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Urinary TMPRSS2:ERG and PCA3 in an active surveillance cohort: results from a baseline analysis in the Canary Prostate Active Surveillance Study

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Abstract

Purpose—Active surveillance is used to manage low risk prostate cancer. Both PCA3 and TMPRSS2-ERG are promising biomarkers that may be associated with aggressive disease. This study examines the correlation of these biomarkers with higher cancer volume and grade determined at the time of biopsy in an active surveillance cohort.

Experimental Design—Post-DRE urine was collected prospectively as part of the multi-institutional Canary Prostate Active Surveillance Study (PASS). PCA3 and TMPRSS2-ERG levels were analyzed in urine collected at study entry. Biomarker scores were correlated to clinical and pathologic variables.

Results—In 387 men, both PCA3 and TMPRSS2-ERG scores were significantly associated with higher volume disease. For a negative repeat biopsy, and 1–10%, 11–33%, 34% positive cores, median PCA3 and TMPRSS2-ERG scores increased incrementally ($P < 0.005$). Both PCA3 and

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TMPRSS2-ERG scores were also significantly associated with presence of high grade disease. For a negative repeat biopsy, Gleason 6 and Gleason 7 cancers, the median PCA3 and TMPRSS2-ERG scores also increased incrementally ($P = 0.02$ and $P = 0.001$, respectively). Using the marker scores as a continuous variables, the odds ratio for a biopsy in which cancer was detected versus a negative repeat biopsy (ref) on modeling was 1.41 (95% CI 1.07–1.85), $P = 0.01$ for PCA3 and 1.28 (95% CI 1.10–1.49), $P = 0.001$ for TMPRSS2-ERG.

Conclusions—For men on active surveillance both PCA3 and TMPRSS2-ERG appear to stratify risk of having aggressive cancer as defined by tumor volume or Gleason score.

Keywords

prostate cancer; active surveillance; biomarkers

INTRODUCTION

The PSA screening era has been associated with a well-established stage migration of prostate cancer, such that a high proportion of newly diagnosed prostate cancers exhibit features that associate with a very low risk of invasion, metastasis, and consequent morbidity and mortality (1). Multiple studies have examined the natural history of these low risk neoplasms, showing that the vast majority of men with this diagnosis die of causes other than prostate cancer, even if they are managed without primary curative treatment (2–4). Nevertheless, as a designation of low risk cancer does not equate to complete absence of risk, the majority of contemporary patients with low risk prostate cancer choose to pursue immediate curative therapy such as surgery or radiotherapy with the attendant costs and side effects (1, 5–7). These practice patterns have spawned substantial debate regarding overdiagnosis, overtreatment and the utility of PSA-based prostate cancer screening (8–10).

To address the problem of overtreatment, a deferred treatment strategy termed active surveillance has been used by clinicians as an approach to manage low risk prostate cancer. Active surveillance incorporates serial PSA measurements, physical examinations, and repeat prostate biopsies to monitor for either the presence of occult aggressive disease or progression to a phenotype more commonly associated with metastasis and mortality. Acceptance of active surveillance has been limited for several reasons including the lack of consensus on optimal selection criteria and triggers for intervention, lack of long-term outcomes data, inconsistent study designs in the current active surveillance series, and fear among both patients and providers of losing the window of curability. Of importance, prostate cancer is well-described to exhibit a pattern of multifocality which can manifest as independent lesions with different pathological grades and distinct molecular features (11). Undersampling of the prostate by standard biopsy techniques, the lack of knowledge regarding the rates of cancer progression, and a lack of diagnostic imaging modalities capable of accurately assessing tumor volume and histology have prompted the incorporation of repeat tissue assessments by biopsy into active surveillance protocols (12–16). Though morbidity is low (17, 18), the discomfort, cost, and continued undersampling problem inherent in the prostate biopsy procedure advocate for the development of non-invasive biomarkers capable of reflecting events throughout the prostate gland and suitable for repeat measurements over time.

