EUROPEAN UROLOGY XXX (2017) XXX–XXX

available at www.sciencedirect.com
journal homepage: www.europeanurology.com

European Association of Urology

Platinum Priority – Prostate Cancer
Editorial by XXX on pp. x–y of this issue

The Single-parameter, Structure-based IsoPSA Assay Demonstrates Improved Diagnostic Accuracy for Detection of Any Prostate Cancer and High-grade Prostate Cancer Compared to a Concentration-based Assay of Total Prostate-specific Antigen: A Preliminary Report

Eric A. Klein [a,*], *Arnon Chait* [b], *Jason M. Hafron* [c], *Kenneth M. Kernen* [c], *Kannan Manickam* [d], *Andrew J. Stephenson* [a], *Mathew Wagner* [e], *Hui Zhu* [f], *Aimee Kestranek* [b], *Boris Zaslavsky* [b], *Mark Stovsky* [a,b]

[a] *Department of Urology, Glickman Urological and Kidney Institute, Cleveland Clinic, Cleveland, OH, USA;* [b] *Cleveland Diagnostics, Cleveland, OH, USA;* [c] *Pathology and Research Department, Michigan Institute of Urology, St Clair Shores, MI, USA;* [d] *Chesapeake Urology Associates, Baltimore, MD, USA;* [e] *Kaiser Permanente Northwest, Clackamas, OR, USA;* [f] *Louis Stokes VA Medical Center, Cleveland, OH, USA*

Article info

Article history:
Accepted March 17, 2017

Associate Editor:
James Catto

Keywords:
Biomarker
Prostate cancer
Prostate-specific antigen

Abstract

Background: IsoPSA is a serum-based assay that predicts prostate cancer (PCa) risk by partitioning isoforms of prostate-specific antigen (PSA) with an aqueous two-phase reagent.
Objectives: To determine the diagnostic accuracy of IsoPSA in identifying the presence or absence of PCa and the presence of high-grade disease in a contemporary biopsy cohort.
Design, setting, and participants: Multicenter prospective study of 261 men scheduled for prostate biopsy at five academic and community centers in the USA enrolled between August 2015 and December 2016.
Intervention: Performance of the IsoPSA assay.
Outcome measurements and statistical analysis: Discrimination power was evaluated using receiver operating characteristic (ROC) analysis. The outcome of the IsoPSA assay was transformed into risk probability using logistic regression. Decision curve analysis (DCA) was used to compare the net benefit of IsoPSA against other clinical protocols.
Results and limitations: The overall prevalence was 53% for any PCa and 34% for high-grade PCa. The area under the ROC curve was 0.79 for any cancer versus none and 0.81 for high-grade PCa versus low-grade PCa/benign histology. In this preliminary study, DCA revealed a superior net benefit of IsoPSA against no biopsy, all biopsy, and the modified Prostate Cancer Prevention Trial Risk Calculator 2.0. At a cutoff selected to recommend biopsy, IsoPSA demonstrated a 48% reduction in false-positive biopsies; at a cutoff selected to identity men at low risk of high-grade disease, there was a 45% reduction in the false-positive rate.
Conclusion: The structure-based IsoPSA assay outperformed concentration-based PSA measurement, and provided a net benefit against other protocols. Once validated, clinical use of IsoPSA could significantly reduce unnecessary biopsies while identifying patients needing treatment.
Patient summary: The IsoPSA assay outperformed prostate-specific antigen in predicting the overall risk of prostate cancer and the risk of clinically significant cancer in a preliminary study. The IsoPSA assay could assist in determining the need for prostate biopsy for patients.

* Corresponding author. Glickman Urological and Kidney Institute, Cleveland Clinic, 9500 Euclid Ave, Cleveland, OH 44106, USA. Tel. +1 216 4445601; Fax: +1 216 6364492.
E-mail address: kleine@ccf.org (E.A. Klein).

http://dx.doi.org/10.1016/j.eururo.2017.03.025
0302-2838/

1. Introduction

Prostate-specific antigen (PSA) is arguably the most successful blood-based cancer biomarker to date. Despite criticism [1,2], PSA has transformed the landscape of early detection, screening, and management of prostate cancer (PCa) in the last few decades. PSA is distinct from virtually all other cancer biomarkers because of its almost exclusive specificity to the prostate, allowing direct assessment of physiological conditions in the gland with a simple blood test. Unfortunately, PSA is tissue- but not cancer-specific, and overdiagnosis and overtreatment of PSA-detected, biologically insignificant cancers are widely recognized as key limitations in its clinical utility [3].

The vast majority of currently available protein-based cancer biomarkers are defined as normal or abnormal according to their concentration in body fluids. This definition owes more to the wide availability of low-cost and convenient technology such as enzyme-linked immunosorbent assays than to biological reasons. Indeed, an increase in biomarker concentration in blood or other body fluids could be due to a plethora of unrelated physiological mechanisms such as increased cell membrane permeability or inflammation resulting in cell death. Furthermore, many cancer-related proteins undergo alterations to their structure, including conformational changes due to point mutations, truncations, and post-translational modifications such as glycosylation [4–7] resulting from the altered metabolism of cancer cells. These structural changes may result in modified interactions with other proteins in the blood, offering an opportunity for improved methods of detection.

