Is Ki67 prognostic for aggressive prostate cancer? A multicenter real-world study

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Aim: To test if Ki67 expression is prognostic for biochemical recurrence (BCR) after radical prostatectomy (RP). **Methods:** Ki67 immunohistochemistry was performed on tissue microarrays constructed from specimens obtained from 464 men undergoing RP at the Durham and West LA Veterans Affairs Hospitals. Hazard ratios (HR) for Ki67 expression and time to BCR were estimated using Cox regression. **Results:** Ki67 was associated with more recent surgery year ($p < 0.001$), positive margins ($p = 0.001$) and extra-capsular extension ($p < 0.001$). In center-stratified analyses, the adjusted HR for Ki67 expression and BCR approached statistical significance for west LA (HR: 1.54; $p = 0.06$), but not Durham (HR: 1.10; $p = 0.74$). **Conclusion:** This multi-institutional 'real-world' study provides limited evidence for the prognostic role of Ki67 in predicting outcome after RP.

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Differentiating between indolent and aggressive prostate cancer (PC) is critical to optimal disease management [1,2]. The advent of prostate specific antigen (PSA) testing, while invaluable, has increased the potential for over-diagnosis and over-treatment [3,4]. Since all therapies carry some risks that may encompass life altering morbidity [5], good prognostic biomarkers are needed to identify those who could benefit from aggressive management [6]. Ideally, a useful biomarker adds prognostic information in a cost-effective manner. Ki67 is a well-studied protein exclusively expressed in proliferating cells throughout all cell cycle phases, except for G0 (the resting cycle) [7]. This allows for quick, easy and cost-effective determination of the 'growth-fraction' of cells. This property of Ki67 has generated substantial interest in using this antigen as a prognostic biomarker in cancer progression and metastasis [8].

Multiple studies [9–15] and a recent systematic review and meta-analysis [8] have provided compelling evidence for the prognostic value of Ki67 in determining the PC progression and disease-free survival risk. Although similar results were reported for breast cancer [16,17], the utility of Ki67 in breast cancer has been questioned due to its marginal reproducibility and lack of analytic standardization across laboratories [18,19]. It is noteworthy that most Ki67 studies in PC were conducted in single centers or in single pathology laboratories. Whether the inconsistencies reported in the reproducibility and validity of Ki67 expression scoring in breast cancer [18,19], also plague Ki67 estimation in PC is unknown.

We determined Ki67 expression using tissue microarrays (TMAs) of radical prostatectomy (RP) specimens from two centers. Our objectives were to determine if Ki67 expression predicted biochemical recurrence (BCR; primary outcome) and other markers of aggressive PC (e.g., castration resistant PC [CRPC], metastasis and PC-specific

death; secondary outcomes). We hypothesized that while Ki67 would predict outcome in each center, the lack of standardization in Ki67 staining and scoring across centers would result in only modest prognostic value when the data were pooled.

Materials & methods

Study population

Approval was obtained for the construction of TMAs, data abstraction and analyses from the Internal Review Boards for Durham and West Los Angeles Veterans Affairs. RP specimens from 479 patients who underwent RP from the West Los Angeles Veterans Affairs Medical Center (WLAVAMC) and Durham, North Carolina Veterans Affairs Medical Center (DVAMC) between 1991 and 2004 and followed-up until November 2013 were used to construct TMAs. Exclusion criteria included preoperative hormonal ablation or radiation therapy. Eight patients missing follow-up and seven missing pathological data were excluded resulting in 464 subjects (WLAVAMC, $n = 293$; DVAMC, $n = 171$).

