

Prognostic and clinicopathologic value of Ki-67 and profilin 1 immunohistochemical expression in primary pT1 urothelial bladder cancer

ABSTRACT

Purpose: To investigate the prognostic and clinicopathologic value of Ki-67 and profilin 1 immunohistochemical expression in primary pT1 papillary urothelial bladder cancer.

Materials and Methods: This study included 88 male and 13 female pT1 primary bladder cancer patients. Demographic characteristics, tumor histological grade, tumor number, presence of concomitant carcinoma *in situ*, tumor size, and status of recurrence or progression were recorded for each patient. Expression of Ki-67 and profilin 1 was evaluated by immunohistochemical analysis of paraffin-embedded tumor tissues. The Pearson's Chi-square test was used for the analysis of qualitative data, and the Kaplan–Meier method and the log-rank test were used for the survival analysis.

Results: In the mean follow-up period of 52 months, 52 (51.5%) patients experienced recurrence, 24 (23.8%) patients experienced progression, and 17 (16.8%) patients died from bladder cancer-related causes. Ki-67 expression was significantly associated with tumor histological grade ($P = 0.001$). In multivariate analysis, Ki-67 positivity had significantly worse outcome for recurrence ($P = 0.006$) and mortality ($P = 0.022$). Ki-67-positive (Ki-67 index $\geq 15\%$) patients had shorter recurrence-free ($P = 0.003$), progression-free ($P = 0.002$), and cancer-specific ($P = 0.003$) survival. However, no statistically significant relationship was found between profilin 1 expression and clinicopathologic features and prognosis.

Conclusions: Ki-67 is a highly predictive biomarker for recurrence-free, progression-free, and cancer-specific survival in pT1 bladder cancer patients, in whom prediction of recurrence and progression are difficult. Ki-67 expression can be safely combined with other prognostic factors. However, in pT1 bladder cancer patients, no significant relationship was found between profilin 1 expression and tumor characteristics or prognostic parameters.

KEY WORDS: Bladder cancer, Ki-67, profilin 1, progression, recurrence

INTRODUCTION

Urothelial bladder cancer (UBC) is one of the most common cancers worldwide, with 429,723 new cases diagnosed annually. In Europe, especially in North and South Europe, the incidence rate of UBC is up to 27/100 000 according to GLOBOCAN 2012 data.^[1] The increasing global incidence of UBC and the necessity of lifelong follow-up result in a concomitant increase in health-care expenditures. Patient-based risk classification has become an important topic of UBC research in an effort to optimize diagnosis, treatment, and follow-up strategies.

Many recent studies have aimed to identify the subtypes of UBC based on clinical, histomorphological, and molecular tumor characteristics, and from these data, they determine the etiologic and prognostic features. Most of these studies have examined all of the nonmuscle invasive stages of bladder cancer (pTa, carcinoma *in situ* [CIS], and pT1), and stage-specific studies

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have rarely been done. However, the clinicopathologic and prognostic differences among the stages are well-known, and molecular analyses have shown that pT1 tumors have a higher level of genomic abnormalities than pTa tumors.^[2]

A marked prognostic variability is evident in pT1 bladder cancer. Some pT1 tumors show rapid progression or early recurrence, but others show no recurrence or progression. Currently, there are no acceptable prognostic markers that can reliably predict progression and determine the need for early radical cystectomy. Therefore, in UBC patients, standard follow-up and treatment regimens can compromise the clinical results, and individualized risk classifications and treatment strategies are required.^[3] To date, the number of studies investigating molecular or immunohistochemical markers in pT1 UBC is very limited. Most studies do not differentiate between invasive and noninvasive tumors and compare the results across all stages of bladder tumors (BTs), ranging from pTa to pT4.

Several studies have evaluated the influence of Ki-67, a reliable marker of cell proliferation, on bladder cancer aggressiveness; the data have suggested that Ki-67 is a promising marker for prognosis.^[4] However, only one such study has addressed profilin 1, one of the earliest-identified actin-binding proteins that is ubiquitously distributed in the cytosol; thus, the validity of profilin 1 as a potential biomarker in UBC needs to be investigated and verified.^[5]

In this study, we aimed to evaluate the prognostic and clinicopathologic value of Ki-67 and profilin 1 immunoexpression in primary pT1 papillary UBC.

