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Original contribution

Mitotic activity in noninvasive papillary urothelial carcinoma: its value in predicting tumor recurrence and comparison with the contemporary 2-tier grading system ☆

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Summary Measures of mitotic activity predict behavior of noninvasive papillary urothelial carcinoma of the urinary bladder, but it is unclear what role these should have in tumor grading. In this article, we compare measures of mitotic activity to contemporary tumor grading, specifically in their association with recurrence of noninvasive papillary urothelial carcinoma. The study uses a retrospective cohort of 199 tumors from 124 patients. Mitotic activity was treated as a categorical variable (mitotic-inert, mitotic-low, or mitotic-high). Evaluating only first-occurrence tumors, recurrence was more frequent in mitotic-high (hazard ratio [HR], 8.8; $P < .0001$, Cox model) and mitotic-low tumors (HR, 3.7; $P = .017$) compared with mitotic-inert tumors, when controlling for treatment with intravesical bacillus Calmette-Guerin, age, and sex. Recurrence was likewise more frequent in high-grade tumors (HR, 3.1; $P = .00019$, Cox model) compared with low-grade tumors, controlling for these factors. However, mitotic group, but not tumor grade, was significantly associated with recurrence in a multivariate Cox model including mitotic group, tumor grade, and treatment status (HR, 6.5 [$P = .0025$] for mitotic-high versus reference; HR, 3.7 [$P = .018$] for mitotic-low versus reference). Frailty models including both first-occurrence and recurrent tumors showed similar results. Isolating the analysis to first occurrence, low-grade tumors, recurrence was more frequent in mitotic-high (HR, 6.8; $P = .0044$, Cox model) and mitotic-low (HR, 3.4; $P = .027$) tumors compared with mitotic-inert

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tumors. The findings indicate that mitotic activity is associated with behavior of noninvasive papillary urothelial carcinoma and may be valuable as an adjunct to the contemporary grading system.

1. Introduction

The annual incidence rate of bladder cancer is 20 cases per 100 000 people in the United States [1]. Most new diagnoses are noninvasive papillary urothelial carcinoma (NI-PUC) [2]. These noninvasive cancers do not metastasize but often recur. Treatment is thus conservative in most cases, typically transurethral resection of cystoscopically visible tumor. However, a subset of NI-PUC progress to muscle-invasive bladder cancer, which may metastasize and has high mortality rates. The risk of progression and recurrence is reduced by installation of intravesical bacillus Calmette-Guerin (BCG) [3]. However, although BCG is an effective adjuvant therapy, it may cause treatment-related morbidity, including bladder pain, voiding dysfunction, and occasionally BCG sepsis [4]. Treatment with BCG is thus reserved for patients at high risk of recurrence and progression to muscle invasion [3]. Specifically, risk of recurrence and progression is gauged based on tumor grade, assigned as either low grade or high grade, the latter being higher risk, and thus typically treated with BCG [5]. Furthermore, patients with high-grade NI-PUC who recur multiple times after treatment with BCG have a high risk of progressing to muscle invasion and dying from the disease [6]. Such patients are candidates for early cystectomy [5], which may reduce risk of death from bladder cancer. In contrast, patients with low-grade NI-PUC who recur have a relatively low risk for progression and are managed conservatively [2]. Clinicians therefore depend heavily on tumor grade to make vital clinical decisions.

Although tumor grade is used to make critical decisions in patients with NI-PUC, reproducibility of the 2-tier grading system is only moderate among pathologists, leading to significant inconsistency in patient care [7]. Much of this irreproducibility stems from the tremendous histologic diversity of NI-PUC, which makes it difficult to draw clear thresholds between high-grade and low-grade tumors.

Several molecular studies have shown cell cycle activity strongly predicts recurrence and progression of NI-PUC [8-14]. Similarly, high Ki-67 labeling index [15,16] and high mitotic index [16-19] are associated with recurrence and progression of NI-PUC. However, per the current World Health Organization (WHO) manual, grade of NI-PUC is determined by the degree of architectural disorder and cytologic atypia [2]. Although mitotic figures are mentioned in these criteria, mitotic count does not feature prominently, and no specific cutoff is presented. Likewise, consensus criteria from the WHO/International Society of Urologic Pathologists state that mitotic figures are frequent in high-grade tumors but infrequent in low-grade tumors, but offer no further guidance for using mitotic activity in tumor grading [20]. No study to our knowledge has systematically evaluated the clinical value of mitotic index in NI-PUC in a well-annotated cohort with long-term follow up, as directly compared with the 2-tier grading system recommended by the WHO. In this article, we describe such a study.