Tissue acquisition/analysis

Durham, North Carolina Veterans Affairs Medical Center

From formalin fixed and paraffin embedded RP specimens, four core needle biopsies of cancer were used to construct TMAs. TMAs were sectioned into five micron sections, followed by immunohistochemical staining for Ki67 expression. A positive control tissue slide was included (human tonsil). The sections were dried for 30 min at 60°C. Slides were deparaffinized in xylene, followed by a series of graded ethanol solutions, and rehydration in dH₂O for 30 s. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide for 10 min. Slides were heated at 80°C in Dako citrate (pH 6.1) antigen-retrieval buffer for 20 min, cooled to room temperature, rinsed with water and placed in Tris-Buffered Saline and Tween 20 (TBST). Background Buster (Innovex Biosciences, CA, USA) was applied and incubated for 30 min at room temperature. The excess was drained without a wash. A 1:200 diluted rabbit monoclonal anti-Ki67 (Cat# RM-9106-S1, Thermo Fisher Scientific, MA, USA) or rabbit IgG, as a negative control antibody, was applied, incubated for 45 min at room temperature, then washed twice in TBST for 1 min, and the excess buffer drained. A 1:200 diluted biotinylated secondary antibody (goat, antirabbit, Vector, CA, USA) was applied and incubated for 30 min followed by two 1 min washes with TBST. Vectastain Elite ABC was applied and incubated for 30 min followed again by two 1 min washes with TBST. The DAB chromogen (Innovex Biosciences, CA, USA) was applied and incubated for 3 min, followed by three changes of dH₂O, then rinsed with tap water for 2 min. The sample was counter stained in hematoxylin for 30 s and rinsed with tap water. Slides were dipped four times in ammonium water and rinsed for 2 min to counterstain the nuclei in blue. Slides were dehydrated, cleared and coverslips were added.

West Los Angeles Veterans Affairs Medical Center

TMAs were constructed using a Manual Tissue Arrayer (Beecher Instruments, Inc., WI, USA). Coring needles having diameter 0.6 mm were used to abstract representative areas from formalin fixed paraffin embedded RP blocks. Tissue was sampled from the primary pathological Gleason grade, the secondary Gleason grade and benign tissue. Three cores of each histology type (primary Gleason grade, secondary Gleason grade and benign tissue) were taken from the blocks and placed into the TMA block. Four micron sections were cut from the TMA blocks and stained for Ki67, as previously described [20]. The slides were scanned using an Aperio slide scanner (Aperio, CA, USA).

TMA reading

TMAs were manually read by a board-certified pathologist at each respective center, blinded to clinical data and outcome. The average percent of positively stained cancer cells across the four core biopsies was calculated. At each site, 300 cancer cells were reviewed and the number of Ki67 positive cells were divided by 300 to compute the percent of positive cells. If the tissue was too scant for analysis, or no tumor was present, the core was considered nonevaluable and that core was excluded. The remaining cores were averaged.

BCR definition

At both sites, BCR was defined as a single PSA value of >0.2 ng/ml, two values of 0.2 ng/ml or need for secondary treatment for elevated postoperative PSA.

Statistical analysis

Since the distributions of percent Ki67 positive cells varied substantially between centers (Supplementary Figure 1), we divided subjects into tertiles of percent Ki67 positive staining within each center.

The patient demographic and clinical variables were compared using t-tests for continuous normally distributed variables, rank-sum for continuous non-normally distributed variables, and χ^2 for categorical variables. Crude and multivariable Cox proportional hazard models were used to test the association between Ki67 tertile and BCR risk. Proportional hazard assumptions were examined and met for the center-specific and combined regression models using Schoenfeld residuals. Analyses were center-stratified as well as pooled. Multivariable analyses were adjusted for age (continuous), prognostic grade groups (GG I–V) [21], race (black/nonblack), surgery year (continuous), positive margins (yes/no), extracapsular extension (yes/no), seminal vesicle invasion (yes/no) and surgical center. An interaction between race and Ki67 was tested by including main effect terms and the cross-product, and evaluated using the Wald test. Kaplan–Meier curves were used to show the BCR-free survival between Ki67 tertiles by center. Statistical significance of these differences was assessed using the log-rank test.

On secondary analysis, we tested the prognostic value of Ki67 for other PC outcomes. We first examined the association between Ki67 and prognostic GG. We calculated median, 25th percentile, and 75th percentile of Ki67 positive staining values within each GG (I–V). To examine differences between centers, we calculated these values in both stratified by center and combined. Kruskal–Wallis test was used to test for an association between Ki67 and GG. We also used Cox regression models to test the association between Ki67 tertile and long-term PC outcomes: CRPC, metastases, and PC-specific death. CRPC was defined as having a PSA increase of 2 ng/ml and 25% greater than the nadir after hormone treatment despite continuous therapy with a luteinizing hormone-releasing hormone agonist, antagonist or after orchiectomy. Metastases were diagnosed by bone scan and other imaging. PC-specific death was defined as having metastatic progressive CRPC at death with no obvious other cause. Because event rates were low, centers were combined and all models were unadjusted.

To address potential concerns of tumor tissue under-sampling that could have led to misclassification of Ki67 status, we conducted sensitivity analyses where we excluded patients with negative Ki67 staining across all cores and repeated the above analyses.

