PTEN Status Assessment in the Johns Hopkins Active Surveillance Cohort

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Abstract

Background: Up to half of men with Gleason score 6 (GS6) prostate cancers initially managed with active surveillance (AS) will eventually require definitive therapy, usually due to tumor grade reclassification during follow-up. We examined the association between PTEN status on biopsy and subsequent clinicopathologic outcomes in men with GS6 cancers who enrolled in AS.

Methods: We performed a case-control study of men enrolled in the Johns Hopkins AS cohort with diagnostic biopsy tissue available for immunohistochemical (IHC) staining. IHC was performed for PTEN using genetically-validated protocols for all patients. Cases included men who underwent grade reclassification to GS 3+4=7 on biopsy within two years of follow-up (i.e. early reclassification) or reclassification to GS 4+3=7 on biopsy or radical prostatectomy during follow-up (i.e. extreme reclassification). Control patients were diagnosed with GS6 cancer and monitored on AS for at least eight years without undergoing biopsy reclassification.

Results: Among 67 cases with adequate tissue, 31 men underwent early reclassification and 36 men underwent extreme reclassification. Cases were compared to 65 control patients with adequate tissue for assessment. On initial prostate biopsy, cases were older (median age 67 vs. 65, p=0.024) and were less likely to meet very low risk criteria (64% vs 79%, p=0.042) as compared to controls. Although not statistically significant, PTEN loss was observed in only one (2%) of 65 controls as compared to six (9%) of 67 cases (p=0.062).

Conclusions: PTEN loss was rare among men with GS6 prostate cancer enrolled in AS at Johns Hopkins. Despite this, PTEN loss was more frequent among men who underwent early or extreme reclassification to higher-grade cancer as compared to controls. Additional studies in larger low-risk cohorts may better elucidate a potential role for PTEN in selecting patients for AS.

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Keywords

Prostatic adenocarcinoma; active surveillance; PTEN

Introduction:

Active surveillance (AS) has emerged as a primary management option for men with very low and low risk prostate cancer (PCa). The specific approach to AS varies by provider and practice, but monitoring has traditionally included routine assessment with serum prostate-specific antigen (PSA), clinical examination, and prostate tissue biopsy.[1] More recently, several groups have explored the utility of multi-parametric magnetic resonance imaging (mpMRI) in this setting, with varying results.[2,3] Acknowledging the cost and morbidity associated with frequent biopsy and imaging, there is great need for practical tools that can better inform individual patients as to their risk of adverse outcomes when electing AS.

Deletion of the tumor suppressor gene *PTEN* is one of the most common alterations in PCa, occurring in 20–50% of primary tumors in most studies.[4] PTEN loss leads to downstream activation of the AKT/mammalian target of rapamycin pathway, thereby promoting aberrant cell growth and proliferation. The clinical significance of PTEN loss has been widely demonstrated in the setting of radical prostatectomy, whereby PTEN loss appears to be associated with adverse pathology, shorter time to biochemical recurrence, and increased frequency of metastatic disease.[5–12] Moreover, an inexpensive and genetically validated immunohistochemical (IHC) assay for PTEN inactivation has been developed and validated in several cohorts.[5,8,13]

We hypothesized that PTEN testing could provide a simple and potentially actionable data point for patients and providers considering AS. Contemporary data exploring this possibility are promising but remain limited.[14] As such, we sought to retrospectively explore the association of PTEN status with clinical outcomes in a large AS cohort.