Recognition of structural changes to PSA, such as free PSA (which signifies differences in interaction of PSA with α_1-antichymotrypsin [8,9]) and pro-PSA, a specific isoform of PSA [10], have better diagnostic accuracy than measurement of the PSA parent protein alone. However, as the molecular evolution of cancer may result in changes in structural isoforms of a biomarker over time in the same patient, and in differences in which isoforms are present among individual patients, the diagnostic accuracy of even these structurally altered PSA proteins has limitations. The lack of perfect sensitivity of the currently available next-generation PSA assays such as PHI and 4Kscore may be attributable to the fact that they measure only a few known isoforms of PSA that are informative only if they are present in a given patient at a given time. Thus, since it is known that multiple isoforms of PSA that are not measured by current assays exist [11–13], a method that detects multiple PSA isoforms without a priori knowledge of which are present in a given sample is likely to have better diagnostic accuracy than existing assays.

Here we describe our initial clinical experience with IsoPSA, previously known as PSA/SIA [14], as a new blood-based assay for detection of PCa. IsoPSA is a structure-based (rather than concentration-based) assay that agnostically interrogates the entire spectrum of structural changes of complex PSA (ACT-PSA). We report on the performance of IsoPSA in a multi-institutional prospective study of US men referred for prostate biopsy on the basis of currently accepted clinical criteria. The endpoints of the study were the ability of the IsoPSA assay to identify the risk of any PCa (defined as Gleason $\geq$6) versus no cancer and of high-grade PCa (defined as Gleason $\geq$7) versus low-grade PCa or benign disease in comparison to a standard concentration-based assay for total PSA.

2. Patients and methods

2.1. Patient population and specimen collection

This institutional review board–approved, multicenter prospective study enrolled men scheduled for prostate biopsy because of a rising PSA level or suspicious digital rectal examination (DRE). Five academic and community urology centers across the USA (Cleveland Clinic; Louis Stokes VA Medical Center; Kaiser Permanente Northwest; Michigan Institute of Urology; and Chesapeake Institute of Urology) collected heparin-plasma for IsoPSA between August 2015 and December 2016. Samples were collected within 30 d before to biopsy, processed according to Early Detection Research Network (EDRN) guidelines [15], and frozen at −80 °C until analysis. The primary study endpoints of this preliminary study were the presence or absence of cancer and cancer grade as detected by 12-core transrectal ultrasound (TRUS) or magnetic resonance imaging (MRI)-TRUS fusion biopsy. Exclusion criteria included serum PSA <2 ng/ml; recent (<72 h) prostate manipulation, including DRE; recent (<2 wk) urinary tract infection and/or prostatitis; recent (<30 d) prostate surgery, urinary catheterization, prostate infarction, or endoscopic evaluation; and other urinary tract malignancy. Because IsoPSA measures PSA structure rather than concentration, men on 5α-reductase inhibitors (5ARIs), which are known to affect PSA concentration, were not excluded. Histopathologic evaluation of the biopsy specimens was performed by each site according to local standards. Overall, 434 samples were collected, with 173 exclusions: 84 because of prolonged storage (>90 d), 22 because of canceled biopsies, 21 because of serum PSA <2 ng/ml, 19 because of a breach in sample collection protocol, 21 because of shipping delays, and six because of other reasons, leaving a final analytical cohort of 261 samples. Signed informed consent was obtained from all enrollees. Demographic data and clinical information for the analytical cohort are shown in Table 1.

2.2. Laboratory methods

Frozen plasma samples were shipped to Cleveland Diagnostics (Cleveland, OH, USA) and all testing was performed and reported naïve to pathology outcome. On receipt, the samples were thawed and immediately added to IsoPSA reagent tubes. The reagent tubes were vortexed, centrifuged, and subjected to the IsoPSA assay (the IsoPSA assay is for research use only in the USA as of February 2017), which comprises two steps: partitioning of plasma samples in an aqueous two-phase system (IsoPSA RUO reagent, Cleveland Diagnostics), followed by measurement of free and total PSA concentrations in each of the two aqueous phases (referred to as top or bottom). An aliquot was removed from each phase and the total and free PSA concentrations were measured using US Food and Drug Administration–approved clinical assays (Cobas e411, Roche Diagnostics, Indianapolis, IN, USA). The relative robustness of the IsoPSA assay and its reliance on only standard clinical PSA assays is a distinct advantage for its eventual use in distributed environments.

The IsoPSA assay readout, or test parameter K, is calculated as:

$$K = \frac{[\text{complex PSA}]_{\text{bottom}}}{[\text{complex PSA}]_{\text{top}}} = \frac{[\text{total PSA}]_{\text{bottom}} - [\text{free PSA}]_{\text{bottom}}}{[\text{total PSA}]_{\text{top}} - [\text{free PSA}]_{top}}$$

Please cite this article in press as: Klein EA, et al. The Single-parameter, Structure-based IsoPSA Assay Demonstrates Improved Diagnostic Accuracy for Detection of Any Prostate Cancer and High-grade Prostate Cancer Compared to a Concentration-based Assay of Total Prostate-specific Antigen: A Preliminary Report. Eur Urol (2017), http://dx.doi.org/10.1016/j.eururo.2017.03.025

Table 1 – Demographic data and clinical parameters for the patient cohort by clinical status category