Results

Patient characteristics

There were 215 black and 249 nonblack men in the study. There were no racial differences between surgical centers (Table 1) ($p = 0.27$). DVAMC men were more likely to be treated more recently ($p < 0.001$), have GG III or greater ($p < 0.001$), positive margins ($p < 0.001$), extracapsular extension ($p < 0.001$) and higher Ki67 scores ($p < 0.001$). There were no differences between centers in age, preoperative PSA, seminal vesicle invasion, positive nodes or mean follow-up (all p -values > 0.18).

We did not find associations between Ki67 tertiles and age, PSA and GG (Table 2). However, a lower percentage of men were classified in pathological stage T0–T2 (67%) in tertile 3 compared with tertiles 1 and 2 ($p = 0.010$).

Center specific risk of BCR

Median PSA follow-up times were 115 months (Q1–Q3: 56–162 months) and 117 months (Q1–Q3: 79–144 months) for WLAVAMC and DVAMC, respectively. During follow-up, 200 (43%) patients had BCR: 122 patients from WLAVAMC and 78 from DVAMC. In WLAVAMC, BCR was not significantly related to Ki67 tertile (log-rank, $p = 0.205$) (Figure 1A). However, there was an increasing risk with increasing tertile which approached but did not reach statistical significance for the highest versus lowest tertile in crude (hazard ratio [HR]: 1.48; 95% CI: 0.96–2.28; $p = 0.08$) and adjusted analyses (HR: 1.54; 95% CI: 0.98–2.44; $p = 0.064$) (Table 3).

Within the DVAMC cohort, Ki67 tertile was significantly related to BCR, but the second tertile actually had the best outcome (log-rank; $p = 0.006$) (Figure 1B). When examined in two-way comparisons, the second tertile was associated with a significantly lower BCR risk in crude analysis (HR: 0.55; $p = 0.0496$), which was attenuated in multivariable analysis (HR: 0.64; $p = 0.17$). The highest tertile was unrelated to BCR in both crude and adjusted analyses (all p -value > 0.2).

BCR risk in pooled data

When the data from both centers were combined, Ki67 tertile was significantly associated with BCR with men in the third tertile having the worst outcomes (log-rank, $p = 0.009$, Figure 1C). Indeed, men in the third Ki67 tertile

Figure 1. Kaplan–Meier estimation for biochemical recurrence-free survival by tertile of percent of cancer cells staining positive for Ki67 at each surgical center and combined.VAMC: Veterans Affairs Medical Center.

Table 1. Demographic, clinical and pathological characteristics of patients by surgical center.

Patient characteristics	WLAVAMC (n = 293)	DVAMC (n = 171)	p-value
Age, mean $\pm$ SD	62.8 $\pm$ 6.3	62.3 $\pm$ 6.2	0.39 [†]
Race, n (%):			0.27 [‡]
– Nonblack	163 (56)	86 (50)	
– Black	130 (44)	85 (50)	
Year of surgery, M (Q1–Q3)	1997 (1994–1999)	2000 (1997–2002)	<0.001 [§]
PSA (ng/ml), M (Q1–Q3)	8.4 (5.5–12.6)	7.7 (5.1–12.0)	0.31 [§]
Post-op grade group, n (%):			<0.001 [‡]
– 1	159 (54)	36 (21)	
– 2–3	89 (30)	89 (52)	
– 4–5	45 (15)	46 (27)	
Positive margins, n (%)	136 (46)	108 (63)	<0.001 [‡]
Seminal vesicle invasion, n (%)	32 (11)	26 (15)	0.18 [‡]
Extracapsular extension, n (%)	30 (10)	47 (27)	<0.001 [‡]
Positive lymph nodes, n (%)	5 (2)	1 (<1)	0.302 [‡]
Average % cells positive for Ki67, M (Q1–Q3):	0.08 (0–0.67)	3.03 (1.02–5.67)	<0.001 [§]
– Tertile 1, minimum–maximum	0–0	0–1.66	
– Tertile 2, minimum–maximum	0.03–0.50	1.67–4.58	
– Tertile 3, minimum–maximum	0.53–10.29	4.67–18.67	
Follow-up (months), M (Q1–Q3)	115 (56–162)	117 (79–144)	0.83

[†]p-values calculated using t-test.[‡]p-values calculated using χ^2 test.[§]p-values calculated using rank sum test.

DVAMC: Durham, North Carolina Veterans Affairs Medical Center; M: Median; PSA: Prostate specific antigen; Q1: 25th percentile; Q3: 75th percentile; SD: Standard deviation; WLAVAMC: West Los Angeles Veterans Affairs Medical Center.