Materials and Methods:

Active Surveillance Cohort

The Johns Hopkins AS program was established in 1995 and includes 1822 patients. Monitoring traditionally included bi-annual clinical examination and PSA testing, as well as yearly prostate biopsy. More recently, the incorporation of multiparametric MRI (mpMRI) has allowed for an increased interval between surveillance biopsies.[3] All patients harbored GS6 cancer at enrollment. The majority (72%) of patients enrolled in AS at our institution meet very low risk disease criteria (clinical stage T1c, PSA density < 0.15 ng/ml/g, biopsy GS 6, 2 positive biopsy cores, and 50% involvement of any core with cancer), while 28% enrolled with low risk disease (clinical stage T2a, PSA <10 ng/ml, and biopsy GS 6).[15]

Study Design

We performed a case-control study of the Johns Hopkins active surveillance (AS) cohort. Cases were defined as men enrolled in AS who underwent either early reclassification (reclassification to GS 3+4=7 or higher on biopsy within 2 years of enrollment) or extreme reclassification (reclassification to GS 4+3=7 or higher on biopsy or radical prostatectomy at any time during follow-up). Controls were defined as patients that underwent monitoring for at least 8 years with no evidence of biopsy reclassification.

Patients and tissue samples

With institutional review board approval, the Johns Hopkins AS database was queried for cases matching these criteria, and attempts to obtain unstained slides from the diagnostic GS6 tumor were made. All cases with adequate unstained tumor tissue (>3 glands of tumor, an unvalidated but conventional threshold to enable both tumor diagnosis and PTEN immunostaining interpretation) available for PTEN staining were included in the study cohort. Diagnostic biopsy tissue was used in all cases when available; tissue from a second, confirmatory GS6 biopsy was used if diagnostic biopsy tissue was insufficient. Grading was performed as specified in the 2005 ISUP Gleason Grading Consensus Conference in all specimens from 2004 onwards[16].

We identified 125 cases that underwent early reclassification, of which 58 (46%) had diagnostic biopsies performed at our institution. Of these, 14 had adequate tumor tissue available after re-sectioning. Of 67 diagnostic biopsies performed at another institution, we were able to obtain biopsy tissue from 41 (62%). Of these, 17 (39%) had adequate tumor tissue available for analysis. An additional 53 cases underwent extreme reclassification, of which 38 (71%) had adequate tumor tissue for staining, yielding a total of 69 cases.

A total of 223 subjects met control group criteria. To roughly match the number of tumors with tissue available in the cases, we requested unstained slides on 98 patients, of which 69 (70%) had adequate tumor tissue remaining after requesting and re-cutting the tissue blocks.

For cases, we attempted to retrieve radical prostatectomy specimens after biopsy reclassification for PTEN immunostaining. We were able to obtain a single section of the dominant tumor nodule (nodule with highest grade) in 47 cases (68%; 21 cases with early reclassification and 26 cases with extreme reclassification) where the radical prostatectomy was performed at Johns Hopkins. The remaining patients were treated elsewhere or received radiation therapy.

PTEN Immunohistochemistry and interpretation

A single biopsy block (usually comprising two cores from the same anatomic location) containing the largest percentage of involvement by GS6 tumor was selected for retrospective PTEN immunostaining in each case. For the radical prostatectomy samples, a single section of the dominant tumor nodule was selected for analysis. PTEN IHC was performed using an automated assay as previously described.[5,8,13] The assay was blindly scored by two uropathologists (LBG and TLL) using a validated scoring system (Figure 1). In brief, a tumor biopsy or nodule was considered to have PTEN protein loss if the intensity

of cytoplasmic and nuclear staining for PTEN was markedly decreased or entirely negative in all or a subset of sampled tumor cells compared to surrounding benign glands and/or stroma. Some cases were scored as having ambiguous PTEN IHC results when the intensity of the tumor cell staining was light or absent in the absence of evaluable benign glands or stroma. We have shown previously that PTEN loss by IHC is sensitive and specific for underlying PTEN genomic deletions. [5,8,13]

Statistical analysis

Baseline clinical and pathological characteristics were assessed in the study population. Comparisons were made between cases and controls using the Mann-Whitney U test for continuous variables and Chi-squared or Fisher's exact test for categorical variables, as appropriate. Considering limited sample size, one-sided tests were used for a priori hypotheses that were directional, and a type I error of 0.05 was used to define statistical significance. Statistical analysis was performed using SAS (Version 9.4, Cary, NC, USA) and Stata Intercooled v13.1 (College Station, TX).