	Negative biopsy	Low-grade PCa (Gleason 6)	High-grade PCa (Gleason ≥7)
Number	122	51	88
Age at blood draw (yr)	63.00 (58–69)	63. 00 (57–70)	64.65 (60–72)
<50 yr	5 (4)	0 (0)	2 (2)
50–75 yr	115 (94)	48 (94)	76 (86)
>75 yr	2 (2)	3 (6)	10 (11)
Race			
African American	12 (10)	6 (12)	12 (14)
Caucasian	108 (89)	44 (86)	72 (82)
Hispanic	0 (0)	0 (0)	0 (0)
Other	0 (0)	0 (0)	3 (3)
Unknown	2 (2)	1 (2)	1 (1)
Abnormal digital rectal examination[a]	13 (11)	38 (75)	19 (22)
Prior prostate biopsy	35 (29)	17 (33)	19 (22)
Medications			
None	79 (64.8)	41 (8.4)	72 (81.8)
Other/unspecified	5 (4.1)	2 (3.9)	1 (1.1)
5-α reductase	2 (1.6)	0 (0.0)	1 (1.1)
α-Blockers	32 (26.2)	8 (15.7)	13 (14.8)
5α-Reductase + α-blockers	4 (3.28)	0 (0.0)	1 (1.1)
Total PSA (ng/ml)	5.68 (4.45–7.89)	5.45 (4.29–7.54)	7.45 (5.83–11.04)
2–4 ng/ml	18 (15)	11 (22)	6 (7)
4–10 ng/ml	87 (71)	36 (71)	52 (59)
10–25 ng/ml	14 (11)	2 (4)	25 (28)
>25 ng/ml	2 (2)	1 (2)	4 (5)
Free PSA (ng/ml)	1.02 (0.66–1.41)	0.74 (0.56–1.29)	0.79 (0.56–1.29)
Prostate volume (g)	49 (40–60)	40(31–44)	40 (30–50)
Free/total PSA ratio (%)	17 (13–22)	14 (11–18)	10 (8–16)
KR-CNC (%)	36 (28–51)	52 (37–74)	74 (51–87)
Quintile 1 (<30.5%)	43 (35)	7 (14)	2 (2)
Quintile 2 (30.5–42.1%)	36 (30)	10 (20)	6 (7)
Quintile 3 (42.1–56.2%)	19 (16)	15 (29)	19 (22)
Quintile 4 (56.2–79.6%)	17 (14)	8 (16)	27 (31)
Quintile 5 (>79.6%)	7 (6)	11 (22)	34 (29)
KR-HG (%)	16 (12–27)	34 (17–49)	50 (27–70)
Quintile 1 (<10.0%)	43 (35)	7 (14)	2 (2)
Quintile 2 (10.0–16.4%)	36 (30)	10 (20)	6 (7)
Quintile 3 (16.4–28.6%)	19 (16)	15 (29)	19 (22)
Quintile 4 (28.6–53.8%)	17 (14)	8 (16)	27 (31)
Quintile 5 (>53.8%)	7 (6)	11 (22)	34 (39)
Biopsy Gleason grade			
6		46 (100)	0 (0)
3 + 4		0 (0)	44 (61)
4 + 3		0 (0)	15 (21)
8		0 (0)	8 (11)
9		0 (0)	3 (4)
10		0 (0)	2 (3)

PCa = prostate cancer; KR = *K* risk value result; CNC = cancer versus no cancer; HG = high-grade PCa versus benign/low-grade PCa.
Data are presented as median (interquartile range) for continuous variables and *n* (%) for categorical variables.
[a] Recent digital rectal examination result not available for 35 of the patients included.

K is obtained ratiometrically and is not directly connected to the corresponding level of serum PSA, except for the fact that both *K* and the PSA concentration generally increase with cancer. The *K* parameter can be used directly to classify patients via binary analysis (eg, cancer present or absent), or converted by logistic regression to an individual risk probability, KR, for the two study indications.

2.3. *IsoPSA clinical performance evaluation*

Two key clinical performance objectives were tested: discriminatory power between PCa (Gleason ≥6) and benign prostate conditions (cancer vs no cancer), and between high-grade PCa (Gleason ≥7) and low-grade cancer (Gleason 6) or benign histology (high grade). Two receiver operating characteristic (ROC) analyses were developed to evaluate the discriminatory power of *K*. Since the subjects were already selected for biopsy, the sample size was calculated according to the length of the 95% confidence interval (CI) for the estimated sensitivity using the formula $N_c = \frac{Z^2_{\alpha/2} V(\theta)}{L^2}$; where N_c is one-half of the cancer cohort, θ is the expected sensitivity estimate, L denotes the desired one-half of the CI, and $V(\theta) = \theta(1-\theta)$ (V. Kipnis, personal communication). Setting the expected sensitivity estimate to $\theta = 0.95$ and the 95% CI to 0.9–1.0, making $L = 0.05$, and with $Z_{\alpha/2} = Z_{0.025} = 1.96$, we obtain $N_c \approx 70$ and thus a total sample size of ~140. More directly, the confidence interval of the area under the ROC curve (AUC) for each ROC analysis was determined using 1000 bootstrapped samples with replacement. A calibration curve for each model was constructed to explore the relationship between the observed and predicted outcome. Decision curve analysis (DCA) [16] was used to investigate the clinical utility of the models in comparison to the two extreme limits of all-biopsy (as for the current patient cohort) and no-biopsy, as well as against the modified Prostate Cancer Prevention

Please cite this article in press as: Klein EA, et al. The Single-parameter, Structure-based IsoPSA Assay Demonstrates Improved Diagnostic Accuracy for Detection of Any Prostate Cancer and High-grade Prostate Cancer Compared to a Concentration-based Assay of Total Prostate-specific Antigen: A Preliminary Report. Eur Urol (2017), http://dx.doi.org/10.1016/j.eururo.2017.03.025

