Clinical Validation of IsoPSA, a Single Parameter, Structure Based Assay for Improved Detection of High Grade Prostate Cancer

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Purpose: Current prostate specific antigen markers to detect prostate cancer are limited by low specificity for high grade disease. IsoPSA™ is a blood based, structure focused assay which predicts risk by partitioning the isoforms of prostate specific antigen that are linked to cancer in an aqueous 2-phase reagent system. We validated the clinical performance of this assay for identifying high grade disease in a new contemporary biopsy cohort.

Materials and Methods: We performed a multicenter prospective validation in 271 men scheduled for prostate biopsy at a total of 7 academic and community centers who were enrolled between May 2017 and March 2018. Blood samples were obtained for assay prior to biopsy. The discrimination power of the assay to detect high grade prostate cancer (Gleason 7 or greater) was evaluated by ROC analysis and compared to prior results. Clinical performance was further improved by comparison with multiparametric magnetic resonance imaging-ultrasound vs transrectal ultrasound guided biopsies.

Results: The assay AUC was 0.784 for high grade vs low grade cancer/benign histology, which was superior to the AUCs of total prostate specific antigen and percent free prostate specific antigen. If 1,000 patients were biopsied, the assay would have reduced the number of unnecessary biopsies from 705 to 402 (43%) with only 22 missed high grade cancers, of which 7 would have been Gleason sum 4 + 3 or higher. Subset analysis of multiparametric magnetic resonance imaging guided biopsy produced a substantial improvement of the AUC to 0.831.

Conclusion: Validation of the structure based IsoPSA assay demonstrated statistical concordance with previously reported results and verified its superior performance vs concentration based prostate specific antigen and the free-to-total prostate specific antigen ratio. The assay improvement in detecting high grade PCa using multiparametric magnetic resonance imaging-ultrasound guided biopsy may help define a new diagnostic paradigm.

Key Words: prostatic neoplasms; prostate specific antigen; protein isoforms; neoplasm grading; biomarkers, tumor

Abbreviations and Acronyms

%fPSA = free-to-total PSA ratio
K = test parameter value
KR-HG = high grade cancer transformed K
mpMRI = multiparametric magnetic resonance imaging
PCa = prostate cancer
PSA = prostate specific antigen
tPSA = total PSA
TRUS = transrectal ultrasound

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THE IsoPSA™ structure based assay interrogates changes in the entire spectrum of PSA protein isoforms in blood in a disinterested manner. In a previous study we reported the clinical performance results of a preliminary trial in 261 men which demonstrated high sensitivity and specificity for the detection of high grade (Gleason 7 or greater) disease.[1] That study also showed that using IsoPSA could reduce unnecessary biopsies by almost 50%.[1]

We now report the results of a validation study in a new patient cohort, which again demonstrated the robust performance of this assay using contemporary triggers for biopsy. We also found improved performance for detecting high grade disease when the IsoPSA assay was coupled with mpMRI guided biopsies.

METHODS

Patient Population and Specimen Collection

This institutional review board approved, multicenter prospective study enrolled men scheduled for prostate biopsy because of elevated or rising total PSA and/or suspicious digital rectal examination (IRB No. 16-1399). Heparin plasma was collected for IsoPSA between May 2017 and March 2018 at 4 academic and 3 community urology centers across the United States and Israel, including Cleveland Clinic, Kaiser Permanente Northwest, Chesapeake Institute of Urology, Advanced Urology Institute, University of Texas Southwestern Medical Center, Johns Hopkins University and Rabin Medical Center.

Samples were collected within 30 days prior to biopsy, processed according to the EDRN (Early Detection Research Network) guidelines[2] and frozen at −80C until analyzed. The primary end point of the study was cancer classification as high grade disease (Gleason 7 or greater) vs benign or low grade disease (Gleason 6) as defined by histopathology findings on biopsy reported at each study center. There was no central pathology review.