Results:

We identified 69 cases and 69 controls with adequate tumor tissue available for IHC staining. Cases were defined as men enrolled in AS who underwent either early reclassification (reclassification to GS 3+4=7 or higher on biopsy within 2 years of enrollment) or extreme reclassification (reclassification to GS 4+3=7 or higher on biopsy or radical prostatectomy at any time during follow-up). Controls were defined as patients that underwent monitoring for at least 8 years with no evidence of biopsy reclassification. Of the cases, 67 (97%) had non-ambiguous PTEN immunostaining and were included in analysis. Thirty-one of these patients met the case definition for early reclassification and 36 underwent extreme reclassification. Of the 69 control patients without grade reclassification after 8 years of follow-up, 65 (94%) had non-ambiguous immunostaining results.

Demographic, clinical, and pathological characteristics of the case and control groups are noted in Table 1. Overall, cases were significantly older than controls (median age 67 vs. 65 years, p=0.024), while race, PSA, and PSA density did not significantly differ between groups. Although both cases and controls harbored a median of one core positive for cancer on biopsy, cases had a significantly higher number of positive cores than controls (median 1 [IQR 1–1] vs. median 1 [IQR 1–2]; p<0.001); the maximum percentage of core involvement with tumor did not significantly differ between the groups (p=0.069). The proportion of cases with very low risk cancer was lower than that of the control group (64% vs 79%; p=0.042). Consistent with the study design, the control group was diagnosed at an earlier year and underwent a greater number of surveillance biopsies as compared to cases (Table 1).

Although PTEN loss overall was rare in these low or very low risk patients (5.3%, Figure 1), and the association did not meet conventional standards of statistical significance (p=0.062), PTEN loss was observed in only one control patient (1.5%) versus six cases (9.0%). A total of 3 cases (50%) and the only control with PTEN loss showed heterogeneous loss in some but not all sampled tumor glands, suggesting subclonal PTEN loss which is a common event

in primary prostate tumors (Figure 1) [5,8,13]. Among controls, PTEN loss was detected in one (2%) of 51 met who met very low risk criteria and none of the 14 men who met low risk criteria. PTEN loss was detected in 3 (7%) of 43 very low risk cases and 3 (13%) of 24 low risk cases.

To examine issues of tumor heterogeneity, we also performed PTEN immunostaining on a subset of radical prostatectomy specimens from cases performed after biopsy reclassification. Tumor tissue was available for 47 cases (68%) and in 87% of cases (41/47) the PTEN results were concordant between biopsy and dominant tumor nodule. Two cases had PTEN loss present on the needle biopsy and both (100%) had PTEN loss in the sampled section of dominant tumor nodule in the radical prostatectomy. Of the 45 cases with intact PTEN on the needle biopsy, 39 (87%) showed intact PTEN in the radical prostatectomy dominant tumor nodule and 6 (13%) showed PTEN loss in the radical prostatectomy only. Of these 6 cases, 4 (67%) had heterogeneous PTEN loss in some but not all tumor glands at radical prostatectomy. Two cases (33%) had PTEN loss in all sampled glands of a single tumor section of the dominant nodule. However, we cannot exclude that these cases had heterogeneous loss of PTEN elsewhere in the dominant tumor nodule at radical prostatectomy or that a non-dominant tumor nodule was sampled on needle biopsy.

Discussion:

Reports from multiple institutions have suggested long-term safety and efficacy in utilizing active surveillance in appropriately selected men.[17–20] Consequently, the use of AS has increased in recent years[21], with AS now serving as a primary management option for men with low risk disease.[22] Nonetheless, the practice of AS varies widely within and across institutions, and the observed incidence of adverse outcomes diverges accordingly.[1] This finding implies that the "optimal" approach to selection and monitoring in AS remains unclear.

One potential avenue to improving the practice of AS lies in better understanding the underlying biology of each patient's cancer. In recent years, several tissue-based molecular assays have been developed to support in patient decision-making, particularly in the postoperative setting.[23,24] These promising tools have been developed and validated in various low-risk cohorts[25,26], but prospective data from contemporary AS cohorts are largely absent. We therefore sought to explore the potential utility of a simple immunohistochemical assay in better risk-stratifying patients enrolled in AS at our institution.