2. Materials and methods

This study was approved by the Human Subjects Protection Office at Penn State College of Medicine (institutional reviewer board). To identify subjects, we performed a natural language search in Copath, the informatics system used by our anatomic pathology department, including specimens from years 2001 to 2016. Only cases of NI-PUC were included. Clinical history was collected by chart review, including time to each recurrence, time to progression to muscle invasive bladder cancer, and treatment with BCG.

Histology slides from all cases were reviewed, first reviewed by a senior pathology resident (M. Z.), then a practicing, fellowship-trained urologic pathologist (J. I. W.). Tumor grade was assigned to each case, per WHO 2004 grading criteria, based on the highest-grade area of the tumor. Neither urothelial papilloma nor papillary urothelial neoplasm of uncertain malignant potential was included. Tumor grades were assigned based on the opinion of a single pathologist (J. I. W.) to provide maximum consistency in tumor grading. In cases with both high-grade and low-grade histology, a cutoff of 5% was used to assign grade: tumors with at least 5% high-grade histology were considered high-grade and those with less than 5% were considered low-grade. Mitotic index was then determined as number of mitotic figures per 10 high-powered fields (HPF), based on the most mitotically active area in the tumor.

In our analysis of recurrence, mitotic activity was used to define a categorical variable ("mitotic group") based on thresholds defined by receiver operating characteristic analysis (see Supplemental Protocol). Thresholds were chosen to create 3 mitotic groups: mitotic-inert (0-1 mitotic figures per 10 HPF), mitotic-low (2-5 mitotic figures per 10 HPF), and mitotic-high (>5 mitotic figures per 10-HPF). Representative microphotographs are presented in Fig. 1.

To evaluate time to tumor recurrence, we performed the analysis on 3 separate sets of tumors from the cohort: (1) first-occurrence tumors only, (2) all tumor samples including both first-occurrence and recurrent tumors (some patients had several recurrences), and (3) low-grade, first-occurrence
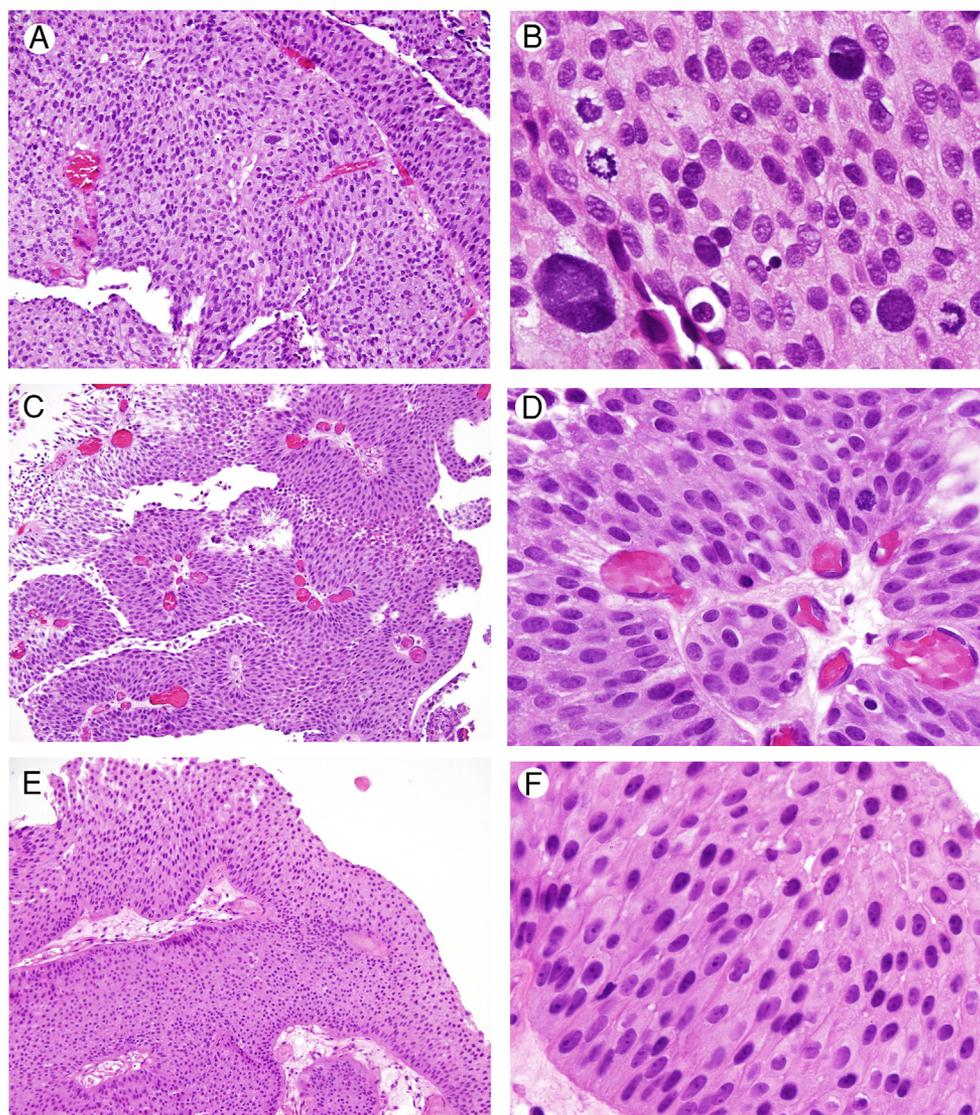
Fig. 1 Histology of NI-PUC. Frankly high-grade carcinoma has a disordered architecture at medium power (A; hematoxylin and eosin [H&E], original magnification ×100) and atypical, hyperchromatic nuclei and multiple mitotic figures at high-power (B; H&E, ×400). Low-grade carcinoma lacks frankly disordered architecture at low power (C; H&E, ×40). In this example, mitotic figures are identified, and there is relatively mild nuclear atypia at high power, keeping with low-grade neoplasia (D; H&E, ×400). Another example of low-grade carcinoma similarly lacks marked architectural disorder at medium power (E; H&E, ×100) and has relatively mild nuclear atypia at high power, but mitotic figures are not identified (F; H&E, ×400).

