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Soluble CD105 is prognostic of disease recurrence in prostate cancer patients

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Abstract

While the overall five-year survival rate for prostate cancer is near 100%, up to 35% of patients will develop recurrent disease. At the time of prostatectomy, prostate specific antigen (PSA) is used to guide primary therapy with the goal of curative intervention. It can be valuable to know when primary therapy may not in fact be curative, so that subsequent adjuvant therapy can be administered at an early stage to limit progression. We examined prostate cancer patients with PSA ≥ 10 ng/ml that were all subjected to prostatectomy with at least five years of follow up ($n = 181$). Based on data that endoglin (CD105) signaling in the tumor can contribute to prostate cancer progression, we examined the expression of soluble CD105 (sCD105) in the patient plasma. To determine the relation of plasma sCD105 measures to cellular CD105 in tissues, we tested an independent set of prostate cancer tissues and paired plasma ($n = 31$). Elevated sCD105 was found to be associated with recurrence-free survival of prostate cancer patients. Further, sCD105 levels in patient plasma were inversely correlated with cellular CD105 expression. This translational study supported preclinical data demonstrating the pro-tumorigenic capacity of cellular CD105 and provide a blood-based biomarker, sCD105, for prostate cancer recurrence in prostatectomy patients with PSA levels ≥ 10 ng/ml.

Keywords

Prostate cancer; biochemical-recurrence; CD105; endoglin; blood-based biomarker

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Declaration of interest

The authors NAB, AM, and VRPH are included in a patent entitled "Sensitization of tumors to therapies through endoglin antagonism" dated January 18, 2019.

Introduction

Prostate cancer (PCa) was estimated to be the leading newly diagnosed cancer type in men, accounting for 19% of all newly diagnosed cancers in 2017 (Siegel et al., 2017). While the overall five year survival rate is near 100%, approximately 35% of patients will develop biochemical recurrence, as determined by the detection of serum prostate specific antigen (PSA) following primary intervention (Freedland et al., 2005). As high-risk patients are increasingly undergoing radical prostatectomy, a reliable prognostic biomarker to identify patient recurrence status at the time of surgical intervention would be invaluable in supporting early adjuvant therapy. For patients that have PSA ≤ 10 ng/ml, indicative of low to intermediate risk for recurrence, there are no additional blood-based markers to more accurately identify post-surgical recurrence. Currently, adjuvant therapy is not prescribed for prostatectomy patients until there is evidence of a biochemical recurrence. Such adjuvant therapy includes androgen receptor signaling inhibitors or salvage pelvic floor irradiation for a possible local tumor expansion. Adjuvant therapy prescribed following biochemical recurrence is curative in only a minority of patients. Although biochemical recurrence is considered a clinical indication of surviving PCa cells in the body, it is a surrogate for metastatic disease progression due to the limitations of current imaging modalities. Clinical trials have indicated that immediate external beam irradiation following radical prostatectomy significantly reduced local PCa recurrence in patients with positive surgical margins (Bolla et al., 2005; Bolla et al., 2012; Gandaglia et al., 2017). However, since most men are cured following prostatectomy alone, aggressive therapies with substantial comorbidities is not warranted unless there is a high probability of local recurrence. Furthermore, even biopsies with evidence of extra-prostatic extension provided limited prognostic value, in that 52% of the patients developed biochemical recurrence and 25% had detectable metastasis within 3 years after radical prostatectomy ($n = 83$) (Faisal et al., 2018). Thus, current presurgical and even post-surgical markers do not provide patients at risk for recurrence adequate guidance for treatment decisions.

Although somewhat controversial prior to primary intervention, PSA is the gold standard biomarker for adjuvant treatment decisions (Prensner et al., 2012). Other markers have emerged to correlate with PCa progression and could be incorporated in a screening panel for improved prognostic capacity, however, they are not used routinely (Prensner et al., 2012; Schmidt, 2017; Terada et al., 2017). These potential markers include PCA3, TMPRSS2-ERG, AR mutations or splice variants, circulating tumor cells, and others. Specifically, one marker previously identified in preoperative plasma levels, soluble CD105 (sCD105), caught our attention (Svatek et al., 2008). CD105 (endoglin) is a cell surface transforming growth factor- β co-receptor that dimerizes with bone morphogenetic protein receptors to activate downstream signaling and enhance cell migration and invasion. Classically, the transmembrane CD105 is expressed by proliferative endothelial cells associated with epithelial cancer cells (Fonsatti et al., 2003; Paauwe et al. 2018; (Madhav et al., 2018). Recently we showed CD105 to be expressed by cancer associated fibroblasts and promote tumor progression in a paracrine manner (Fonsatti et al., 2003; Paauwe et al., 2018; Kato et al., 2019). When the extracellular domain of CD105 is cleaved, sCD105 is released and enters the bloodstream. The detection of sCD105 is a recognized marker for