MATERIALS AND METHODS

We commenced our study after obtaining approval from the institutional ethics committee (reference number 2015/0174). We examined tumor tissue from 101 primary pT1 UBC patients (88 men and 13 women) who were treated between January 2008 and December 2014. Tumor grade and stage were assessed according to the WHO 2016 TNM classification system by two pathologists experienced in urogenital pathology (SŞÖ and TZ).^[6]

After the initial transurethral resection of BT (TUR-BT), a second resection was done after 4–8 weeks, and intravesical bacillus Calmette–Guerin (BCG) instillations were recommended to all patients according to the EAU guidelines on nonmuscle invasive urothelial carcinoma of the bladder.^[7] All patients participated in follow-up by cystoscopy and urine cytology every 3 months for a period of 2 years after initial TUR-BT, every 6 months thereafter until 5 years, and then yearly. After complete resection of the initial tumor, biopsy-proven tumor detection at follow-up was classified as recurrence, and progression to muscle-invasive disease, distant organ metastasis, or death due to UBC were classified as progression.

Radical cystectomy was recommended to all patients who experienced progression to muscle-invasive disease or were at a high risk for tumor recurrence following BCG treatment.

Demographic data, tumor grade, presence of CIS, tumor size, number of tumors, status of recurrence or progression, time to recurrence or progression, and death were recorded retrospectively. Patients having incomplete data sets or unavailable pathology specimens were excluded from the study. Furthermore, patients who did not have muscularis propria tissue in initial or second TUR-BT specimens and had tumor in muscularis propria were excluded from the study.

Pathology specimens from the initial TUR-BT surgeries were located by archive scans, and appropriate paraffin blocks were selected. Pathology specimens were reevaluated by two pathologists for tumor stage and grade [Figure 1]. The 4µ thick sections obtained from the paraffin-embedded tissues were mounted on poly-L-lysine-coated slides. Immunohistochemical staining was performed with the LEICA BOND-MAX immunostainer (New Castle, United Kingdom) using the avidin-biotin peroxidase method with diaminobenzidine as the chromogen, according to the manufacturer's instructions. The Ki-67 antibody (clone SP6: CELL MARQUE, Rocklin, California, USA) was used at a dilution of 1:100, and the profilin 1 antibody (mouse monoclonal, EPR6304, EMD MILLIPORE, Darmstadt, Germany) was used at a dilution of 1:200.

The immunohistochemical expression of Ki-67 and profilin 1 was determined from the areas where the highest staining intensity was seen. Ki-67 immunoreactivity was considered low (negative) if the nuclear staining of tumor cells was <15% and was considered high (positive) if the staining was ≥15% [Figure 2a and b]. We chose the cutoff value according to previous studies.^[8] A semi-quantitative assessment of profilin 1 immunoreactivity was performed separately in stromal and tumoral epithelial tissue. According

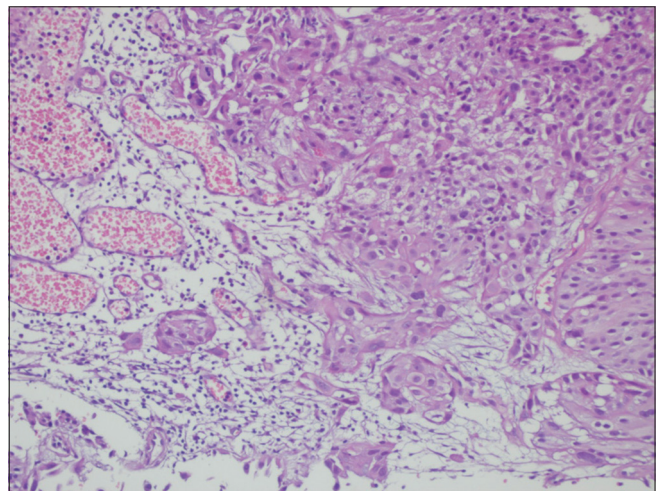


Figure 1: Hematoxylin and eosin staining (pT1 urothelial bladder cancer)

to the patterns of staining intensity, profilin 1 expression was divided into the score categories of 0, 1, 2, and 3. A score of 0 (no expression) was considered negative, while the scores of 1, 2, and 3 were considered positive [Figure 2c-f]. Evaluation of the immunohistochemical data was performed by two experienced uropathologists (SŞÖ and TZ); in the presence of inconsistent findings, the data were discussed until agreement was reached.

Statistical analysis was performed with NCSS 2007 (Number Cruncher Statistical System, Kaysville, Utah, USA). Pearson's Chi-square test was used to compare qualitative data and evaluate descriptive statistical data (mean, standard deviation, median, frequency, ratio, minimum, and maximum). The Kaplan–Meier method and the log-rank test were used to assess survival analysis. Cox regression analysis was used to examine the risk factors for recurrence, progression, and mortality. $P < 0.05$ was considered statistically significant.

RESULTS

A total of 101 UBC patients, 13 (12.9%) females and 88 (87.1%) males, were included in this study. The median age was 66 years (range, 44–89), and the median follow-up period was 51 months (range, 6–96).