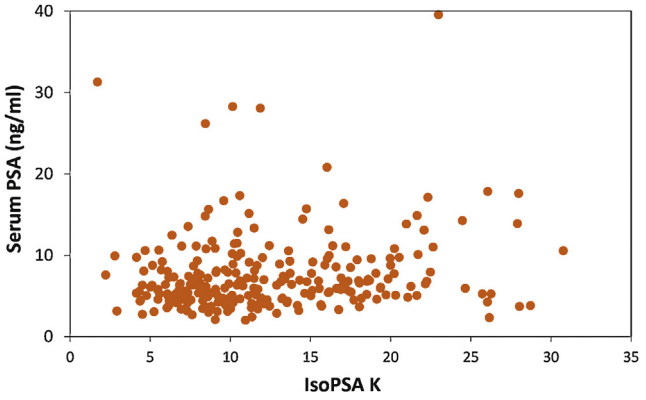

Fig. 1 – Serum prostate-specific antigen (PSA) concentration versus IsoPSA *K* value for all patients.

Trial Risk Calculator (PCPTRC) 2.0 risk calculator [17], providing risk estimates for low-grade versus high-grade cancer with PSA, age, DRE, race, and prior biopsy as parameters. Performance parameters including specificity, sensitivity, negative predictive value (NPV), and positive predictive value (PPV) were examined, and their clinical consequences for each of the two indications were recorded in terms of biopsies avoided as defined according to selected cutoff values. Statistical analysis was conducted using either Analyse-It for Microsoft Excel v.4.65.2 (Analyse-it Software, Leeds, UK) or Stata/MP 13.1 for Windows (StataCorp, College Station, TX, USA).

Table 2 – Area under the ROC curve for IsoPSA versus total PSA for high-grade cancer versus low-grade cancer/benign histology and for cancer versus no cancer

Model	Area under the ROC curve (95% CI)		*p* value[a]
	IsoPSA K	Total PSA	
KR-HG	0.81 (0.74–0.86)	0.69 (0.61–0.75)	0.005
KR-CNC	0.79 (0.73–0.84)	0.61 (0.54–0.67)	<0.001

KR-CNC = IsoPSA *K* result for cancer versus no cancer; KR-HG = IsoPSA *K* result for high-grade cancer (Gleason ≥7) versus benign/low-grade cancer; PSA = prostate-specific antigen; ROC = receiver operating characteristic.
[a] Delong and Delong test.

3. Results

The overall prevalence of PCa in the entire cohort was 53.3% (139/261), while the prevalence of high-grade PCa was 33.7% (88/261). As expected, there was no significant correlation between IsoPSA *K* and serum PSA levels (Fig. 1; Pearson correlation coefficient 0.2). As measured by ROC, IsoPSA outperformed standard PSA for both study endpoints (Table 2 and Fig. 2). For the cancer versus no cancer endpoint, the AUC was 0.79 (95% CI 0.73–0.84) for IsoPSA versus 0.61 (95% CI 0.54–0.67) for total PSA ($p < 0.001$). For high-grade cancer versus low-grade cancer/benign histology, the AUC was 0.81 (95% CI 0.74–0.86) for IsoPSA versus 0.69 (95% CI 0.61–0.75) for total PSA ($p < 0.005$). Calibration curves for both endpoints demonstrated very good correspondence between predicted and observed results, with a Hosmer-Lemeshow statistic of 8.21 ($p = 0.41$, >0.05) for cancer versus no cancer and 9.86 ($p = 0.28$, >0.05) for high-grade cancer versus low-grade cancer/benign histology (Fig. 2). Table 3 shows the sensitivity, specificity, PPV, and NPV for IsoPSA versus total PSA at selected cutoff values for risk probability for the endpoints cancer versus no cancer (KR-CNC) and high-grade PCa versus low-grade PCa/benign histology (KR-HG). Recognizing that the decision to perform or forgo a biopsy depends on clinical suspicion and both patient and physician risk tolerance, Table 3 suggests some clinically relevant scenarios illustrating the superior predictive power of IsoPSA. For example, as an exclusion test for separating PCa from benign disease, a risk probability cutoff value of KR-CNC = 35% for IsoPSA provides a good balance of high sensitivity (90%) and specificity (48%), whereas at similar sensitivity (87%) standard PSA (at 4 ng/ml) has significantly inferior specificity (15%). As an exclusion test to identify patients at high risk of high-grade PCa, a KR-HG cutoff of 17% yields NPV of 96%, while KR-HG of 70% yields PPV of 76%.

DCA also demonstrated superior performance for IsoPSA compared to the modified PCPTRC 2.0 risk calculator. For expression as net benefit, Fig. 3 illustrates DCA results for a relevant risk probability range for the two study endpoints. If the goal is to identify all patients with cancer of any grade, KR-CNC = 35% results in a 48% reduction in unneeded biopsies (from 122 to 63). For the objective of identifying

Table 3 – Performance metrics versus PSA at selected cutoff values for risk probability

	Cancer vs no cancer		High-grade PCa vs low-grade PCa/BH		
	Total PSA	KR-CNC	Total PSA	IsoPSA KR-HG	
				Low RP	High RP
Prevalence (%)	53		34		
Cutoff	4 ng/ml	35%	4 ng/ml	17%	70%
Sensitivity (%)	87	90	93	96	25
Specificity (%)	15	48	17	43	96
NPV (%)	50	81	83	95	72
PPV (%)	54	66	36	46	76
AUC	0.61	0.79	0.69	0.81	0.81

PSA = prostate-specific antigen; PCa = prostate cancer; BH = benign histology; KR-CNC = IsoPSA *K* result for cancer versus no cancer; KR-HG = IsoPSA *K* result for high-grade cancer (Gleason ≥7) versus benign/low-grade cancer (Gleason 6); RP = risk probability; NPV = negative predictive value; PPV = positive predictive value; AUC = area under the receiver operating characteristic curve.