PCA3 and the TMPRSS2-ERG fusion are two prostate cancer-specific biomarkers that hold promise for stratifying risk in an active surveillance setting. PCA3 is a prostate-specific noncoding mRNA that is significantly over expressed in prostate carcinoma compared to benign prostatic tissue (19, 20). Urinary PCA3 levels have been investigated for prostate cancer early detection (21, 22) and importantly are correlated with histological grade and tumor volume in prostatectomy specimens (23–26). Of the genomic alterations involving

ETS oncogene family members, a rearrangement involving the androgen-regulated TMPRSS2 gene with the ERG transcription factor (TMPRSS2:ERG) is the most prevalent (27), occurring in approximately half of the prostate cancers diagnosed in Caucasians (28) and have been correlated in some reports with aggressive disease (29, 30). A clinical-grade, quantitative TMPRSS2:ERG urine assay has been developed and measurements of TMPRSS2:ERG transcript levels associate with cancer volume and grade at prostatectomy, and upgrading from biopsy histological assessments (31). The combination of both TMPRSS2:ERG and PCA3 improved the performance of PSA for detection of prostate cancer and predicting clinically significant cancer (31). The goal of the present study was to determine whether urinary PCA3 and TMPRSS2:ERG mRNA levels are associated with higher volume or grade prostate cancer in a multi-institutional active surveillance cohort.

MATERIALS AND METHODS

Canary Prostate Active Surveillance Study (PASS) Cohort

The PASS clinical protocol (clinicaltrials.gov NCT00756665) was approved by institutional review boards at Stanford University, University of British Columbia, University of California at San Francisco, University of Texas Health Sciences Center at San Antonio, University of Washington, Veterans Affairs Puget Sound Health Care System, and Fred Hutchinson Cancer Research Center (Coordinating Center), and the study opened for enrollment in late 2008; subsequently the protocol was approved and enrollment opened at Beth Israel Deaconess Medical Center, Eastern Virginia Medical School, and University of Michigan (32). At the time of the present analysis, November 10, 2010, 413 men provided written informed consent for entry into this prospective, observational, active surveillance study. The enrollment criteria for PASS include: histologically confirmed adenocarcinoma of the prostate, ECOG performance status of 0 or 1, clinical T1 - 2 disease, no previous treatment for prostate cancer including hormonal therapy, radiation therapy surgery or chemotherapy, and the willingness to undergo serial prostate biopsies. Participants enrolled in Canary PASS are followed with serum PSA measurements every 3 months, clinical exam and digital rectal examination (DRE) every 6 months, and serial repeat prostate biopsy 6–12 months after the initial diagnosis, 24 months after the initial diagnosis, and every other year thereafter. In an attempt to make this multi-center study reflect community practice, standard biopsy templates were not mandated, however, at least 10-core biopsy regimens are required and 97% of repeat biopsy regimens were greater than or equal to 12-core regimens. At study entry and each follow-up visit, blood (plasma and serum) and post-DRE urine are collected, DNA is collected from peripheral blood at study entry. De-identified demographic, clinical, and pathologic data is stored in a central data repository at the Fred Hutchinson Cancer Research Center (FHCRC) managed by the NCI's Early Detection Research Network Data Management and Coordination Center (EDRN DMCC), and specimens are housed in a central biospecimen repository at FHCRC. A collaboration agreement that governs study conduct and specimen and data use has been executed at all participating institutions. Specimens are available to the research community upon approval of the PASS Biomarker Review Committee.

The initial 413 consecutive men enrolled in PASS were included in this study. Of these, two were excluded due to problems with sample preservation, 10 participants did not provide a urine specimen, and 14 were excluded because their specimens yielded uninformative results, leaving 387 with evaluable specimens. At study entry the median time since diagnosis was 10.4 months (range of 6 days to 18 years); 284 (54%) participants were within one year of their diagnosis. One hundred ninety six men (51%) had undergone a single prostate biopsy (i.e. diagnostic biopsy) and 49% of men had previously been using active surveillance to manage their prostate cancer and had repeat surveillance biopsies performed since their diagnosis - 106 men (27%) had undergone two biopsies on or after diagnosis, 55

(14%) had undergone 3 prior biopsies, and 29 (8%) had undergone 4 or more biopsies. Although all subjects enrolled had at least one biopsy with carcinoma, 20% of participants had a subsequent prostate biopsy session that did not identify cancer. In 302 participants (78%), the biopsy that was associated with study entry was performed a mean of 6.5 months (range of 0.2–46.2 months, $s = 5.5$) prior to study entry. In the remaining 85 participants (22%), the biopsy associated with study entry was a surveillance biopsy performed the day of study entry and specimen collection was performed immediately before the biopsy. Importantly, 91% of urine samples were obtained within 12 months of the biopsy. In this study, biopsies were evaluated for Gleason score by a local GU-trained study pathologist using the 2005 WHO/ISUP modified Gleason system (33). Tumor volume was defined as the percent of biopsy cores with cancer involvement.