preeclampsia and heart disease (Kapur et al., 2013; Nikuei et al., 2017). In addition, sCD105 has been described as a serum biomarker in breast, colon, and prostate cancer (Li et al., 2000; Takahashi et al., 2001; Svatek et al., 2008; Fujita et al., 2009). In this study, we examined sCD105 as a blood-based prognostic biomarker for disease recurrence following prostatectomy. Based on the mechanism of CD105 action, sCD105 may be used as a prognostic biomarker in PCa patients.

Materials and Methods

Study Population

Of the 212 prostate cancer patient plasma samples from the Prostate Cancer Biorepository Network (PCBN) at University of Washington, 4 patients with missing data in sCD105 were excluded from analyses. The plasma tested was collected from 2002 to 2012 from prostatectomy cases by the PCBN. Clinical and pathologic values for each patient were collected with the blood by consent and provided to us de-identified. The blood was collected in lavender EDTA tubes. This retrospective study sought to determine if sCD105 has a prognostic capacity in concert with PSA for PCa biochemical recurrence following prostatectomy. With the goal of predicting biochemical recurrence free survival in a group of patients that may be presumed to be in the lower risk category prior to prostatectomy, we chose patients with PSA ≤ 10 ng/mL. Of 208, 181 patients with PSA ≤ 10 ng/ml were included in the analyses. There were 36 plasma samples obtained from the Cedars-Sinai Medical Center Biobank core facility with matching tissue in an annotated tissue microarray used to correlate circulating sCD105 levels with cellular CD105. However, since immunohistochemical staining was viable in 31 specimens on the tissue array, only 31 corresponding plasma sCD105 values were evaluated. These specimens were collected from prostatectomy patients before surgery between 2010 to 2015 at Cedars-Sinai Medical Center. Patient samples were obtained after informed consent regarding use of specimens for research purposes. Deidentified specimens were used in these studies under the Cedars-Sinai Medical Center IRB Protocol 35976 and 29278.

sCD105 ELISA

Human plasma was diluted 1:100 in assay buffer provided in the CD105 (soluble) human instant ELISA kit (BMS2105INST, eBioscience, USA). sCD105 was measured per the manufacturer's directions.

CD105 immunohistochemistry

The prostate cancer tissue array was incubated with anti-CD105 (AB114052, Abcam, USA) antibody at 4°C overnight. Secondary antibody development was performed with Dako Cytomation Envision+ HRP mouse kit (K4001, Agilent, Santa Clara, USA) and visualized using 3,3'-diaminobenzidinetetrahydrochloride (DAB) substrate (K3468, Agilent, Santa Clara, USA). Slides were scanned with a Leica Biosystems Aperio AT.

Statistical analysis

Patient characteristics are presented for patients with and without developing biochemical recurrence in those with a prostate-specific antigen (PSA) level ≤ 10 . Univariate associations

between the level of sCD105 and other variables were examined with Kruskal-Wallis test, Wilcoxon rank-sum test, or Spearman rank correlation, where appropriate. The primary outcome was biochemical recurrence-free survival (RFS) defined as the time in years from the date of prostatectomy to either biochemical recurrence or last follow-up if the patient did not experience a recurrence. Patients who did not experience a recurrence were censored at the last follow-up time. The main prognostic variable was sCD105. Median follow-up was calculated using the reverse Kaplan-Meier method (Schemper and Smith, 1996). Biochemical RFS function was estimated by the Kaplan-Meier method. Univariate and multivariable analyses of biochemical RFS were carried out using a Cox proportional hazards model (Cox, 1986). The proportional hazards assumption was assessed graphically and analytically with scaled Schoenfeld residuals (Grambsch and Therneau, 1994). Unadjusted 5-year biochemical recurrence rates were estimated using a Cox proportional hazards model and plotted against values of sCD105 or PSA to graphical display recurrence estimates with respect to the continuous variables, sCD105 and PSA (Allmer and Sargent, 2000). In a separate cohort of patients, the strength and direction of the linear relationship between plasma levels of sCD105 and cellular CD105 levels was determined with Spearman's rank correlation. As analyses did not involve multiple simultaneous statistical tests, no adjustment for multiple comparisons was made. Analyses were performed using SAS 9.4 (SAS Institute, Inc., Cary, North Carolina) and R package version 3.5.3 (The R Foundation for Statistical Computing) with two-sided tests and a significance level of 0.05.

