statistical significance was defined as $p<0.05$. All analyses were performed using SAS version 9.2 (SAS Institute, Cary, North Carolina).

Individual Patient Risk Assessment

A 25% PCa detection rate has been previously reported in men with PSA of 2.0–10.0 ng/mL.[3] For this study, cancer patients were over-sampled by design, resulting in 48.2% of study participants with PCa. Since the proportion of PCa was determined by design, direct calculation of PCa probability would result in inflated estimates for detecting PCa. Therefore, to obtain more accurate risk estimates for PCa, we adjusted the proportion of PCa to 25% by repetitively sampling the study population 1000 times with each replicate dataset consisting of 462 (75%) benign and 154 (25%) cancer participants.[23–25] The mean probability of cancer in the bootstrapped datasets for each *phi* range was used as the point estimate, and bootstrap-estimated standard errors were used to calculate 95% confidence intervals. Likewise, relative risk estimates were calculated for each replicate dataset by dividing the probability of PCa in each *phi* range to that of *phi* 0–24.9. The mean relative risk and bootstrap-estimated standard errors were used to calculate the risk estimate and 95% confidence intervals. In addition, age-stratified probability estimates for PCa were calculated to determine whether observed trends persist in all age groups.

Association of phi with Gleason Score

Among participants with PCa, the probability of a Gleason score ≥7 was calculated directly from the proportion of participants in each *phi* range with Gleason score ≥7. Risk ratios were estimated by dividing the probability of Gleason score ≥7 in each *phi* range to that of *phi* 0–24.9. Confidence intervals were calculated using the normal approximation of the binomial distribution. The Cochrane-Armitage test for trend was used to determine whether increasing *phi* ranges corresponds to increasing probability of PCa with Gleason score ≥7. ROC analysis was used to evaluate the clinical utility of *phi* in detecting PCa with Gleason scores 4+3 or higher.

RESULTS

Participants

Table 1 shows the demographics and results for each assay. Both *phi* and p2PSA were significantly higher in PCa than controls; whereas, fPSA and %fPSA were lower in PCa than controls. Total PSA and age were comparable between groups.

Of the participants, 89.8% had ≥12-core biopsy, and 98% had ≥10 cores. Overall, 30.6%, 49.9%, and 19.6% of participants were aged 50–59, 60–69 and 70–84 years, respectively. Mean age and PSA were similar across the 7 clinical sites. In addition, none of the interaction terms in the statistical model for evaluating heterogeneity by site was significant, supporting data pooling across sites. There were no significant differences in age (P=0.123), PSA (P=0.106), p2PSA (P=0.088), %fPSA (P=0.125), or *phi* (P=0.848) between Caucasians and African-Americans.

Receiver Operating Characteristic (ROC) Results

Figure 1 shows the sensitivity and specificity for all observed PSA, fPSA, p2PSA, %fPSA, and *phi* cutoffs in the 2.0–10.0 ng/mL PSA range. At a given sensitivity, *phi* demonstrated greater specificity than the other analytes (Table 2). At 95% sensitivity, the specificity of *phi* was 16.0% compared to 8.4% for %fPSA (P=0.015), 7.6% for p2PSA, 6.5% for PSA, and 3.5% for fPSA, rejecting the primary null hypothesis. Moreover, at lower sensitivities (90%, 85%, and 80%) for PCa detection, the specificity of *phi* was significantly greater than %fPSA (i.e., unnecessary biopsies possibly avoided: 26% vs. 18%, P= 0.036; 39% vs. 28%, P= 0.006; 45% vs. 37%, P= 0.031, respectively).

The AUC for PCa detection was significantly greater for *phi* (AUC=0.703) than for %fPSA (0.648, P=0.004), fPSA (0.615), p2PSA (0.557), or PSA (0.525), rejecting the secondary null hypothesis.

Individual Patient Risk Assessment

Higher *phi* values were associated with an increased risk of PCa detection based upon the adjusted 25% proportion of PCa cases (Table 3). Of the study population, 25%, 33%, 30%, and 13% had *phi* values of 0–24.9, 25.0–34.9, 35.0–54.9, and ≥ 55.0, respectively. Compared to *phi* < 25.0, the relative risk of PCa detection on biopsy was 1.6-, 3.0-, and 4.7-fold higher at *phi* values of 25.0–34.9, 35.0–54.9, and ≥ 55.0, respectively. Overall, a *phi*≥ 55.0 was associated with a 52.1% probability of PCa.

Age and Probability of PCa

Higher *phi* values were also associated with higher bootstrapped risk estimates of PCa within each age group. The probability (and relative risk [RR]) of PCa ranged from 10.9% (*phi* 0–24.9) to 53.4% (*phi* ≥ 55) (RR 4.9) for the 50–59 age group, 12.5% (*phi* 0–24.9) to 54.5% (*phi* ≥ 55) (RR 4.4) for the 60–69 age group, and 5.8% (*phi* 0–24.9) to 44.8% (*phi* ≥ 55) (RR 7.7) for the > 70 age group.

Association of phi with Gleason Score

Phi also had a significant relationship with biopsy Gleason score (r=0.138, P=0.004). Among participants with PCa, biopsy Gleason score was <7 in 290 (67.6%) and ≥7 in 139 (32.4%) Compared to *phi* < 25.0, the relative risk of Gleason ≥ 7 PCa increased to 1.08 for *phi* values from 25.0–34.9, 1.15 for *phi* values from 35.0–54.9, and 1.61 for *phi* ≥ 55.0. The corresponding proportion of cancers with a Gleason score ≥ 7 increased from 26.2% to 28.2%, 30.1%, and 42.1% at *phi* values of 0–24.9, 25.0–34.9, 35.0–54.9, and ≥ 55.0, respectively (Cochran-Armitage test for trend, P=0.013) (Table 4). The AUC for *phi* (0.724) exceeded that of %fPSA (0.670) in discriminating between Gleason ≥ 4+3 vs. lower Gleason grade PCa or negative biopsies.

Relationship of TRUS volume and phi

The AUCs for *phi* exceeded those of %fPSA in all three prostate volume tertiles (≤38, 39–53, and ≥54cc): 1^{st} tertile: AUC 0.693 for *phi* vs. 0.614 for %fPSA; 2^{nd} tertile: 0.707 vs. 0.593; 3^{rd} tertile: 0.642 vs. 0.559.

Evaluation of Excluded Participants

AUCs for *phi* with and without the excluded site were 0.696 and 0.703, respectively. Similarly, AUCs for %fPSA were 0.634 and 0.648, respectively.

COMMENT

Prostate biopsy is routinely recommended for suspicious DRE results regardless of PSA.[3] Biopsy is also recommended using PSA thresholds ranging from 2.5 to 4.0 ng/mL.[1, 2, 15] However, this has led to unnecessary biopsies and possible over-detection of some cancers.[15–17] To elucidate whether *phi* PSA-isoform measurement can improve PCa early detection, we examined a large, prospective cohort to predict biopsy findings in patients with moderate PSA elevations (2.0–10.0 ng/mL) and benign DRE findings. Such men are at higher risk of PCa (25% cancer detection rate compared with 4% in the general male population aged ≥50 years).[3] Our bootstrapped population was designed to mirror this 25% incidence of PCa on biopsy.

Prostate biopsy may be associated with discomfort, anxiety, and financial costs. Minor complications occur frequently, and major complications are possible, underscoring the need for more specific markers to reduce unnecessary biopsies. We sought to determine the utility of p2PSA and *phi* for this clinical goal.

Precursor forms of PSA have been shown to improve the accuracy of PSA for detecting PCa.[5, 6, 9–12, 28, 29] Specifically, preliminary reports suggest that p2PSA may be useful at PSA concentrations from 2.0–10.0 ng/mL.[6, 9–12, 28, 29] Some, but not all, studies have suggested an association between proPSA and PCa aggressiveness.[10, 12, 20] Thus, p2PSA and *phi* are being investigated in active surveillance programs to help overtreatment of insignificant PCa.[19, 30]

Catalona et al. previously reported in the PSA range of 2.0–10.0 ng/mL, the proPSA-to-fPSA ratio (%proPSA) yielded a higher specificity than %fPSA.[9] Results from a separate multi-site study also supported the role of p2PSA, in combination with PSA and fPSA, in reducing unnecessary biopsies.[12, 13]

In the current study, the specificity for *phi* was higher than %fPSA at all pre-specified sensitivities, and PCa risk increased directly with increasing *phi* values. This suggests a role for *phi* as a patient monitoring tool, since increasing *phi* values reflect PCa risk.[19] For example, at 95% sensitivity, the specificity of *phi* was 16.0% compared to 8.4% for %fPSA. Moreover, at lower sensitivities (90%, 85%, and 80%) for PCa detection that might be preferred to reduce the detection of possibly "insignificant" tumors, *phi* had a significantly greater specificity than %fPSA. These results were consistent across age groups, PSA concentrations, and ethnic groups, suggesting that they are representative of the intended-use population.

For individual risk assessment, the probability of PCa varied considerably based upon *phi* values. For example, a man with a *phi* ≥ 55 (13% of the study population) had a > 52% probability of PCa and 4.7-fold increased relative risk of positive biopsy. In contrast, at approximately 90% sensitivity, a patient with a *phi* < 25 had an 11% probability of PCa.

For the PCa group, higher *phi* values were also significantly associated with a higher percentage of biopsy Gleason grade ≥ 7, ranging from 26% to 42% for *phi* concentrations < 25 and ≥ 55, respectively. For the entire study population, the AUC for *phi* (0.724) exceeded that of %fPSA (0.670) in discriminating Gleason ≥ 4+3 PCa vs. lower Gleason grade PCa or negative biopsies. Using a *phi* cutoff of 21.3 (95% sensitivity), 25% of missed cancers were Gleason score ≥7; therefore, careful surveillance is necessary. The AUCs for *phi* also exceeded those of %fPSA in all three prostate volume tertiles, suggesting that *phi* provides better discrimination of PCa from benign disease than %fPSA across the spectrum of prostate volumes. Because *phi* did not differ by age and race these results suggest that *phi* may be applicable to a broad spectrum of men as an adjunct to predict clinically-significant PCa.

The large number of subjects in the present validation study provides confidence in the *phi* cutoffs determined. *Phi* is highly effective when used in patients with moderately elevated PSA concentrations who may be most likely to benefit from early diagnosis and curative PCa treatment. A physician might recommend biopsy for a patient with a *phi* ≥ 55.0 (risk = 52.1%) and surveillance for some men with a *phi* <25.0 (risk = 11.0%). For patients reluctant to undergo prostatic biopsy, a high *phi* might increase compliance with the appropriate follow-up.

We conclude that the *phi* measurement ([−2]proPSA / fPSA) × $PSA^{1/2}$) may be useful to reduce unnecessary biopsies with improved specificity at various sensitivities for PCa detection in men age ≥50 years with PSA concentrations from 2.0–10.0 ng/mL, and negative DRE findings.****

****Our results apply to the Access Hybritech p2PSA, PSA and fPSA assays on the Beckman Coulter Access Immunoassay Systems, as studies have shown that results differ when assays from different manufacturers or standardization are used.[31]

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REFERENCES

1. Krumholtz JS, Carvalhal GF, Ramos CG, et al. Prostate-specific antigen cutoff of 2.6 ng/mL for prostate cancer screening is associated with favorable pathologic tumor features. Urology. 2002; 60:469. [PubMed: 12350486]
2. Thompson IM, Pauler DK, Goodman PJ, 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; 350:2239. [PubMed: 15163773]
3. Catalona WJ, Richie JP, Ahmann FR, et al. Comparison of digital rectal examination and serum prostate specific antigen in the early detection of prostate cancer: results of a multicenter clinical trial of 6,630 men. J Urol. 1994; 151:1283. [PubMed: 7512659]
4. Lilja H, Christensson A, Dahlen U, et al. Prostate-specific antigen in serum occurs predominantly in complex with alpha 1-antichymotrypsin. Clin Chem. 1991; 37:1618. [PubMed: 1716536]
5. Catalona WJ, Partin AW, Slawin KM, et al. Use of the percentage of free prostate-specific antigen to enhance differentiation of prostate cancer from benign prostatic disease: a prospective multicenter clinical trial. Jama. 1998; 279:1542. [PubMed: 9605898]
6. Mikolajczyk SD, Catalona WJ, Evans CL, et al. Proenzyme forms of prostate-specific antigen in serum improve the detection of prostate cancer. Clin Chem. 2004; 50:1017. [PubMed: 15054080]
7. Mikolajczyk SD, Grauer LS, Millar LS, et al. A precursor form of PSA (pPSA) is a component of the free PSA in prostate cancer serum. Urology. 1997; 50:710. [PubMed: 9372880]
8. Chan TY, Mikolajczyk SD, Lecksell K, et al. Immunohistochemical staining of prostate cancer with monoclonal antibodies to the precursor of prostate-specific antigen. Urology. 2003; 62:177. [PubMed: 12837462]
9. Catalona WJ, Bartsch G, Rittenhouse HG, et al. Serum pro prostate specific antigen improves cancer detection compared to free and complexed prostate specific antigen in men with prostate specific antigen 2 to 4 ng/ml. J Urol. 2003; 170:2181. [PubMed: 14634374]
10. Catalona WJ, Bartsch G, Rittenhouse HG, et al. Serum pro-prostate specific antigen preferentially detects aggressive prostate cancers in men with 2 to 4 ng/ml prostate specific antigen. J Urol. 2004; 171:2239. [PubMed: 15126794]
11. Sokoll LJ, Chan DW, Mikolajczyk SD, et al. Proenzyme psa for the early detection of prostate cancer in the 2.5–4.0 ng/ml total psa range: preliminary analysis. Urology. 2003; 61:274. [PubMed: 12597929]
12. Sokoll LJ, Wang Y, Feng Z, et al. [−2]proenzyme prostate specific antigen for prostate cancer detection: a national cancer institute early detection research network validation study. J Urol. 2008; 180:539. [PubMed: 18550118]
13. Sokoll LJ, Sanda MG, Feng Z, et al. A prospective, multicenter, National Cancer Institute Early Detection Research Network study of [−2]proPSA: improving prostate cancer detection and correlating with cancer aggressiveness. Cancer Epidemiol Biomarkers Prev. 19:1193. [PubMed: 20447916]

