Association of [−2]proPSA with Biopsy Reclassification During Active Surveillance for Prostate Cancer

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Purpose: Previous studies have suggested an association between [−2]proPSA expression and prostate cancer detection. Less is known about the usefulness of this marker in following patients with prostate cancer on active surveillance. Thus, we examined the relationship between [−2]proPSA and biopsy results in men enrolled in an active surveillance program.

Materials and Methods: In 167 men from our institutional active surveillance program we used Cox proportional hazards models to examine the relationship between [−2]proPSA and annual surveillance biopsy results. The outcome of interest was biopsy reclassification (Gleason score 7 or greater, more than 2 positive biopsy cores or more than 50% involvement of any core with cancer). We also examined the association of biopsy results with total prostate specific antigen, %fPSA, [−2]proPSA/%fPSA and the Beckman Coulter Prostate Health Index phi ([−2]proPSA/free prostate specific antigen) × (total prostate specific antigen)$^{1/2}$).

Results: While on active surveillance (median time from diagnosis 4.3 years), 63 (37.7%) men demonstrated biopsy reclassification based on the previously mentioned criteria, including 28 (16.7%) of whom had reclassification based on Gleason score upgrading (Gleason score 7 or greater). Baseline and longitudinal %fPSA, %[−2]proPSA, [−2]proPSA/%fPSA and phi measurements were significantly associated with biopsy reclassification, and %[−2]proPSA and phi provided the greatest predictive accuracy for high grade cancer.

Conclusions: In men on active surveillance, measures based on [−2]proPSA such as phi appear to provide improved prediction of biopsy reclassification during followup. Additional validation is warranted to determine whether clinically useful thresholds can be defined, and to better characterize the role of %[−2]proPSA and phi in conjunction with other markers in monitoring patients enrolled in active surveillance.

Key Words: prostate-specific antigen, prostatic neoplasms, biopsy, watchful waiting

Abbreviations and Acronyms

fPSA = free PSA

phi = Beckman Coulter Prostate Health Index

proPSA = proenzyme PSA

PSA = prostate specific antigen

tPSA = total PSA

Submitted for publication September 13, 2011.

Study received institutional review board approval.

Supported by the Johns Hopkins University Prostate Cancer SPORE (Grant number P50CA58236), the Prostate Cancer Foundation, the Patana Fund and the Patrick C. Walsh Prostate Cancer Research Fund.

* Equal study contribution.

† Nothing to disclose.

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§ Financial interest and/or other relationship with Beckman Coulter, Inc.

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Prostate cancer is the most commonly diagnosed noncutaneous malignancy in United States men.[1] Although PSA is widely used in prostate cancer screening, benign conditions may result in increased serum PSA which limits its specificity. However, recently characterized free PSA isoforms may improve the specificity of PSA. These isoforms include BPSA, a

0022-5347/12/1884-1131/0
THE JOURNAL OF UROLOGY®

http://dx.doi.org/10.1016/j.juro.2012.06.009
Vol. 188, 1131-1136, October 2012
Printed in U.S.A.

degraded form increased in benign prostatic hyperplasia,[2] as well as proPSA, an inactive PSA precursor containing a 7 amino acid pro leader peptide, which has been associated with prostate cancer.[3] Additional forms of proPSA that contain truncated leader sequences of 5, 4 or 2 amino acids have also been described.[4]

Previous studies have demonstrated an increased proportion of proPSA in prostate cancer tissue and in the serum of patients with prostate cancer.[3,5] Other data have suggested that the proPSA-to-free PSA ratio (%proPSA) may be superior to total PSA and percent free PSA in prostate cancer detection in select subgroups of patients.[6,7] An additional application of these markers is the recently described Beckman Coulter Prostate Health Index (phi), which combines [−2]proPSA with free and total PSA.[8,9] In a recent multicenter study phi outperformed total and %fPSA in the detection of prostate cancer.[9]

Although the role of proPSA has been examined in the early detection of cancer, less is known about its potential applications for patients with prostate cancer undergoing active surveillance. [−2]proPSA has been associated with prostate cancer aggressiveness,[9,10] and proPSA has been reported to be specifically associated with high grade (Gleason 7 or greater) disease among men with PSA between 2 and 4 ng/ml.[11] Based on these findings and our initial results in tissue and serum,[12,13] we determined whether [−2]proPSA was associated with biopsy reclassification in a larger cohort of very low risk patients enrolled in active surveillance.