Results

This retrospective study sought to determine if sCD105 has a prognostic capacity in concert with PSA for PCa biochemical recurrence following prostatectomy. PCa patients with a PSA 10 ng/mL have otherwise ambiguous odds for recurrent disease following prostatectomy; the goal was to test a blood-based marker for disease free survival. Of the 181 PCBN patients, 83 patients developed a biochemical recurrence after prostatectomy and 98 patients did not experience a biochemical recurrence, in an overall follow-up of up to 11 years post-surgery (Table 1). Of note, 64 patients developed biochemical recurrence within 5 years after prostatectomy. Total Gleason scores ranged from 5–9 with the majority being 7. Of these Gleason 7 subjects, 58% developed biochemical recurrence. Out of 42 patients with positive surgical margins, 79% of these patients developed recurrent disease. Pathologic T stage of T2 represented 78% of the patients of which 63% remained recurrent-free. But, 79% of T3 patients developed recurrent disease. The median pre-surgical serum PSA was 5.00 ng/mL and the median plasma sCD105 was 5.54 ng/mL (Figure 1). All but 10 of the subjects stratified to the Low to Intermediate risk category by NCCN (National Comprehensive Cancer Network) guidelines for PCa diagnostic recommendations. The majority of patient population (97 subjects) fell into the Intermediate-Favorable NCCN category, yet 57% of this group developed recurrent disease. As each of the NCCN categories had patients that developed biochemical recurrence, it would support the need for improved risk stratification.

To determine the extent to which sCD105 is associated with other clinical variables, univariate analysis was performed (Table 2). There was no significant association of sCD105 with either Gleason grade or pathologic stage of the disease. Interestingly, there was an association of sCD105 and age of the patients at the time of prostatectomy (p value = 0.006).

More importantly, there was a significant association of sCD105 with surgical margin status (p value = 0.037) and pre-surgery PSA (p value = 0.015), respectively. Overall, the presence of surgical margin, younger age at surgery, and lower levels of PSA were significantly associated with elevated concentrations of plasma sCD105. Univariate and multivariable analyses of RFS are presented in Table 3. The median follow-up was 6.28 years, and median biochemical recurrence-free survival (RFS) was 6.94 years (Figure 2A). Apart from age, Gleason scores 5–7, negative surgical margin status, T2 stage, reduced PSA, and elevated sCD105 were significantly associated with an improved biochemical RFS on univariate analysis. Age at surgery and pathologic stage did not significantly affect risk of biochemical recurrence by multivariate analysis. But, Gleason grade, surgical margin status, PSA and sCD105 remained as significant prognostic indicators of improved biochemical RFS in multivariate models.

We independently examined the recurrence rate 5 years following surgery, as opposed to overall recurrence based on the biological implications of the detection of a biomarker just prior to surgery. Elevated PSA was associated with biochemical recurrence and lower plasma sCD105 concentrations prior to prostatectomy was associated with recurrent disease within 5 years, in this lower risk cohort of subjects. The estimated 5-year recurrence rate with respect to PSA and sCD105 concentrations was calculated and plotted on a biaxial graph to highlight the differences between these two markers (Figure 2B). There was an inverse correlation of pre-surgical PSA and sCD105 within 5-years of prostatectomy. Further studies, with greater number of patients will be required to prove this novel relationship revealed for sCD105 levels, biochemical recurrence, and pre-surgical PSA for prostatectomy patients.