14. Jemal A, Thun MJ, Ries LA, et al. Annual report to the nation on the status of cancer, 1975–2005, featuring trends in lung cancer, tobacco use, and tobacco control. J Natl Cancer Inst. 2008; 100:1672. [PubMed: 19033571]
15. Schroder FH, Hugosson J, Roobol MJ, et al. Screening and prostate-cancer mortality in a randomized European study. N Engl J Med. 2009; 360:1320. [PubMed: 19297566]
16. Andriole GL, Grubb RL 3rd, Buys SS, et al. Mortality results from a randomized prostate-cancer screening trial. N Engl J Med. 2009; 360:1310. [PubMed: 19297565]
17. Welch HG, Albertsen PC. Prostate Cancer Diagnosis and Treatment After the Introduction of Prostate-Specific Antigen Screening: 1986–2005. J Natl Cancer Inst. 2009
18. Hugosson J, Carlsson S, Aus G, et al. Mortality results from the Goteborg randomised population-based prostate-cancer screening trial. Lancet Oncol. 2010; 11:725. [PubMed: 20598634]
19. Makarov DV, Isharwal S, Sokoll LJ, et al. Pro-prostate-specific antigen measurements in serum and tissue are associated with treatment necessity among men enrolled in expectant management for prostate cancer. Clin Cancer Res. 2009; 15:7316. [PubMed: 19934305]
20. Jansen FH, van Schaik RH, Kurstjens J, et al. Prostate-Specific Antigen (PSA) Isoform p2PSA in Combination with Total PSA and Free PSA Improves Diagnostic Accuracy in Prostate Cancer Detection. Eur Urol. 2010; 50:921. [PubMed: 20189711]
21. Semjonow A, Kopke T, Eltze E, et al. Pre-analytical in-vitro stability of [−2]proPSA in blood and serum. Clin Biochem. 43:926. [PubMed: 20450900]
22. Qin G, Hsu YS, Zhou XH. New confidence intervals for the difference between two sensitivities at a fixed level of specificity. Stat Med. 2006; 25:3487. [PubMed: 16345124]
23. Hosmer, DW.; Lemeshow, S. Applied Logistic Regression. New York, NY: John Wiley & Sons Inc.; 1989.
24. Efron, B. The Jackknife, the Bootstrap, and Other Resampling Plans. Philadelphia, PA: Society for Industrial and Applied Mathematics; 1982.
25. Efron B. Better bootstrap confidence intervals. J Am Stat Assoc:. 1993; 171
26. Pepe, MS. The Statistical Evaluation of Medical Tests for Classification and Prediction. New York, NY: Oxford University Press; 2003.
27. DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. Biometrics. 1988; 44:837. [PubMed: 3203132]
28. de Vries SH, Raaijmakers R, Blijenberg BG, et al. Additional use of [−2] precursor prostate-specific antigen and "benign" PSA at diagnosis in screen-detected prostate cancer. Urology. 2005; 65:926. [PubMed: 15882725]
29. Naya Y, Fritsche HA, Bhadkamkar VA, et al. Evaluation of precursor prostate-specific antigen isoform ratios in the detection of prostate cancer. Urol Oncol. 2005; 23:16. [PubMed: 15885578]
30. Isharwal S, Makarov DV, Sokoll LJ, et al. Prostate Health Index and diagnostic biopsy tissue DNA content combination improves accuracy to predict the need for prostate cancer treatment among men enrolled in a proactive surveillance program. Cancer Epidemiol Biomarkers Prev. 2010 in press.
31. Loeb S, Chan DW, Sokoll L, et al. Prostate specific antigen assay standardization bias could affect clinical decision making. J Urol. 2008; 180:1959. [PubMed: 18801532]

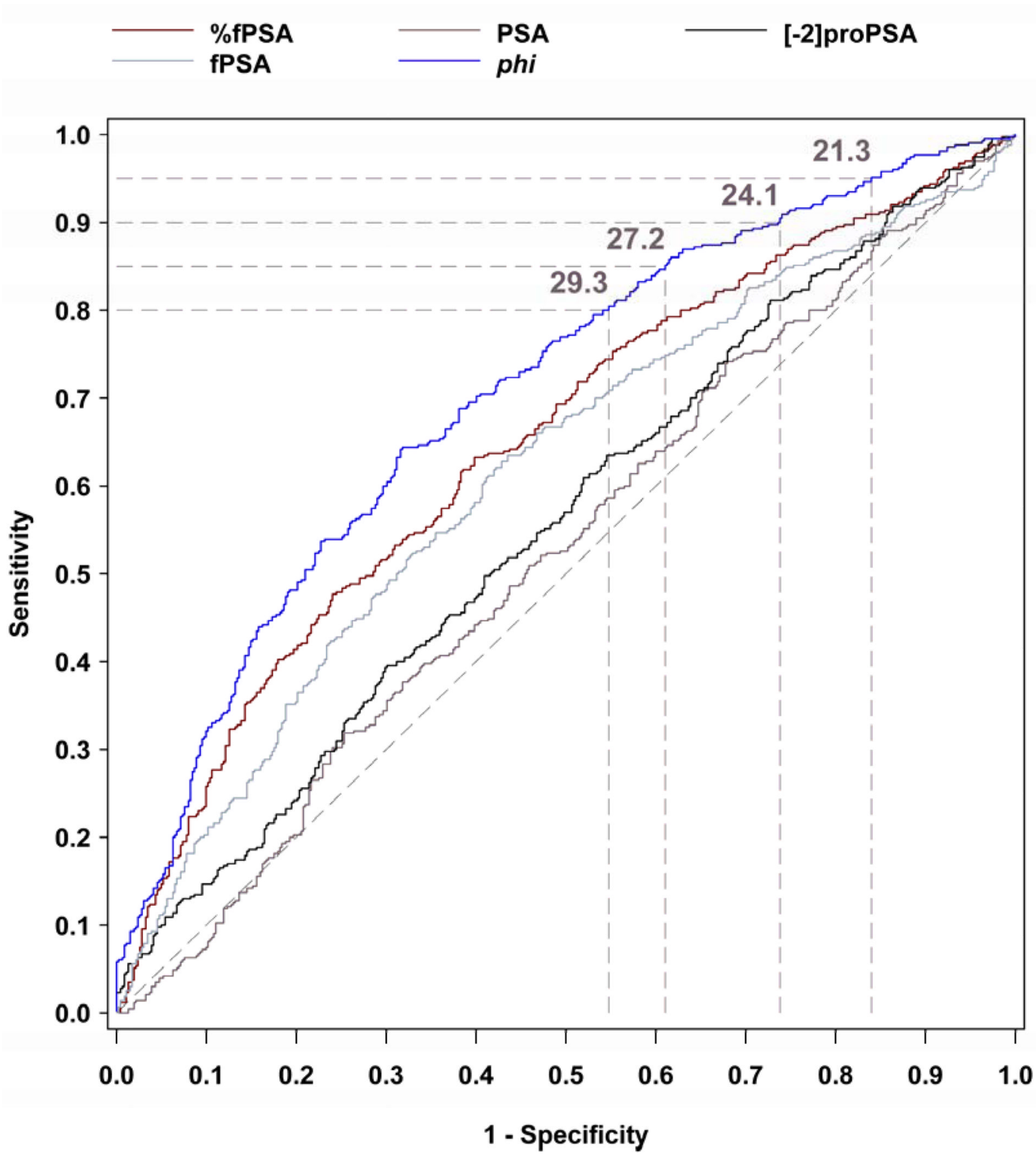

Figure 1.
PSA, fPSA, [−2]proPSA, %fPSA, and *Phi* ROC Curves in the 2–10 ng/mL PSA Range
Sensitivity × 1-Specificity for Sequential Cutpoints

TABLE 1

Clinical Characteristics of the Study Population

Characteristic		Benign N=462	Cancer N=430	p-value	Total N=892
Age	**Median**	63.0	63.0		63.0
	Mean ± SD	62.6 ± 7.0	63.0 ± 7.1		62.8 (7.0)
	Range	50 – 84	50 – 84		50 – 84
				0.477	
Race, n(%)	**Caucasian**	361 (78.1)	365 (84.9)		726 (81.4)
	African-American	24 (5.2)	22 (5.1)		46 (5.2)
	Other	22 (4.8)	9 (2.1)		31 (3.5)
	Unknown	55 (11.9)	34 (7.9)		89 (10.0)
				0.025	
Ethnicity, n(%)	**Hispanic**	14 (3.0)	6 (1.4)		20 (2.2)
	Not Hispanic	187 (40.5)	153 (35.6)		340 (38.1)
	Unknown	261 (56.5)	271 (63.0)		532 (59.6)
				0.059	
Prostate Volume	**Median**	51.0	40.0		45.0
	Mean ± SD	55.1 ± 23.2	44.3 ± 19.4		50.1 ± 22.2
	Range	16 – 209	14 – 120		14 – 209
				<0.001	
Prior Biopsy, n(%)	**No prior biopsy**	345 (74.7)	361 (84.0)		706 (79.2)
	Prior biopsy	105 (22.7)	54 (12.6)		159 (17.8)
	Unknown	12 (2.6)	15 (3.5)		27 (3.0)
				<0.001	
Gleason Score, n(%)	**5**	Not Applicable	1 (0.2)		1 (0.2)
	6		289 (67.2)		289 (67.2)
	7		119 (27.7)		119 (27.7)
	8		9 (2.0)		9 (2.0)
	9		11 (2.6)		11 (2.6)

Characteristic		Benign N=462	Cancer N=430	p-value	Total N=892
	Unknown		1 (0.2)	Not Applicable	1 (0.2)
PSA (ng/mL)	**Median**	5.1	5.3		5.1
	Mean ± SD	5.3 ± 1.9	5.4 ± 1.9		5.4 ± 1.9
	Range	2.0 – 10.0	2.0 – 9.8	0.199	2.0 – 10.0
fPSA (ng/mL)	**Median**	1.0	0.7		0.9
	Mean ± SD	1.0 ± 0.5	0.9 ± 0.5		1.0 ± 0.5
	Range	0.1 – 4.3	0.2 – 3.9	<0.001	0.1 – 4.3
[−2]proPSA (pg/mL)	**Median**	12.9	14.1		13.3
	Mean ± SD	14.4 ± 7.1	16.8 ± 11.1		15.5 ± 9.3
	Range	2.9 – 43.5	2.9 – 93.5	0.003	2.9 – 93.5
%fPSA	**Median**	18.8	15.1		17.0
	Mean ± SD	20.0 ± 8.0	16.4 ± 7.6		18.3 ± 8.0
	Range	3.1 – 53.2	3.7 – 51.1	<0.001	3.1 – 53.2
phi	**Median**	30.3	42.2		34.7
	Mean ± SD	33.9 ± 15.0	49.2 ± 31.3		41.3 ± 25.5
	Range	13.7 – 98.2	10.2 – 325.8	<0.001	10.2 – 325.8

TABLE 2

Sensitivity and Specificity for PCa Using Various *phi* Cutoffs in Men with Non-Suspicious DRE

% Sensitivity	*phi* Cutoff	% Specificity (n)
99	**17.2**	5.2 (24)
98	**18.4**	8.4 (39)
95	**21.3**	16.0 (74)
90	**24.1**	26.2 (121)
89.1	**25.0**	29.4 (136)
85	**27.2**	39.0 (180)
80	**29.3**	45.2 (209)
75	**31.1**	52.6 (243)
70	**33.4**	60.0 (277)
65	**35.0**	65.2 (301)
60	**37.5**	70.3 (325)
55	**39.1**	74.2 (343)
50	**42.2**	79.0 (365)
45	**44.3**	82.7 (382)
40	**46.7**	85.7 (396)
35	**49.3**	87.4 (404)
30	**52.6**	90.7 (419)
25	**55.9**	91.8 (424)
20	**61.9**	93.7 (433)
15	**67.6**	95.2 (440)
10	**78.1**	97.6 (451)
5	**104.2**	100 (462)

TABLE 3

Risk Assessment Probability of PCa using *phi*

phi Range	Probability of Cancer (95% Confidence Interval)	Relative Risk (95% Confidence Interval)	Percent of patients in *phi* range
0–24.9	11.0% (6.5% – 15.8%)	1.0	24.9%
25.0–34.9	18.1% (13.7% – 22.6%)	1.6 (1.0 – 3.1)	32.8%
35.0–54.9	32.7% (27.3% – 38.0%)	3.0 (1.9 – 5.3)	29.5%
55.0+	52.1% (42.0% – 62.1%)	4.7 (3.0 – 8.3)	12.8%

TABLE 4

Relationship of *phi* with Biopsy Gleason Score

	Gleason Score on Biopsy		
***phi* Range**	**Less than 7 n (%)**	**≥7 n (%)**	**Risk Ratio (95% CI)**
0–24.9	34 (73.9)	12 (26.1)	1.0
25.0–34.9	74 (71.8)	29 (28.2)	1.08 (0.61, 1.92)
35.0–54.9	116 (69.9)	50 (30.1)	1.15 (0.67, 1.98)
55.0+	66 (57.9)	48 (42.1)	1.61 (0.95, 2.75)

Note: One participant excluded with missing Gleason score.

Cochran-Armitage test for trend, p=0.01

Review

Xavier Filella* and Nuria Giménez

Evaluation of [–2] proPSA and Prostate Health Index (phi) for the detection of prostate cancer: a systematic review and meta-analysis

Abstract: The usefulness of %[–2] proPSA and Prostate Health Index (phi) in the detection of prostate cancer are currently unknown. It has been suggested that these tests can distinguish prostate cancer from benign prostatic diseases better than PSA or %fPSA. We performed a systematic review and meta-analysis of the available scientific evidence to evaluate the clinical usefulness of %[–2] proPSA and phi. Relevant published papers were identified by searching computerized bibliographic systems. Data on sensitivity and specificity were extracted from 12 studies: 10 studies about %[–2] proPSA (3928 patients in total, including 1762 with confirmed prostate cancer) and eight studies about phi (2919 patients in total, including 1515 with confirmed prostate cancer). The sensitivity for the detection of prostate cancer was 90% for %[–2] proPSA and phi, while the pooled specificity was 32.5% (95% CI 30.6–34.5) and 31.6% (95% CI 29.2–34.0) for %[–2] proPSA and phi, respectively. The measurement of %[–2] proPSA improves the accuracy of prostate cancer detection in comparison with PSA or %fPSA, particularly in the group of patients with PSA between 2 µg/L and 10 µg/L. Similar results were obtained measuring phi. Using these tests, it is possible to reduce the number of unnecessary biopsies, maintaining a high cancer detection rate. Published results also showed that %[–2] proPSA and phi are related to the aggressiveness of the tumor.

Keywords: evidence-based laboratory medicine; meta-analysis; prostate cancer; Prostate Health Index (phi); prostate specific antigen (PSA); ProPSA; systematic review.

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Introduction

Prostate specific antigen (PSA) is a serum tumor marker that is widely used in the early detection of prostate cancer. However, since the specificity (Sp) of PSA is limited, biopsy is positive in approximately 25% of patients with PSA in the range between 2 µg/L and 10 µg/L [1]. Furthermore, prostate cancer is detected on repeated biopsy in 10%–35% of patients with a negative first biopsy. So, according to the guidelines of the European Association of Urology, it is necessary to repeat the biopsy in these patients [2].