PCA3 and TMPRSS2:ERG Urine Assay

Urine specimens were collected at each clinical site at the time of study entry. Specimens were collected after attentive DRE involving three sweeps of each lateral prostate lobe, put on ice, and processed within four hours by mixing with an equal volume of urine transport medium (detergent-based stabilization buffer; PROGENSA PCA3 Urine Specimen Kit, Hologic Gen-Probe Inc.). Specimens were stored at -70°C until analysis, with grouped shipments on dry ice to the Central Repository and to Hologic Gen-Probe. Assays were performed by Hologic Gen-Probe to determine amounts of PCA3, TMPRSS2:ERG, and PSA mRNAs in each specimen using the PROGENSA PCA3 assay or the second generation developmental TMPRSS2:ERG assay as described previously (22, 31). Operators were blinded with respect to subject clinical information at the time of testing and did not participate in data analysis. PCA3 and PSA RNA measurements were performed in duplicate, and TMPRSS2:ERG RNA levels were measured in triplicate. Samples with an average PSA transcript level of > 7500 copies/ml were considered informative. PCA3 scores were calculated as $1,000 \times (\text{average urine PCA3 copies/ml})/(\text{average PSA copies/ml})$. TMPRSS2:ERG scores were calculated as $100,000 \times (\text{average urine TMPRSS2:ERG copies/ml})/(\text{average PSA copies/ml})$.

Statistical Analysis

Statistical analyses were performed at the EDRN DMCC using SAS version 9.2 (Cary, NC, USA). Descriptive statistics summarized clinical factors. Spearman rank correlation coefficients were calculated between PCA3 and TMPRSS2:ERG scores and continuous clinicopathological variables. Disease volume and grade were divided into clinically meaningful categories and nonparametric Mann-Whitney and Kruskal Wallis tests were performed to compare PCA3 and TMPRSS2:ERG among the groups. Univariate logistic regression models with log-transformed PCA3 and log-transformed TMPRSS2:ERG were fit separately to provide odds ratios for prediction of positive disease and high grade disease, respectively. Receiver operating characteristic (ROC) curves were plotted for serum PSA, PCA3, and TMPRSS2:ERG and the area under the curves (AUC) were analyzed using the DeLong method for comparing correlated ROC curves (34). Multivariable logistic regression models included PCA3, TMPRSS2:ERG, PSA, and other study covariates commonly associated with prostate cancer including DRE results, family history of prostate cancer, race, and age. The linear scores from these multivariable models were used to plot ROC curves.

RESULTS

Characteristics of participants at the time of initial urine specimen collection are given in Table 1. The majority of participants were Caucasian (91%), 4% were African American, 3% were Asian, and 2% have other or unknown racial backgrounds. The Gleason score of

the biopsy associated with urine specimen collection was 6 in 72% of the participants, with one participant having a Gleason score reported as 5, and the Gleason sum was 7 in 8% of participants; 20% of the participants had a negative repeat biopsy associated with specimen collection. Ninety-three percent of participants had PSAs of <10, 84% were clinical stage T1c disease, and 94% of participants with a known number of positive cores had <34% of cores involved with cancer.

In this active surveillance cohort, the mean urine PCA3 score was 49 with a median of 31 (IQR 42). The mean urine TMPRSS2:ERG score was 55 with a median of 12 (IQR 60). We examined the correlations of both markers to clinicopathologic variables of disease (Tables 2 and 3). Both PCA3 and TMPRSS2:ERG scores were significantly correlated to biopsy Gleason score and tumor volume, assessed by percentage of biopsy cores with cancer ($P < 0.01$ for all comparisons). While others have looked at linear lengths, biopsy Gleason score and percentage of cores with cancer have been shown to independently predict outcome in men who undergo surgery (35–37). There was no significant correlation of the urine markers to serum PSA, prostate volume, body mass index, number of prior biopsies, time from biopsy to urine collection, time from initial prostate cancer diagnosis (Table 2), family history, or clinical stage (Table 3). We also found no significant correlations between urine PCA3 or TMPRSS2:ERG scores with IPSS score, PSA doubling time, or the use of statins, diabetes medications, 5 α -reductase inhibitors, or NSAIDs (data not shown). TMPRSS2:ERG score was not correlated with age, but PCA3 levels were positively correlated with advancing age ($P < 0.0001$), as has been observed by others (38).