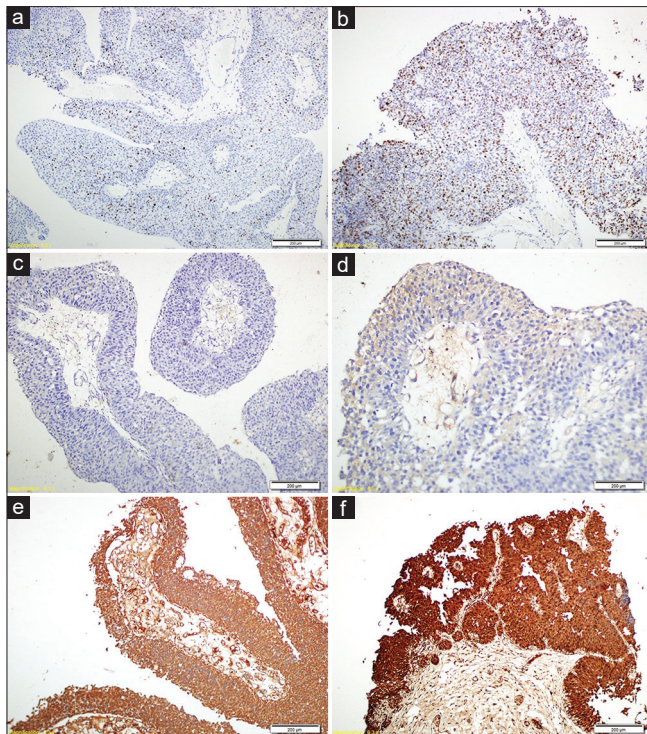


Figure 2: (a and b) Ki-67 immunohistochemistry. (a) Low proliferation index: <15% positive cells. (b) High proliferation index: ≥15% positive cells. (c-f) Profilin 1 immunohistochemistry. (c) Negative epithelial expression of profilin 1. (d) + epithelial and stromal expression of profilin 1. (e) ++ epithelial and stromal expression of profilin 1. (f) +++ epithelial and stromal expression of profilin 1

According to the WHO 2016 TNM classification system, 45.5% ($n = 46$) of tumors were low and 54.5% ($n = 55$) of tumors were high grade. The average tumor diameter was 46.44 ± 27.05 mm (range 5–140), and 81.2% ($n = 82$) of the tumors were ≥3 cm in diameter. The rates of concomitant CIS and multifocality were 12.9% ($n = 13$) and 31.7% ($n = 32$), respectively. A second resection was performed to all patients and in re TUR-BT 17 patients (16.8%) have had T1 and 25 patients have had Ta tumor. Fifty-nine (58.4%) patients had no tumor in the second resection. After the initial TUR-BT, 53.5% ($n = 54$) of the patients received intravesical BCG instillation, and 13.8% ($n = 14$) received intravesical mitomycin C instillation. During the follow-up period, disease recurrence, progression, and UBC-related death were observed in 51.5% ($n = 52$), 23.8% ($n = 24$), and 16.8% ($n = 17$) of the patients, respectively [Table 1].

Association of Ki-67 and profilin 1 expression with clinicopathologic features, recurrence, and progression

The immunohistochemical expression of Ki-67 and profilin 1 was not significantly related to gender, multifocality, tumor size, concomitant CIS, or adjuvant treatment. However, Ki-67 positivity was significantly related to high-grade tumors ($P = 0.001$), but this relationship was not observed for profilin 1 expression [Table 2].

Ki-67 expression and tumor grade were significantly related to recurrence, progression, and mortality ($P = 0.038$, $P = 0.023$ for recurrence; $P = 0.006$, $P = 0.001$ for progression; and $P = 0.007$, $P = 0.020$ for mortality, respectively), but neither stromal nor epithelial profilin 1 expression was significantly related to these parameters [Table 3]. In multivariate Cox

Table 1: Patient clinicopathologic features

	<i>n</i> (%)
Tumor grade	
Low	46 (45.5)
High	55 (54.5)
Tumor diameter (cm)	
<3	19 (18.8)
≥3	82 (81.2)
Concomitant CIS	
Negative	88 (87.1)
Positive	13 (12.9)
Tumor Number	
Unifocal	69 (68.3)
Multifocal	32 (31.7)
Intravesical therapy	
None	33 (32.7)
MMC	14 (13.8)
BCG	54 (53.5)
Recurrence	
Negative	49 (48.5)
Positive	52 (51.5)
Progression	
Negative	77 (76.2)
Positive	24 (23.8)
Deaths	
Tumor related	17 (16.8)
Other causes	15 (14.9)