The measurement of the several fractions of PSA (free PSA, complexed PSA) has been proposed with the aim to improve the Sp of total PSA. A meta-analysis, published in 2005, showed that the use of the percentage of free PSA (%fPSA) is useful to improve the detection of prostate cancer [3]. More recently, fPSA has been found to include the subforms BPSA, iPSA and proPSA [4, 5]. BPSA and iPSA are associated with benign tissue, but proPSA is associated with cancer. It is possible to detect three truncated forms of proPSA in serum, [–2], [–4] and [–5,–7], with [–2] proPSA being the most stable form. Several studies suggested the clinical usefulness of proPSA in the detection of prostate cancer using different non-commercial assays, including the measurement of the cumulative sum of all truncated forms [6, 7] and the measurement of [–5,–7] proPSA [8, 9]. However, these tests have not been shown to be as useful as the new assay for the measurement of [–2] proPSA. Also, the use of a panel of four kallikrein markers – total PSA, free PSA, intact PSA and hK2 – in the detection of prostate cancer has been proposed by recent studies [10, 11].

The development of the [–2] proPSA assay by Beckman Coulter opens a new field of study in the detection of prostate cancer. Currently, several studies have suggested that in men with a total PSA between 2.5 μg/L and 10 μg/L, the percentage of [−2] proPSA to fPSA (%[−2] proPSA) can distinguish between malignant and benign prostate diseases better than total PSA or %fPSA. Also, several studies underlined the usefulness of the Prostate Health Index (phi), a mathematical combination of total PSA, fPSA and [−2] proPSA according to the formula [−2] proPSA/fPSA)×√tPSA.

The objective of this systematic review was to assess the usefulness of %[−2] proPSA and phi in the detection of prostate cancer. A critical analysis of results referring to the relationship between these tests and the aggressiveness of prostate cancer was also performed.

Methods

Meta-analysis was performed in accordance with the preferred reporting items from systematic reviews and meta-analysis (consensus PRISMA) adapted to studies of diagnostic tests [12]. In short, the PRISMA statement is a consensus that intends to inform by evidence whenever possible and consists of a 27-item checklist and a four-phase flow diagram that are available for researchers on internet for free (http://www.prisma-statement.org/).

Search strategy and study selection

A systematic search of several electronic databases was performed: MedLine, Embase, Cancerlit, Cochrane Library, Web of Science and Scopus. A strategy search in title, abstract or keyword lists was done looking for combinations of the following search terms: as medical subject headings MeSH ("Prostatic Neoplasms", "Sensitivity and Specificity", "Diagnosis", "Evidence-Based Medicine") and as free search terms ("proPSA", "p2PSA", "[−2]proPSA", "[−2]proenzyme prostate specific antigen", "Prostate Health Index", "phi", "Prostate tumor", "Prostate tumour"). This literature search was complemented with the review of three specialized journals in Urology (European Urology, Journal of Urology and Prostate) from January 1990 to December 2011. Furthermore, the authors checked the cited bibliographies of selected studies and contacted experts.

To avoid duplication of information, when the same population was reported in several publications, priority was given to scientific articles over meeting abstracts or in case there was more than a scientific article, the most complete study was chosen.

Eligibility criteria

All the studies about diagnostic tests and systematic review about %[−2] proPSA and phi were considered eligible for inclusion if they met the following criteria: original data and confirmation of prostate cancer on biopsy. There were no language restrictions.

Data extraction

All the studies were assessed independently by both researchers to determine study inclusion. Both reviewers, separately, screened all titles and excluded studies if obviously irrelevant and removed duplicate citations. When there was any doubt concerning the eligibility of a study, the abstract was examined and, if necessary, the full text. After selecting relevant studies, data extraction was carried out using a standardized form. The analysis of the concordance between both researchers about the eligibility of a study and the values of true positive (TP), false-positive (FP), false negative (FN) and true negative (TN) was done by calculating the kappa index. Disagreements about eligibility and data extraction were resolved by consensus.

Assessment of risk of bias

The quality of the selected studies was assessed by using quality assessment of diagnostic accuracy studies (QUADAS) [13]. The QUADAS tool consists of a set of 14 items, phrased as questions, each of which should be scored as yes, no or unclear. Possible sources of heterogeneity between studies were examined. Methodological heterogeneity or differences in design or quality were assessed during the selection of relevant studies and statistical heterogeneity was measured using I^2 scores and the χ^2-test.

The protocol was prepared a priori and this study was done in accordance with the Research Ethics Committee of Mútua Terrassa Hospital, Barcelona, Spain.

Data analysis

For each study, 2×2 tables for each test with TP, FP, FN and TN results using data extraction from the original referred scientific articles were performed. Pooled estimates of sensitivity (Se) and Sp as the main outcome measures were calculated as well as the limits of the 95% confidence intervals for such values. Forest plot was represented as figures. Methodological heterogeneity was assessed during selection.

The threshold effect is a characteristic source of heterogeneity in the meta-analysis of diagnostic tests and arises when the included studies uses different cut-off points to define what is considered as a positive result of a diagnostic test. The analysis of diagnostic threshold was assessed through receiver operating characteristic (ROC) plane and correlation coefficient Spearman. The ROC plane is the graphic representation of the pairs of Se and Sp and, characteristically its points show a curvilinear pattern if the threshold effect exists. Statistical heterogeneity was measured using the χ^2-test and I^2 scores. I^2 score was used as a measure of the inconsistency between studies in the meta-analysis and was interpreted as low (25%–50%), moderate (51%–75%) and high (>75%).

Data were analyzed using a free statistical software package Metadisc version 1.4 [14], with the only exception of the analysis of the concordance between reviewers and kappa index which was performed using SPSS 17.0 (SPSS Inc., Chicago, IL, USA).

Assays used in the references evaluated in this study

In the studies corresponding to references [15–27] the concentrations of [–2] proPSA were measured in a Beckman Coulter ACCESS® immunoassay system, using dual monoclonal antibodies. [–2] proPSA was measured in references [28, 29] using a dual monoclonal sandwich assay in a microtiter plate. PSA and fPSA were measured using a Beckman Coulter ACCESS® immunoassay system in references [15–24] or Hybritech Tandem PSA and Tandem free PSA assays in reference [28]. The measurement of PSA and fPSA in reference [29] was determined with Hybritech Tandem PSA and Tandem free PSA assays (Beckman Coulter, Inc.) in site 2 (Washington University) and with the Abbott total and free PSA assays (Abbott Laboratories, Chicago, IL, USA) in site 1 (Innsbruck University).

Phi was calculated in studies corresponding to references [16–21, 25, 27] using the formula [–2] proPSA/fPSA)×√tPSA.

Results

Two hundred and thirteen potentially relevant references were obtained by electronic databases and supplementary sources in our systematic search. The results of the search and study selection process are shown in Figure 1. There were 31 articles requiring full-text review, and 12 studies were finally included in the meta-analysis. Data on Se and Sp were pooled from 10 studies for %[–2] proPSA (3928 patients in total, including 1762 with confirmed prostate

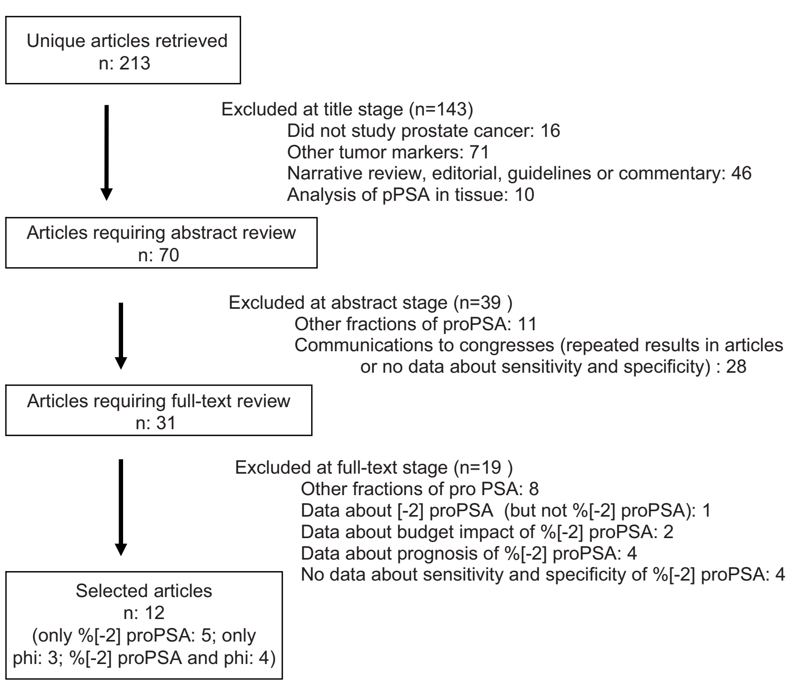

Figure 1 Summary of literature search and selection of studies included.

cancer) and eight studies about phi (2919 patients in total, including 1515 with confirmed prostate cancer).

The study by Jansen et al. [15] contained two different populations (Rotterdam and Innsbruck), and was treated as two separate studies.

The results about concordance between both reviewers had a coincidence of 94% and a kappa index of 0.812 (95% CI 0.635–0.990).

The quality assessment of the eligible studies was moderate-high according to QUADAS scale (Table 1) [15–24, 28, 29]. The main characteristics about the selected studies are shown in Table 2 including the description of the population of each study, the sampling frame and the criteria and characteristics of prostate biopsy. Table 3 shows the performance of %[–2] proPSA and phi and compares the area under the curve (AUC) corresponding to these tests with the AUC for PSA and %fPSA. The accuracy of %[–2] proPSA and phi in the detection of prostate cancer is reported in Table 4. Data presented in this table were extracted from the included studies. Of the 12 studies included, only three specified the cut-off value. The cut-off level for %[–2] proPSA at a Se of 90% was 2.5% for Mikolajczyk et al. [28] and 1.06% for Miyakubo et al. [19]. The cut-off reported for phi at a Se of 90% was 24.9% for Miyakubo et al. [19] and 21.1% for Catalona et al. [16].

Methodological heterogeneity was assessed before analyses and no studies were excluded due to this reason. The existence of a threshold effect was ruled out after examining the ROC plane and Spearman's correlation coefficient (r=0.636 and p-value=0.048 for %[–2] proPSA and r=0.262 and p-value=0.531 for phi).

When revising the studies, it was found that they had in common the results for sensibility of 90% and therefore it was decided to extract the data and perform calculations to this Se. There was a high degree of statistical heterogeneity (I^2 score ≥75%) in Sp of %[–2] proPSA (χ^2=84.24; p<0.0001) and phi (χ^2=36.07; p<0.0001). Results are shown in Figure 2. For this selected Se of 90%, the pooled Sp of %[–2] proPSA was 32.5% (95% CI 30.6–34.5%, I^2 score=89.3%, p<0.001, Figure 2A) and the pooled Sp of phi was 31.6% (95% CI 29.2–34.0%, I^2 score=80.6%, p<0.001, Figure 2B).

Discussion

A low %fPSA has been shown to be associated with prostate cancer and several studies have indicated that this test is useful in reducing the number of negative biopsies [3]. However, currently, we know that fPSA is composed of three distinct molecular forms, which are associated differently with cancer. Initial clinical studies showed that proPSA may be a useful marker for the detection of prostate cancer, and more recently Beckman Coulter introduced a new immunoassay for the measurement of the [–2] proPSA, a stable form of proPSA [30].

This meta-analysis is the first study that shows the available information on the clinical usefulness of this tumor marker in the detection of prostate cancer. Data on Se and Sp about %[–2] proPSA and the derivative test phi were extracted from 12 eligible studies. At Se of 90%, which is clinically acceptable, the Sp was 32% for %[–2] proPSA, ranging between 21% and 49%, and 32% for phi, ranging between 26% and 43%. The AUCs obtained by ROC analysis were also clinically acceptable, with results between 0.635 and 0.780 for %[–2] proPSA and between 0.703 and 0.77 for phi.

This study has some limitations. For one, information about the cut-offs used was showed only in three studies [16, 19, 28]; therefore, there was heterogeneity in primary studies. The high level of inconsistency in the global Sp for %[–2] proPSA (89%) and for phi (81%) shows the heterogeneity of the studies included in this meta-analysis. Differences in recruitment strategy, in population characteristics, and in the number of cores obtained in biopsies may contribute to these variations. We must underline that the same assay was used in the majority of studies, with only two exceptions, corresponding to the earlier references [28, 29] that uses a non-commercial assay for the measurement of [–2] proPSA. This factor may influence in part in the heterogeneity of results. PSA and fPSA were measured using an equivalent assay (Beckman Coulter ACCESS® immunoassay or Hybritech Tandem assays) in all studies, only with a partial exception in reference [29], that used the Abbott total and free PSA assays in part of the measurements.

%[–2] proPSA and phi have a similar performance for patients with PSA between 2 µg/L and 4 µg/L and for patients with PSA between 4 µg/L and 10 µg/L according to different studies [17, 22, 24, 29]. So, Guazzoni et al. [17] showed that the AUC for %[–2] proPSA is 0.76 for patients with PSA between 2 µg/L and 4 µg/L and 0.78 for patients with PSA between 4 µg/L and 10 µg/L. For both groups of patients the AUC for phi was 0.76. Similar results were indicated for %[–2] proPSA in other studies [22, 24, 29].

The majority of studies reported in this meta-analysis showed that the AUC for %[–2] proPSA (ranging between 0.635 and 0.78) was higher than the AUC for %fPSA. Sokoll et al. [22] communicated an exception to this criteria, but in this study, too, the AUC for %[–2] proPSA was higher to %fPSA in the group of patients with PSA between 2 µg/L

Study	Patients		Test			Results	
Author	Patients are representative of the question	Selection criteria according DRA and PSA serum levels	Biopsy is performed in all patients	Number of cores per biopsy ≥ 10	Assays for the measurement of [−2] proPSA and phi are described	Blinded	Cut-off reported
Catalona et al., 2011 [16]	Yes	Yes (Normal DRE, PSA 2–10 μg/L)	Yes	Yes	Yes	Yes	Yes (only for phi)
Guazzoni et al., 2011 [17]	Yes	Yes (Normal DRE, PSA 2–10 μg/L)	Yes	Yes	Yes	Yes	No
Houlgatte et al., 2011 [18]	Yes	Yes (Normal DRE, PSA 2–10 μg/L)	Yes	Yes	Yes	Yes	No
Miyakubo et al., 2011 [19]	Yes	Only according to PSA 2–10 μg/L; No information about DRE	Yes	Age- and prostate volume-adjusted multiple-core biopsies	Yes	No	Yes
Vincendeau et al., 2011 [20]	Yes	Yes (Normal DRE, PSA 2–10 μg/L)	Yes	Yes	Yes	No	No
Jansen et al., 2010 Site 1 (Rotterdam) [15]	Yes	Yes (PSA >4 μg/L or abnormal DRE or abnormal TRUS)[a]	Yes	No	Yes	No	No
Jansen et al., 2010 Site 2 (Innsbruck) [15]	Yes	Yes (according to estimation of prostate cancer risk using ANN)[b]	Yes	No	Yes	No	No
Le et al., 2010 [21]	Yes	Yes (Normal DRE, PSA 2–10 μg/L)	No[c]	Not reported	Yes	Yes	No
Sokoll et al., 2010 [22]	Yes	Yes (Normal and abnormal DRE, PSA 0.29–310.6 μg/L)	Yes	Yes	Yes	Yes	No
Stephan et al., 2009 [23]	Yes	Normal DRE, PSA 0.26–28.4 μg/L	Yes	8–12 cores	Yes	No	No
Sokoll et al., 2008 [24]	Yes	PSA 0.48–33.18 μg/L; No information about DRE	Yes	Yes	Yes	Yes	No
Mikolajczyk et al., 2004 [28]	Yes	PSA 4–10 μg/L; No information about DRE	Yes	Not reported	Yes[d]	Yes	Yes (only for %[−2] proPSA)
Catalona et al., 2003 [29]	Yes	PSA 2–10 μg/L; No information about DRE	Yes	Yes in Innsbruck site; No in Washington site	Yes[d]	Yes	No

Table 1 Quality of 12 studies included in the meta-analysis according to the questionnaire QUADAS.
[a]In 1997, this combination was replaced by PSA testing only; [b]Indication for biopsy based on the estimation of prostate cancer by an artificial neural network (ANN) including PSA, fPSA, age, DRE, and TRUS. In addition, PSA velocity was incorporated in 2005; [c]Biopsy was performed in all patients included for the calculation of the sensitivity and specificity of the tests; [d]Non-commercial assay. DRE, digital rectal examination; TRUS, transrectal ultrasound.