We further evaluated the associations between PCA3 and TMPRSS2:ERG and tumor histology. We found a significant sequential increase in both PCA3 and TMPRSS2:ERG as Gleason grade increased. For negative repeat biopsy, Gleason 5 to 6, and Gleason 7, the median PCA3 scores were 27 (95% CI 24–31), 31 (95% CI 27–35), 48 (95% CI 31–92), $P = 0.02$, and median TMPRSS2-ERG scores were 5 (95% CI 2–8), 14 (95% CI 9–18), 29 (95% CI 13–78), $P = 0.001$, respectively (Table 3). Using log-transformed biomarker scores as continuous predictors, both PCA3 and TMPRSS2:ERG urine measurements associated with a positive biopsy versus a negative biopsy (reference) with odds ratios for PCA3 of 1.41 (95% CI 1.07–1.85; $P = 0.01$) and for TMPRSS2:ERG of 1.28 (95% CI 1.10–1.49; $P = 0.001$). The odds ratios for a Gleason score of seven or above versus less than 7 for PCA3 and TMPRSS2:ERG are 1.67 (95% CI 1.10–2.52; $P = 0.02$) and 1.24 (95% CI 1.01–1.53; $P = 0.05$) respectively. We also observed a sequential increase in the marker scores as volume increased. For a negative repeat biopsy, and 1–10%, 11–33%, 34% positive cores, median PCA3 scores were 27 (95% CI 24–31), 28 (95% CI 22–35), 40 (95% CI 31–51), 46 (95% CI 18–90), $P = 0.004$, and median TMPRSS2-ERG scores were 3 (95% CI 2–8), 10 (95% CI 4–14), 20 (95% CI 14–31), 27 (95% CI 4–115), $P < 0.0001$, respectively. The odds ratios for a biopsy with 34% positive cores versus <34% (reference) are 1.64 (95% CI 0.97–2.74; $P = 0.06$) for PCA3 and 1.16 (95% CI 0.98–1.63; $P = 0.08$) for TMPRSS2:ERG.

In ROC analysis (Figure 2), we compared the area of the curve (AUC) for the prediction of Gleason 7 disease at study entry of serum PSA alone or with the urine biomarkers. The AUC for PSA alone was 0.68, the AUC for the two markers alone 0.66 and the AUC for the combination of both markers and PSA was 0.70. The addition of the markers was not significantly different from the AUC for PSA alone, ($P = 0.08$) although there was a trend toward significance. Similar results were found in ROC analysis for the prediction of >34% positive cores (see supplementary material). Results from multivariable logistic regression models were not significant after adjusting for covariates (see supplementary material).

DISCUSSION

We report the correlation of urinary levels of PCA3 and TMPRSS2:ERG transcripts with clinical characteristics at the time of study entry in a multi-institutional, prospective active surveillance cohort. We find that in univariate analyses both markers appear to stratify for baseline risk of disease aggressiveness as defined by biopsy Gleason score or volume of tumor (% of positive cores). However, although there is a trend towards these biomarkers improving the power of PSA to predict high grade or volume disease (Figure 2), the increase of the markers is not significant.

Men diagnosed with clinically localized prostate cancer are offered a variety of treatment strategies including active surveillance or primary therapies with curative intent. However, decision making for these men is currently impacted by the lack of high specificity for detection of occult aggressive disease or identification of a disease that is likely to progress to an aggressive phenotype, and the majority of men with newly diagnosed low risk prostate cancer opt for primary curative treatment (1, 6, 7), despite a growing body of evidence that treatment may often be safely delayed (13–15, 39) or avoided all together (2–4). Greater acceptance of active surveillance is limited by several factors. For example, entry into active surveillance programs and triggers for intervention are currently based on a number of clinical parameters including PSA (value, density, kinetics), clinical stage, and biopsy results (Gleason score, core involvement) (13–16, 32), however, there is not a consensus as to the optimal criteria for safely or effectively using active surveillance (40). Furthermore, prostate biopsies, which are an integral part of active surveillance regimens, are invasive and frequently underestimate the grade and extent of disease (41, 42).

