Published in final edited form as:
J Urol. 2012 October ; 188(4): 1131–1136. doi:10.1016/j.juro.2012.06.009.

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

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Abstract

Purpose—Previous studies have suggested an association between [-2]proPSA expression and prostate cancer detection. Less is known about the utility of this marker in following prostate cancer patients on active surveillance. Thus, our objective was to examine 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 >2 positive biopsy cores, or >50% involvement of any core with cancer). We also examined the association of biopsy results with total PSA, %fPSA, [-2]proPSA/%fPSA, and the Beckman Coulter Prostate Health Index [*phi*=([-2]proPSA/fPSA) x $(tPSA)^{½}$].

Results—While on active surveillance (median time from diagnosis 4.3 years), 63 (37.7%) men demonstrated biopsy reclassification based on the above criteria, including 28 (16.7%) of whom had reclassification based on Gleason score upgrading (Gleason score≥7). 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 follow-up. 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.

Keywords

proPSA; PSA; prostate cancer; biopsy; active surveillance

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Introduction

Prostate cancer is the most commonly diagnosed non-cutaneous malignancy in U.S. men.[1] Although prostate-specific antigen (PSA) is widely used in prostate cancer screening, benign conditions may result in elevated serum PSA levels which limits its specificity. However, the recently characterized free PSA (fPSA) isoforms may improve the specificity of PSA. These isoforms include BPSA, a degraded form elevated in BPH[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 prostate cancer patients.[3,5] Other data have suggested that the proPSA to free PSA ratio (%proPSA) may be superior to both total PSA and percent free PSA (%fPSA) 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 for prostate cancer detection.[9]

Although the role of proPSA has been examined for the early detection of cancer, less is known about its potential applications for prostate cancer patients 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) disease among men with PSA levels between 2 and 4 ng/ml.[11] Based on these findings and our initial results in tissue and serum,[12,13] we sought to determine 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 (NCCN).[17] Enrollment criteria include: clinical stage T1c disease, PSA density <0.15 ng/ml/cm^3, Gleason score ≤6, ≤2 biopsy cores with cancer, and a maximum of 50% involvement of any core with cancer. All patients provide written informed consent prior to enrolling in the IRB-approved program.

Follow-up for men in the program includes 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 is used as a trigger for intervention. Curative intervention is recommended after evidence of biopsy reclassification (Gleason score ≥7, or >2 positive biopsy cores, or >50% involvement of any biopsy core with cancer) taking into consideration patient preferences and the presence or absence of comorbidities. Additionally, 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 had enrolled in our active surveillance program. Of these, 214 had a minimum of two serum samples (mean 3.5 samples, range 2-10) collected prior to 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 follow-up biopsy data, and one 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 (tPSA), free PSA (fPSA), and [-2]proPSA (Beckman Coulter p2PSA) were measured on the Beckman Coulter Access 2 immunoassay analyzer in samples stored at -80 °C. The three dual monoclonal sandwich assays use Hybritech antibodies and a chemiluminescent detection system. The research-use [-2]proPSA assay has < 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) x (tPSA ng/mL)^{½}.

Statistical Analysis

Baseline clinical characteristics and changes from baseline to last follow-up were compared using the Wilcoxon rank-sum (Mann-Whitney) test in men who did and did not eventually demonstrate biopsy reclassification. Patients who did not experience biopsy reclassification were censored at the time of most recent biopsy, and follow-up 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 both 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). 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 (c-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, the median age at diagnosis was 65.7 years (range 50.6-76.1) and median follow-up after diagnosis was 4.30 years (range 0.96-10.47). The majority of men were Caucasian. Table 1 compares characteristics of men who did or did not demonstrate biopsy reclassification. Overall, 63 (37.7%) men had biopsy reclassification on follow-up, 29 (17.4%) of which revealed Gleason score upgrading (Gleason score ≥7). The remaining 104 (62.3%) men did not have biopsy reclassification during follow-up.