Biopsy was performed using TRUS alone or under mpMRI guidance according to local institutional standards. Study exclusion criteria included serum PSA less than 2 ng/ml; recent (less than 72 hours) prostate manipulation, including digital rectal examination; recent (less than 2 weeks) urinary tract infection and/or prostatitis; recent (less than 30 days) prostate surgery, urinary catheterization, prostate infarction or endoscopic evaluation; and another urinary tract malignancy. Because the IsoPSA assay measures PSA structure rather than concentration, men on 5ARIs (5α-reductase inhibitors), which are known to affect PSA concentration, were not excluded from analysis.

A total of 305 samples were collected with 34 exclusions, including 9 due to prolonged storage (more than 90 days), 4 due to canceled biopsies, 15 due to confirmation serum PSA less than 2 ng/ml and 6 due to another unrelated reason, leaving a final analytical cohort of 271 samples.

Signed informed consent was obtained from all enrollees. The supplementary table (https://www.jurology.com) shows cohort demographics, and clinical and analytical information.

Laboratory Methods

Frozen plasma samples were shipped elsewhere and all testing was performed and reported naïve to the pathology outcome. Upon receipt the samples were thawed and immediately added to IsoPSA reagent tubes. The reagent tubes were vortexed, centrifuged and subjected to the assay. The 2 assay steps are partitioning plasma samples in an aqueous 2-phase system, IsoPSA RUO (Research Use Only), followed by measurement of the free and total PSA concentrations in each of the 2 aqueous phases, referred to as the top or the bottom. An aliquot was removed from each phase, and the total and free PSA concentrations were measured elsewhere using United States FDA (Food and Drug Administration) approved clinical assays. As of June 2018 in the United States the IsoPSA assay is for research use only.

The IsoPSA assay readout or test parameter, K, is calculated by the equation, $K = ([\text{total PSA}]_{\text{bottom}} - [\text{free PSA}]_{\text{bottom}})/([\text{total PSA}]_{\text{top}} - [\text{free PSA}]_{\text{top}})$. As described previously,[1] multiple isoforms and complexes of PSA forming an intricate admixture in serum are individually partitioned between the top and bottom aqueous phases of the assay based on differences in structure and protein-protein interactions. The unique combination of isoforms in the 2 phases, reflecting the difference between the readings by the total and free PSA assay antibodies in each of the 2 phases, is expressed as the ratio, K, which is the assay test parameter. The difference between the immunoassay readings using the total and free PSA assays should not be construed as the concentration of the ACT (α-1-antichymotrypsin)-PSA complex.

K is a ratiometric parameter not directly connected with the corresponding level of serum PSA.[1] However, K and serum PSA concentrations generally increase in accord with the presence of cancer. The cutoff value of K is selected to optimize the diagnostic performance specific to each clinical application. It is generally determined using conventional binary statistical techniques. Alternatively the unbounded range of K can be converted to a bounded risk parameter, KR-HG, which ranges from 0 to 100 for convenience and clinical interpretation to estimate the risk of high grade cancer.

Statistical Analysis

The key clinical performance objective tested in this study was the discriminating power of IsoPSA for the dichotomous outcome of the presence on biopsy of high grade PCa (Gleason 7) vs low grade cancer (Gleason 6) or benign histology. To validate the performance of K we first used logistic regression with K and a constant term as the independent variable. The dependent variable, outcome, was high grade PCa (Gleason 7 or greater) vs low grade cancer (Gleason 6) or benign histology using only the cohort in the preliminary study.[1] The coefficients of the logistic regression model from that preliminary cohort were then applied to the validation cohort.