Please cite this article in press as: Klein EA, et al. The Single-parameter, Structure-based IsoPSA Assay Demonstrates Improved Diagnostic Accuracy for Detection of Any Prostate Cancer and High-grade Prostate Cancer Compared to a Concentration-based Assay of Total Prostate-specific Antigen: A Preliminary Report. Eur Urol (2017), http://dx.doi.org/10.1016/j.eururo.2017.03.025

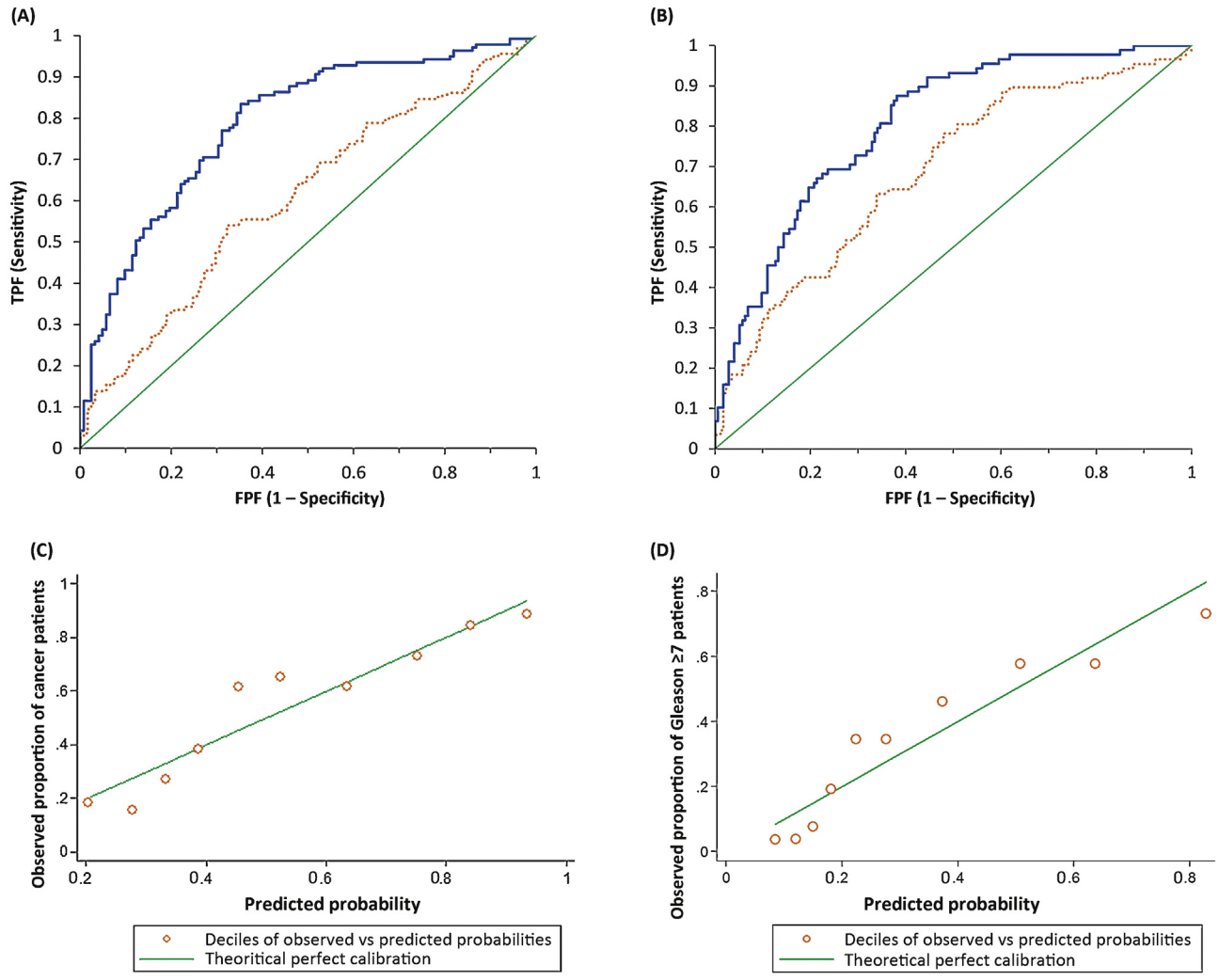

Fig. 2 – Receiver operating characteristic (ROC) analysis and calibration plots for the models. ROC for (A) cancer versus no cancer and (B) high-grade cancer versus low-grade cancer/benign histology. Calibration plot for (C) cancer versus no cancer and (D) high-grade cancer versus low-grade cancer/benign histology. Solid line = IsoPSA; dotted line = standard prostate-specific antigen; TPF = true positive fraction; FPF = false positive fraction.

only those with high grade PCa, KR-HG = 17% results in a 45% reduction in unneeded biopsies (from 173 to 95). A summary of the number of biopsies avoided and delayed (false negatives) versus risk probability cutoff values is provided in Table 4.