Table 1 Clinicopathological details, patients, and tumor occurrences

Feature	Value
Patient features (n = 124 patients)	
Age at first occurrence (y), median (range)	68 (34-92)
Recurred, no. (%)	56 (45)
Progressed to muscle invasion or metastasis (%)	5 (4)
Tumor features (n = 199 tumor occurrences)	
High-grade tumors, no. of cases (%)	74 (37)
Mitotic index, median (range)	3 (0-60)
Mitotic group, no. of cases (%)	
Mitotic-high	77 (39)
Mitotic-low	60 (30)
Mitotic-inert	62 (31)
Intravesical BCG, no. (%)	64 (32)

tumors only. Cases with no recurrences were censored at the time of last follow-up. For the analysis of first-occurrence tumors, Kaplan-Meier curves and log-rank tests were used to perform univariate analyses of the time from first occurrence of NI-PUC to first recurrence, if any. Similarly, multivariate Cox proportional hazards models were used to analyze the

Table 2 Mitotic group *vs* 2-tier tumor grade

Mitotic group	High-grade (n = 74)	Low-grade (n = 125)
Mitotic-high	64 (86.5%)	13 (10%)
Mitotic-low	9 (12%)	51 (41%)
Mitotic-inert	1 (1.5%)	61 (49%)

NOTE. Values are number of cases and percent of cases for each grade (total n = 199).
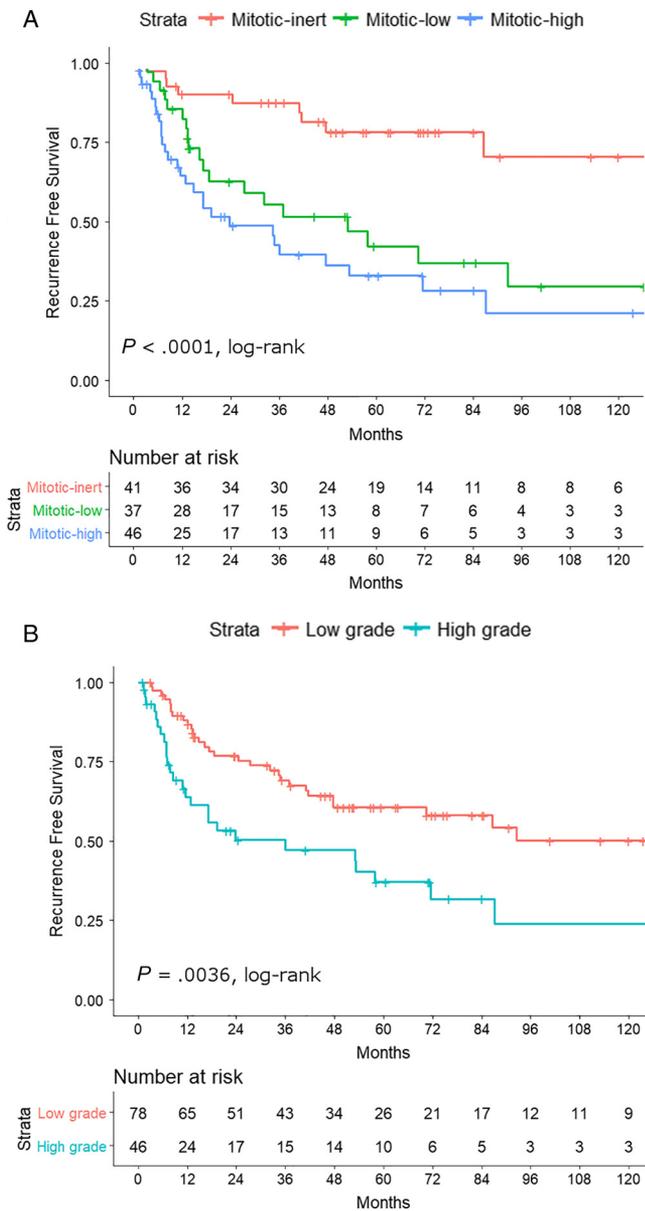
Fig. 2 Kaplan-Meier plots for recurrence-free survival, including all first-occurrence tumors. Lower recurrence-free survival was found in the mitotic-high and mitotic-low groups compared with the mitotic-inert group (A) and in high-grade compared with low-grade tumors (B).