Finally, we chose to determine the relationship of cellular CD105 and circulating sCD105. Cellular CD105 expression in PCa epithelia and associated fibroblasts mediate increased PCa progression (Sharma et al., 2002; Madhav et al., 2018; Kato et al., 2019). However, there is some controversy as to the relationship between cellular CD105 and sCD105 found in circulation. There were two possibilities: one being that greater cellular CD105 translated to greater circulating sCD105 or two, that the presence of sCD105 in circulation meant there was less on the cell surface. To address this, we needed paired plasma and tumor tissue. As this was not available to us for the initial PCBN patient population we analyzed, we garnered a tissue array of primary radical prostatectomy tissues with 31 corresponding plasma specimens from the Cedars-Sinai Biobank. Of note, these patients did not have neoadjuvant treatment. The median concentration of sCD105 found in the plasma was 8.91 ng/mL, with a mean of 9.56 ng/ml (Figure 3A). Interestingly, CD105 localization by immunohistochemistry in prostate tissues was exclusively observed in the stromal compartment (Figure 3B). Quantitation of tissue expression of CD105 was interestingly inversely correlated to the level of plasma sCD105 measured by Spearman's correlation ($r_s = -0.52692$, p value = 0.002, Figure 3C). Together, these findings indicated that lower blood sCD105 concentration was associated with elevated stromal expression of CD105 and elevated risk of biochemical recurrence within 5-years of prostatectomy.

Discussion

This study demonstrated that elevated plasma sCD105 was associated with an improved biochemical recurrence-free survival in PCa patients with PSA levels ≥ 10 ng/mL. Using strictly blood-based markers prior to prostatectomy, the combination of PSA and sCD105 had greater risk for biochemical recurrence, compared to the current use of PSA alone (Table 3). Based on the analysis of the PCBN patient data for sCD105 as an independent predictor of recurrence-free survival, correlation of sCD105 levels found in the plasma with cellular levels of stromal CD105 was investigated. In a separate cohort of patients, the plasma levels of sCD105 were found to inversely associated with cellular CD105 levels for a matched subset of patients (Figure 3). In contrast to the well described role of CD105 in the promotion of angiogenesis, its effects on PCa epithelia are less understood. Elevated expression of CD105 in PCa cell lines have been attributed to increased motility and metastasis (Liu et al., 2002; Sharma et al., 2002). Such progression is ultimately a result of PCa cells escaping the boundaries of the surgically resected tissue, contributing to local or distant metastasis. We have also demonstrated that PCa epithelial CD105 expression contributed to radiation therapy resistance and via prostate cancer associated fibroblasts contributes to PCa androgen receptor signaling inhibitor resistance (Madhav et al., 2018; Kato et al., 2019). Interestingly, the prostatectomy tissues we evaluated in this study had primarily fibroblastic and endothelial associated CD105 expression seen by immunohistochemical localization (Figure 3). This first demonstration of the inverse relationship between cellular CD105 capable of signaling and the proteolytically cleaved sCD105 accessible by a simple blood draw provides the needed mechanistic insight to reconcile the past understanding of CD105 biology for its application as a biomarker for PCa patients undergoing radical prostatectomy.

Increased CD105 expression in esophagus, lung, breast, ovary, endometrium, and prostate cancer tissues has been associated with poor outcome (Kumar et al., 1999; Tanaka et al., 2001; Wikstrom et al., 2002; Dales et al., 2003; Saad et al., 2005; Erdem et al., 2006; Taskiran et al., 2006). Importantly, blood sCD105 was described as a serum biomarker in breast, colon, and prostate (Li et al., 2000; Takahashi et al., 2001; Svatek et al., 2008; Fujita et al., 2009). Our findings demonstrated that sCD105 can serve as a surrogate for the expression of the full-length transmembrane receptor expression. However, unlike our study, Svatek et al. suggested that lower preoperative plasma levels of sCD105 correlated with PCa progression (Svatek et al., 2008). As PCa cancer populations differ, such differences are not uncommon especially in relatively small patient cohorts in both studies. It is important to note that our sCD105 measurements were made in subjects with lower risk subjects as characterized by NCCN guidelines (Table 1). The prediction of biochemical recurrence of such patients is significant. This study expands on the utility of sCD105 by showing that in patients with PSA levels ≥ 10 ng/mL; reduced sCD105 concentrations strongly correlate with PCa recurrence. Those patients that exhibit PSA levels ≥ 10 ng/mL do not have a reliable blood-based prognostic indicator. However, tissue-based indicators apart from standard pathologic Gleason score include transcriptomic expression panels that have more recently emerged to have significant predictive capacity. For example, the PAM50 gene set initially developed for breast cancer, has proven to have prognostic capacity in PCa patients