Participants with and without biopsy reclassification during follow-up were similar at baseline with respect to age, %[-2]proPSA, prostate volume, and the median number of biomarker measurements. However, men who demonstrated biopsy reclassification had a significantly lower initial %fPSA (p=0.0016), as well as 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 follow-up 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 indices revealed improved discrimination (predictive accuracy) when baseline %[-2]proPSA, [-2]proPSA/%fPSA and *phi* were included in the models, or using %fPSA (baseline or longitudinal) as 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 differ 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). 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 c-indices, for both baseline and longitudinal measures. For all biomarkers, using longitudinal measures provided increased discriminant accuracy compared to the measure at baseline. Both %[-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 the reason why biopsy upgrading was better predicted by longitudinal than baseline biomarker values, we compared the absolute increase in each biomarker from baseline to last follow-up 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; 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, as 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] Underutilization of surveillance may be due to lack of biomarkers that can reliably predict which men will demonstrate reclassification on biopsy and may subsequently require treatment. Moreover, current methods of monitoring disease (i.e. 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 has 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 follow-up 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 levels between 2.5 and 4.0 ng/mL.[6] An additional study demonstrated similar results in a larger population of men with PSA levels from 2 to 10 ng/ml.[7] Furthermore, in men with tPSA levels 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, and screening[29] studies have 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 two 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, it was shown that proPSA levels were associated with high-grade disease (Gleason score ≥7) and/or extra-capsular 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. That notwithstanding, 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 aimed to expand upon 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 lower the risk associated with surveillance. In the current study, we found that both 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). Total PSA, however, was not significantly associated with biopsy reclassification.

For biopsy upgrading, but not overall biopsy reclassification, longitudinal biomarker measures provided greater predictive accuracy than only using the baseline measure. The absolute biomarker change between baseline and last biopsy was somewhat higher for men with biomarker upgrading than 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 are likely to represent undergrading 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 follow-up 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 endpoints achieved; clinical application should therefore be reserved until these findings can be validated within 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. Also, the associations observed in this study may vary in surveillance programs utilizing other eligibility and surveillance criteria. For these reasons, 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

In conclusion, baseline and longitudinal %[-2]proPSA, [-2]proPSA/%fPSA, and *phi* measurements were significantly higher, and %fPSA measurements were significantly lower among men in 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

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

Reagents were provided for this study by Beckman Coulter, Inc.

References

1. American Cancer Society: Cancer Facts & Figures 2012. Atlanta: American Cancer Society; 2012.
2. Linton HJ, Marks LS, Millar LS, et al. Benign prostate-specific antigen (BPSA) in serum is increased in benign prostate disease. Clin Chem. 2003; 49:253. [PubMed: 12560348]
3. Mikolajczyk SD, Rittenhouse HG. Pro PSA: a more cancer specific form of prostate specific antigen for the early detection of prostate cancer. Keio J Med. 2003; 52:86. [PubMed: 12862359]
4. Peter J, Unverzagt C, Krogh TN, et al. Identification of precursor forms of free prostate-specific antigen in serum of prostate cancer patients by immunosorption and mass spectrometry. Cancer Res. 2001; 61:957. [PubMed: 11221890]
5. Mikolajczyk SD, Millar LS, Wang TJ, et al. A precursor form of prostate-specific antigen is more highly elevated in prostate cancer compared with benign transition zone prostate tissue. Cancer Res. 2000; 60:756. [PubMed: 10676664]
6. 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]