	Sampling frame	Years of recruitment of patients	Population	Age of Patients	Inclusion criteria	Indication for biopsy	Number of cores in biopsy	Patients with biopsy	Patients with cancer	%[−2] proPSA Assay	Algorithms
Catalona et al., 2011 [16]	Multi-center: Prospective and retrospective[a]	2003–2009	Selected	62.8±7.0 (mean±S.D.)	≥50 year, PSA 2–10 µg/L & biopsy	All patients included in the study	89.8% had ≥12 cores; 98% had ≥10 cores	892	430	Beckman Coulter	Phi
Guazzoni et al., 2011 [17]	Prospective	2010	Referral patients/ consecutive	63.3±8.2 (mean±S.D.)	PSA 2–10 µg/L & DRE -	All patients included in the study	18–22 biopsy cores	268	107	Beckman Coulter	Phi
Houlgatte et al., 2011 [18]	Retrospective	Not reported	Selected	Not reported	PSA 2–10 µg/L	All patients included in the study	12 or more cores	452	243	Beckman Coulter	Phi
Miyakubo et al., 2011 [19]	Retrospective	2004–2007	Consecutive	Not reported	PSA 4–10 µg/L	All patients included in the study	Age- and prostate volume-adjusted multiple-core biopsies	239	53	Beckman Coulter	Phi
Vincedeau et al., 2011 [20]	Retrospective	Not reported	Early detection/ selected	Not reported	PSA 2–10 µg/L & DRE -	All patients included in the study	≥10 cores	250	143	Beckman Coulter	Phi
Jansen et al., 2010 Site 1 (Rotterdam) [15]	Retrospective	1994–1997	Screening/non serial	55–75 (66) range (median)	≥50 year, PSA 2–10 µg/L & biopsy[a]	PSA >4, DRE + or TRUS + (In 1997 replaced by PSA only)	6 or more cores	405	226	Beckman Coulter	Phi
Jansen et al., 2010 Site 2 (Innsbruck) [15]	Retrospective	Started in 1993	Screening/non serial	50–77 (69) range (median)	≥50 year, PSA 2–10 µg/L & biopsy[a]	ANN including PSA, fPSA, age, DRE and TRUS (PSA velocity was incorporated in 2005)	6 or more cores	351	174	Beckman Coulter	Phi
Le et al., 2010 [21]	Prospective	2007	Screening/ consecutive	65 (median)	PSA 2.5–10 µg/L & DRE −	PSA ≥2.5 µg/L & DRE +	Not reported	63	26	Beckman Coulter	Phi
Sokoll et al., 2010 [22]	Prospective multicenter	Not reported	Early detection/ consecutive	61.7±8.6 (mean ± S.D.)	>40 year, no prior prostate surgery, biopsy or history of PCa	All patients included in the study	≥10 cores	566 With PSA between 2 and 10 µg/L: 429	245 With PSA between 2 and 10 µg/L: 195	Beckman Coulter	LR including age, race, DRE, prostate cancer family history, log PSA, log %fPSA and log %[−2] proPSA
Stephan et al., 2009 [23]	Retrospective	2002–2006	Referral patients	62.1±5.63 (PCa) 67.2±7.01 (subjects with negative biopsy) (mean±S.D.)	Referred to department of Urology for suspected PCa	All patients included in the study	8–12 cores	586 With PSA between 2 and 10 µg/L: 475	311 With PSA between 2 and 10 µg/L: 264	Beckman Coulter	ANN and LR models including [−2] proPSA, %fPSA, tPSA and age
Sokoll et al., 2008 [24]	Retrospective, multicenter	Not reported	Early detection/ selected	62.2±8.2 (mean±S.D.)	Indication for prostate biopsy	All patients included in the study	≥10 cores	123 With PSA between 2 and 10 µg/L: 89	63 With PSA between 2 and 10 µg/L: 50	Beckman Coulter	LR including PSA, BPSA, %fPSA, %[−2] proPSA, [−2] proPSA/ BPSA, testosterone
Mikolajczyk et al., 2004 [28]	Retrospective	1995–2001	Screening/non serial	66 (median)	PSA 4–10 µg/L	All patients included in the study	Not reported	380	238	Research assay	No
Catalona et al., 2003 [29]	Retrospective, 2 institutions (Innsbruck & Washington)	Innsbruck: 1999–2002 Washington: 1995–2001	Screening/non serial	Not reported	PSA 2–10 µg/L	All patients included in the study	Innsbruck: 10 core biopsy Washington: 6 core biopsy	1091	456	Research assay	No

Table 2 Characteristics of the studies included in the review.
ANN, artificial neural network; CaP, prostate cancer; DRE, digital rectal examination; LR, logistic regression; TRUS, transrectal ultrasound. [a]Only 3.1% were retrospective samples.

	AUC PSA (95% CI)	AUC %fPSA (95% CI)	AUC %[−2] proPSA (95% CI)	AUC phi (95% CI)	Relationship of %[−2] proPSA and Gleason score	Relationship of phi and Gleason score
Catalona et al., 2011 [16]	0.525	0.648	Not reported	0.703	Not reported	Yes The probability of Gleason score ≥7 was 26.1% when phi <25, and 42.1% when phi ≥55.
Guazzoni et al., 2011 [17]	0.53 (0.47–0.59)	0.58 (0.52–0.64)	0.76 (0.71–0.81)	0.76 (0.70–0.81)	%[−2] proPSA was significantly associated with Gleason score (Spearman r: 0.303; p<0.002), but it did not improve the prediction of Gleason score ≥7 PCa in multivariable accuracy analyses	Phi was significantly associated with Gleason score (Spearman r: 0.387; p<0.002), but it did not improve the prediction of Gleason score ≥7 PCa in multivariable accuracy analyses
Houlgatte et al., 2011 [18]	0.56 (0.51–0.64)	0.59 (not reported)	0.72 (not reported)	0.73 (0.67–0.77)	Not reported	Not reported
Miyakubo et al., 2011 [19]	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported
Vincedeau et al., 2011 [20]	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported
Jansen et al., 2010, Site 1 (Rotterdam) [15]	0.585 (0.535–0.634)	0.675 (0.627–0.721)	0.716 (0.669–0.759)	0.750 (0.704–0.791)	%[−2] proPSA discriminates Gleason score ≥7 (with biopsy Gleason score, p:0.002; with pathologic Gleason score, p:0.09)	Phi discriminates Gleason score ≥7 (with biopsy Gleason score, p:<0.0001; with pathologic Gleason score, p:0.02)
Jansen et al., 2010, Site 2 (Innsbruck) [15]	0.534 (0.473–0.594)	0.576 (0.523–0.629)	0.695 (0.644–0.743)	0.709 (0.658–0.756)	No (neither with biopsy or pathologic Gleason score)	No (neither with biopsy or pathologic Gleason score)
Le et al., 2010 [21]	0.50	0.68	0.76	0.77	Not reported	Not reported
Sokoll et al., 2010 [22]	0.66 (0.62–0.71) For PSA 2–10 µg/L: 0.58 (0.53–0.64)	0.70 (0.65–0.74) For PSA 2–10 µg/L: 0.66 (0.61–0.71)	0.67 (0.62–0.71) For PSA 2–10 µg/L: 0.70 (0.65–0.75)	Not reported LRM[a]: 0.79 (0.75–0.82) For PSA 2–10 µg/L: 0.76 (0.72–0.81)	Yes %[-2] proPSA increased with increasing Gleason score (p<0.001 for all patients and 0.02 for patients with PSA between 2 µg/L and 10 µg/L	Not reported
Stephan et al., 2009 [23]	0.56 (0.51–0.61)	0.77 (0.73–0.81)	0.78 (0.74–0.82)	Not reported (ANN[b]: 0.85; 0.81–0.88) (LR[b]: 0.84; 0.80–0.87)	Yes: %[−2] proPSA is significantly elevated in PCa (p<0.0001)	Not reported
Sokoll et al., 2008 [24]	0.52 (0.42–0.63) For PSA 2 10 µg/L 0.52 (0.40–0.64)	0.61 (0.51–0.71) For PSA 2–10 µg/L 0.53 (0.41–0.65)	0.69 (0.60–0.79) For PSA 2–10 µg/L 0.73 (0.63–0.84)	Not reported LRM[c]: 0.73; 0.64–0.83 For PSA 2–10 µg/L: 0.73 (0.62–0.84)	Not reported	Not reported
Mikolajczyk et al., 2004 [28]	0.526	0.627	0.635	Not reported	Not reported	Not reported
Catalona et al., 2003 [29]	Not reported	0.602	0.638		Not reported	Not reported

Table 3 AUCs for PSA, %fPSA, %[−2] proPSA and phi, and relationship of %[−2] proPSA and phi with Gleason score.
[a]Logistic regression model (LRM) including PSA, BPSA, %fPSA, %[−2] proPSA, [−2] proPSA/BPSA, testosterone; [b]Artificial Neural Network (ANN) and logistic regression (LR) models including %[−2] proPSA, %fPSA, tPSA and age; [c]Logistic regression model (LRM) including age, race, DRE, prostate cancer family history, log PSA, log%fPSA and log %[−2] proPSA. CI, confidence interval.

Table 4A %[−2] proPSA

Studies %[−2] proPSA	TP	FP	FN	TN	Se	Sp
Guazzoni et al., 2011 [17]	96	99	11	62	90%	39%
Miyakubo et al., 2011 [19]	48	139	5	47	90%	25%
Jansen et al., 2010, Site 1 (Rotterdam) [15]	204	122	22	57	90%	32%
Jansen et al., 2010, Site 2 (Innsbruck) [15]	154	117	17	60	90%	34%
Le et al., 2010 [21]	23	19	3	18	88.5%	48.6%
Sokoll et al., 2010 [22]	196	177	49	144	80%	44.9%
Stephan et al., 2009 [23][a]	238	123	26	88	90%	41.7%
Sokoll et al., 2008 [24]	56	38	7	22	90%	37%
Mikolajczyk et al., 2004 [28]	128	152	14	86	90%	36%
Catalona et al., 2003 [29]	410	502	46	133	90%	21%

Table 4B Phi

Studies phi	TP	FP	FN	TN	Se	Sp
Catalona et al., 2011 [16]	387	341	43	121	90%	26.2%
Guazzoni et al., 2011 [17]	96	92	11	69	90%	43%
Houlgatte et al., 2011 [18]	219	149	24	59	90%	28.2%
Miyakubo et al., 2011 [19]	48	125	5	61	90%	33%
Vincendeau et al., 2011 [20]	129	79	14	28	90%	26%
Jansen et al., 2010, Site 1 (Rotterdam) [15]	204	117	22	62	90%	35%
Jansen et al., 2010, Site 2 (Innsbruck) [15]	157	122	17	55	90%	31%
Le et al., 2010 [21]	23	13	3	24	88.5%	64.9%

Table 4 Diagnostic accuracy: sensitivity and specificity. Data were extracted from included studies.
[a]Results for patients with PSA between 2 µg/L and 10 µg/L. FN, false negative; FP, false positive; Se, sensitivity; Sp, specificity; TN, true negative; TP, true positive.

and 10 µg/L. These results underline that %[−2] proPSA may be a useful test in the detection of prostate cancer in men with PSA between 2 µg/L and 10 µg/L.

The derivative test phi showed similar or slightly better results than %[−2] proPSA, with AUCs between 0.703 and 0.77. The performance of other derivative tests obtained by artificial neural network (ANN) or logistic regression (LR) analysis was better than %[−2] proPSA. The best results were reported by Stephan et al. [23] using ANN and logistic regression models with AUCs of 0.85 and 0.84, respectively. According to this author, the ANN model, including %[−2] proPSA, %fPSA, tPSA and age, performs significantly better than %fPSA or %[−2] proPSA, enhancing the Sp of 17%–28% at sensitivities of 90% and 95%.

These results show that the measurement of %[−2] proPSA and phi increases the specificity of the detection of prostate cancer hence reducing the number of unnecessary biopsies. However, information about the recommended cut-offs for these tests were not shown in the majority of papers included in our review. The cut-off level for %[−2] proPSA at Se of 90% was 2.5% for Mikolajczyk et al. [28] and 1.06% for Miyakubo et al. [19]. More similar are the cut-offs suggested for phi by Miyakubo et al. [19] and Catalona et al. [16] showing, respectively that 24.9% and 21.1% of phi corresponds to Se of 90%. Published results showed that while the accuracy of PSA declines with age, the %fPSA increases the predictive value of PSA in older patients [31]. Results communicated by Catalona et al. [16] indicated that phi does not differ by age, and this test may be applicable to young and older men in the detection of prostate cancer.

However, although the unit cost of [−2] proPSA is two to three times higher than both PSA or fPSA, the use of %[−2] proPSA and phi for the detection of prostate cancer decreases global costs. The additional blood test costs were compensated by the savings on the costs of physician office visits and the avoidance of unnecessary biopsies [32, 33].

Several authors showed that %[−2] proPSA and phi may be related to prostate cancer aggressiveness, with higher levels of these tests in patients with Gleason score higher than 7 and in patients with locally advanced tumors [15, 17, 22, 23]. This is relevant information because about one-third of new diagnosed tumors have features of insignificant prostate cancer [34] and these patients can be candidates to active surveillance. However, the identification of these patients using the standard markers, including PSA, biopsy, Gleason score and number of positive biopsy cores, fails to predict accurately the prostate cancer aggressiveness and to choose the more adequate treatment. This point has been confirmed recently by the PIVOT study [35] comparing the effectiveness of radical prostatectomy versus observation in 731 men with localized prostate cancer. The authors showed absolute reductions in all-cause mortality with radical prostatectomy in patients with PSA higher than 10 µg/L and possibly for patients with intermediate- or high-risk tumors, but not in patients with low-risk prostate cancer.