(Zhao et al., 2017). Similarly, the PCS gene set has also demonstrated prognostic efficacy in late stage disease (You et al., 2016). This study supports the use of sCD105 as an independent risk factor of recurrence in a patient cohort where pathologic stage was not a statistically significant predictor following radical prostatectomy by multivariable analysis. This is significant since past trials where adjuvant external beam irradiation was given at an early stage after surgery was based on the patient having stage T3 disease (Bolla et al., 2005; Bolla et al., 2012). In our PCa patient population the majority of patients are T2 stage (78%) and Gleason grade 7 (63%, Table 1). Thus, in this small cohort the analysis of preoperative plasma utilizing sCD105 as a biomarker can support receiving local irradiation therapy prior to biochemical recurrence in even lower risk subjects. This study highlights the need for a biomarker driven clinical trial to test the use of traditional clinical factors including PSA, Gleason, and pathologic stage compared to or in combination with sCD105 for patients.

Intriguingly, an inverse correlation of lower plasma sCD105 levels was associated with higher stromal cellular CD105 immunohistochemical localization (Figure 3). This would suggest that the lower sCD105 levels found in patients with PCa recurrence likely had elevated cellular CD105 and bone morphogenic protein signaling in the tumors. Of note, the median sCD105 of the PCBN subjects were considerably greater than that of the Cedars-Sinai Medical Center Biorepository (Table 1 and Figure 3). A possible explanation would be that the patient population that seek treatment at Cedars-Sinai Medical Center are generally of lower disease progression risk than those participating in the PCBN, a broader specimen biorepository involving six independent institutions (in the USA and UK). Further studies are needed to address the role of BMP/CD105 signaling in PCa metastatic progression. In our previous study, fibroblastic expression of CD105 contributed to elevated epithelial proliferation by way of paracrine secreted frizzled receptor 2 (SFRP2) activity in the context of androgen receptor signaling inhibition (Kato et al., 2019). The importance of stromal CD105 was corroborated by a colon cancer study, demonstrating its contribution to cancer progression and metastasis (Paauwe et al., 2018). In conclusion, this study suggests that sCD105 may serve as a prognostic biomarker to determine PCa recurrence in patients with PSA levels ≥ 10 ng/mL to aid in treatment decision making following radical prostatectomy.

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Figure 1. Prostatectomy PCa patients characterized with pre-surgical PSA levels (ng/mL) and sCD105 (ng/mL). The non-recurrent (NR) and recurrent (R) patients are indicated with the blue and red bar, respectively with the p value calculated by two tailed Mann-Whitney test to determine significance of the PSA (z score = 2.99) and sCD105 (z score = 1.970) concentrations. The box whisker plot represents the median (horizontal line), upper and lower quartiles, and mean (marked by the 'x'). Outliers of the box-whisker plots are shown for individual patients indicated with individual dots (n=181).

Figure 2.

Recurrence free survival of prostatectomy patients with presurgical PSA levels ≤ 10 were characterized for blood sCD105 and PSA concentrations ($n = 181$). **(A)** Kaplan-Meier estimates of biochemical recurrence-free survival (RFS) along with 95% confidence intervals over 11 years post-surgery. The number of patients at risk at each year is shown at the bottom of the graph. A vertical dashed line highlights recurrent-free survival 5 years post-surgical intervention. **(B)** The estimated unadjusted 5-year recurrence rate percentage are plotted for sCD105 values (ng/mL, solid line) and pre-surgical PSA (ng/mL, dashed line) in patients with a PSA level ≤ 10 .

Figure 3.

Plasma levels of sCD105 are inversely correlated with stromal CD105 immunohistochemical staining. **(A)** sCD105 levels of 31 pre-surgical prostatectomy patients were ordered by their sCD105 concentrations measured (ng/mL). Their corresponding primary, secondary, and sometimes tertiary Gleason scores (GS) are shown. **(B)** Representative images chosen from prostatectomy tissue immunohistochemistry for cellular CD105 (brown) counterstained with hematoxylin (blue). The upper panels represent relatively low level of staining of fibroblastic cells (arrows) compared to that in the lower panels. Vascular endothelial localization of CD105 are indicated with arrowheads. Scale bar indicates 32 μ m. **(C)** Dot plot shows the Spearman's correlation of patient samples measured for sCD105 levels with quantitated cellular CD105 immunohistochemical staining.

Table 1.

PCa patient characteristics for those with PSA 10 that developed biochemical recurrence or those that remained recurrence-free 5 years following radical prostatectomy.