7. 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]
8. 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; 57:921. [PubMed: 20189711]
9. 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; 185:1650. [PubMed: 21419439]
10. 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; 19:1193. [PubMed: 20447916]
11. 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]
12. 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]
13. Isharwal S, Makarov DV, Sokoll LJ, et al. ProPSA and diagnostic biopsy tissue DNA content combination improves accuracy to predict the need for prostate cancer treatment among men enrolled in an active surveillance program. Urology. 2011; 77:763 e1-6. Epub 2011 Jan 8. [PubMed: 21216447]
14. Carter HB, Walsh PC, Landis P, et al. Expectant management of nonpalpable prostate cancer with curative intent: preliminary results. J Urol. 2002; 167:1231. [PubMed: 11832703]
15. Tosoian JJ, Trock BJ, Landis P, et al. Active surveillance program for prostate cancer: an update of the Johns Hopkins experience. J Clin Oncol. 2011; 29:2185. [PubMed: 21464416]
16. Epstein JI, Walsh PC, Carmichael M, et al. Pathologic and clinical findings to predict tumor extent of nonpalpable (stage T1c) prostate cancer. JAMA. 1994; 271:368. [PubMed: 7506797]
17. Mohler J, Bahnson RR, Boston B, et al. NCCN clinical practice guidelines in oncology: prostate cancer. J Natl Compr Canc Netw. 2010; 8:162. [PubMed: 20141676]
18. Fisher LD, Lin DY. Time-dependent covariates in the Cox proportional-hazards regression model. Annu Rev Public Health. 1999; 20:145. [PubMed: 10352854]
19. Twisk J, de Vente W. Attrition for longitudinal studies. How to deal with missing data. J Clin Epidemiol. 2002; 55:329. [PubMed: 11927199]
20. Newson RB. Comparing the predictive power of survival models using Harrell's *c* or Somers' *D*. Stata Journal. 2010; 10:339.
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; 28:126. [PubMed: 19917860]
22. Warlick C, Trock BJ, Landis P, et al. Delayed versus immediate surgical intervention and prostate cancer outcome. J Natl Cancer Inst. 2006; 98:355. [PubMed: 16507832]
23. van den Bergh RC, Steyerberg EW, Khatami A, et al. Is delayed radical prostatectomy in men with low-risk screen-detected prostate cancer associated with a higher risk of unfavorable outcomes? Cancer. 2010; 116:1281. [PubMed: 20066716]
24. Miller DC, Gruber SB, Hollenbeck BK, et al. Incidence of initial local therapy among men with lower-risk prostate cancer in the United States. J Natl Cancer Inst. 2006; 98:1134. [PubMed: 16912266]
25. Tseng KS, Landis P, Epstein JI, et al. Risk stratification of men choosing surveillance for low risk prostate cancer. J Urol. 2010; 183:1779. [PubMed: 20304433]
26. Tosoian JJ, Loeb S, Kettermann A, et al. Accuracy of PCA3 measurement in predicting short-term biopsy progression in an active surveillance program. J Urol. 2010; 183:534. [PubMed: 20006883]

27. Khan MA, Partin AW, Rittenhouse HG, et al. Evaluation of proprostate specific antigen for early detection of prostate cancer in men with a total prostate specific antigen range of 4.0 to 10.0 ng/ml. J Urol. 2003; 170:723. [PubMed: 12913682]

28. 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]

29. Le BV, Griffin CR, Loeb S, 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. [PubMed: 20171670]

30. Sheridan TB, Carter HB, Wang W, et al. Change in prostate cancer grade over time in men followed expectantly for stage T1c disease. J Urol. 2008; 179:901. [PubMed: 18207195]

Table 1
Baseline characteristics of the study population

Variable	Overall (n=167)	No biopsy reclassification (n=104)	Biopsy reclassification (n=63)	p-value
Age (yr)				
Mean ± SD	65.7 ± 4.8	65.6 ± 4.5	65.8 ± 5.2	0.7164
Median (Range)	65.8 (50.6-76.1)	65.8 (55.0-74.9)	66.3 (50.6-76.1)	
tPSA (ng/ml)				
Mean ± SD	4.9 ± 3.1	4.5 ± 3.4	5.6 ± 2.3	0.0004
Median (Range)	4.6 (0.40-18.6)	3.86 (0.4-18.6)	5.31 (1.47-13.1)	
Prostate volume				
Mean ± SD	50.88±22.38	51.95 ± 25.31	49.07 ± 16.34	0.7663
Median (Range)	48.0 (10-145.3)	49.0 (10.0-145.3)	48.0 (21.9-90.0)	
PSA density				
Mean ± SD	0.10±0.06	0.09 ± 0.06	0.12 ± 0.06	<0.0001
Median (Range)	0.08 (0.02-0.39)	0.07 (0.02-0.39)	0.12 (0.04-0.32)	
%fPSA				
Mean ± SD	20.68 ± 7.91	22.26 ± 8.03	18.08 ± 7.03	0.0016
Median (Range)	19.45 (5.65-41.49)	21.12 (7.92-41.49)	17.52 (5.65-36.71)	
%[-2]proPSA				
Mean ± SD	1.54 ± 0.54	1.49 ± 0.43	1.63 ± 0.69	0.3587
Median (Range)	1.48 (0.45-4.54)	1.48 (0.52-2.64)	1.48 (0.45-4.54)	
[-2]proPSA/%fPSA				
Mean ± SD	0.72±0.48	0.60±0.39	0.90±0.55	0.0001
Median (Range)	0.62 (0.07-2.81)	0.52 (0.07-2.18)	0.76 (0.26-2.81)	
phi				
Mean ± SD	31.56±14.42	27.99±10.07	37.45±18.21	0.0002
Median (Range)	29.08 (10.55-104.62)	26.87 (10.55-64.77)	32.23 (11.14-104.62)	
Number of biomarker measurements				
Mean ± SD	**3.32±1.54**	**3.47±1.51**	**3.11±1.57**	**0.067**
Median (Range)	**3.0 (2-10)**	**3.0 (2-10)**	**3.0 (2-8)**	
Time from prostate cancer diagnosis to 1st [-2]proPSA (yr)				
Mean ± SD	1.27±0.95	1.39±0.92	1.07±0.97	0.001
Median (Range)	1.12 (-0.1-5.91)	1.28 (-0.04-5.91)	0.98 (-0.1-5.14)	
Time from prostate cancer diagnosis to biopsy reclassification or censoring (yr)				