We compared 67 cases with favorable-risk cancer who underwent early grade reclassification (within two years of initiating AS, n=31) or extreme grade reclassification (to Gleason score   4+3=7 at any time during follow-up, n=36) to 65 control patients who did not undergo reclassification within eight years of follow-up. Overall, PTEN loss was particularly rare among control patients (1.5%) as compared to cases (9.0%). Consistent with previous observations, however, cases were older than controls and more likely to harbor low risk (as compared to very low risk) disease.[20,27] While these data suggest that PTEN loss on biopsy is associated with adverse short-term outcomes on AS, the clinical

utility of assessing PTEN status and what it adds to conventional clinicopathologic data is unclear.

To examine issues of tumor heterogeneity, we also looked at PTEN status in highest grade (dominant) tumor nodule of the accompanying radical prostatectomy for a subset of cases that were reclassified in our study with available tissue. We found PTEN status was concordant in the needle biopsy and in a single sampled section of the dominant tumor nodule from the radical prostatectomy in the majority (87%) of cases. In cases where PTEN loss was seen in the needle biopsy, this was always concordant with loss in the dominant tumor nodule. In cases where PTEN was intact in the needle biopsy, 13% had PTEN loss in the dominant tumor nodule. These discordant cases likely represent undersampling of the dominant tumor nodule or sampling of an additional, non-dominant tumor nodule by needle biopsy. Overall, these data reinforce the importance of tumor sampling and tumor heterogeneity for any molecular biomarker utilized in the context of prostate needle biopsies. Although we correctly predicted PTEN status in dominant tumor nodule in the majority of the cases using the needle biopsy, we missed the presence of PTEN loss in 13% of the cases overall.

One other group has assessed PTEN in a contemporary AS population.[14] Lokman and colleagues performed PTEN IHC in 190 men enrolled in the Prostate Cancer Research International: Active Surveillance (PRIAS) cohort. In contrast to the Johns Hopkins AS program, the PRIAS cohort allows for some higher risk features, including clinical stage T2c, PSA density 0.20, and positive biopsy cores with >50% cancer involvement.[28] Overall, PTEN loss was observed on diagnostic biopsy in 29 men (15%). Over median follow-up of 46.2 months, PTEN loss was significantly associated with each measured outcome: grade group upgrading to GG >1 at re-biopsy (HR 2.57, 95% CI 1.16–5.70, p=0.02), protocol-based treatment change (GG >1, >2 positive biopsy cores, PSA doubling-time < 3 years, or clinical stage >T2) during follow-up (HR 2.31, 95% CI 1.264–4.189, p=0.006), and adverse pathology (GG 3 or pathological stage T3) in men who underwent prostatectomy (HR 4.745, 95% CI 1.84–12.232, p=0.001).

Taken together, these data corroborate previous reports that PTEN loss is a rare event among men with clinically low-risk disease. Nonetheless, these studies suggest that the clinical relationships established in higher-risk cohorts are likely to persist in the low-risk population. Specifically, PTEN loss portends a higher likelihood of adverse outcomes, in this case failing management with AS, though our study failed to reach statistical significance. These data are also consistent with our prior work which demonstrated that PTEN loss is associated with a significantly higher Ki-67 proliferation index in prostate cancer[29]. Acknowledging the rarity of PTEN loss, even among cases with early or extreme reclassification, it is apparent that a negative test result (PTEN intact) has limited practical utility in the clinical setting, or may best be interpreted with additional testing using RNA-based commercial prognostic tests or Ki-67 proliferation index. However, all molecular testing will be subject to issues of tumor heterogeneity and under-sampling which we documented in at least 13% of our cases where we examined the dominant tumor nodule at radical prostatectomy. On the other hand, a positive test result (PTEN loss) appears to identify a phenotype better suited for immediate definitive therapy, as six of seven patients