4. Discussion

In selecting a diagnostic test, especially for cancer, it is paramount to focus on its inherent ability to reflect underlying disease-related biology. Specificity is the weakness of the test

Table 4 – Biopsies avoided and delayed for high-grade cancer versus low-grade cancer/benign history according to IsoPSA cutoff value

IsoPSA KR-HG cutoff	Biopsies performed (*n*)	Reduction in FPs, *n* (%)	Biopsies avoided, *n* (%)	GS ≥7 cancer, *n* (%)		Gleason score for delayed Dx of GS ≥7 cancer, *n* (%)		
				Detected	Dx delayed	3 + 4	4 + 3	≥4 + 4*
≥0%	261	0 (0)	0 (0)	88 (34)	0 (0)	0 (0)	0 (0)	0 (0)
≥10%	238	23 (13)	24 (9.2)	87 (33)	1 (0.4)	0 (0)	0 (0)	1 (0.4)
≥15%	197	65 (38)	67 (26)	86 (33)	2 (0.8)	1 (0.4)	0 (0)	1 (0.4)
≥17%	183	78 (45)	84 (32)	83 (32)	5 (1.9)	2 (0.8)	2 (0.8)	1 (0.4)
≥20%	166	96 (55)	103 (39)	81 (31)	7 (2.7)	4 (1.5)	2 (0.8)	1 (0.4)
≥22%	157	105 (61)	116 (44)	77 (30)	11 (4.2)	6 (2.3)	3 (1.1)	2 (0.8)
≥25%	148	113 (65)	131 (50)	70 (27)	17 (6.5)	10 (3.8)	4 (1.5)	3 (1.1)

KR-HG = IsoPSA *K* result for high-grade cancer versus benign/low-grade cancer; GS Gleason score; FPs = false positives; Dx = diagnosis.

Please cite this article in press as: Klein EA, et al. The Single-parameter, Structure-based IsoPSA Assay Demonstrates Improved Diagnostic Accuracy for Detection of Any Prostate Cancer and High-grade Prostate Cancer Compared to a Concentration-based Assay of Total Prostate-specific Antigen: A Preliminary Report. Eur Urol (2017), http://dx.doi.org/10.1016/j.eururo.2017.03.025

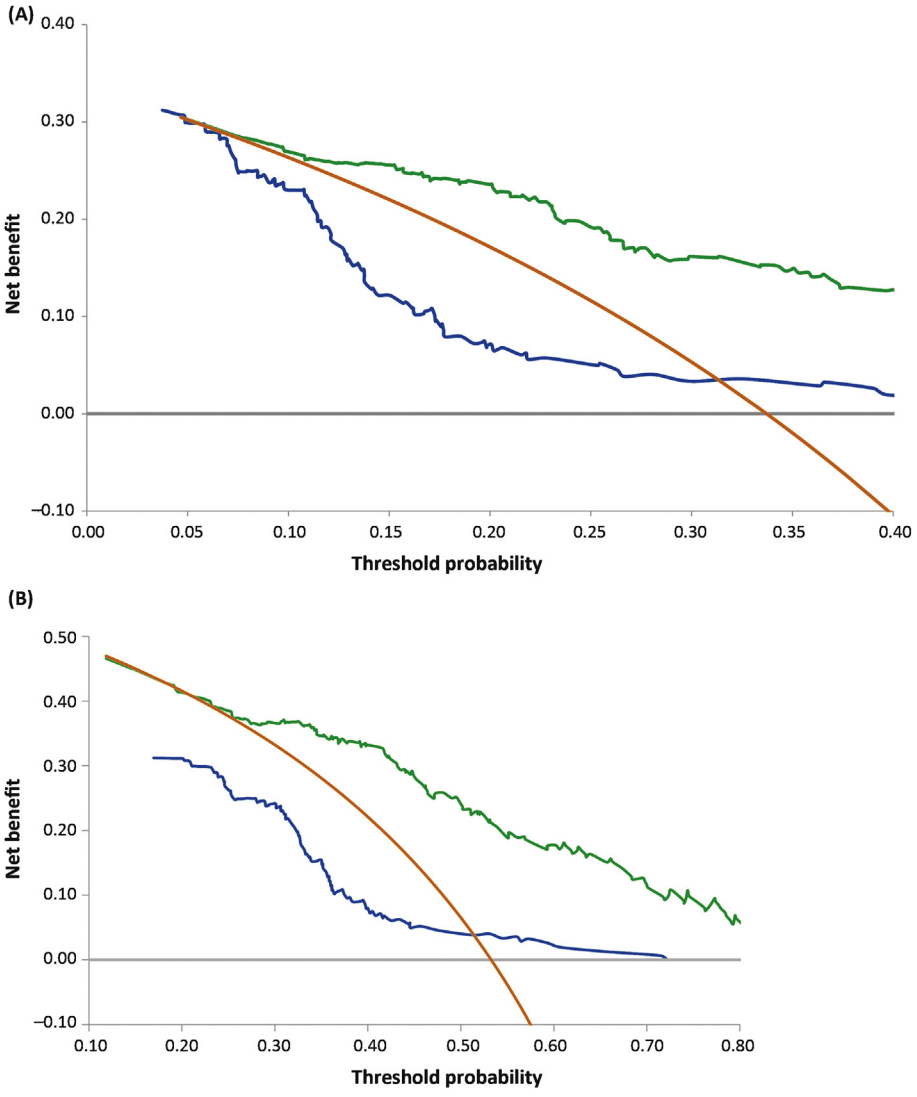

Fig. 3 – (A) Decision curve analysis (DCA) comparing IsoPSA (green line) to the modified Prostate Cancer Prevention Trial Risk Calculator (PCPTRC) 2.0 (blue line) for high-grade prostate cancer (PCa) versus low-grade PCa/benign histology, and two extreme protocols: biopsy no patients (gray line) and biopsy all patients (yellow line) for the study cohort. In the DCA, at any given threshold probability, the model with the best clinical outcome is associated with the highest net benefit. (B) DCA comparing IsoPSA (green line) to the modified PCPTRC 2.0 (blue line) for all cancer versus no cancer, and two extreme protocols: biopsy no patients (gray line) and biopsy all patients (yellow line) for the study cohort. In the DCA, at any given threshold probability, the model with the best clinical outcome is associated with the highest net benefit.