CIS=Carcinoma *in situ*, MMC=Mitomycin C, BCG=Bacillus Calmette-Guerin

Table 2: Association of Ki-67 and profilin 1 expression with clinicopathologic features

	<i>n</i>	Ki-67 ≥15%, <i>n</i> (%)	Stromal profilin 1 (+), <i>n</i> (%)	Epithelial profilin 1 (+), <i>n</i> (%)
Gender				
Female	13	6 (46.2)	9 (69.2)	13 (100.0)
Male	88	54 (61.4)	57 (64.8)	77 (87.5)
<i>P</i> ^a		0.297	0.753	0.177
Tumor grade				
High	55	48 (87.3)	37 (67.3)	48 (87.3)
Low	46	12 (26.1)	29 (63.0)	42 (91.3)
<i>P</i> ^a		0.001**	0.656	0.517
Tumor number				
Multifocal	32	16 (50.0)	22 (68.8)	30 (93.8)
Unifocal	69	44 (63.8)	44 (63.8)	60 (87.0)
<i>P</i> ^a		0.190	0.624	0.308
Tumor diameter (cm)				
<3	19	11 (57.9)	16 (84.2)	19 (100.0)
≥3	82	49 (59.8)	50 (61.0)	71 (86.6)
<i>P</i> ^a		0.882	0.055	0.091
Concomitant CIS				
Positive	13	6 (46.2)	6 (46.2)	11 (84.6)
Negative	88	54 (61.4)	60 (68.2)	79 (89.8)
<i>P</i> ^a		0.297	0.119	0.577
Intravesical therapy				
Positive	68	39 (57.4)	42 (61.8)	60 (88.2)
Negative	33	21 (63.6)	24 (72.7)	30 (90.9)
<i>P</i> ^a		0.546	0.278	0.686

^aPearson's Chi-square test, ***P*<0.01**Table 3: Association of clinicopathologic features with recurrence and progression**

	<i>n</i>	Recurrence	Progression	Mortality
Gender				
Female	13	4 (30.8)	2 (15.4)	2 (18.2)
Male	88	48 (54.5)	22 (25.0)	15 (20.0)
<i>P</i> ^a		0.109	0.447	1.000b
Tumor grade				
High	55	34 (61.8)	20 (36.4)	13 (29.5)
Low	46	18 (39.1)	4 (8.7)	4 (9.5)
<i>P</i> ^a		0.023*	0.001**	0.020*
Multifocality				
Positive	32	14 (43.8)	7 (21.9)	3 (10.7)
Negative	69	38 (55.1)	17 (24.6)	14 (24.1)
<i>P</i> ^a		0.289	0.762	0.143
Tumor diameter (cm)				
<3	19	6 (31.6)	3 (15.8)	3 (20.0)
≥3	82	46 (56.1)	21 (25.6)	14 (19.7)
<i>P</i> ^a		0.054	0.365	1.000b
Concomitant CIS				
Positive	13	8 (61.5)	3 (23.1)	1 (11.1)
Negative	88	44 (50.0)	21 (23.9)	16 (20.8)
<i>P</i> ^a		0.437	0.950	0.681b
Intravesical therapy				
Positive	68	37 (54.4)	13 (19.1)	9 (15.0)
Negative	33	15 (45.5)	11 (33.3)	8 (30.8)
<i>P</i> ^a		0.398	0.115	0.092
Ki-67 expression				
<15	41	16 (39.0)	4 (9.8)	2 (5.7)
≥15	60	36 (60.0)	20 (33.3)	15 (29.4)
<i>P</i> ^a		0.038*	0.006**	0.007**
Profilin 1 stromal expression				
Negative	35	19 (54.3)	8 (22.9)	6 (19.4)
Positive	66	33 (50.0)	16 (24.2)	11 (20.0)
<i>P</i> ^a		0.682	0.876	0.942
Profilin 1 epithelial expression				
Negative	11	5 (45.5)	1 (9.1)	0 (0)
Positive	90	47 (52.2)	23 (25.6)	17 (22.4)
<i>P</i> ^a		0.672	0.226	0.200b

^aPearson's Chi-square test, ^bFisher's exact test, **P*<0.05, ***P*<0.01. CIS=Carcinoma *in situ*

regression analysis of various clinicopathologic parameters, Ki-67 expression (≥ 15) was significant independent predictor of worse outcome for recurrence (odds ratio (OR): 2.307; 95% confidence interval [CI], 1.268–4.196; $P = 0.006$) and mortality (ODDS: 5.699; 95% CI, 1.282–25.339; $P = 0.022$). The only significant independent parameter for progression was tumor grade in multivariate analysis (ODDS: 5.331; 95% CI, 1.813–15.674; $P = 0.002$) [Table 4].