Table 2. Patient characteristics by Ki67 tertiles.

Patient characteristics	Ki67 tertile 1 (n = 175)	Ki67 tertile 2 (n = 145)	Ki67 tertile 3 (n = 144)	p-value [†]
Age, median (IQR)	64 (59–68)	63 (58–66)	64 (59–68)	0.30
PSA, median (IQR)	7.8 (5.5–11.5)	7.6 (5.4–12.0)	8.4 (5.6–13.8)	0.51
Post-op grade group, n (%):				0.49
– 1	83 (47)	56 (39)	56 (39)	
– 2–3	81 (46)	79 (55)	77 (53)	
– 4–5	11 (6)	10 (7)	11 (8)	
Pathological stage, n (%):				0.010
– T0–T2	141 (81)	117 (81)	96 (67)	
– T3	25 (14)	25 (17)	41 (28)	
– T4	9 (5)	3 (2)	7 (5)	

[†]p-values calculated using Kruskal–Wallis for age and PSA and χ^2 test for grade group and pathological stage.

IQR: Interquartile range; PSA: Prostate specific antigen.

had a statistically significant increased BCR risk versus men in the first tertile on crude analysis (HR: 1.50; 95% CI: 1.08–2.09; $p = 0.015$) (Table 4). After multivariable adjustment, the HR was attenuated and no longer significant (HR: 1.33; 95% CI: 0.94–1.89; $p = 0.11$) (Table 4). The interaction term for race and Ki67 was not statistically significant (p -interaction = 0.56); hence, the relation between Ki67 and BCR was not further stratified by race.

Long-term secondary outcomes

During a median overall follow-up of 117 and 115 months, in DVAMC and WLAVAMC, respectively, 26 men developed CRPC, 29 developed metastases, and 18 PC deaths occurred. When stratified by site, Ki67 was significantly associated with GG in DVAMC ($p = 0.043$), but not WLAVAMC ($p = 0.62$) (Supplementary Table 1). In pooled analyses, Ki67 was significantly associated with GG ($p < 0.001$). The associations between Ki67

Table 3. Hazard ratios for risk of biochemical recurrence by tertile of percent of cancer cells staining positive for Ki67 stratified by surgical center.

Tertile of Ki67	WLAVAMC (n = 293)			DVAMC (n = 171)		
	BCR/N	HR (95% CI)	p-value	BCR/N	HR (95% CI)	p-value
Crude:						
– T1	43/119	Reference		27/56	Reference	
– T2	40/87	1.23 (0.80–1.89)	0.35	18/58	0.55 (0.30–0.999)	0.0496
– T3	39/87	1.48 (0.96–2.28)	0.08	33/57	1.38 (0.83–2.31)	0.21
Adjusted: [†]						
– T1	43/119	Reference		27/56	Reference	
– T2	40/87	1.01 (0.64–1.60)	0.95	18/58	0.64 (0.34–1.21)	0.17
– T3	39/87	1.54 (0.98–2.44)	0.064	33/57	1.10 (0.63–1.94)	0.74

[†]Adjusted for PSA, age, pathological Gleason score, race, year of surgery, positive margins, extracapsular extension, seminal vesicle invasion and surgical center.
BCR: Biochemical recurrence; DVAMC: Durham, North Carolina Veterans Affairs Medical Center; HR: Hazard ratio; PSA: Prostate specific antigen; WLAVAMC: West Los Angeles Veterans Affairs Medical Center.

Table 4. Hazard ratios for risk of biochemical recurrence by tertile of percent of cancer cells staining positive for Ki67 data combined from centers (n = 464).

Tertile of Ki67	BCR/N	HR (95% CI)	p-value
Crude:			
– T1	70/175	Reference	
– T2	58/145	0.92 (0.65–1.31)	0.66
– T3	72/144	1.50 (1.08–2.09)	0.015
Adjusted: [†]			
– T1	70/175	Reference	
– T2	58/145	0.88 (0.62–1.26)	0.43
– T3	72/144	1.33 (0.94–1.89)	0.11

[†]Adjusted for PSA, age, pathological Gleason score, race, year of surgery, positive margins, extracapsular extension, seminal vesicle invasion and surgical center.
BCR: Biochemical recurrence; HR: Hazard ratio; PSA: Prostate specific antigen.

tertiles and all long-term outcomes were null on univariable analysis ($p \geq 0.65$) (Supplementary Table 2) (there were an insufficient number of events to perform multivariable analyses).