MATERIALS AND METHODS

Active Surveillance Program

Since 1995 active surveillance has been offered to patients who present to our institution with very low risk prostate cancer,[14,15] as defined by Epstein et al[16] and endorsed by the National Comprehensive Cancer Network.[17] Enrollment criteria included clinical stage T1c disease, PSA density less than 0.15 ng/ml/cm^3, Gleason score 6 or less, 2 or fewer biopsy cores with cancer and a maximum of 50% involvement of any core with cancer. All patients provided written informed consent before enrolling in the institutional review board approved program.

Followup of men in the program included semiannual PSA measurements (free and total), digital rectal examination and an annual surveillance biopsy (typically 14-core, including transition zone biopsies since 2009). Neither total PSA nor PSA kinetics were used as a trigger for intervention. Curative intervention was recommended after evidence of biopsy reclassification (Gleason score 7 or greater, more than 2 positive biopsy cores or more than 50% involvement of any biopsy core with cancer), taking into consideration patient preferences and the presence or absence of comorbidities. In addition, some men request curative therapy in the absence of biopsy reclassification.

Selection of Study Cohort

From 1995 to the initiation of this study 689 men enrolled in our active surveillance program. Of these men 214 had a minimum of 2 serum samples (mean 3.5, range 2 to 10) collected before any of the study biopsies, and available for PSA and isoforms testing. We excluded 29 men with a history of finasteride or dutasteride use, 17 men with unavailable followup biopsy data, and 1 subject with documented infection at the time of blood draw. The remaining 167 men formed our study population.

Measurement of PSA and Isoforms

Total PSA, free PSA and [−2]proPSA (Beckman Coulter p2PSA) were measured on the Beckman Coulter Access® 2 immunoassay analyzer in samples stored at −80C. The 3 dual monoclonal sandwich assays use Hybritech antibodies and a chemiluminescent detection system. The [−2]proPSA assay has less than 1% cross-reactivity with other PSA forms. %[−2]proPSA was calculated as ([−2]proPSA pg/ml/10)/fPSA ng/ml, and phi as ([−2]proPSA pg/ml/fPSA ng/ml) × (tPSA ng/ml)$^{1/2}$.

Statistical Analysis

Baseline clinical characteristics and changes from baseline to last followup were compared using the Wilcoxon rank sum (Mann-Whitney) test in men who did and those who did not eventually demonstrate biopsy reclassification. Patients who did not experience biopsy reclassification were censored at the time of most recent biopsy and followup was defined as the time from diagnosis to disease reclassification or censoring.

Separate Cox proportional hazards models were used to evaluate the association between surveillance biopsy reclassification, and the baseline and longitudinal[18] marker measurements. Additional Cox models were used to assess the relationship between these analytes and biopsy reclassification based only on Gleason score upgrading (Gleason score 7 or greater). For longitudinal analyses, if a specimen was not available for biomarker measurements at the last biopsy, biomarker results were imputed using the method of last observation carried forward.[19] All models were adjusted for age, date of diagnosis and PSA density (continuous variables).

Finally, the concordance index was used to compare the discrimination of biopsy reclassification among analytes. The concordance index for longitudinal data was calculated using the approach described by Newson.[20] All analyses were performed using Stata® v11.0.

RESULTS

Of the 167 men included in this analysis median age at diagnosis was 65.7 years (range 50.6 to 76.1) and median followup after diagnosis was 4.30 years (range 0.96 to 10.47). The majority of men were white. Table 1 compares the characteristics of men who did or did not demonstrate biopsy reclassification. Overall 63 (37.7%) men had biopsy reclassification on followup, 29 (17.4%) of whom had Gleason score upgrading (Gleason score 7 or greater). The remaining 104 (62.3%) men did not have biopsy reclassification during followup.