Variable	All patients (N=181)	Recurrence status at 5 years post-surgery	
		Non-Recurrence (N=98)	Recurrence (N=83)
Age at surgery (Years)			
Mean (± SD)	59.98 (± 6.86)	59.24 (± 7.04)	60.86 (± 6.58)
Total Gleason			
5–6	60 (33.15)	50 (83.33)	10 (16.67)
7	114 (62.98)	48 (42.11)	66 (57.89)
8–9	7 (3.87)	0 (0)	7 (100)
Surgical margin			
No	139 (76.80)	89 (64.03)	50 (35.97)
Yes	42 (23.20)	9 (21.43)	33 (78.57)
T stage			
T2	142 (78.45)	90 (63.38)	52 (36.62)
T3	39 (21.55)	8 (20.51)	31 (79.49)
NCCN Risk Stratification			
Low	13 (7.18)	12 (92.31)	1 (7.69)
Interm.-Favorable	97 (53.59)	42 (43.30)	55 (56.70)
Interm.-Unfavorable	39 (21.55)	10 (25.64)	29 (74.36)
High	7 (3.87)	1 (14.29)	6 (85.71)
Very High	3 (1.66)	1 (33.33)	2 (66.66)
Pre-surgery PSA (ng/mL)			
Median (IQR)	5.00 (4.30 – 6.72)	4.64 (3.90 – 6.30)	5.60 (4.60 – 7.50)
sCD105 (ng/ml)			
Median (IQR)	5.54 (3.51 – 7.32)	5.71 (4.06 – 7.63)	5.2 (2.96 – 6.97)

Data for Non-Recurrence or Recurrence columns are presented as number of patients (row %), mean ($\pm$ SD) or median (IQR, interquartile range) as appropriate. The data for All patients are presented as number of patients (column %), mean ($\pm$ SD) or median (IQR, interquartile range) as appropriate.

Table 2.

Univariate associations of sCD105 with pre-surgical and pathologic covariants in PCa patients with a pre-surgical PSA ≥ 10 .

Variable	N	sCD105 (ng/ml) Median (IQR) or Spearman CC	P value
Pre-surgery PSA (ng/mL)	181	−0.181	0.015
Age at surgery (Years)	181	−0.206	0.006
Total Gleason			
5–6	60	6.07 (4.17 – 7.95)	0.077
7	114	5.31 (3.44 – 7.10)	
8–9	7	3.17 (1.96 – 6.80)	
Surgical margin			
Yes	42	6.42 (4.88 – 7.95)	0.037
No	139	5.31 (3.24 – 7.10)	
T stage			
T2	142	5.68 (3.84 – 7.42)	0.066
T3	39	4.97 (3.08 – 6.78)	

P-value is calculated by Kruskal-Wallis test, Wilcoxon rank-sum test, or Spearman rank correlation as appropriate.

Abbreviation: IQR, interquartile range; CC, correlation coefficient.

Table 3.

Univariate and multivariate Cox proportional-hazards models of biochemical recurrence-free survival in PCa patients PSA ≥ 10 prior to radical prostatectomy.

Variable	N	Univariate model		Multivariate model	
		Hazard Ratio (95% CI)	P value	Hazard Ratio (95% CI)	P value
Log-transformed sCD105 [†]	181	0.58 (0.43–0.80)	<.001	0.63 (0.45–0.88)	0.007
Pre-surgery PSA (ng/mL) [†]	181	1.30 (1.15–1.46)	<.001	1.26 (1.10–1.44)	<.001
Age at surgery (Years) [†]	181	1.02 (0.99–1.05)	0.246	0.99 (0.96–1.02)	0.558
Total Gleason					
5–6	60	0.05 (0.02–0.13)	<.001	0.08 (0.03–0.22)	<.001
7	114	0.20 (0.09–0.44)	<.001	0.19 (0.08–0.45)	<.001
8–9	7	1 (Reference)		1 (Reference)	
Surgical margin					
Yes	42	2.77 (1.77–4.33)	<.001	3.33 (2.06–5.37)	<.001
No	139	1 (Reference)		1 (Reference)	
T stage					
T2	142	0.34 (0.22–0.54)	<.001	0.65 (0.39–1.06)	0.087
T3	39	1 (Reference)		1 (Reference)	

Cox proportional hazards models were fit modeling biochemical recurrence-free survival.

181 observations were used in the multivariable model.

[†] Hazard Ratio (95% CI) was expressed as 1-unit increment.