Analyses excluding patients with no positive Ki67 staining

There were 119 patients at WLAVAMC and 14 patients at DVAMC with no Ki67 staining; following their exclusion 331 remained. Results were similar to those observed for the entire sample (Supplementary Tables 3 & 4).

Comments

Ki67 is a marker of cellular proliferation expressed in all but the G0 phase of the cell cycle. Since uncontrolled proliferation is a hallmark of cancer [22], Ki67 expression has generated substantial interest for its potential as a cancer biomarker [8,23]. Although it shows promise in determining the aggressiveness of breast and prostate cancers [9–17], its quantification is compromised by poor reproducibility [18,19,24]. This limitation raises questions with respect to its clinical utility and prompted our interest in evaluating its performance in predicting PC aggressiveness across two institutions where TMAs were evaluated by different pathology laboratories. Null to weak associations were observed between Ki67 and BCR in center-stratified and pooled analyses, while no associations were observed between Ki67 and long-term PC outcomes: CRPC, metastases and PC-specific death. Although these findings do not rule out an association between Ki67 expression and aggressive PC, they suggest a modest association of limited clinical utility. Importantly our results demonstrate the substantial variability in immunohistological staining and scoring between centers under real-world conditions. As such, they further substantiate concerns pertaining to the application of Ki67 to current clinical practice.

The over-riding implications of our findings are that there is a lack of standardization in the technical and analytical methods used to quantify Ki67 expression. Manual and automated laboratory methods are available for the determination of Ki67 staining with each method currently associated with distinct advantages and inherent limitations. In this study, a board-certified pathologist manually counted cells for positive Ki67 staining. Although feasible in most clinical centers, this approach is known to be subject to intra- and interobserver variability [25] which likely played a role in our discrepant Ki67 distributions. In addition, the method is limited by the number of cells that can realistically be counted in larger studies without over-extending resources. We included 300 cells per core in the evaluation of Ki67 status. However, manual counting of as many as 1000 cells has been suggested to optimally evaluate Ki67 status [18]. This may be too labor-intensive for most laboratories. Automated digital image analysis is an alternate approach that is becoming more accessible and is proposed for increasing the precision, reproducibility and rapidity of Ki67 quantification [25]. The technology is promising, but remains limited by its poor ability to distinguish lymphocytes from tumor cells. On the other hand, with visual inspection, lymphocytes are easily differentiated from tumor cells, thus avoiding the potential for misclassified [26]. In prostate cancer studies, a comparison of digital image analysis with visual assessment has led to conflicting results with respect to the concordance of Ki67 staining and its relation to PC outcomes [25,27]. Similarly, in breast cancer, superiority of one method over the other has not emerged, prompting an international multicenter collaboration to focus on the standardization of Ki67 expression scoring [24,28]. Clearly, standardization of a readily available Ki67 staining quantification method is essential prior to its widespread use as a cancer biomarker.

Another issue critical to the clinical utility of Ki67 as a cancer biomarker is the establishment of threshold cut-points that risk-stratify patients for PC outcomes. Although Ki67 expression is a continuous measure [18,23,29] that is often studied as such in relation to cancer-related outcomes [25], continuous markers do not readily translate into clinical decisions that align with treatment strategies. In the absence of consensus pertaining to universal cut-points, we classified subjects by Ki67 tertiles. The differences in Ki67 score distributions between centers resulted in vastly different tertile cut-points. A weak association between Ki67 and BCR was observed for WLAVAMC where the upper tertile cut-point was 0.53%, while the results were null for DVAMC where the upper tertile cut-point was 4.67%. In PC studies with dichotomized Ki67 scores, cut-points between 1 and 26% have been used to examine the relation between Ki67 and BCR [11,14,15] and between 1 and 11.3% for disease-specific survival [8,9]. Studies that have used multiple cut-points indicate that cut-points of 10% or lower may be most effective [12–14]. Although the percent cut-points for the highest tertiles in this study are in line with those reported in recent studies, we observed predominantly null to borderline statistically significant associations for the relation between Ki67 and BCR.