Association of Ki-67 and profilin 1 expression with recurrence-free survival

Ki-67 expression was significantly related to recurrence-free survival (RFS), but no statistically significant relationship was found between either stromal or epithelial profilin 1 expression and RFS ($P = 0.003$, $P = 0.740$, and $P = 0.892$, respectively). The mean RFS was 39.52 ± 4.75 months in the Ki-67-positive group and 65.41 ± 5.33 months in the Ki-67-negative group [Figure 3].

Association of Ki-67 and profilin 1 expression with progression-free survival

Ki-67 expression was significantly related to progression-free survival (PFS), but no statistically significant relationship was found between either stromal or epithelial profilin 1 expression and PFS ($P = 0.002$, $P = 0.836$, and $P = 0.281$, respectively). The mean PFS was 62.71 ± 4.81 months in the Ki-67-positive group and 88.90 ± 3.43 months in the Ki-67-negative group [Figure 4].

Association of Ki-67 and profilin 1 expression with cancer-specific survival

Ki-67 expression was significantly related to cancer-specific survival (CSS), but no statistically significant relationship was found between either stromal or epithelial profilin 1 expression and CSS ($P = 0.003$, $P = 0.968$, and $P = 0.968$,

respectively). The mean CSS was 67.77 ± 4.55 months in the Ki-67-positive group and 88.29 ± 2.58 months in the Ki-67-negative group [Figure 5].

DISCUSSION

The gold standard diagnostic method for bladder cancer is histologic H and E staining and immunohistochemical biomarker examination following TUR-BT. Prognostic biomarkers provide information about patient outcomes independently of treatment, and they may be useful in identifying patients who may benefit from radical therapies. The high rates of prevalence, recurrence, and progression in nonmuscle-invasive bladder cancer (NMIBC), and especially the uncertain clinical course of pT1 bladder cancer, make the search for new biomarkers important. Deferred cystectomy results in a poor oncologic outcome in tumors with a poor prognosis, whereas early cystectomy results in overtreatment in tumors with a favorable prognosis.^[9] Theoretically, a biomarker that can be measured during follow-up can reduce the number of cystoscopies performed, can predict recurrence and progression more accurately than conventional tests, and can determine the need for early radical cystectomy.^[10] The European Organization for Research and Treatment of Cancer scoring system is used to identify patient risk level for recurrence and progression of bladder cancer, but this system seems inadequate to predict the prognosis of pT1 tumors; therefore, additional molecular markers are needed.^[7]

Many proliferation markers are used to assess prognosis in UBC, and one of the most well-studied is Ki-67. Ki-67 can be detected by the monoclonal antibodies-1 antibody in the G1, S, and G2 phases of mitosis and has been investigated in several studies related to the prognosis of UBC.^[4] The relationship between Ki-67 and UBC was first described in

Table 4: Univariate and multivariate Cox regression analysis of clinicopathologic features in relation to recurrence, progression, and mortality

	Univariate analysis				Multivariate logistic regression analysis			
	P	OR	95% CI		P	OR	95% CI	
			Lower	Upper			Lower	Upper
Recurrence								
Gender (male)	0.178	2.018	0.726	5.604				
Tumor grade (high)	0.012*	2.097	1.180	3.726				
Tumor size (≥ 3 cm)	0.086	2.111	0.899	4.955	0.098	2.053	0.875	4.819
Ki-67 expression (≥ 15)	0.005**	2.341	1.286	4.261	0.006**	2.307	1.268	4.196
Progression								
Age (years)	0.067	1.040	0.997	1.085				
Tumor grade (high)	0.002**	5.331	1.813	15.674	0.002**	5.331	1.813	15.674
Intravesical treatment (none)	0.090	2.007	0.898	4.486				
Ki-67 expression (≥ 15)	0.006**	4.647	1.564	13.809				
Profilin 1 epithelial expression (+)	0.305	2.853	0.385	21.156				
Mortality								
Age (years)	0.025*	1.058	1.007	1.112	0.081	1.046	0.994	1.100
Tumor grade (high)	0.019*	3.827	1.244	11.772				
Multifocality (no)	0.118	2.711	0.777	9.467				
Intravesical treatment (none)	0.066	2.442	0.941	6.333				
Ki-67 expression (≥ 15)	0.011*	6.839	1.552	30.134	0.022*	5.699	1.282	25.339