The present study begins to address an unmet need for a non-invasive biomarker test that can provide a higher degree of specificity for detecting aggressive disease than currently available clinical metrics. This study is based on the PASS cohort, which is a contemporary, multi-institutional active surveillance cohort with prospective collection and centralized data and specimen storage. In PASS, high quality specimens and data are maintained by on-site training for standardized specimen collection and processing procedures along with regular site visits and data audits. The clinical study is designed to meet the primary objective of confirming biomarkers that predict the presence of or progression to aggressive disease (32).

Broad eligibility criteria were used in PASS to allow most men who choose to manage their prostate cancer using active surveillance to enroll in the study, including men with primary disease features that are not currently considered low risk. This broad scope of disease characteristics allows for biomarker studies, such as the one presented here, that should provide greater insight into the natural history of prostate cancer and be more informative than studies performed using strict entry criteria. Another aspect of the PASS design is that it allows participants who were diagnosed with low grade/stage disease to enroll in the study on the day of a serial repeated biopsy, with specimen collection immediately prior to the biopsy. In this situation, the repeat biopsy may show evidence of disease progression (e.g. higher grade or volume of disease), yet the participant samples are still included in this present study, and the Gleason score from the biopsy at the baseline visit is used in the association analyses. This study includes 85 such participants, accounting for 15 of the 30 participants with a Gleason score ≥ 7 associated with specimen collection.

A limitation of this study is the inherent and well-recognized undersampling of the prostate by current biopsy procedures. There are several studies that report lack of correlation of PCA3 score with initial biopsy Gleason grade or progression (31, 43), despite strong correlations with prostatectomy Gleason grade (23–26). However, in this study nearly half of the participants had at least one repeat biopsy, suggesting more adequate sampling in our

cohort when compared to previous studies. As many of the participants in this study had undergone multiple prostate biopsy sessions at study entry, when we evaluated our data for the highest Gleason score at any timepoint (versus the single biopsy closest to study entry), the TMPRSS2:ERG score was not found to be statistically significant ($P = 0.40$) although PCA3 remained so ($P = 0.0019$). Similarly, using the highest Gleason score, the odds ratio for a Gleason score of seven or above versus less than 7 for TMPRSS2:ERG was not significant (1.08, 0.91–1.30, $P = 0.39$) and for PCA3 remained significant (1.63, 1.14–2.34, $P = 0.0007$), suggesting that PCA3 may perform better in predicting aggressive disease than TMPRSS2:ERG. A further limitation involves the inter-observer variability in Gleason scoring, especially for a relevant subset of cancers in which it is difficult to distinguish tangentially sectioned pattern 3 vs. poorly formed pattern 4 glands (44). However, in PASS, most biopsies are read by a study pathologist at each site, and the study pathologists have routine consensus meetings in which questionable cases are reviewed. Finally, the power of this study is limited by a relatively uniform cohort and a small number of Gleason grades

7. As such, the ROC analysis in Figure 2 revealed a trend towards statistical significance, but was likely underpowered due the lack of high grade disease at study entry.

In conclusion, both PCA3 and TMPRSS2-ERG appear to stratify risk at time of enrollment, for men on active surveillance, of having aggressive cancer as defined by tumor volume or Gleason score. While there is a statistically valid trend towards these markers, especially PCA3, predicting higher grade and volume cancer, further work is needed to determine their clinical utility for men on active surveillance. The results presented here are encouraging, but the clinically relevant question is how these biomarkers aid in the prediction of the presence of occult aggressive disease or progression to an aggressive phenotype over time. To address these important questions, we are continuing to expand our cohort, collect and analyze longitudinal clinical data and specimens, and follow participants to collect long-term disease status.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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STATEMENT OF TRANSLATIONAL RELEVANCE

The identification of biomarkers that, at the time of diagnosis, associate with the presence of – or progression to – aggressive prostate cancer will transform the clinical management of this malignancy. If patients and their physicians have reliable and valid tools for estimating the risk of disease-specific morbidity, then more patients might opt for and adhere to active surveillance regimens, and consequently reduce overtreatment and the attendant substantial costs and harms. Also, a marker or marker panel with high accuracy for progression on active surveillance will identify those men who could be placed on less intensive surveillance protocols with fewer repeated prostate biopsies, reducing the risks and costs of invasive procedures. The study presented here is a step toward validating such biomarkers.

Figure 1. A.

Figure 1. B.

Figure 1. C.

Figure 1. D.