Using the predicted probabilities from the validation data we used ROC curves[3] to measure the discriminatory power of K. Given the result of the preliminary study (AUROC = 0.805),[1] we estimated that 199 subjects would be required in each group to detect a 0.07 difference in ROC AUC values with the 95% CI and 80% power.[4] To

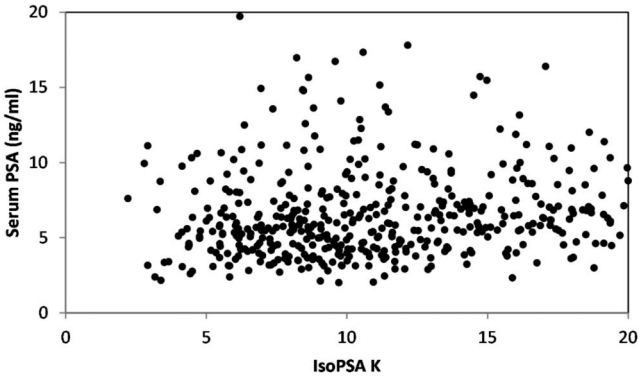

Figure 1. Serum PSA and IsoPSA K in all patients.

detect a smaller effect of 0.05 with 95% CIs and 80% power required 445 subjects. Given the desire to maintain similar sample sizes in studies, we determined that approximately 270 participants would ensure that the current study was adequately powered to detect a detect a difference in the ROC AUC of between 0.05 and 0.07.

The bias corrected CI of the AUC in each ROC analysis was determined using 1,000 bootstrapped samples with replacement. Performance parameters, including specificity, sensitivity, and negative and positive predictive values as well as the clinical consequences in terms of avoided biopsies were examined as defined by the cutoff value selected in the preliminary study.[1] Statistical analysis was done with Analyse-t® for Microsoft® Excel®, version 4.96 or Stata/MP™ 15.1 for Windows®.

RESULTS

In the entire validation cohort of 271 subjects 80 (29.5%) had high grade PCa. In agreement with our preliminary study finding[1] IsoPSA K only weakly correlated with serum total PSA (fig. 1). Also as reported previously[1] IsoPSA in the 271 patients in the validation set outperformed standard PSA to detect high grade cancer (AUC 0.784, 95% CI 0.724–0.843 vs 0.657, 95% CI 0.587–0.726, p <0.005) with the CI corrected based on 1,000 bootstrapped samples (table 1 and fig. 2).

Table 1 shows the sensitivity, specificity, positive and negative predictive values, and clinical outcomes of the assay vs total PSA at the cutoff value of 17% selected in our previous study. The 17% value was the predicted probability of high grade PCa (Gleason 7 or greater) selected in our previous study.[1] The logistic regression coefficients to transform the assay index K into a bounded range of 0% to 100%, KR-HG, were determined from the preliminary study. As an exclusion test to identify patients at high risk for high grade cancer, the KR-HG cutoff of 17% yielded 93% negative predictive value. We estimated that in 1,000 patients undergoing biopsy using IsoPSA at this cutoff value would reduce unnecessary biopsies in 303 men from 705 to 420 (43%) with only 22 missed high grade cancers, of which only 7 would have been Gleason sum 4 + 3 or higher. Subset analysis of the 60 African American participants showed an AUC of 0.824 (95% CI 0.719–0.928).

Statistical comparison of the AUC ROC values between the current and the previous study[1] using the method of Delong et al[5] resulted in a chi-square statistic of 0.29 (p = 0.593). This means that there was no significant difference in AUC ROC values in the 2 studies.

Using the combined data from the current study and the previous study[1] we also compared clinical outcomes for IsoPSA, total PSA, %fPSA and a model using the same data for training and validation including total and %fPSA. Table 2 shows the clinical outcome performance of all assays set at 93% sensitivity, corresponding to IsoPSA at the predetermined 17% cutoff. The assay outperformed total PSA, % fPSA evaluated at the clinically approved range of 4 to 10 ng/ml total PSA and total %fPSA (AUC 0.794, 0.670, 0.727 and 0.767, respectively). Moreover, at the same 93% sensitivity level the assay demonstrated a significantly reduced unnecessary biopsy