Table 1. *Baseline characteristics of the study population*

	Overall	No Biopsy Reclassification	Biopsy Reclassification	p Value
No. pts		104	63	
Age:				0.7164
Mean ± SD	65.7 ± 4.8	65.6 ± 4.5	65.8 ± 5.2	
Median (range)	65.8 (50.6–76.1)	65.8 (55.0–74.9)	66.3 (50.6–76.1)	
tPSA (ng/ml):				0.0004
Mean ± SD	4.9 ± 3.1	4.5 ± 3.4	5.6 ± 2.3	
Median (range)	4.6 (0.40–18.6)	3.86 (0.40–18.6)	5.31 (1.47–13.1)	
Prostate vol:				0.7663
Mean ± SD	50.88 ± 22.38	51.95 ± 25.31	49.07 ± 16.34	
Median (range)	48.0 (10–145.3)	49.0 (10.0–145.3)	48.0 (21.9–90.0)	
PSA density:				<0.0001
Mean ± SD	0.10 ± 0.06	0.09 ± 0.06	0.12 ± 0.06	
Median (range)	0.08 (0.02–0.39)	0.07 (0.02–0.39)	0.12 (0.04–0.32)	
%fPSA:				0.0016
Mean ± SD	20.68 ± 7.91	22.26 ± 8.03	18.08 ± 7.03	
Median (range)	19.45 (5.65–41.49)	21.12 (7.92–41.49)	17.52 (5.65–36.71)	
%[−2]proPSA:				0.3587
Mean ± SD	1.54 ± 0.54	1.49 ± 0.43	1.63 ± 0.69	
Median (range)	1.48 (0.45–4.54)	1.48 (0.52–2.64)	1.48 (0.45–4.54)	
[−2]proPSA/%fPSA:				0.0001
Mean ± SD	0.72 ± 0.48	0.60 ± 0.39	0.90 ± 0.55	
Median (range)	0.62 (0.07–2.81)	0.52 (0.07–2.18)	0.76 (0.26–2.81)	
phi:				0.0002
Mean ± SD	31.56 ± 14.42	27.99 ± 10.07	37.45 ± 18.21	
Median (range)	29.08 (10.55–104.62)	26.87 (10.55–64.77)	32.23 (11.14–104.62)	
No. biomarker measurements:				0.067
Mean ± SD	3.32 ± 1.54	3.47 ± 1.51	3.11 ± 1.57	
Median (range)	3.0 (2–10)	3.0 (2–10)	3.0 (2–8)	
Yrs from prostate Ca diagnosis to 1st [−2]proPSA:				0.001
Mean ± SD	1.27 ± 0.95	1.39 ± 0.92	1.07 ± 0.97	
Median (range)	1.12 (−0.1–5.91)	1.28 (−0.04–5.91)	0.98 (−0.1–5.14)	
Yrs from prostate Ca diagnosis to biopsy reclassification or censoring:				0.0004
Mean ± SD	4.43 ± 2.10	4.81 ± 1.93	3.81 ± 2.23	
Median (range)	4.30 (0.96–10.47)	4.78 (1.00–10.00)	3.41 (0.96–10.47)	

Participants with and those without biopsy reclassification during followup were similar at baseline with respect to age, %[−2]proPSA, prostate volume and median number of biomarker measurements. However, men who demonstrated biopsy reclassification had a significantly lower initial %fPSA ($p = 0.0016$), as well as a significantly higher initial tPSA ($p = 0.0004$), PSA density ($p < 0.0001$), [−2]proPSA/%fPSA ($p = 0.0001$) and phi ($p = 0.0002$). The duration of followup was significantly longer in men who did not demonstrate biopsy reclassification (median 4.78 vs 3.41, $p = 0.0004$).

Cox proportional hazards models for risk of biopsy reclassification are shown in table 2. After adjusting for age, date of diagnosis and PSA density, baseline tPSA was not significantly associated with biopsy reclassification ($p = 0.061$). However, risk of reclassification was significantly associated with lower baseline %fPSA ($p = 0.002$), and higher %[−2]proPSA ($p < 0.0001$), [−2]proPSA/%fPSA ($p = 0.026$) and phi ($p < 0.0001$). Similarly, Cox models with longitudinal measurements of %fPSA ($p = 0.002$), %[−2]proPSA ($p < 0.0001$), [−2]proPSA/%fPSA ($p = 0.005$) and phi ($p < 0.0001$) demonstrated significant associations with biopsy reclassification. Concordance indexes revealed improved discrimination (predictive accuracy) when baseline %[−2]proPSA, [−2]proPSA/%fPSA and phi were in-

Table 2. *Cox proportional hazards models and concordance indices to predict biopsy reclassification using baseline and longitudinal measurements*

	Cox Proportional Hazards Models		
	HR (95% CI)	p Value	C Index
Baseline:			
tPSA	0.90 (0.80–1.00)	0.061	0.630
%fPSA	0.93 (0.89–0.97)	0.002	0.664
%[−2]proPSA	2.44 (1.51–3.94)	<0.0001	0.651
[−2]proPSA/%fPSA	2.13 (1.09–4.16)	0.026	0.652
phi	1.04 (1.02–1.06)	<0.0001	0.662
Longitudinal:			
tPSA	0.96 (0.88–1.05)	0.366	0.703
%fPSA	0.94 (0.90–0.98)	0.002	0.722
%[−2]proPSA	1.92 (1.36–2.73)	<0.0001	0.647
[−2]proPSA/%fPSA	2.12 (1.25–3.59)	0.005	0.654
phi	1.04 (1.02–1.06)	<0.0001	0.635