(86%) with PTEN loss in the current study demonstrated early or extreme progression during follow-up. The low cost and ease of use increase the possibility that PTEN testing demonstrates clinical utility in AS despite the low prevalence of PTEN loss. Additional studies are needed to establish both the clinical accuracy and potential cost-effectiveness of PTEN testing in the AS setting. It is likely that PTEN testing may be more useful in AS cohorts with a larger proportion of low risk disease than seen in the Hopkins cohort or perhaps even in low intermediate risk patients.[30]

The current study has limitations worth noting. As previously acknowledged, PTEN loss is a rare event in the low-risk population and occurred in only 5% of this cohort. For this reason, a case-control analysis was performed. At the same time, the overall number of samples was limited by practical and technical considerations, precluding us from adequately matching cases and controls for clinical-pathologic characteristics. Given the low-volume cancers monitored at our institution, many cases that otherwise met study criteria lacked sufficient tumor volume for analysis. Indeed, tumor tissue availability remains a major barrier to tissue-based molecular testing among patients eligible for AS. Compared to sequencing or RNA-based testing, analysis by IHC requires less tissue; yet we still had difficulty finding available cases and ultimately used two definitions of reclassification (early reclassification and extreme reclassification) for this analysis. In part, this is because our tissue collection occurred retrospectively after the tissue block had been recut. Prospective tissue collection efforts at the time of diagnosis for patients in AS may be more fruitful. In addition, because several men underwent initial diagnostic biopsies at outside institutions, some tissue specimens were obtained from outside institutions where careful sectioning protocols to preserve limited AS tissue had not been implemented. To address these issues, since 2014, we have been prospectively banking unstained tissue sections between the diagnostic hematoxylin and eosin-stained slides from all AS patients at Johns Hopkins and storing them at −20°C to preserve tissue antigens and nucleic acids.[31] These slides will serve as a valuable resource for future testing.

In conclusion, this study represents one of the first assessments of tissue-based biomarkers in a prospective AS program with careful clinical follow-up. We found that PTEN loss on diagnostic biopsy was more common among men who underwent grade reclassification during active surveillance. As a simple, inexpensive, and informative tool, immunohistochemical PTEN testing holds promise. Indeed, PTEN loss may help to identify men with more aggressive biology than is apparent in traditional clinical parameters. Additional studies are needed to confirm these findings and better characterize the optimal use of PTEN in the setting of AS.