Table 1. *Clinical performance metrics for detecting high grade disease*

	Total PSA Study		IsoPSA Study	
	Preliminary[1]	Validation	Preliminary[1]	Validation
No. pts	261	271	261	271
No. actionable:				
Yes	88	80	88	80
No	173	191	173	191
AUC (95% CI)	0.684 (0.615–0.753)	0.657 (0.587–0.726)	0.805 (0.747–0.851)	0.784 (0.724–0.843)
% Prevalence	33.7	29.5	33.7	29.5
Cutoff:	4 ng/ml		17%	
% Sensitivity	93	94	96	93
% Specificity	18	22	43	40
% Pos predictive value	36	34	46	39
% Neg predictive value	83	89	95	93
% Unnecessary biopsy reduction	20.8	24.6	45.1	42.9
% Delayed diagnosis*	3.8	1.8	2.3	2.2

* Missed actionable cancer.

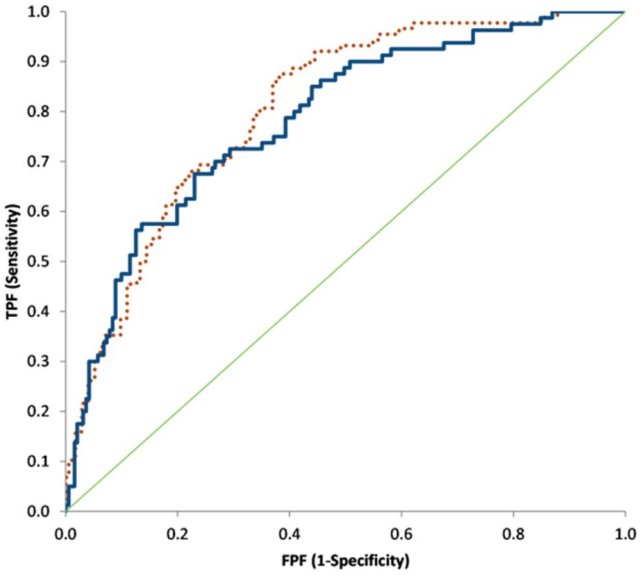

Figure 2. ROC curve of validation study (solid curve) in 271 patients vs preliminary study (dotted curve) in 261 (AUC 0.805). ROC analysis of validation study demonstrated statistically equivalent assay performance (AUC 0.784, 95% CI 0.724–0.843) compared to preliminary study (AUC 0.805, 0.74-0.851) with 95% bias corrected CI on AUC calculated for 1,000 bootstrapped samples. *TPF*, positive fraction. *FPF*, false-positive fraction.

rate of 46% vs 21% for %fPSA and 19% for the tPSA plus %fPSA model.

We also examined the influence of biopsy technique on assay performance. When segregating the data into 2 groups based on technique (TRUS alone vs mpMRI guided), we observed significant improvement in the IsoPSA AUC when mpMRI was used [F3] (fig. 3). For TRUS vs mpMRI-ultrasound guided biopsies the IsoPSA AUC was 0.784 (95% CI 0.738–0.831) in 402 patients and 0.831 (95% CI 0.749–0.913) in 121, respectively, when the 2 cohorts combining the preliminary cohort of 261 participants[1] and the validation cohort of 271 were aggregated.

DISCUSSION

The use of PSA as a screening test for prostate cancer is limited by its lack of specificity for cancer and high grade cancer, leading to well quantitated diagnostic inaccuracies and high rates of unnecessary biopsies due to false-positive results.[6] Recognition of this fundamental limitation has led to attempts to develop new biomarker assays based on biological changes in protein structure which better represent the underlying biology of the disease process.[7–17]

Changes to the structure of PSA and other proteins formed in cancer cells include but are not limited to alterations in the primary sequence[7] and post-translational modifications such as glycosylation,[8–11] potentially resulting in differential interaction with other serum proteins.[12] Chief among the latter is the interaction between PSA and the protease inhibitor ACT.[13,14] However, PSA also forms complexes with other carrier proteins, including human serum albumin, transferrin and γ-globulin.[12,15–17] All of these changes are differential to the disease process and could potentially be exploited for improved specificity of prostate cancer diagnostics. Indeed, they form the basis of secondary and tertiary assays in the field, including %fPSA, the 4KScore®,[18] the PHI (Prostate Health Index)[19] and IsoPSA.[1]