* $P < 0.05$, ** $P < 0.01$. CI=Confidence interval

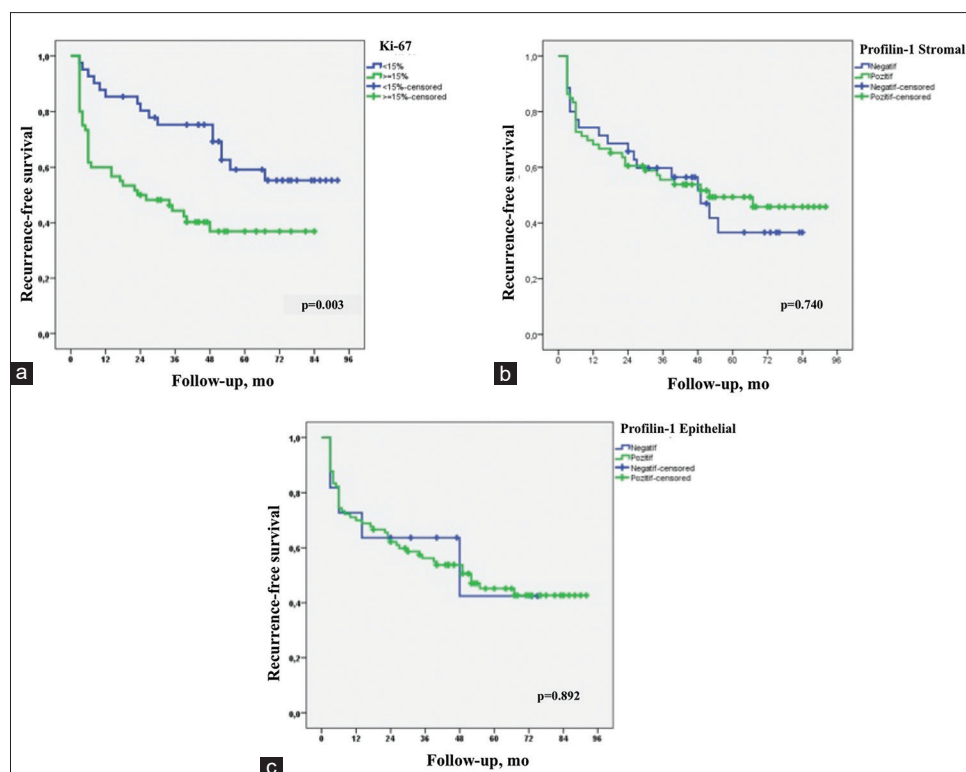


Figure 3: Kaplan–Meyer analysis of recurrence-free survival regarding Ki-67 and profilin 1 stromal and epithelial expressions. (a) Ki-67 $\geq 15\%$ is statistically significantly correlated with worse recurrence-free survival ($P = 0.003$). (b) There is no statistically significant correlation between profilin 1 stromal expressions and recurrence-free survival ($P = 0.740$). (c) There is no statistically significant correlation between profilin 1 epithelial expressions and recurrence-free survival ($P = 0.892$)

the English literature in 1990, when an increase in the Ki-67 index was reported in 55 UBC tissues. Okamura *et al.* found that this increase correlated with tumor grade and stage and suggested that these findings could be useful for deciding treatment methods.^[11] Similar studies in the following years have shown that an increase in the Ki-67 index is related to tumor histological grade and stage, and it has been reported that the Ki-67 index is increased in histologically high-grade and advanced tumors.^[12–15]

In fact, the most important subject of investigation about Ki-67 in UBC is its relationship with prognosis. Many studies investigating the relationship between recurrence and Ki-67 expression have demonstrated a statistically significant association with recurrence at different Ki-67 index thresholds.^[14,16,17] However, data regarding the relationship of Ki-67 expression and progression remain more controversial. Some studies have reported that a high Ki-67 index was associated with recurrence, but not statistically associated with progression.^[16,17] Contrary to these findings, Wang *et al.* reported that a high Ki-67 index was statistically associated with both recurrence and progression.^[15] We also found in our study that a Ki-67 index $\geq 15\%$ was statistically associated with both recurrence and progression in univariate analysis but in multivariate analysis not statistically associated with

progression. However, it is important to keep in mind that, contrary to the abovementioned studies, only pT1 UBC patients are included in our study.

A total of 5147 UBC patients were studied in a systematic review and meta-analysis in 2016, and this study concluded that high Ki-67 expression was significantly associated with shorter RFS (hazard ratios [HR]: 1.69, 95% CI: 1.33–2.14), PFS (HR: 1.89, 95% CI: 1.43–2.51), and CSS (HR: 1.69, 95% CI: 1.47–1.95).^[18] However, in the subgroup analyses performed, these data were stated to be valid only for European-American patients.