Acknowledgments

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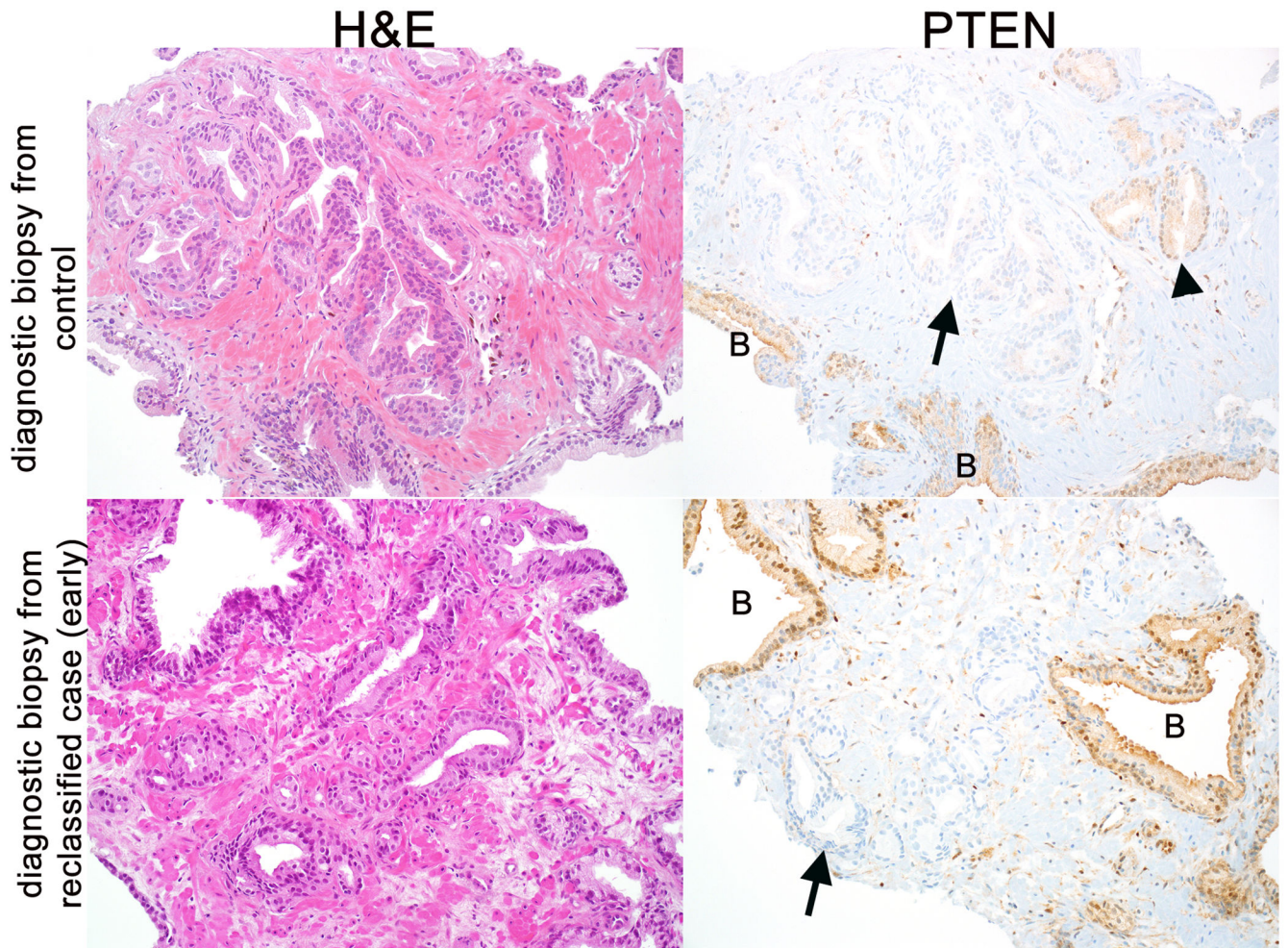
Figure 1: Microscopic images of case and control diagnostic active surveillance biopsies with PTEN loss by immunohistochemistry.

Hematoxylin and eosin (H&E)-stained diagnostic biopsy slides show small foci of Gleason score 6 tumor in both the control and the reclassified case. PTEN immunostaining is intact in surrounding benign glands (B) in both biopsies, providing an internal positive control. In the control biopsy, the tumor shows heterogeneous PTEN loss (arrow) in some tumor glands with intact PTEN in others (arrowhead). In the case biopsy, all tumor glands within the microscopic field show PTEN loss (arrow).

Table 1.

Cohort Characteristics

	Controls (n=65)	Cases (n=67)	P-value
	Median (IQR)	Median (IQR)	
Age	65 (61 - 69)	67 (64 -71)	0.024
Race, n (%)			
Caucasian	60 (92)	58 (87)	0.330
African-American	1 (2)	5 (7)	
Other	4 (6)	4 (6)	
PSA (ng/ml)	5.0 (3.1 - 6.3)	4.6 (3.5 – 5.7)	0.356
PSAD	0.09 (0.07 – 0.14)	0.11 (0.07 – 0.13)	0.280
No. of cores positive	1 (1-1)	1 (1-2)	<0.001
Max % core involvement	5 (1-15)	10 (1-30)	0.069
Year of diagnosis	2003 (2001- 2004)	2009 (2005 -2011)	<0.001
Biopsies since diagnosis	7 (5 -10)	3 (2 - 4)	< .0001
Risk group, n (%)			
Very low risk	51(79)	43 (64)	0.042
Low risk	14 (21)	24 (36)	
PTEN status, n (%)			
Lost	1 (2)	6 (9)	0.062
Intact	64 (98)	61 (91)	