A key distinction between IsoPSA and other structure specific biomarkers rests in its approach to the scope of the definition of the disease biology. While all other biomarkers which include structural information do so by focusing on a predefined single isoform (eg the PHI) or by combining several PSA

Table 2. *Clinical performance metrics of IsoPSA, total PSA, %fPSA, and combined total PSA and %fPSA models*

	IsoPSA K		Total PSA		%fPSA*		Total PSA + %fPSA	
Cutoff	17%		4 ng/ml		23.5		12%	
% Prevalence	31.5%		31.2%		29.5%		31.1%	
No. pts	532		522		346		518	
No. actionable:								
Yes	168		163		102		161	
No	364		359		244		357	
AUC (95% CI)	0.794 (0.755–0.834)		0.670 (0.621–0.719)		0.727 (0.667–0.787)		0.767 (0.722–0.813)	
No. pos	156		152		95		150	
No. false-pos	210		288		199		271	
No. neg	154		71		45		86	
No. false-neg	12		11		7		11	
% Sensitivity	92.9		93.3		93.1		93.2	
% Specificity	42.3		19.8		18.4		24.1	
% Predictive value:								
Pos	43		35		32		37	
Neg	93		87		87		90	
No. unnecessary biopsy reduction (%)	166	(45.6)	82	(15.7)	52	(21.3)	97	(18.7)
No. delayed diagnosis (%)†	12	(2.3)	11	(2.1)	7	(2)	11	(2.1)

* Free-to-total PSA ratio evaluated at clinically approved 4 to 10 ng/ml range of total PSA.
† Missed cancer.

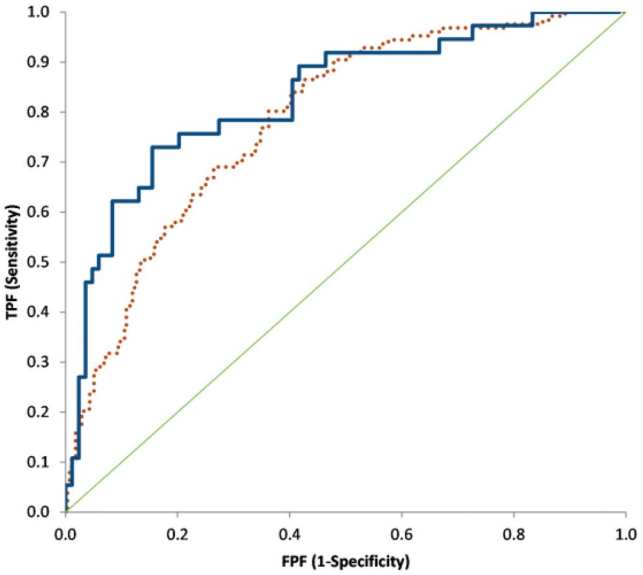

Figure 3. ROC curves of TRUS guided biopsy (dotted curve) in 402 patients (AUC 0.784, 95% CI 0.738–0.831) vs mpMRI guided biopsy (solid curve) in 121 (AUC = 0.831). *TPF,* positive fraction. *FPF,* false-positive fraction.

isoforms (eg the 4KScore), IsoPSA makes no a priori assumption about isoform composition. Compared to the IsoPSA assay, the underlying molecular heterogeneity of cancer cells from populations of patients and in individuals at different phases of stage and grade progression represents a key limitation for tests based on measuring predefined isoforms. Instead, IsoPSA is based on a 2-phase chemical system which agnostically interrogates the entire isoform population in serum to identify patients at increased risk for the cancer phenotype.