Variable	Overall (n=167)	No biopsy reclassification (n=104)	Biopsy reclassification (n=63)	p-value
Mean ± SD	4.43±2.10	4.81±1.93	3.81±2.23	0.0004
Median (Range)	4.30 (0.96-10.47)	4.78 (1.00-10.00)	3.41 (0.96-10.47)	

Table 2

Cox proportional hazards models and concordance indices to predict biopsy reclassification during active surveillance using (a) baseline and (b) longitudinal measurements of total PSA, %fPSA, %[-2]proPSA, [-2]proPSA/%fPSA, and *phi* after adjusting for age, date of diagnosis, and PSA density (n=167; 63 biopsy reclassification events).

(a)

	Cox Proportional Hazards Models		
	HR (95% CI)	**P-value**	**C-index**
Baseline tPSA	**0.90 (0.80-1.00)**	**0.061**	**0.630**
Baseline %fPSA	**0.93 (0.89-0.97)**	**0.002**	**0.664**
Baseline %[-2]proPSA	**2.44 (1.51-3.94)**	**<0.0001**	**0.651**
Baseline [-2]proPSA/%fPSA	**2.13 (1.09-4.16)**	**0.026**	**0.652**
Baseline *phi*	**1.04 (1.02-1.06)**	**<0.0001**	**0.662**

(b)

	Cox Proportional Hazards Models		
	HR (95% CI)	**P-value**	**C- index**
Longitudinal tPSA	**0.96 (0.88-1.05)**	**0.366**	**0.703**
Longitudinal %fPSA	**0.94 (0.90-0.98)**	**0.002**	**0.722**
Longitudinal %[-2]proPSA	**1.92 (1.36-2.73)**	**<0.0001**	**0.647**
Longitudinal [-2]proPSA/%fPSA	**2.12 (1.25-3.59)**	**0.005**	**0.654**
Longitudinal *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 (Gleason score ≥7) during active surveillance using (a) baseline and (b) longitudinal measurements of total PSA, %fPSA, %[-2]proPSA, [-2]proPSA/%fPSA, and *phi* after adjusting for age, date of diagnosis, and PSA density (n=167; 28 biopsy Gleason upgrade events).

(a)			
	Cox Proportional Hazards Models		
	HR (95% CI)	**P-value**	**C-index**
Baseline tPSA	**0.90 (0.77-1.05)**	**0.192**	**0.705**
Baseline %fPSA	**0.92 (0.86-0.99)**	**0.025**	**0.743**
Baseline %[-2]proPSA	**4.02 (1.90-8.49)**	**<0.0001**	**0.784**
Baseline [-2]proPSA/%fPSA	**3.48 (1.26-9.59)**	**0.016**	**0.762**
Baseline *phi*	**1.06 (1.03-1.09)**	**<0.0001**	**0.788**

(b)			
	Cox Proportional Hazards Models		
	HR (95% CI)	**P-value**	**C-index**
Longitudinal tPSA	**0.95 (0.84-1.08)**	**0.445**	**0.771**
Longitudinal %fPSA	**0.93 (0.88-0.99)**	**0.025**	**0.786**
Longitudinal %[-2]proPSA	**2.49 (1.51-4.10)**	**<0.0001**	**0.832**
Longitudinal [-2]proPSA/%fPSA	**2.49 (1.16-5.34)**	**0.019**	**0.786**
Longitudinal *phi*	**1.05 (1.02-1.07)**	**<0.0001**	**0.820**