These results underline the usefulness of risk factors in the management of patients with prostate cancer in order to select between a radical treatment and active surveillance. Results reported about %[−2] proPSA and phi suggest that these tests may distinguish low- and high-risk prostate cancer. Using a multivariate analysis, Guazzoni et al. [25] showed that the inclusion of %[−2] proPSA and phi significantly increased the predictive accuracy of a model based on patient age, PSA, %fPSA, clinical stage and biopsy Gleason score in the prediction of high pathologic stage or high pathologic Gleason

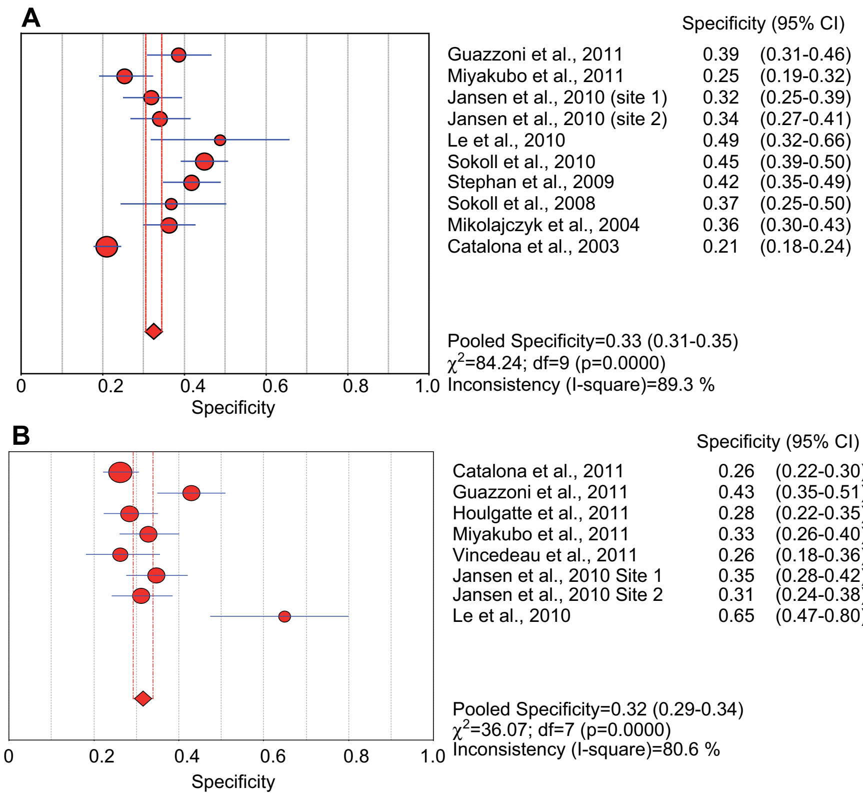

Figure 2 Specificities of %[−2] proPSA and phi. Forest plots showing pooled specificity results of %[−2] proPSA (A) and phi (B). Studies are ordered by author and year of publication. The circles and horizontal lines correspond to the recorded percentage of TN results among patients without prostate cancer and their respective 95% CIs. The area of circles reflects the weight each study contributes to the analysis. The diamond represents the pooled value with its 95% CI.

score. Similarly, de Vries et al. [26] indicated promising results for %[−2] proPSA in selecting treatment strategies for men with prostate cancer using Epstein's criteria to differentiate between non-aggressive and aggressive tumors. Finally, in a recently published study Isharwal et al. [27] described that %[−2] proPSA and phi predicts unfavorable biopsy conversion at an annual surveillance biopsy examination among men enrolled in an active surveillance program. According to this study, the probability of an unfavorable biopsy conversion is higher in patients with %[−2] proPSA higher than 0.7 or with phi higher than 34.2.

Conclusions

The available data shows that %[−2] proPSA and the derivative test phi may be useful in the detection of prostate cancer reducing the number of negative biopsies and improving results obtained with %fPSA and total PSA. Recent published data, concerning cost-effectiveness of these tests also suggests a positive budget impact of their generalized implementation in the management of prostate cancer. Results about the relationship of %[−2] proPSA and phi with the aggressiveness of the tumor corroborate the clinical usefulness of these tests. However, more studies are necessary in order to confirm these data and, specially, in order to define the most appropriate cut-off for %[−2] proPSA and phi.

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References

1. 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. J Am Med Assoc 1998;279:1542–7.
2. Heidenreich A, Bellmunt J, Bolla M, Joniau S, Mason M, Matveev V, et al. EAU guidelines on prostate cancer. Part 1: screening, diagnosis, and treatment of clinically localised disease. Eur Urol 2011;59:61–71.
3. Roddam AW, Duffy MJ, Hamdy FC, Ward AM, Patnick J, Price CP, et al. Use of prostate-specific antigen (PSA) isoforms for the detection of prostate cancer in men with a PSA level of 2–10 ng/ml: systematic review and meta-analysis. Eur Urol 2005;48:386–99.
4. Mikolajczyk SD, Marks LS, Partin AW, Rittenhouse HG. Free prostate-specific antigen in serum is becoming more complex. Urology 2002;59:797–802.
5. Mikolajczyk SD, Marker KM, Millar LS, Kumar A, Saedi MS, Payne JK, et al. A truncated precursor form of prostate-specific antigen is a more specific serum marker of prostate cancer. Cancer Res 2001;61:6958–63.
6. Sokoll LJ, Chan DW, Mikolajczyk SD, Rittenhouse HG, Evans CL, Linton HJ, et al. Proenzyme PSA for the early detection of prostate cancer in the 2.5–4.0 ng/ml total PSA range: preliminary analysis. Urology 2003;61:274–6.
7. Khan MA, Sokoll LJ, Chan DW, Mangold LA, Mohr P, Mikolajczyk SD, et al. Clinical utility of proPSA and 'benign' PSA when percent free PSA is less than 15%. Urology 2004;64:1160–4.
8. Filella X, Alcover J, Molina R, Luque P, Corral JM, Augé JM, et al. Usefulness of proprostate-specific antigen in the diagnosis of prostate cancer. Anticancer Res 2007;27:607–10.
9. Stephan C, Meyer HA, Paul EM, Kristiansen G, Loening SA, Lein M, et al. Serum (−5, −7) proPSA for distinguishing stage and grade of prostate cancer. Anticancer Res 2007;27:1833–6.
10. Vickers AJ, Gupta A, Savage CJ, Pettersson K, Dahlin A, Bjartell A, et al. A panel of kallikrein marker predicts prostate cancer in a large, population-based cohort followed for 15 years without screening. Cancer Epidemiol Biomarkers Prev 2011;20:255–61.
11. Vickers AJ, Cronin AM, Roobol MJ, Savage CJ, Peltola M, Pettersson K, et al. A four-kallikrein panel predicts prostate cancer in men with recent screening: data from the European Randomized Study of Screening for Prostate Cancer, Rotterdam. Clin Cancer Res 2010;16:3232–9.
12. Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gøtzsche PC, Ioannidis JP, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: explanation and elaboration. BMJ 2009;339:b2700.
13. Whiting P, Rutjes AW, Reitsma JB, Bossuyt PM, Kleijnen J. The development of QUADAS: a tool for the quality assessment of studies of diagnostic accuracy included in systematic reviews. BMC Med Res Methodol 2003;3:25.
14. Zamora J, Abraira V, Muriel A, Khan K, Coomarasamy A. Meta-DiSc: a software for meta-analysis of test accuracy data. BMC Med Res Methodol 2006;6:31.
15. Jansen FH, van Schaik RH, Kurstjens J, Horninger W, Klocker H, Bektic J, et al. Prostate-specific antigen (PSA) isoform p2PSA in combination with total PSA and free PSA improves diagnostic accuracy in prostate cancer detection. Eur Urol 2010;57:921–7.
16. 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;185:1650–5.
17. 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;60:214–22.
18. Houlgatte A, Vincendeau S, Desfemmes F, Ramirez J, Benoist N, Bensalah K, et al. Place du −2proPSA et de l'index phi dans la détection précoce du cancer de prostate: évaluation sur unes série de 452 patients. Prog Urol 2011;22:279–83.
19. Miyakubo M, Ito K, Yamamoto T, Suzuki K. Diagnostic significance of [−2]proPSA, total and transition zone prostate volume adjusted PSA-related indices in Japanese men with total PSA in the 2.0 to 10.0 ng/ml range. Eur Urol Suppl 2011;10:65.
20. Vincendeau S, Stephan C, Houlgatte A, Semjonow A. The Beckman Coulter Prostate Health Index (phi) increases the specificity of detection of prostate cancer and reduces the number of negative biopsies. IFCC, WorldLab, EuroMedLab Berlin 2011. Berlin, 15–19 May 2011. Clin Chem Lab Med 2011;49:S874.
21. Le BV, Griffin CR, Loeb S, Carvalhal GF, Kan D, Baumann NA, et al. [−2]Proenzyme prostate specific antigen is more accurate than total and free prostate specific antigen in differentiating prostate cancer from benign disease in a prospective prostate cancer screening study. J Urol 2010;183:1355–9.
22. Sokoll LJ, Sanda MG, Feng Z, Kagan J, Mizrahi IA, Broyles DL, et al. A prospective, multicenter, National Cancer Institute Early Detection Research Network study of [−2]proPSA: improving prostate cancer detection and correlating with cancer aggressiveness. Cancer Epidemiol Biomarkers Prev 2010;19:1193–200.
23. Stephan C, Kahrs AM, Cammann H, Lein M, Schrader M, Deger S, et al. A [−2]proPSA-based artificial neural network significantly improves differentiation between prostate cancer and benign prostatic diseases. Prostate 2009;69:198–207.
24. Sokoll LJ, Wang Y, Feng Z, Kagan J, Partin AW, Sanda MG, et al. [−2]proenzyme prostate specific antigen for prostate cancer detection: a National Cancer Institute early detection research network validation study. J Urol 2008;180:539–43.
25. Guazzoni G, Lazzeri M, Nava L, Lughezzani G, Larcher A, Scattoni V, 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. Eur Urol 2012;61:455–66.
26. de Vries SH, Raaijmakers R, Blijenberg BG, Mikolajczyk SD, Rittenhouse HG, Schröder FH. Additional use of [−2] precursor prostate-specific antigen and "benign" PSA at diagnosis in screen-detected prostate cancer. Urology 2005;65:926–30.
27. Isharwal S, Makarov DV, Sokoll LJ, Landis P, Marlow C, Epstein JI, et al. ProPSA and diagnostic biopsy tissue DNA content

combination improves accuracy to predict need for prostate cancer treatment among men enrolled in an active surveillance program. Urology 2011;77:763.e1–6.
28. Mikolajczyk SD, Catalona WJ, Evans CL, Linton HJ, Millar LS, Marker KM, et al. Proenzyme forms of prostate-specific antigen in serum improve the detection of prostate cancer. Clin Chem 2004;50:1017–25.
29. Catalona WJ, Bartsch G, Rittenhouse HG, Evans CL, Linton HJ, Amirkhan A, et al. Serum pro prostate specific antigen improves cancer detection compared to free and complexed prostate specific antigen in men with prostate specific antigen 2 to 4 ng/ml. J Urol 2003;170:2181–5.
30. Semjonow A, Köpke T, Eltze E, Pepping-Schefers B, Bürgel H, Darte C. Pre-analytical in-vitro stability of [−2]proPSA in blood and serum. Clin Biochem 2010;43:926–8.
31. Vickers AJ, Ulmert D, Serio AM, Björk T, Scardino PT, Eastham JA, et al. The predictive value of prostate cancer biomarkers depends on age and time to diagnosis: towards a biologically-based screening strategy. Int J Cancer 2007;121:2212–7.
32. Nichol MB, Wu J, An JJ, Huang J, Denham D, Frencher S, et al. Budget impact analysis of a new prostate cancer risk index for prostate cancer detection. Prostate Cancer Prostatic Dis 2011;14:253–61.
33. Nichol MB, Wu J, Huang J, Denham D, Frencher SK, Jacobsen SJ. Cost-effectiveness of Prostate Health Index for prostate cancer detection. BJU Int 2011;110:353–62.
34. Roemeling S, Roobol MJ, Postma R, Gosselaar C, van der Kwast TH, Bangma CH, et al. Management and survival of screen-detected prostate cancer patients who might have been suitable for active surveillance. Eur Urol 2006;50:475–82.
35. Wilt TJ, Brawer MK, Jones KM, Barry MJ, Aronson WJ, Fox S, et al. Prostate Cancer Intervention versus Observation Trial (PIVOT) Study Group. Radical prostatectomy versus observation for localized prostate cancer. N Engl J Med 2012;367:203–13.

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The Prostate Health Index: a new test for the detection of prostate cancer

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***Abstract*:** A major focus in urologic research is the identification of new biomarkers with improved specificity for clinically-significant prostate cancer. A promising new test based on prostate-specific antigen (PSA) is called the Prostate Health Index (PHI), which has recently been approved in the United States, Europe and Australia. PHI is a mathematical formula that combines total PSA, free PSA and [-2] proPSA. Numerous international studies have consistently shown that PHI outperforms its individual components for the prediction of overall and high-grade prostate cancer on biopsy. PHI also predicts the likelihood of progression during active surveillance, providing another noninvasive modality to potentially select and monitor this patient population. This article reviews the evidence on this new blood test with significant promise for both prostate cancer screening and treatment decision-making.

Keywords: prostate health index, PHI, prostate cancer, PSA, free PSA, screening, prognosis

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Introduction

In 2013, there will be an estimated 238,590 new cases of prostate cancer and 29,720 deaths, making it the second leading cause of cancer death in US men [ACS, 2013]. Widespread prostate cancer screening with prostate-specific antigen (PSA) has led to a dramatic reduction in the proportion of men diagnosed with metastatic disease and prostate cancer death rates [Schroder *et al.* 2012]. However, PSA screening continues to be highly controversial due to its limited specificity for clinically significant prostate cancer, resulting in unnecessary biopsies for false positive results as well as detection of some indolent tumors that would not have caused harm during the patient's lifetime.

To preserve the benefits of screening and early detection and to reduce these harms, there has been great progress into alternate ways of using the PSA test with better performance characteristics. In the early 1990s, several studies showed that a greater percentage of PSA circulating in the unbound or form ('free PSA') indicated a greater likelihood that the elevation was from benign conditions rather than prostate cancer [Lilja *et al.* 1991; Stenman *et al.* 1991].

More recently, several PSA isoforms have been identified that can further increase the specificity for prostate cancer [Mikolajczyk *et al.* 2004]. In particular, the [-2] form of proPSA ('p2PSA') has become commercially available, with improved performance over either total or free PSA for prostate cancer detection on biopsy [Catalona *et al.* 2003; Sokoll *et al.* 2010].

The Prostate Health Index (PHI) is a new formula that combines all three forms (total PSA, free PSA and p2PSA) into a single score that can be used to aid in clinical decision-making [Catalona *et al.* 2011]. PHI is calculated using the following formula: ([-2]proPSA/free PSA) × $\sqrt{\text{PSA}}$. Intuitively, this formula makes sense, in that men with a higher total PSA and p2PSA with a lower free PSA are more likely to have clinically significant prostate cancer. In this article, we review the evidence on PHI in prostate cancer screening and management.