Beyond the issues discussed above, if Ki67 is to be of clinical utility, its measurement must demonstrate robust prognostic value. Notably, two separate recent multicenter studies examined Ki67 and outcomes after RP (similar to our study) and found Ki67 predicted recurrence (unlike our study) [13,15]. However, there are notable differences between these studies and ours. First, the studies performed Ki67 staining and scoring in single laboratories, thereby eliminating differences between laboratories. Although studying the biomarker potential of Ki67 under optimal conditions is appropriate, this approach is not indicative of ‘real-world’ practice as exemplified in our study wherein tissue preparation, staining and reading were done separately at each center. Second, these two studies had larger sample sizes ($n = 1004$ and $n = 3123$) than ours ($n = 464$). If a biomarker requires >1000 men to demonstrate clinical significance, the clinical utility is likely low. As such, though our study was smaller, it was sufficiently powered to detect clinically meaningful differences. Indeed, in the larger of the two studies ($n = 3123$), a dichotomized Ki67 score $\geq 20\%$ increased recurrence risk by a mere 19% in multivariable analysis ($p = 0.019$) [15]. 3-year BCR-free rates were 85 versus 82.1% – again a statistically significant difference, but one with minimal clinical utility. In the second study ($n = 1004$), the effect of Ki67 was larger, but nonetheless modest [13]. A Ki67 score $>5\%$ increased the relative risk of recurrence by 47% ($p = 0.007$), again a threshold that is unlikely to have significant effects on overall risk stratification. Third, the cut-points used in the two prior multicenter studies differed (5 and 20%) further demonstrating the lack of consensus with respect to how to use Ki67 clinically. In summary, even when analyzed in a single laboratory, the clinical utility of Ki67 appears to be limited at best.

A major strength of our study is that we conducted a multicenter analysis with noncentralized Ki67 immunostaining and scoring, in contrast to larger studies where single laboratories with specially trained genitourinary pathologists were employed, often with sophisticated imaging technologies [13,15]. Our methods resemble contemporary ‘real-world’ scenarios. In addition, all patients in this study underwent RP, and the results are most relevant

to this group. Additional strengths of the study are the long follow-up period and the multiethnic mix of patients coming from two different centers.

Of note, there are limitations in our study. First, there was no centralized determination of the prognostic GG of RP specimens or standardization of Ki67 tissue acquisition, preparation or analysis. However, this allowed us to illustrate the impact of unstandardized methods on the determination of Ki67 expression. Second, our sample size was limited by the number of TMAs available from the centers and may have limited our power to detect true associations between Ki67 and PC outcomes.

Clearly there is a need for robust clinically feasible biomarkers that identify patients with high-risk disease and those with high risk of recurrence. It is with this aim that prognostic biomarkers continue to be investigated. For example, while there has been interest in the prognostic ability of premalignant prostatic lesions such as high-grade prostatic intraepithelial neoplasia (HGPIN), a recent review, concluded that the risk of cancer after a diagnosis of HGPIN is about 20–30%. This is similar to the risk of cancer on repeat needle biopsy among men with benign diagnosis on initial biopsy [30]. Nonetheless, the data to date are limited, warranting ongoing research into the potential role of HGPIN in the identification of patients with high risk disease.

Given the limited clinical utility of Ki67, it is incumbent on the field to identify valuable markers. Current efforts are focused on multiplex markers, often based upon genomic testing. Other markers, however, should be explored either alone or in combination such as p53, centrosomal protein 57, phosphatase and tensin homolog, and p27.

Conclusion

The PC field continues to be rife with decisions on treatment options and the need for further risk stratification remains paramount. There is an urgent need for biomarker-driven clinical decisions to better define surveillance protocols and specifically to determine the prognostic outcomes of those having received curative surgery [8]. We performed a multi-institutional analysis of the Ki67 expression assay as a marker for aggressive PC using BCR as our end point. In this study, Ki67 was not associated with a higher risk for developing BCR, in part owing to the great Ki67 expression variation between centers. To truly examine the clinical utility of Ki67 in assessing PC aggressiveness, standardization of staining, reading, and analysis, readily available in ‘real world’ clinical settings is required.

Summary points

- There is an urgent need to identify prognostic biomarkers that differentiate between indolent and highly aggressive prostate cancers.
- Ki67 has generated substantial interest as a prognostic biomarker in cancer progression.
- Poor reproducibility and lack of standardization raise questions regarding its clinical utility.
- Two pathology laboratories were used for the construction of tissue microarrays, immunohistochemical staining and Ki67 expression scoring.
- Prostate cancer outcomes were assessed after a median of about 10 years of follow-up.
- Null to weak associations were observed between Ki67 and biochemical recurrence.
- Ki67 was not associated with castration-resistant prostate cancer, metastases and prostate cancer-specific mortality.
- We observed substantial variability in immunohistological staining and scoring between centers.
- This ‘real-world’ study substantiates current concerns pertaining to the application of Ki67 to clinical practice.

Supplementary data

To view the supplementary data that accompany this paper please visit the journal website at:
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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

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