We report validation of the clinical performance of IsoPSA in a multi-institutional and multinational prospective study in a new cohort of men referred for prostate biopsy based on currently accepted clinical criteria. We used the same cutoff values of the K index defined in the preliminary study.[1] The end point of the study was the ability of the assay to identify the risk of high grade PCa (defined as Gleason 7 or greater) vs low grade PCa or benign disease compared to a concentration based assay for total PSA. The results of this validation study confirm that IsoPSA outperforms standard PSA to identify clinically significant PCa and concomitant mpMRI based imaging augments its overall diagnostic accuracy. The results suggest that clinical use of IsoPSA could reduce the rate of unnecessary biopsies by 43% with an acceptable rate of false-negative findings at the cutoff value of KR-HG of 17% defined in the preliminary study[1] and directly applied to the validation cohort.

Based on these data in a theoretical cohort of 1,000 men undergoing biopsy the use of IsoPSA would have reduced unnecessary biopsies by 43% from 705 to 402 with only 22 missed high grade cancers, of which only 7 would have been Gleason sum 4 + 3 or higher. Importantly, in a head-to head comparison IsoPSA outperformed total PSA, %fPSA and a model including tPSA and %fPSA to detect high grade cancer with a similar 2% rate of missed cancers, while significantly reducing unnecessary biopsy by 46% vs 21% for %fPSA and 19% in the tPSA plus % fPSA model.

The clinical performance consistency of IsoPSA at the same cutoff level makes its clinical use as a stand-alone test easy and appealing to exclude high grade disease. We are also encouraged by the robust performance of IsoPSA in African American men and seek to validate this observation in future studies.

Another important finding is the improved performance of IsoPSA in men undergoing mpMRI guided biopsy compared with TRUS alone. The AUC improved from 0.784 to 0.831 with magnetic resonance imaging guidance. Notably more biopsies were done under mpMRI guidance in the validation study than in our preliminary study (41% vs 4%), reflecting a change in practice patterns during the sequential years of study completion.

Multiple prior studies have demonstrated the limitations of TRUS based biopsy with respect to under sampling high grade disease with an estimated 30% upgrade rate upon subsequent pathological inspection of radical prostatectomy and saturation biopsy specimens[20–22] as well as the superiority of mpMRI guided approaches to reduce this detection bias.[23,24] As such, the improved performance of IsoPSA in men undergoing mpMRI guided biopsies adds confidence that the biomarker accurately identifies those at highest risk for high grade disease and establishes the performance of the assay in contemporary clinical practice. To our knowledge this is the first study to explicitly compare the performance of a blood based biomarker as a function of 2 biopsy techniques with different accuracy for identifying the primary end point of high grade disease.

Strengths of this study include 1) a prospective multicenter and international design, 2) use of prespecified, fixed outcome measures defined by the results of the previously published development study, 3) reliance on standard and widely accepted clinical indications for prostate biopsy and 4) inclusion of a significant number of men undergoing mpMRI guided biopsy, reflecting contemporary urological practice. Limitations include the lack of standardized biopsy techniques and the lack of a central pathology review. These weaknesses are mitigated by the fact that the results represent the performance of the IsoPSA assay under real world conditions, adding confidence that the observed results are generalizable.

Another potential weakness is that IsoPSA performance was not directly compared to that of other commercially available tests shown to have improved accuracy over PSA. However, the study was not designed as a head-to-head comparison of other available tests.

CONCLUSIONS

Independent prospective evaluation of the IsoPSA assay in a new patient cohort has validated the use of structure rather than concentration as the basis of defining a new cancer specific biomarker assay to detect high grade prostate cancer. The results were in statistical accord with those obtained in our preliminary work.[1] Furthermore, performance was enhanced when a diagnostically superior mpMRI guided biopsy technique was used to evaluate the assay. Now that it is validated, IsoPSA may improve the diagnostic accuracy of early detection paradigms for PCa, reduce the number of unneeded biopsies with the attendant clinical risks and costs, and lower the likelihood of over detection of nonlethal cancer.

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