Results

US studies on PHI in prostate cancer screening

In 2011, Catalona and colleagues published the results of a large multicenter trial of PHI for prostate cancer detection in 892 men with total PSA levels from 2 to 10 ng/ml and normal digital rectal examination (DRE) who were undergoing prostate biopsy [Catalona *et al.* 2011]. The mean PHI scores were 34 and 49 for men with negative and positive biopsies, respectively. Setting the sensitivity at 80–95%, PHI had greater specificity for distinguishing prostate cancer on biopsy compared with PSA or percentage free PSA (%fPSA). On receiver operating characteristic analysis, PHI had an area under the curve (AUC) of 0.70, compared with 0.65 for %fPSA and 0.53 for PSA. Although the PHI test has been approved by the US Food and Drug Administration only in the 4 -10 ng/ml PSA range, this study showed that PHI performed well in the 2-10 ng/ml PSA range. [Loeb *et al.* 2013].

More recently, Sanda and colleagues showed that not only did PHI outperform free and total PSA for prostate cancer detection, but it also improved the prediction of high-grade and clinically-significant prostate cancer [Sanda *et al.* 2013]. In 658 men with PSA levels of 4 to 10 ng/ml from the multicenter study population, this study showed a significant relationship between PHI and the Gleason score on prostate biopsy. PHI had a higher AUC (0.698) compared with %fPSA (0.654), p2PSA (0.550) and PSA (0.549) for clinically significant prostate cancer based on the Epstein criteria. Furthermore, a quarter of the study population had PHI levels <27, and only a single patient in this PHI range had a biopsy Gleason score ≥4+3 = 7. These combined findings suggest that the use of PHI could significantly reduce unnecessary biopsies and the overdetection of nonlethal disease.

Since the aforementioned results came from a large multicenter trial, it is important to note that PHI has also been examined in a grassroots population with consistent findings. Specifically, Le and colleagues compared PHI with to its individual components in men undergoing a prostate biopsy with PSA levels from 2.5 to 10 ng/ml and negative DRE from a prospective screening population of 2034 men [Le *et al.* 2010]. On ROC analysis, PHI had the highest AUC (0.77) compared with p2PSA (0.76), %fPSA (0.68) and PSA (0.50) for prostate cancer detection.

International studies on PHI in prostate cancer screening

Several large international studies have also reported on PHI, including the PRO-PSA Multicentric European Study. Among 646 European men from five centers undergoing prostate biopsy for a PSA of 2–10 ng/ml or suspicious DRE, Lazzeri and colleagues showed that using p2PSA or PHI significantly improved the prediction of biopsy outcome over total and free PSA [Lazzeri *et al.* 2013b]. While the use of %p2PSA or PHI would reduce the number of unnecessary biopsies by ≥15% at 90% sensitivity, PHI would miss the fewest high-grade tumors.

The same authors also reported a subset of men from this multicenter PROMEtheuS trial to specifically evaluate men with a positive family history of prostate cancer [Lazzeri *et al.* 2013a]. They found that proPSA and PHI were significant independent predictors of prostate cancer in this high-risk population. When added to a model containing PSA and prostate volume, p2PSA and PHI led to a 8.7% and 10% increase in accuracy, respectively ($p < 0.0001$). In addition, p2PSA and PHI were associated with Gleason score on biopsy, suggesting their potential utility to reduce unnecessary biopsies in men with a positive family history. Additional study is warranted to further examine the potential utility of PHI in other high-risk populations, including men of African descent.

Several groups have also compared the performance of PHI with other prostate cancer biomarkers leading up to a prostate biopsy. For example, Scattoni and colleagues reported on a comparison between PHI and PCA3 in European men undergoing initial or repeat biopsy. Overall, PHI had a higher AUC (0.70) than either PCA3 (0.59) or %fPSA (0.60) [Scattoni *et al.* 2013]. Another series of 300 patients undergoing first biopsy in Italy had a 36% prostate cancer detection rate [Ferro *et al.* 2013]. They reported an AUC of 0.77 for PHI, which compared favorably with 0.73 for PCA3 and 0.62 for free PSA. On decision curve analysis, PHI had greater net benefit at threshold probabilities >25%. Stephan and colleagues also performed a comparison of PHI with both PCA3 and the urinary TMPRSS2:ERG test in 246 men undergoing either initial or repeat prostate biopsy [Stephan *et al.* 2013]. In the overall population, PHI and PCA3 had a statistically similar AUC for prostate cancer detection on biopsy, and in general, the inclusion of both variables led to significant net benefit compared with standard parameters. However, their comparative performance differed between clinical scenarios, with PCA3 performing best in men undergoing repeat biopsy. Nevertheless, only PHI correlated with Gleason score among men with prostate cancer, while PCA3 and TMPRSS2:ERG did not.

PHI for risk stratification and treatment outcomes

The recent Melbourne Consensus Statement discusses the importance of dissociating diagnosis from treatment and considering active surveillance as a way to reduce overtreatment for men with low-risk disease [Murphy *et al.* 2013]. There is currently no consensus over the optimal patient selection and follow-up protocol for patients on active surveillance. Some programs use PSA kinetics to help determine the need for intervention, but others have found that changes in total PSA are not always reliable predictors of histological findings, at least in the short term [Ross *et al.* 2010]. The Johns Hopkins active surveillance program includes men with very low-risk prostate cancer (clinical stage T1c, PSA density<0.15, **Gleason ≤6** in a maximum of 2 positive cores with **≤50%** involvement) and has traditionally used annual repeat prostate biopsies to assess for signs of progression. Increasing recognition of the risks of prostate biopsy highlights the need for other noninvasive modalities that can be used to monitor patients during active surveillance [Loeb *et al.* 2012]. Numerous recent studies have suggested that magnetic resonance imaging (MRI) may be helpful during active surveillance [Morgan *et al.* 2011]. In addition, Tosoian and colleagues showed that both baseline and longitudinal values of PHI predicted which men would have reclassification to higher-risk disease on repeat biopsy during a median follow up of 4.3 years after diagnosis [Tosoian *et al.* 2012]. Baseline and longitudinal measurements of PHI had C-indices of 0.788 and 0.820 for upgrading on repeat surveillance biopsy, respectively. In contrast, an earlier study in the Johns Hopkins active surveillance, PCA3 did not reliably predict short-term biopsy progression during active surveillance [Tosoian *et al.* 2010]. Additional studies are warranted to further examine the use of PHI in different active surveillance populations.

Risk stratification is also important for men undergoing definitive treatment and those with more advanced disease. Although relatively fewer **studies** have been studied using phi in this clinical context, a recent pilot study of men with biochemical recurrence reported significantly higher p2PSA and phi in men with metastatic progression compared those without clinical metastasis [Sottile *et al.* 2012]. Future studies are necessary to further evaluate and validate a role for PHI in the management of more advanced disease.

Conclusion

Although no single marker in isolation has perfect performance characteristics, PHI is a simple and inexpensive blood test that should be used as part of a multivariable approach to screening. In multiple prospective international trials, this composite measurement has been shown to outperform conventional PSA and free PSA measurements. Unlike PCA3 and TMPRSS2:ERG, PHI is also consistently associated with Gleason score and upgrading during active surveillance. PHI should be considered as part of the standard urologic armamentarium for biopsy decisions, risk stratification and treatment selection.

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Conflict of interest statement

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References

American Cancer Society (ACS) (2013) Cancer facts & figures 2013 [online]. Atlanta, GA: American Cancer Society. Available at: http://www.cancer.org/acs/groups/content/@epidemiologysurveilance/documents/document/acspc-036845.pdf (accessed 1 August 2013).

Catalona, W., Bartsch, G., Rittenhouse, H., Evans, C., Linton, H., Amirkhan, A. *et al.* (2003) Serum pro prostate specific antigen improves cancer detection compared to free and complexed prostate specific antigen in men with prostate specific antigen 2 to 4 ng/ml. *J Urol* 170: 2181–2185.

Catalona, W., Partin, A., Sanda, M., Wei, J., Klee, G., Bangma, C. *et al.* (2011) 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* 185: 1650–1655.

Ferro, M., Bruzzese, D., Perdona, S., Marino, A., Mazzarella, C., Perruolo, G. *et al.* (2013) Prostate Health Index (PHI) and Prostate Cancer Antigen

3 (PCA3) significantly improve prostate cancer detection at initial biopsy in a total PSA range of 2–10 ng/ml. *PLoS One* 8: e67687.

Lazzeri, M., Haese, A., Abrate, A., de la Taille, A., Redorta, J., McNicholas, T. *et al.* (2013a) 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* 112: 313–321.

Lazzeri, M., Haese, A., de la Taille, A., Palou Redorta, J., McNicholas, T., Lughezzani, G. *et al.* (2013b) Serum isoform [-2]proPSA derivatives significantly improve prediction of prostate cancer at initial biopsy in a total PSA range of 2–10 ng/ml: a multicentric European study. *Eur Urol* 63: 986–994.

Le, B., Griffin, C., Loeb, S., Carvalhal, G., Kan, D., Baumann, N. *et al.* (2010) [-2]Proenzyme prostate specific antigen is more accurate than total and free prostate specific antigen in differentiating prostate cancer from benign disease in a prospective prostate cancer screening study. *J Urol* 183: 1355–1359.

Lilja, H., Christensson, A., Dahlen, U., Matikainen, M., Nilsson, O., Pettersson, K. *et al.* (1991) Prostate-specific antigen in serum occurs predominantly in complex with alpha 1-antichymotrypsin. *Clin Chem* 37: 1618–1625.

Loeb, S., Carter, H., Berndt, S., Ricker, W. and Schaeffer, E. (2012) Is repeat prostate biopsy associated with a greater risk of hospitalization? Data from SEER-Medicare. *J Urol* 189: 867–870.

Loeb, S., Sokoll, L., Broyles, D., Bangma, C., van Schaik, R., Klee, G. *et al.* (2013) Prospective multicenter evaluation of the Beckman Coulter Prostate Health Index using WHO calibration. *J Urol* 189: 1702–1706.

Mikolajczyk, S., Catalona, W., Evans, C., Linton, H., Millar, L., Marker, K. *et al.* (2004) Proenzyme forms of prostate-specific antigen in serum improve the detection of prostate cancer. *Clin Chem* 50: 1017–1025.

Morgan, V., Riches, S., Thomas, K., Vanas, N., Parker, C., Giles, S. *et al.* (2011) Diffusion-weighted magnetic resonance imaging for monitoring prostate cancer progression in patients managed by active surveillance. *Br J Radiol* 84: 31–37.

Murphy, D., Costello, T., Walsh, P., Ahlering, T., Cataolona, W., Santor, O. et al. (2013) The Melbourne Consensus Statement on Prostate Cancer Testing [online], BJU International. Available at: http://www.bjuinternational.com/bjui-blog/the-melbourne-consensus-statement-on-prostate-cancer-testing/ (accessed 15 September 2013).

Ross, A., Loeb, S., Landis, P., Partin, A., Epstein, J., Kettermann, A. *et al.* (2010) Prostate-specific antigen kinetics during follow-up are an unreliable trigger for intervention in a prostate cancer surveillance program. *J Clin Oncol* 28: 2810–2816.

Sanda, M., Wei, J., Broyles, D., Shin, S., Partin, A., Klee, G. *et al.* (2013) Evaluation of the Prostate Health Index (PHI) for improving prostate cancer detection and identification of clinically significant prostate cancer in the 4 to 10 ng/mL PSA range. In: *Proceedings of American Urological Association Annual Meeting*, San Diego.

Scattoni, V., Lazzeri, M., Lughezzani, G., De Luca, S., Passera, R., Bollito, E. *et al.* (2013) Head-to-head comparison of Prostate Health Index and urinary PCA3 for predicting cancer at initial or repeat niopsy. *J Urol* 190: 496–501.

Schroder, F., Hugosson, J., Roobol, M., Tammela, T., Ciatto, S., Nelen, V. *et al.* (2012) Prostate-cancer mortality at 11 years of follow-up. *New Engl J Med* 366: 981–990.

Sokoll, L., Sanda, M., Feng, Z., Kagan, J., Mizrahi, I., Broyles, D. *et al.* (2010) A prospective, multicenter, National Cancer Institute Early Detection Research Network study of [-2]proPSA: improving prostate cancer detection and correlating with cancer aggressiveness. *Cancer Epidemiol Biomarkers Prev* 19: 1193–1200.

Sottile, A., Ortega, C., Berruti, A., Mangioni, M., Saponaro, S., Polo, A. *et al.* (2012) A pilot study evaluating serum pro-prostate-specific antigen in patients with rising PSA following radical prostatectomy. *Oncol Lett* 3: 819–824.

Stenman, U., Leinonen, J., Alfthan, H., Rannikko, S., Tuhkanen, K. and Alfthan, O. (1991) A complex between prostate-specific antigen and alpha 1-antichymotrypsin is the major form of prostate-specific antigen in serum of patients with prostatic cancer: assay of the complex improves clinical sensitivity for cancer. *Cancer Res* 51: 222–226.

Stephan, C., Jung, K., Semjonow, A., Schulze-Forster, K., Cammann, H., Hu, X. *et al.* (2013) Comparative assessment of urinary prostate cancer antigen 3 and TMPRSS2:ERG gene fusion with the serum [-2]proprostate-specific antigen-based prostate health index for detection of prostate cancer. *Clin Chem* 59: 280–88.

Tosoian, J., Loeb, S., Feng, Z., Isharwal, S., Landis, P., Elliot, D. *et al.* (2012) Association of [-2]proPSA with biopsy reclassification during active surveillance for prostate cancer. *J Urol* 188: 1131–1136.

Tosoian, J., Loeb, S., Kettermann, A., Landis, P., Elliot, D., Epstein, J. *et al.* (2010) Accuracy of PCA3 measurement in predicting short-term biopsy progression in an active surveillance program. *J Urol* 183: 534–538.

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

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Abstract

***Background/Aim**: Several efforts have been made to find biomarkers that could help clinicians to preoperatively determine prostate cancer (PCa) pathological characteristics and choose the best therapeutic approach, avoiding over-treatment. On this effort, prostate cancer antigen 3 (PCA3), prostate health index (phi) and sarcosine have been presented as promising tools. We evaluated the ability of these biomarkers to predict the pathologic PCa characteristics within a prospectively collected contemporary cohort of patients who underwent radical prostatectomy (RP) for clinically localized PCa at a single high-volume Institution.*

***Materials and Methods**: The prognostic performance of PCA3, phi and sarcosine were evaluated in 78 patients undergoing RP for biopsy-proven PCa. Receiver operating characteristic (ROC) curve analyses tested the accuracy (area under the curve (AUC)) in predicting PCa pathological characteristics. Decision curve analyses (DCA) were used to assess the clinical benefit of the three biomarkers.*

Results: *We found that:*

PCA3, phi and sarcosine levels were significantly higher in patients with tumor volume (TV) ≥0.5 ml, pathologic Gleason sum (GS) ≥7 and pT3 disease (all p-values ≤0.01).