platforms most widely used today, including X-ray–based imaging (eg, mammography) and blood-based expression of specific proteins (eg, PSA), whose lack of specificity for cancer is in part due to their lack of direct connection to the underlying disease processes. In particular, PSA is prostate-specific but not PCa-specific, leading to significant limitations in diagnostic accuracy and resulting in an excess of unnecessary biopsies and overdetection and overtreatment of nonlethal cancers. More recent PSA-based tests, such as PHI and 4Kscore, have better specificity for high-grade cancer [18–20] but measure only a limited number of PSA isoforms that may not be present in some patients.

In this study we evaluated the clinical performance of a novel blood-based assay, IsoPSA, which measures structural changes in PSA that result directly from disordered cellular processes present in PCa. We demonstrate that IsoPSA has better diagnostic accuracy compared to standard PSA for detection of PCa and high-grade PCa in a cohort of men undergoing biopsy for indications typical in contemporary urologic practice. According to a variety of analytical tools (ROC curves, logistic regression, and DCA), IsoPSA outperformed a standard concentration-based PSA assay in this study. The results show that if adopted clinically, IsoPSA could significantly reduce the rate of unnecessary biopsies by almost 50% while preserving both PPV and NPV for detection of cancer versus no cancer and of high-grade PCa versus low-grade PCa/benign histology.

Use of the IsoPSA assay has many strengths compared to currently available PSA assays. First, by measuring structural changes in PSA that arise specifically in cancer cells, it is less affected by conditions such as benign prostatic hyperplasia, inflammation, and age that reduce the specificity of standard

Please cite this article in press as: Klein EA, et al. The Single-parameter, Structure-based IsoPSA Assay Demonstrates Improved Diagnostic Accuracy for Detection of Any Prostate Cancer and High-grade Prostate Cancer Compared to a Concentration-based Assay of Total Prostate-specific Antigen: A Preliminary Report. Eur Urol (2017), http://dx.doi.org/10.1016/j.eururo.2017.03.025

PSA assays. Second, current PSA assays measure only a few prespecified PSA forms or related molecules (total PSA, free PSA, [−2]proPSA, and/or hk2), which represent only a fraction of the PSA isoforms that are present in patients with PCa, while IsoPSA measures all PSA isoforms without an a priori requirement of knowing which species are present. Since the cellular metabolism of cancer cells may vary during clonal evolution, the molecular species of PSA present in blood may vary between patients and even within the same patient over time. Thus, an assay such as IsoPSA that is agnostic to the presence of specific isoforms is likely to have better sensitivity and specificity in the broadest group of patients. Structural changes in cancer-related PSA are unaffected by drugs such as 5ARIs that lower PSA concentration, and IsoPSA can be used in patients taking these drugs without the need for adjustment of the results. Finally, the effectiveness of IsoPSA should remain uniform over varying total PSA concentrations, potentially directly replacing serum PSA concentration with structure, even in screening applications.

IsoPSA provides clinically useful information in a highly parsimonious manner, using a single test parameter, the *K* value. It is the first test since the advent of PSA itself that demonstrates an improvement in AUC performance simply by changing the definition of the biomarker from PSA concentration to PSA structure. Although clinical and demographic parameters such as age, race, and prostate volume that may affect the clinical performance of IsoPSA were collected for this study, we have not adjusted the results for these variables so that we could focus only on the clinical performance of the single test result, *K*, of the IsoPSA assay. Thus, the results reported here represent the minimal performance envelope to be expected from IsoPSA, which can be improved by considering additional population- and individual-specific parameters. Such analyses will be the subject of a subsequent manuscript.

The strengths of this study include its multicenter design and reliance on standard clinical indications for prostate biopsy in contemporary practice. Its weaknesses include the lack of central or standardized pathology review of the biopsies, a lack of distinction between primary and repeat biopsy, and variability in the use of MRI for decisions on the need for and the technique used for biopsy.

In conclusion, this study demonstrates for the first time that use of a structure-based rather than concentration-based assay of PSA has better diagnostic accuracy for detecting any cancer and high-grade cancer in a cohort of men undergoing biopsy for standard clinical indications. Once validated, use of IsoPSA may substantially reduce the need for biopsy, and may thus lower the likelihood of overdetection and overtreatment of nonlethal PCa.

Author contributions: Eric A. Klein had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Klein, Stovsky, Chait.
Acquisition of data: All authors.
Analysis and interpretation of data: Klein, Stovsky, Kestranek, Chait.
Drafting of the manuscript: Klein, Stovsky, Chait.
Critical revision of the manuscript for important intellectual content: All authors.
Statistical analysis: Carried out by third-party agents.
Obtaining funding: All authors.
Administrative, technical, or material support: All authors.
Supervision: Klein, Stovsky.
Other: None.

Financial disclosures: Eric A. Klein certifies that all conflicts of interest, including specific financial interests and relationships and affiliations relevant to the subject matter or materials discussed in the manuscript (eg, employment/affiliation, grants or funding, consultancies, honoraria, stock ownership or options, expert testimony, royalties, or patents filed, received, or pending), are the following: Eric A. Klein and Andrew J. Stephenson are employed by Cleveland Clinic, which has an equity position in Cleveland Diagnostics, but they have no direct or indirect personal financial interests in the company. Mark Stovsky is a co-founder and Chief Medical Officer of Cleveland Diagnostics and has financial interest in the company. Arnon Chait, Aimee Kestranek, and Boris Zaslavsky are employed by Cleveland Diagnostics and have financial interest in the company. The remaining authors have nothing to disclose.