time from first occurrence of NI-PUC to first recurrence, while controlling for patient age, sex, and combinations of treatment (BCG), grade, and mitotic group. For the analysis of all tumors, including recurrent tumors, multivariate frailty models [21] were used to analyze time from first-occurrence to all recurrences while accounting for tumor grade and mitotic group of each tumor occurrence (additional details are provided in the Supplementary Protocol). For the analysis of first-occurrence, low-grade tumors only, we used the log-rank test and Cox proportional hazards models, similar to above, but isolated to low-grade tumors.

We also evaluated time to stage progression using the log-rank test to evaluate time from first occurrence to either invasion of the muscularis propria or distant metastasis. Mitotic group and tumor grade were based on the first tumor occurrence for that patient. Given the small number of cases with progression (5 cases), we did not perform multivariate analysis for this portion the study.

All analyses were performed using R version 3.4.1 or 3.4.3 [22]. Receiver operating characteristic analysis was performed using the pROC package [23]. The survival and survminer packages [24,25] were used to perform all survival analyses.

3. Results

We evaluated 199 urinary bladder transurethral resection samples, all diagnosed as NI-PUC, from 124 patients. Patient and sample data are presented in Table 1. Tumor grade was associated with mitotic group ($P < .001$, Fisher exact test), as shown in Table 2. Isolating the analysis to first-occurrence tumors, recurrence-free survival was lower in mitotic-high and mitotic-low tumors compared with mitotic-inert tumors (Fig. 2A; $P < .0001$, log-rank test). Likewise, recurrence-free survival was lower in high-grade tumors than in low-grade tumors (Fig. 2B; $P = .0036$, log-rank test). Isolating the analysis to first-occurrence, low-grade tumors, recurrence-free survival was lower in mitotic-high and mitotic-low tumors compared with mitotic-inert tumors (Fig. 3; $P = .0020$, log-rank test). There was no difference in recurrence-free survival between high-grade tumors and low-grade tumors, if mitotic-inert tumors were excluded from the latter group (Fig. 4; $P = .5$, log-rank test, first-occurrence tumors only).

On multivariate analysis including only first-occurrence tumors (Table 3), recurrence was greater in mitotic-high and mitotic-low tumors compared with mitotic-inert tumors, when controlling for treatment with intravesical BCG, sex, and age. Similarly, recurrence was greater in high-grade tumors compared with low-grade tumors, when controlling for treatment, sex, and age. However, mitotic group, but not tumor grade, was significantly associated with recurrence in a model including mitotic group, tumor grade, treatment, sex, and age. Frailty models including first-occurrence and recurrent tumors showed similar results (Table 4). Similarly, Harrell concordance index (C index) was 0.64 for the model including tumor grade and BCG, 0.70 for the model including mitotic group and BCG, and 0.71 for the model including grade, mitotic group, and BCG, indicating mitotic group adds predictive information beyond tumor grade.

On multivariate analysis isolated to low-grade, first-occurrence tumors, recurrence was greater in mitotic-high and mitotic-low tumors compared with the mitotic-inert tumors (Table 5), Among low-grade tumors in the first-occurrence group, there was no difference in recurrence between mitotic-low and mitotic-high cancers ($P = .5$, log-rank test).