ROC curve analysis showed that:

> *PHI is an accurate predictor of high-stage (AUC 0.85 [0.77-0.93]), high-grade (AUC 0.83 [0.73-0.93]) and high-volume disease (AUC 0.94 [0.88-0.99]).*
>
> *Sarcosine showed a comparable AUC (0.85 [0.76-0.94]) only for T3 stage prediction.*
>
> *PCA3 score showed lower AUCs, ranging from 0.74 (for GS) to 0.86 (for TV).*

Conclusion: *PCA3, phi and sarcosine are predictors of PCa characteristics at final pathology. Successful clinical translation of these findings would reduce the frequency of surveillance biopsies and may enhance acceptance of active surveillance (AS).*

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Prognostic accuracy of Prostate Health Index and urinary Prostate Cancer Antigen 3 in predicting pathologic features after radical prostatectomy.

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Abstract

OBJECTIVE:

To compare the prognostic accuracy of Prostate Health Index (PHI) and Prostate Cancer Antigen 3 in predicting pathologic features in a cohort of patients who underwent radical prostatectomy (RP) for prostate cancer (PCa).

METHODS AND MATERIALS:

We evaluated 156 patients with biopsy-proven, clinically localized PCa who underwent RP between January 2013 and December 2013 at 2 tertiary care institutions. Blood and urinary specimens were collected before initial prostate biopsy for [-2] pro-prostate-specific antigen (PSA), its derivates, and PCA3 measurements. Univariate and multivariate logistic regression analyses were carried out to determine the variables that were potentially predictive of tumor volume >0.5ml, pathologic Gleason sum≥7, pathologically confirmed significant PCa, extracapsular extension, and seminal vesicles invasions.

RESULTS:

On multivariate analyses and after bootstrapping with 1,000 resampled data, the inclusion of PHIsignificantly increased the accuracy of a baseline multivariate model, which included patient age, total PSA, free PSA, rate of positive cores, clinical stage, prostate volume, body mass index, and biopsy Gleason score (GS), in predicting the study outcomes. Particularly, to predict tumor volume>0.5, the addition of PHI to the baseline model significantly increased predictive accuracy by 7.9% (area under the receiver operating characteristics curve [AUC] = 89.3 vs. 97.2, P>0.05), whereas PCA3 did not lead to a significant increase. Although both PHI and PCA3 significantly improved predictive accuracy to predict extracapsular extension compared with the baseline model, achieving independent predictor status (all P′s<0.01), only PHI led to a significant improvement in the prediction of seminal vesicles invasions (AUC = 92.2, P<0.05 with a gain of 3.6%). In the subset of patients with GS≤6, PHIsignificantly improved predictive accuracy by 7.6% compared with the baseline model (AUC = 89.7 vs. 97.3) to predict pathologically confirmed significant PCa and by 5.9% compared with the baseline model (AUC = 83.1 vs. 89.0) to predict pathologic GS≥7. For these outcomes, PCA3 did not add incremental predictive value.

CONCLUSIONS:

In a cohort of patients who underwent RP, PHI is significantly better than PCA3 in the ability to predict the presence of both more aggressive and extended PCa.

KEYWORDS:

Active surveillance; PCA3; PHI; Prognostic accuracy; Prostate cancer; Radical prostatectomy

PMID: 25575715 [PubMed - as supplied by publisher]

Prostate Health Index (*phi*)

Regulatory Information

FDA APPROVAL

phi is indicated for use as an aid in distinguishing prostate cancer from benign prostatic conditions, for prostate cancer detection in men aged 50 years and older with total PSA ≥ 4 .0 to ≤ 10.0 ng/mL, and with digital rectal examination findings that are not suspicious for cancer. Peer-reviewed published studies support the use of the *phi* test in men with total PSA values as low as 2 ng. Prostatic biopsy is required for diagnosis of cancer. (See FDA Letter Following this Page)

Recommended by National Comprehensive Cancer Network (NCCN)

phi has been recommended by the National Comprehensive Cancer Network (NCCN) as a blood test to improve specificity for prostate cancer detection in its Clinical Practice Guidelines in Oncology (NCCN Guidelines) for Prostate Cancer Early Detection. Inclusion in the NCCN Guidelines recognizes the benefit and clinical utility of *phi* to help the appropriate use of prostate biopsy, and therefore help bring about better cancer diagnosis.

DEPARTMENT OF HEALTH & HUMAN SERVICES

Food and Drug Administration

10903 New Hampshire Avenue
Silver Spring, MD 20993

JUN 14 2012

Mr. Brent Taber
Staff Regulatory Specialist
Beckman Coulter, Inc.
1000 Lake Hazeltine Dr.
Chaska, MN 55318-1084

Re: P090026
Access® Hybritech® p2PSA on the Access Immunoassay Systems
Filed: November 17, 2009
Amended: January 8, 2010, July 28, 2010, April 7, 2011 and September 6, 2011
Procode: OYA

Dear Mr. Taber:

The Center for Devices and Radiological Health (CDRH) of the Food and Drug Administration (FDA) has completed its review of your premarket approval application (PMA) for the Access® Hybritech® p2PSA on the Access Immunoassay Systems. This device is indicated for:

> The Access Hybritech p2PSA assay is a paramagnetic particle, chemiluminescent immunoassay for the quantitative determination of [-2]proPSA antigen, an isoform of free PSA, in human serum using the Access Immunoassay Systems. Access Hybritech p2PSA is intended to be used in combination with Access Hybritech (total) PSA and Access Hybritech free PSA to calculate the Beckman Coulter Prostate Health Index (*phi*), an In Vitro Diagnostic Multivariate Index Assay (IVDMIA).
>
> Beckman Coulter *phi* as calculated using the Access Hybritech assays is indicated for use as an aid in distinguishing prostate cancer from benign prostatic conditions, for prostate cancer detection in men aged 50 years and older with total PSA $\geq$ 4.0 to $\leq$ 10.0 ng/mL, and with digital rectal examination findings that are not suspicious for cancer. Prostatic biopsy is required for diagnosis of cancer.

We are pleased to inform you that the PMA is approved. You may begin commercial distribution of the device in accordance with the conditions of approval described below.

The sale and distribution of this device are restricted to prescription use in accordance with 21 CFR 801.109 and under section 515(d)(1)(B)(ii) of the Federal Food, Drug, and Cosmetic Act (the act). FDA has determined that this restriction on sale and distribution is necessary to provide reasonable assurance of the safety and effectiveness of the device. Your device is therefore a restricted device subject to the requirements in sections 502(q) and (r) of the act, in addition to the many other FDA requirements governing the manufacture, distribution, and marketing of devices.

Expiration dating for this device has been established and approved at 12 months when stored at 2 to 10°C. Expiration dating for the Access Hybritech p2PSA calibrator has been established and approved at 12 months when stored unopened at ≤ -20°C.

Continued approval of this PMA is contingent upon the submission of periodic reports, required under 21 CFR 814.84, at intervals of one year (unless otherwise specified) from the date of approval of the original PMA. Two copies of this report, identified as "Annual Report" (please use this title even if the specified interval is more frequent than one year) and bearing the applicable PMA reference number, should be submitted to the address below. The Annual Report should indicate the beginning and ending date of the period covered by the report and should include the information required by 21 CFR 814.84.

In addition to the above, and in order to provide continued reasonable assurance of the safety and effectiveness of the device, the Annual Report must include, separately for each model number (if applicable), the number of devices sold and distributed during the reporting period, including those distributed to distributors. The distribution data will serve as a denominator and provide necessary context for FDA to ascertain the frequency and prevalence of adverse events, as FDA evaluates the continued safety and effectiveness of the device.

Before making any change affecting the safety or effectiveness of the device, you must submit a PMA supplement or an alternate submission (30-day notice) in accordance with 21 CFR 814.39. All PMA supplements and alternate submissions (30-day notice) must comply with the applicable requirements in 21 CFR 814.39. For more information, please refer to the FDA guidance document entitled, "Modifications to Devices Subject to Premarket Approval (PMA) - The PMA Supplement Decision-Making Process" (www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm089274.htm).

You are reminded that many FDA requirements govern the manufacture, distribution, and marketing of devices. For example, in accordance with the Medical Device Reporting (MDR) regulation, 21 CFR 803.50 and 21 CFR 803.52, you are required to report adverse events for this device. Manufacturers of medical devices, including in vitro diagnostic devices, are required to report to FDA no later than 30 calendar days after the day they receive or otherwise becomes aware of information, from any source, that reasonably suggests that one of their marketed devices:

1. May have caused or contributed to a death or serious injury; or

2. Has malfunctioned and such device or similar device marketed by the manufacturer would be likely to cause or contribute to a death or serious injury if the malfunction were to recur.

Additional information on MDR, including how, when, and where to report, is available at www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm.

In accordance with the recall requirements specified in 21 CFR 806.10, you are required to submit a written report to FDA of any correction or removal of this device initiated by you to: (1) reduce a risk to health posed by the device; or (2) remedy a violation of the act caused by the device which may present a risk to health, with certain exceptions specified in 21 CFR 806.10(a)(2). Additional information on recalls is available at www.fda.gov/Safety/Recalls/IndustryGuidance/default.htm.

CDRH does not evaluate information related to contract liability warranties. We remind you; however, that device labeling must be truthful and not misleading. CDRH will notify the public of its decision to approve your PMA by making available, among other information, a summary of the safety and effectiveness data upon which the approval is based. The information can be found on the FDA CDRH Internet HomePage located at www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/DeviceApprovalsandClearances/PMAApprovals/default.htm. Written requests for this information can also be made to the Food and Drug Administration, Dockets Management Branch, (HFA-305), 5630 Fishers Lane, Rm. 1061, Rockville, MD 20852. The written request should include the PMA number or docket number. Within 30 days from the date that this information is placed on the Internet, any interested person may seek review of this decision by submitting a petition for review under section 515(g) of the act and requesting either a hearing or review by an independent advisory committee. FDA may, for good cause, extend this 30-day filing period.

Failure to comply with any post-approval requirement constitutes a ground for withdrawal of approval of a PMA. The introduction or delivery for introduction into interstate commerce of a device that is not in compliance with its conditions of approval is a violation of law.

You are reminded that, as soon as possible and before commercial distribution of your device, you must submit an amendment to this PMA submission with copies of all approved labeling in final printed form. Final printed labeling that is identical to the labeling approved in draft form will not routinely be reviewed by FDA staff when accompanied by a cover letter stating that the final printed labeling is identical to the labeling approved in draft form. If the final printed labeling is not identical, any changes from the final draft labeling should be highlighted and explained in the amendment.

All required documents should be submitted in triplicate, unless otherwise specified, to the address below and should reference the above PMA number to facilitate processing. One of those three copies may be an electronic copy (eCopy), in an electronic format that FDA can process, review and archive (general information: http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/HowtoMarketYourDevice/PremarketSubmissions/ucm134508.htm; clinical and statistical data: http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/HowtoMarketYourDevice/PremarketSubmissions/ucm136377.htm)

U.S. Food and Drug Administration
Center for Devices and Radiological Health
PMA Document Mail Center – WO66-G609
10903 New Hampshire Avenue
Silver Spring, MD 20993-0002

If you have any questions concerning this approval order, please contact Maria M. Chan at 301-796-5482.

Sincerely yours,

for Alberto Gutierrez, Ph.D.
Office Director
Office of In Vitro Diagnostic Device Evaluation and Safety
Center for Devices and Radiological Health

Prostate Health Index (*phi*)

Customer Support

Evaluation & Customer Support

MEMORIAL HERMAN HEALTH SYSTEM - evaluation completed 2014

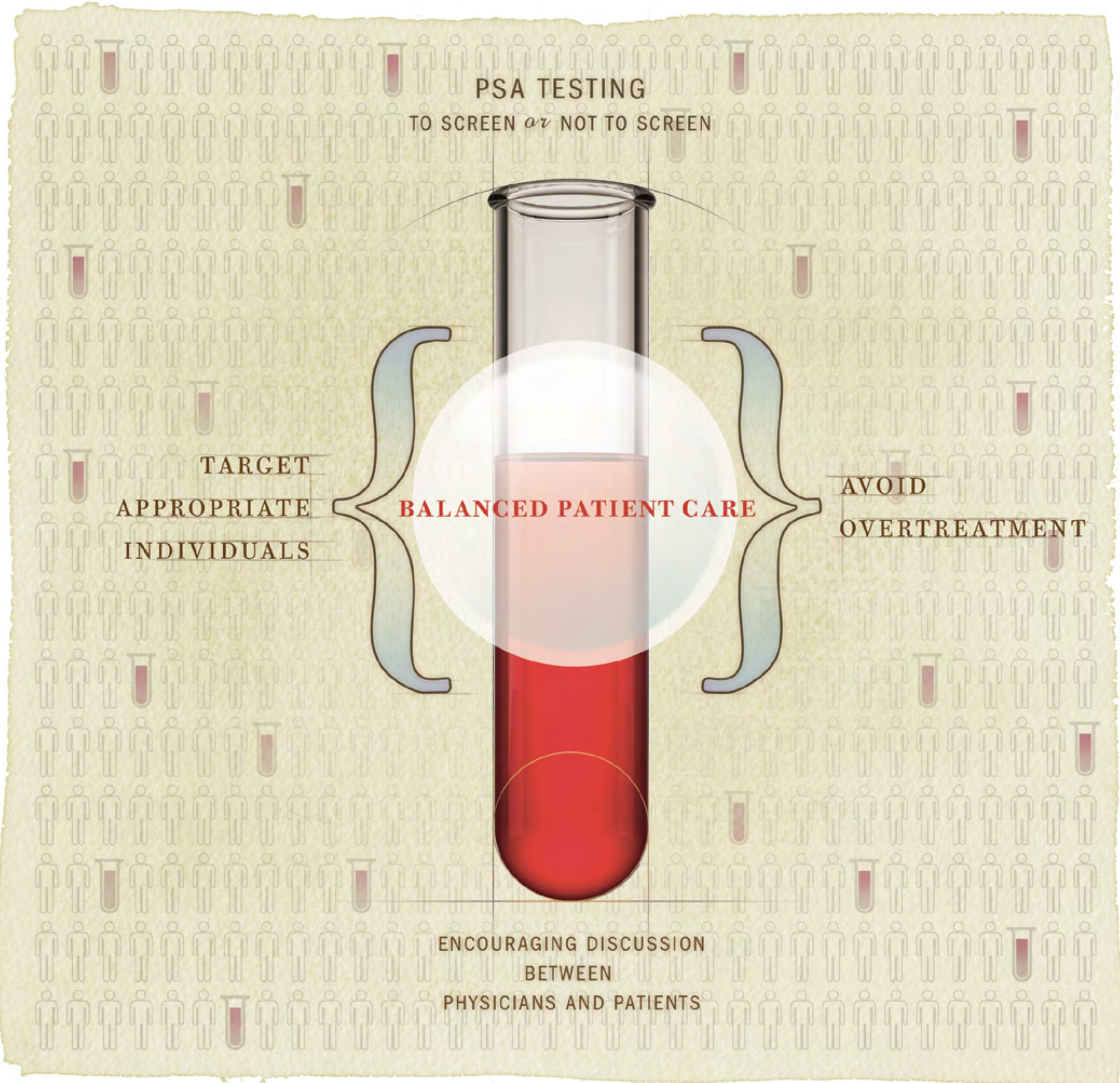
MD ANDERSON - evaluation completed 2015

More than 600 practices have used the *phi* test thoughout the US.