Figure 1.

Box and whisker plots of Kruskal-Wallis correlations between (A) TMPRSS2:ERG and (B) PCA3 scores and Gleason score associated with specimen collection, (C) TMPRSS2:ERG and (D) PCA3 scores and tumor volume, defined by the percent of biopsy cores with tumor involvement, associated with specimen collection.

Figure 2. part 1

Figure 2. part 2.

Figure 2.

ROC analysis of serum PSA, TMPRSS2:ERG, PCA3, alone and in combination, for prediction of high Gleason grade (≥ 7) at time of specimen collection. AUC(PSA) does not differ significantly from AUC(TMPRSS2:ERG) [$P = 0.38$], AUC(PCA3) [$P = 0.51$], AUC(TMPRSS2:ERG + PCA3) [$P = 0.86$], or AUC(TMPRSS2:ERG + PCA3 + PSA) [$P = 0.08$].

Table 1

Participant characteristics at urine specimen collection.

Race	n (%)
Caucasian	351 (91)
African American	15 (4)
Asian	13 (3)
American Indian/Alaska Native	2 (1)
Other or unknown	6 (1)
Ethnicity (Latino/Hispanic)	
Yes	13 (3)
No	366 (95)
Unknown	8
Age at study entry	
<50	13 (3)
50–60	105 (27)
61–70	201 (52)
>70	68 (18)
Median (range)	64 (38–84)
Mean	63.8
Serum PSA	
0–3.99	170 (44)
4.0–10.0	190 (49)
> 10	27 (7)
Mean	4.8
Median (range)	4.4 (0.25–28.8)
Clinical Stage	
T1a	5 (1)
T1c	322 (83)
T2a	55 (14)
T2b	4 (1)
T2c	1
Gleason score	
no cancer detected	79 (20)
5–6	278 (72)
7	27 (7)
8–9	3 (1)
Volume; % positive cores	
no cancer detected	79 (20)
2–10	112 (29)
11–33	108 (28)

Race	n (%)
34	19 (5)
Unknown	69 (18)

Table 2

Spearman rank correlation of clinicopathologic variables with PCA3 and TMPRSS2:ERG scores.

	Variable	N	r_s	p-value
Serum PSA	PCA3 score	387	0.09	0.07
	T2:ERG score	387	0.03	0.5
Age	PCA3 score	387	0.25	< 0.0001
	T2:ERG score	387	0.04	0.47
Prostate volume	PCA3 score	302	0.007	0.9
	T2:ERG score	302	0.03	0.56
Body mass index	PCA3 score	387	-0.03	0.61
	T2:ERG score	387	-0.08	0.13
Number of prior biopsies	PCA3 score	387	0.07	0.16
	T2:ERG score	387	0.09	0.08
Time from biopsy to urine collection	PCA3 score	387	0.009	0.9
	T2:ERG score	387	0.05	0.3
Time from diagnosis to urine collection	PCA3 score	387	0.09	0.07
	T2:ERG score	387	0.07	0.17
Gleason score at study entry	PCA3 score	387	0.13	0.01
	T2:ERG score	387	0.2	0.0001
Tumor volume at study entry (% positive cores)	PCA3 score	294	0.18	0.002
	T2:ERG score	294	0.3	<0.001

Table 3

Correlation of clinicopathologic variables with PCA3 and TMPRSS2:ERG scores

	Parameter	N	PCA3 Score		TMPRSS2:ERG Score	
			Median (95% CI)	p-value	Median (95% CI)	p-value
Family History	Yes	99	34 (26-43)	0.28**	16 (8-25)	0.37**
	No	265	30 (27-35)		11 (7-15)	
Clinical T-stage	T1	327	30 (27-34)	0.18**	12 (8-15)	0.32**
	T2	60	38 (28-49)		22 (3-48)	
Gleason Score	no cancer detected	79	27 (24-31)	0.02*	5 (2-8)	.001*
	5 or 6	278	31 (27-35)		14 (9-18)	
	7	30	48 (31-92)		29 (13-78)	
Tumor Volume	no cancer detected	79	27 (24-31)	0.004*	3 (2-8)	<0.0001*
	1-10	112	28 (22-35)		10 (4-14)	
	11-33	108	40 (31-51)		20 (14-31)	
	34	19	46 (18-90)		27 (4-115)	

* Kruskal-Wallis test,

** Mann-Whitney test