Table 3. *Cox proportional hazards models and concordance indices to predict reclassification by Gleason score upgrading using baseline and longitudinal measurements*

	Cox Proportional Hazards Models		
	HR (95% CI)	p Value	C Index
Baseline:			
tPSA	0.90 (0.77–1.05)	0.192	0.705
%fPSA	0.92 (0.86–0.99)	0.025	0.743
%[−2]proPSA	4.02 (1.90–8.49)	<0.0001	0.784
[−2]proPSA/%fPSA	3.48 (1.26–9.59)	0.016	0.762
phi	1.06 (1.03–1.09)	<0.0001	0.788
Longitudinal:			
tPSA	0.95 (0.84–1.08)	0.445	0.771
%fPSA	0.93 (0.88–0.99)	0.025	0.786
%[−2]proPSA	2.49 (1.51–4.10)	<0.0001	0.832
[−2]proPSA/%fPSA	2.49 (1.16–5.34)	0.019	0.786
phi	1.05 (1.02–1.07)	<0.0001	0.820

cluded in the models, or using %fPSA (baseline or longitudinal) compared to total PSA. For baseline measures the models that included baseline %fPSA and phi yielded the highest discriminative accuracy, while for longitudinal measures %fPSA was the best discriminant. Notably the scales of measurement differed for each of these biomarkers. Thus, comparing the magnitude of the hazard ratio between biomarkers is not indicative of relative strengths of association.

Table 3 shows Cox proportional hazards models for the risk of Gleason score upgrading on biopsy (Gleason score 7 or greater). Baseline and longitudinal measures of all of the PSA isoforms were significantly associated with biopsy upgrading, but tPSA was not associated with upgrading. All of the isoforms also showed improved discriminant accuracy compared to tPSA, with %[−2]proPSA and phi showing the highest concordance indexes for baseline and longitudinal measures. For all biomarkers, using longitudinal measures provided increased discriminant accuracy compared to the measure at baseline. %[−2]proPSA and [−2]proPSA/%fPSA showed much larger hazard ratios for biopsy upgrading than for biopsy reclassification. The other biomarkers demonstrated similar hazard ratios for both outcomes.

To explore why biopsy upgrading was better predicted by longitudinal than baseline biomarker values, we compared the absolute increase in each biomarker from baseline to last followup value for men without vs with biopsy upgrading. For all biomarkers except %fPSA (where smaller values confer higher risk), the magnitude of the change was larger for men with upgrading. The difference was statistically significant only for phi. We also evaluated absolute biomarker change as a predictor in models of biopsy upgrading, and none were statistically significant (data not shown).

DISCUSSION

In men with low risk prostate cancer, active surveillance with delayed curative intervention has been associated with a cause specific survival greater than 97%.[21] In accordance with these data, there have been no deaths due to prostate cancer in our active surveillance cohort.[15] Furthermore, using a pathology based definition of curability, preliminary results from our cohort suggested that the opportunity for cure, if necessary, was not sacrificed in those who underwent treatment after a trial of active surveillance compared to those who underwent immediate treatment.[22] Based on these and other similar findings,[23] active surveillance is considered a reasonable management option for carefully selected older men with low risk prostate cancer.

Despite these results, and the potential morbidity associated with all forms of prostate cancer therapy, the majority of low risk patients choose to undergo immediate treatment rather than surveillance.[24] Underuse of surveillance may be due to the lack of biomarkers that can reliably predict which cases will demonstrate reclassification on biopsy and may subsequently require treatment. Moreover current methods of monitoring disease (ie repeat biopsies) are invasive, such that the discovery of a reliable serum biomarker could improve the quality of care for men undergoing surveillance.

Our group previously examined the relationship between prostate cancer biomarkers and biopsy results during active surveillance.[25,26] When used in combination with other clinical variables, %fPSA at diagnosis was associated with biopsy reclassification,[25] while baseline values of the molecular urine marker PCA3 did not reliably predict reclassification in the short term, although this study was limited by sample size and followup time.[26] Another promising new marker is proPSA, which has been suggested as a means to improve the specificity of PSA based screening.[3]