Funding/Support and role of the sponsor: Funding for this study was provided by Cleveland Diagnostics. The sponsor was involved in the design and conduct of the study and in data collection and statistical analysis.

Acknowledgments: We thank Joseph Briggman for his experience and clinical insights, and Brian Nathanson and Victor Kipnis for conducting statistical analysis of the data.

References

[1] Illic D, Neuberger M, Djulbegovic M, et al. Screening for prostate cancer, 2013. Cochrane Database Syst Rev 2013;2013:CD004720.
[2] Moyer VA. US Preventive Services Task Force. Screening for prostate cancer: U.S. Preventive Services Task Force recommendation statement. Ann Intern Med 2012;157:120–34.
[3] Carter HB, Albertsen PC, Barry MJ, et al. Early detection of prostate cancer: AUA guideline. J Urol 2013;190:419–26.
[4] Gilgunn S, Conroy PJ, Saldova R, Rudd PM, O'Kennedy RJ. Aberrant PSA glycosylation–a sweet predictor of prostate cancer. Nat Rev Urol 2013;10:99–107.
[5] Leymarie N, Griffin PJ, Jonscher K, et al. Interlaboratory study on differential analysis of protein glycosylation by mass spectrometry: the ABRF glycoprotein research multi-institutional study. Mol Cell Proteomics 2013;12:2935–51.
[6] Saldova R, Fan Y, Fitzpatrick JM, Watson RW, Rudd PM. Core fucosylation and alpha2-3 sialylation in serum N-glycome is significantly increased in prostate cancer comparing to benign prostate hyperplasia. Glycobiology 2011;21:195–205.
[7] Vermassen T, Speeckaert MM, Lumen N, Rottey S, Delanghe JR. Glycosylation of prostate specific antigen and its potential diagnostic applications. Clin Chim Acta 2012;413:1500–5.
[8] Higashihara E, Nutahara K, Kojima M, et al. Significance of serum free prostate specific antigen in the screening of prostate cancer. J Urol 1996;156:1964–8.
[9] Okihara K, Cheli CD, Partin AW, et al. Comparative analysis of complexed prostate specific antigen, free prostate specific antigen and their ratio in detecting prostate cancer. J Urol 2002;165:2017–23.
[10] 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;50:1017–25.
[11] Hilz H, Noldus J, Hammerer P, Buck F, Lück M, Huland H. Molecular heterogeneity of free PSA in sera of patients with benign and malignant prostate tumors. Eur Urol 1999;36:286–92.

Please cite this article in press as: Klein EA, et al. The Single-parameter, Structure-based IsoPSA Assay Demonstrates Improved Diagnostic Accuracy for Detection of Any Prostate Cancer and High-grade Prostate Cancer Compared to a Concentration-based Assay of Total Prostate-specific Antigen: A Preliminary Report. Eur Urol (2017), http://dx.doi.org/10.1016/j.eururo.2017.03.025

[12] Chen YT, Tuan LP, Chen HW, et al. Quantitative analysis of prostate specific antigen isoforms using immunoprecipitation and stable isotope labeling mass spectrometry. Anal Chem 2015;87:545.
[13] Llop E, Ferrer-Batallé M, Barrabés S, et al. Improvement of prostate cancer diagnosis by detecting PSA glycosylation-specific changes. Theranostics 2016;24:1190–204.
[14] Stovsky M, Ponsky L, Vourganti S, et al. Prostate-specific antigen/solvent interaction analysis: a preliminary evaluation of a new assay concept for detecting prostate cancer using urinary samples. Urology 2011;78:601–5.
[15] National Cancer Institute. EDRN standard operating procedures (SOP). https://edrn.nci.nih.gov/resources/standard-operating-procedures/standard-operating-procedures.
[16] Vickers AJ, Elkin EB. Decision curve analysis: a novel method for evaluating prediction models. Med Decis Making 2006;26:565–74.
[17] Ankerst DP, Hoefler J, Bock S, et al. Prostate Cancer Prevention Trial Risk Calculator 2.0 for the prediction of low- vs high-grade prostate cancer. Urology 2014;83:1362–8.
[18] Stattin P, Vickers AJ, Sjoberg DD, et al. Improving the specificity of screening for lethal prostate cancer using prostate-specific antigen and a panel of kallikrein markers: a nested case control study. Eur Urol 2015;68:207–13.
[19] Parekh DJ, Punnen S, Sjoberg DD, et al. A multi-institutional prospective trial in the USA confirms that the 4Kscore accurately identifies men with high-grade prostate cancer. Eur Urol 2015;68:464–70.
[20] Loeb S, Sanda MG, Broyles DL, et al. The Prostate Health Index (PHI) selectively identifies clinical-significant prostate cancer. J Urol 2015;193:1163–9.

Please cite this article in press as: Klein EA, et al. The Single-parameter, Structure-based IsoPSA Assay Demonstrates Improved Diagnostic Accuracy for Detection of Any Prostate Cancer and High-grade Prostate Cancer Compared to a Concentration-based Assay of Total Prostate-specific Antigen: A Preliminary Report. Eur Urol (2017), http://dx.doi.org/10.1016/j.eururo.2017.03.025