A fairly limited number of studies exist that, like our study, included only pT1 UBC patients. First, in 2005, a study by Krüger *et al.* that included 73 patients with pT1 UBC reported that Ki-67 (with a threshold value of 10%) was an independent predictor of tumor recurrence.^[19] Similar to our study, Bertz *et al.* investigated Ki-67 associations in pT1 UBC with a Ki-67 index threshold of 15% and reported that high Ki-67 expression was associated with PFS ($P = 0.002$) and CSS ($P < 0.001$), but not RFS ($P = 0.095$).^[8] In our study, we found that Ki-67 was significantly associated with PFS, CSS, and RFS.

Profilin 1 is an actin-binding protein found in the cytosol, and its downregulation is thought to be associated with breast,

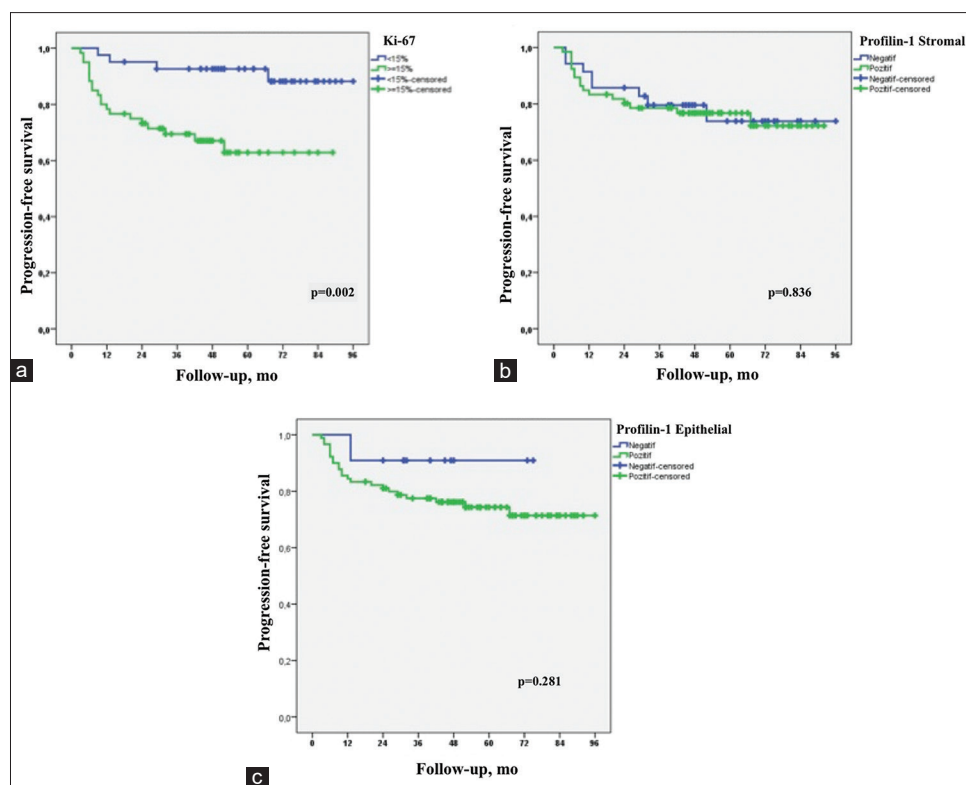


Figure 4: Kaplan–Meyer analysis of progression-free survival regarding Ki-67 and profilin 1 stromal and epithelial expressions. (a) Ki-67 $\geq 15\%$ is statistically significantly correlated with worse progression-free survival ($P = 0.002$). (b) There is no statistically significant correlation between profilin 1 stromal expressions and progression-free survival ($P = 0.836$). (c) There is no statistically significant correlation between profilin 1 epithelial expressions and progression-free survival ($P = 0.281$)

bladder, pancreatic, and renal cancer.^[20] In the first and only clinical study investigating the relationship between UBC and profilin 1, Zoidakis *et al.* investigated the relationship between immunohistological expression patterns of profilin 1 and UBC aggressiveness in 92 nonmuscle-invasive and 102 muscle-invasive UBC patients. This study, in which the stromal and cytological expression of profilin 1 was evaluated separately, reported that invasive tumors had a significant loss of epithelial cytological expression compared to T1G3 tumors and that this pattern was found to be associated with poor prognosis and increased mortality. Stromal expression of profilin 1 was found to be associated with tumor size ($P = 0.012$), recurrence ($P = 0.032$), and poor overall survival ($P = 0.005$) in the NMIBC group. In the MIBC group, stromal expression of profilin 1 was associated with metastases ($P = 0.040$), and the loss of epithelial cytosolic expression of profilin 1 was associated with poor cancer-specific ($P = 0.014$) and overall survival ($P = 0.016$).^[5]

However, contrary to the sole previously published study in the literature, no evidence of an association between the immunohistochemical expression of profilin 1 and tumor characteristics or prognosis in primary pT1 UBC patients was found in our study. We also performed a statistical analysis for low expression (score 0 and 1) versus high expression (score 2 and 3), but could not find any statistically significance

in survival parameters. The difference between the two studies may be because only pT1 patients are included in our study; however, large-scale studies are clearly needed to improve clarity regarding the association of profilin 1 expression and UBC prognosis.