It was previously reported that the percentage of proPSA measured in serum was useful for detecting prostate cancer and reducing unnecessary biopsies in men with tPSA between 2.5 and 4.0 ng/ml.[6] An additional study demonstrated similar results in a larger population of men with PSA from 2 to 10 ng/ml.[7] Furthermore, in men with a tPSA of 4 to 10 ng/ml, proPSA used in combination with PSA and %fPSA increased the specificity for prostate cancer detection more than any other parameter alone.[27] More recently, retrospective[28] and prospective[10] multicenter studies as well as screening studies[29] validated the usefulness of the [−2]proPSA isoform for cancer detection in the 2 or 2.5 to 10 ng/ml tPSA range. In addition, recent studies have reported on the Beckman Coulter phi, which combines

[−2]proPSA, fPSA and tPSA in a mathematical formula. Jansen et al reported that phi had a higher AUC for prostate cancer detection than tPSA or %fPSA in 2 European screening populations.[8] Similarly the AUC for phi was higher than total or %fPSA in a multicenter study of 892 men in which an increasing phi was associated with a 4.7-fold increased risk of prostate cancer.[9]

Several studies have aimed to clarify the potential role of proPSA in predicting prostate cancer severity. In 2004 Catalona et al found that proPSA levels were associated with high grade disease (Gleason score 7 or greater) and/or extracapsular tumor extension.[11] In a prospective, multicenter study %[−2]proPSA increased with increasing biopsy Gleason score and was higher in aggressive cancers.[10] The relationship between phi and Gleason score has been mixed, with no association observed in the European cohorts[8] and an increased risk of Gleason score 4 + 3 = 7 at biopsy with increasing phi observed in a recent multicenter study.[9]

These data suggest that proPSA, in conjunction with other biomarkers, may offer valuable diagnostic and prognostic information. Nevertheless, there are limited data on the usefulness of proPSA in monitoring men on active surveillance. In a previous study of 71 men in our active surveillance program, tissue and serum [−2]proPSA successfully identified those who could safely remain on active surveillance.[12,13] Also, little is known regarding the role of phi in active surveillance. Accordingly, we expanded on previous findings by examining the association of potential biomarkers and biopsy reclassification in a larger population of men on active surveillance.

We believe that failing to identify high grade cancer poses the greatest risk to men on surveillance. Thus, an improved ability to predict such cancers could potentially decrease the risk associated with surveillance. We found that baseline and longitudinal measures of %fPSA, %[−2]proPSA, [−2]proPSA/%fPSA and phi were significantly associated with overall biopsy reclassification. Similar associations were observed for reclassification based specifically on Gleason score upgrading (Gleason score 7 or greater). However, total PSA was not significantly associated with biopsy reclassification.

For biopsy upgrading but not overall biopsy reclassification, longitudinal biomarker measures provided greater predictive accuracy than using the baseline measure only. The absolute biomarker change between baseline and last biopsy was somewhat higher for men with biomarker upgrading than for those without upgrading for all biomarkers except %fPSA, but the difference was significant only for phi. It is possible that serial biomarker measurements characterize the tumor grade phenotype more accurately than a single baseline measure. However, interpretation of this result is tentative as most events of biopsy upgrading likely represent under grading at the initial biopsy rather than true grade progression.[30] Given the cardinal role of tumor grade as an indicator of suitability for active surveillance, it will be important for larger independent studies to validate whether longitudinal biomarker sampling provides improved prediction of grade and, if so, the optimal number and timing of samples.

A notable strength of our analysis is that all participants were subject to a stringent and consistent followup protocol. Furthermore, this study allowed for the comparison of new serum markers with objective histological findings. Nonetheless, this study is limited by its relatively small sample size and number of end points achieved. Therefore, clinical application should be reserved until these findings can be validated in a larger cohort. Validation should also explore threshold values yielding sufficient sensitivity and specificity for potential clinical use such that patients with abnormal values may benefit from a more extensive preliminary evaluation. As previously suggested,[27] these markers must also be studied in the context of other prostate cancer markers and may be most useful in a combined model to improve predictive ability. In addition, the associations observed in this study may vary in surveillance programs using other eligibility and surveillance criteria. Therefore, our analysis should not be considered a formal assessment of a predictive model, but rather that of an association between selected markers and biopsy reclassification in this cohort.

CONCLUSIONS

Baseline and longitudinal %[−2]proPSA, [−2]proPSA/%fPSA and phi measurements were significantly higher and %fPSA measurements were significantly lower among men on active surveillance who demonstrated biopsy reclassification due to extent of tumor or Gleason upgrading on biopsy. Neither baseline nor longitudinal tPSA measurements were significantly associated with biopsy reclassification. Future studies are warranted to better define the potential role of these biomarkers and the optimal sampling scheme for monitoring patients on active surveillance.

ACKNOWLEDGMENTS

Reagents were provided by Beckman Coulter, Inc.

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