14 | FALL

MEMORIAL HERMANN TEXAS MEDICAL CENTER UROLOGY JOURNAL

A Publication from Memorial Hermann-Texas Medical Center and the UTHealth Medical School



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Understanding *and* Implementing PSA Guidelines *into* Practice

By Kevin M. Slawin, M.D.

The individual and societal burden of prostate cancer is enormous. In 2013, the American Cancer Society estimated that nearly 240,000 new cases would be diagnosed in the United States alone, and 29,720 American men – or 1 in 36 – would die of the disease. Prostate cancer is the second leading cause of cancer fatality among American men, second only to lung cancer.

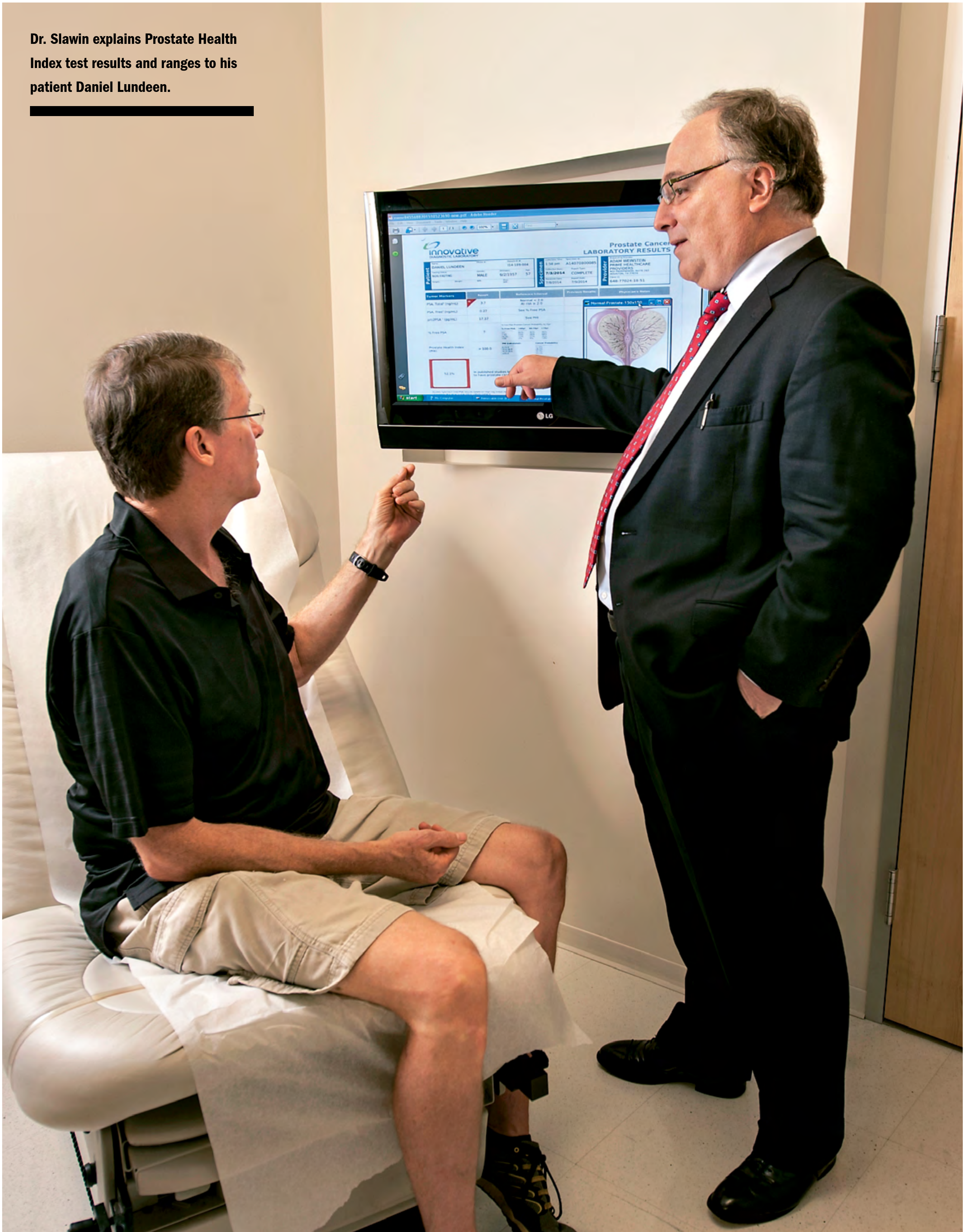
"NEW ADVANCES IN MOLECULAR MARKERS HAVE IMPROVED OUR ABILITY TO BETTER GAUGE THE RISK OF SERIOUS PROSTATE CANCER, AND HELP GUIDE BETTER DECISION-MAKING ABOUT THE DIAGNOSIS AND TREATMENT OF POTENTIALLY LETHAL DISEASE."

In May 2012, the United States Preventive Services Task Force (USPSTF) recommended against PSA-based screening for prostate cancer, noting that there is "a very small potential benefit and significant potential harms." The panel, which did not include urologists or cancer specialists, advised clinicians to "not screen their patients with a PSA test unless the individual being screened understands what is known about PSA screening and makes the personal decision that even a small possibility of benefit outweighs the known risk of harms." The recommendation applies to men in the general U.S. population, regardless of age.

While the recommendation was written with good intent, the fact remains that the introduction of the PSA blood test has resulted in significantly more early stage prostate cancer diagnoses, including high-risk cancers for which potentially curative treatment options can be offered. Studies support an initial PSA test for men between the ages of 40 and 45, before the possibility of the presence of benign prostatic hyperplasia (BPH) may confound the ability of the test to establish the future risk of prostate cancer. A baseline serum PSA level ≥ 1.0 ng/ml at 45 years of age and a baseline serum PSA level ≥ 2.0 ng/ml at 60 years of age are associated with a significantly increased risk of prostate cancer-related mortality and diagnosis of advanced or metastatic disease even 25 years after the initial PSA was obtained. Based on these and other studies, the European Urological Association (EUA) issued sound, evidence-based guidelines for early detection of prostate cancer in July 2013.[1] These guidelines included recommendations that baseline testing be done between the ages of 40 and 45. In a patient with very low PSA and the absence of symptoms, the need for further lifetime screening may be obviated. A PSA of less than 1.0 ng/ml is considered low risk and a good indication of the potential lack of need for intensive screening in the future, whereas men with a higher PSA at that age may need to be followed more closely as they age. The EUA guidelines balance early screening with appropriate surveillance guidelines and appear to be more scientifically nuanced than the USPSTF guidelines.

Prostate-specific antigen testing may be problematic. PSA is not a classic tumor marker – expression is highest in benign cells. At lower levels, it primarily reflects the presence of BPH. While there is persistent debate over the risk-to-benefit ratio of PSA-based screening for prostate cancer, there is general agreement about the need for new markers specifically associated with biologically aggressive prostate cancer for improved diagnosis and staging.

In 2012, the FDA approved a groundbreaking, new prostate cancer screening test called the Prostate Health Index *(phi)*. This new screening test combines the PSA and free PSA with a novel,



Dr. Slawin explains Prostate Health Index test results and ranges to his patient Daniel Lundeen.

clipped form of the precursor to PSA, called [-2]pro-PSA. This precursor form of PSA, which is more elevated in prostate cancer patients and more accurately identifies the disease, was jointly discovered by myself and researchers at Beckman Coulter. Baylor College of Medicine, where I practiced at the time, licensed the technology exclusively to Beckman Coulter, which then developed the new screening test. PSA-screening expert William Catalona, M.D., led a multicenter study that confirmed the improved performance of the *phi* score over the PSA or free PSA tests, the results of which were published in the *Journal of Urology*.[2] The *phi* is approved and available in Europe, and was recently launched in the United States through Innovative Diagnostic Laboratory in Richmond, Virginia.

"WHILE NOT ALL PROSTATE CANCERS ARE POTENTIALLY LETHAL, IF WE DON'T MAINTAIN OUR FOCUS ON THE EARLY DETECTION OF PROSTATE CANCER, WE WILL FAIL TO DETECT THOSE AGGRESSIVE CANCERS THAT WARRANT AGGRESSIVE, POTENTIALLY LIFE-SAVING THERAPY."

The phi test reduces unnecessary biopsies by 26 percent for men with PSA values between 2-10 ng/mL. The test also preferentially detects more aggressive, potentially life-threatening cancers that most agree require treatment. FDA approval of phi has renewed the path to effective screening and offers hope and subsequent treatment to patients in whom disease may have gone previously unidentified. It represents a significant step forward in settling the prostate cancer screening controversy and has the potential to reintroduce screening as a viable and important tool in the overall disease management of prostate cancer, preventing us from losing the considerable ground we've gained since PSA was first introduced.

For men in their 50s and older with an elevated PSA, new advances in molecular markers have improved our ability to better gauge the risk of serious prostate cancer, and to assist in the approach to the diagnosis and treatment of potentially lethal disease. Treatment options include active surveillance for men with smaller, lower-grade tumors who meet rigid criteria. For men who choose surgical removal of the prostate gland as treatment for early prostate cancer, advanced robotic techniques in the hands of an experienced surgeon may reduce the chances of debilitating side effects such as incontinence and impotence, problems too often cited in the media as inevitable complications from prostate cancer surgery.

In the midst of this controversy, there are nine principles supported by most medical evidence[3]:

1) PSA is strongly associated with prostate cancer. There is a strong relationship at the population level between PSA and clinically relevant prostate cancer endpoints. There are few other markers in medicine that can predict disease-specific death at 25 years with an area-under-the-curve of 0.90.
2) Screening can be risk stratified. PSA is highly informative of long-term risk. Screening could focus on the men at highest risk, identified by PSA. Men at lower risk may need less frequent screening or in some cases, the need for subsequent screening may be completely eliminated.
3) The DRE is not an effective screening test. In a man with elevated PSA, a positive DRE does not indicate increased risk of cancer. In low PSA ranges, however, the positive predictive value of DRE is very poor – 4 to 11 percent – and the DRE adds little information.
4) PSA has moderate specificity. Most men with an elevated PSA do not have prostate cancer. This has led to the search for markers to use as a reflex test in men with elevated PSA, including free PSA; a panel of four kallikrein markers in blood; and the recently launched *phi* test that includes [-2]pro-PSA, urinary PCA3, and urinary detection of the TMPRSS2-ERG gene fusion.

5) PSA screening is associated with substantial overdiagnosis. Many of the cancers identified by current approaches to PSA-based screening would never have become apparent in the course of a man's lifetime. PSA screening is recommended in men with a life expectancy of 10 years. It is clear that, given a mean lead time of 12 years, a non-negligible proportion of men would die in the period between screen and clinical cancer detection.
6) PSA screening reduces prostate cancer mortality in men who would not otherwise be screened. The European Randomized Study of Screening for Prostate Cancer (ERSPC) trial reported statistically significant reductions in cancer mortality in the participants randomized to screening compared to unscreened controls.[4]
7) The benefits of screening take time to accrue. The survival curves in ERSPC only separated noticeably after about 10 years.
8) Not all cancers need treatment. Recent long-term studies suggest low risk of prostate cancer death from patients with Gleason 6 tumors, suggesting that many of these patients will not benefit from immediate treatment and could therefore be placed on an active surveillance program. This is especially relevant as, in the ERSPC, nearly three-quarters of the patients diagnosed in the screening arm had a Gleason score of 6 or less.
9) The type of treatment matters. PSA screening in and of itself cannot prevent mortality or lead to physical dysfunction; it is treatment following diagnosis of screen-detected cancer that leads to both benefit and harm. Benefits can be maximized and harms minimized if patients in need of curative therapy are treated by high-volume surgeons, or by radiation oncologists who use high-dose approaches.

While not all prostate cancers are potentially lethal, if we don't maintain our focus on the early detection of prostate cancer, we will fail to detect those aggressive cancers that warrant aggressive, potentially life-saving therapy. We must rely on the urologists caring for these patients to wisely apply these new technologies and knowledge to focus on the early detection and cure of aggressive prostate cancer, not strip them of their ability to effectively manage this common but complex disease.

"THE PHI TEST COMBINES THE PSA AND FREE PSA WITH A NOVEL, CLIPPED FORM OF THE PRECURSOR TO PSA, CALLED [-2]PRO-PSA."

Dr. Slawin is director of the Vanguard Urologic Institute at Memorial Hermann Medical Group, director of urology at Memorial Hermann-Texas Medical Center, adjunct professor at the Center for Clinical and Translational Sciences at The University of Texas Health Science Center at Houston and clinical professor of urology at Baylor College of Medicine. He has devoted his career to the study and clinical care of men with prostate cancer and is a pioneer in robotic prostatectomy, which he first performed in 2001. He emphasizes the importance of minimizing the risks of prostate biopsy and reducing the side effects of prostate cancer treatment.

[1] Heidenreich A, Abrahamsson P, Artibani W, Catto J, Montorsi F, Van Poppel H, Wirth M, Mottet N. Early Detection of Prostate Cancer Recommendation: European Association of Urology Recommendation. European Urology. 2013;64:347-54.

[2] Catalona WJ, Partin AW, Sanda MG, Wei JT, Klee GG, Bangma CH, Slawin KM, Marks LS, Loeb S, Broyles DL, Shin SS, Cruz AB, Chan DW, Sokoll LJ, Roberts WL, van Schaik RHN, Mizrahi IA. A Multi-Center Study of [-2]Pro-Prostate-Specific Antigen (PSA) in Combination with PSA and Free PSA for Prostate Cancer Detection in the 2.0 to 10.0 ng/mL PSA Range. J Urol. 2011 May;185(5):1650-55.

[3] Vickers AJ, Roobol MJ, Lilja H. Screening for Prostate Cancer: Early Detection or Overdetection? Annu Rev Med. 2012;63:161-170.

[4] Schröder FH, Hugosson J, Roobol MJ, Tammela TLJ, Ciatto S, Nelen V, Kwiatkowski M, Lujan M, Lilja H, Zappa M, Denis LJ, Recker F, Berenguer A, Määttänen L, Bangma CH, Aus G, Villers A, Rebillard X, van der Kwast T, Blijenberg BG, Moss SM, de Koning HJ, Auvinen A for the ERSPC Investigators. Screening and Prostate-Cancer Mortality in a Randomized European Study. N Engl J Med. 2009 Mar 26;360:1320-28.

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