The most important limitations of our study are the retrospective nature of the study and the limited number of patients included. In addition, the tumor distribution in the blocks prepared for immunohistochemical examination does not represent the whole tumor. The single-center nature of the study can also be seen as a limitation. The unfortunate inability of all of our patients to receive intravesical BCG treatment due to a global BCG shortage can be considered as a limitation, but we believe that this inconsistency did not affect our results; no difference in the expression of Ki-67 and profilin 1 was observed between the BCG-treated and non-BCG-treated groups. Finally, we have an unexpected high percentage of low-grade pT1 cases, but we reevaluated the pathology specimens, but the results did not change. This can be an another limitation.

CONCLUSIONS

Ki-67 is a highly predictive biomarker of tumor histologic grade, recurrence, RFS, PFS, and CSS in pT1 UBC patients, in

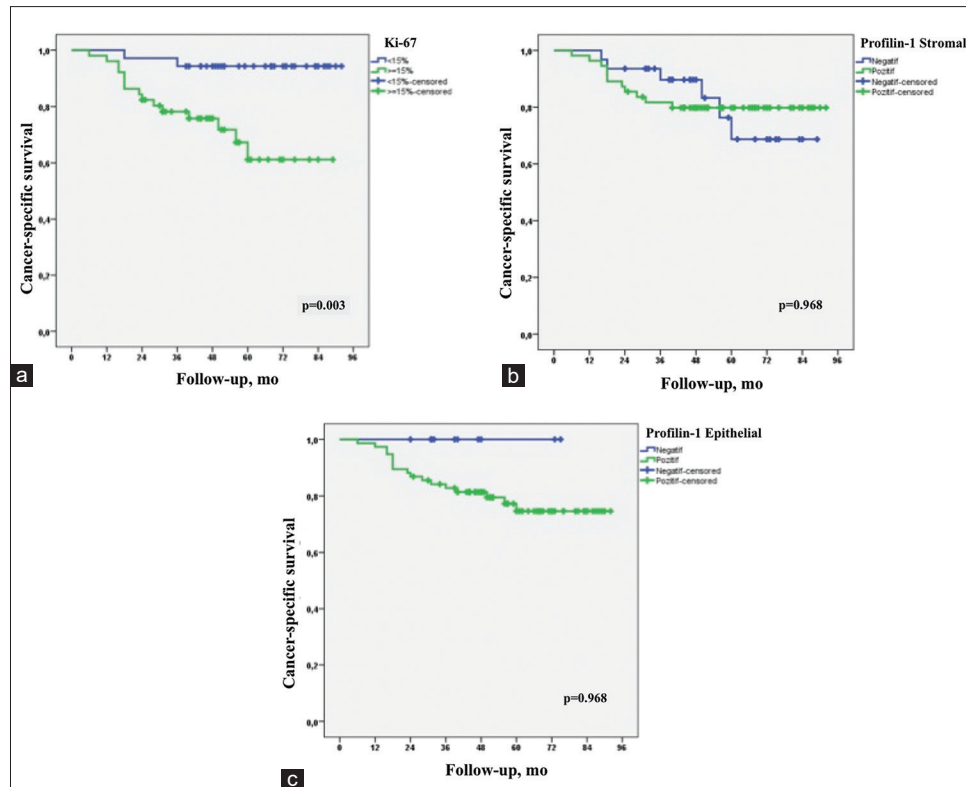


Figure 5: Kaplan–Meyer analysis of cancer-specific survival regarding Ki-67 and profilin 1 stromal and epithelial expressions. (a) Ki-67 $\geq 15\%$ is statistically significantly correlated with worse cancer-specific survival ($P = 0.003$). (b) There is no statistically significant correlation between profilin 1 stromal expressions and cancer-specific survival ($P = 0.968$). (c) There is no statistically significant correlation between profilin 1 epithelial expressions and cancer-specific survival ($P = 0.968$)

whom prediction of prognosis is very difficult. Therefore, Ki-67 expression can be safely combined with other prognostic factors. However, no significant relationship was found between profilin 1 immunohistochemical expressions and tumor characteristics, recurrence, progression, and survival in pT1 bladder cancer patients. The existence of previous publications that present contradictory findings suggests that further studies are needed regarding the suitability of profilin 1 as a potential biomarker in UBC.

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Conflicts of interest

There are no conflicts of interest.

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