Of the 124 patients, 5 (4%) progressed to muscle-invasive bladder cancer or distant metastasis. Progression was greater
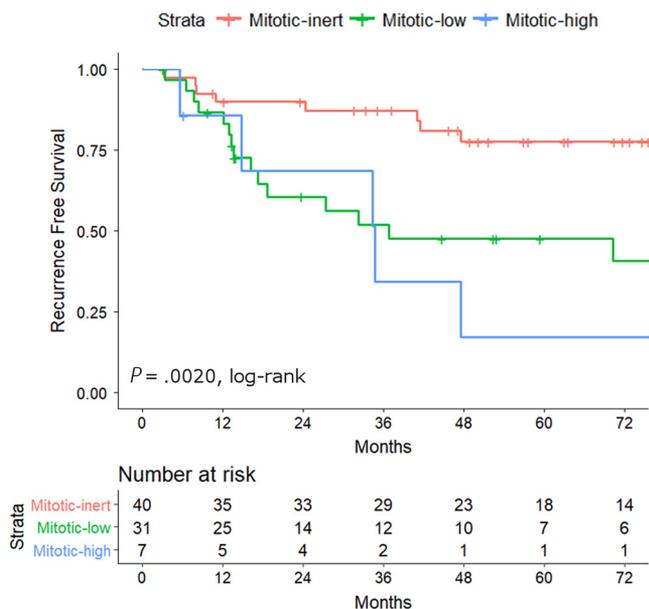
Fig. 3 Kaplan-Meier plots for recurrence-free survival, including only low-grade, first-occurrence tumors. Lower recurrence-free survival was found in the mitotic-high and mitotic-low groups compared with the mitotic-inert group.

in mitotic-high tumors than in mitotic-inert tumors ($P = .029$, log-rank test).

We further evaluated the prognostic value of mitotic groups using cutoffs different from those used above.
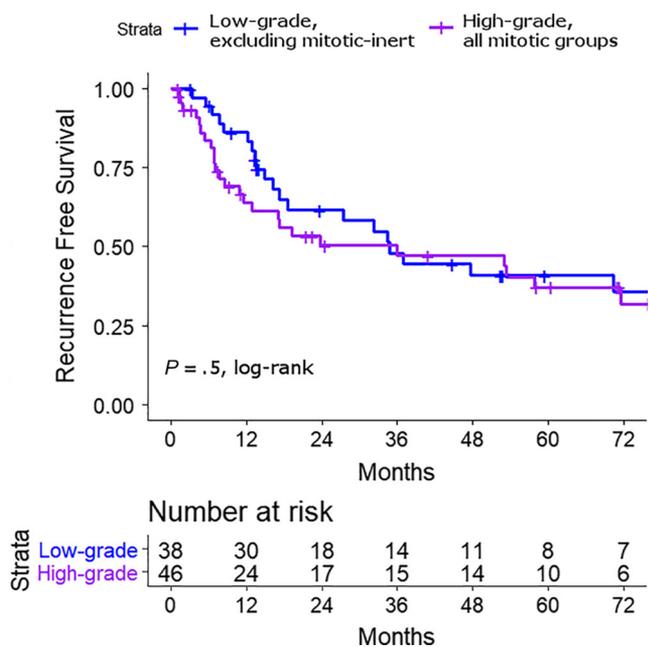
Fig. 4 Kaplan-Meier plots for recurrence-free survival, comparing high-grade tumors with low-grade tumors, in a subgroup analysis excluding all mitotic-inert, low-grade tumors. This analysis only included first-occurrence tumors. The survival curves largely overlap and are not significantly different ($P = .5$, Fisher exact test).

Specifically, we used variations that defined mitotic-inert strictly as zero mitotic figures per 10 HPF and mitotic-high as those with greater than 10 mitotic figures per 10 HPF. The results were similar to the results above. Full results for different cutoffs are presented in Supplementary Table S1.

4. Discussion

The WHO currently recommends that NI-PUC be graded on a 2-tier system as either high-grade or low-grade. In this

Table 3 Risk of recurrence, as shown by Cox proportional hazards models including all first-occurrence NI-PUCs

Variables in model	Hazard ratio (recurrence)	Confidence interval (95%)	P
BCG Status in model alone			
BCG	0.50	0.26-0.96	.037
No BCG	Reference		
Mitotic group + BCG			
Mitotic group			
Mitotic-high	8.01	3.04-21.07	<.0001
Mitotic-low	3.18	1.20-8.43	.020
Mitotic-inert	Reference		
BCG status			
BCG	0.33	0.17-0.66	.0017
No BCG	Reference		
Tumor grade + BCG			
Tumor grade			
High-grade	3.43	1.91-6.15	<.0001
Low-grade	Reference		
BCG status			
BCG	0.30	0.15-0.60	.00067
No BCG	Reference		
Mitotic group + tumor grade + BCG			
Mitotic group			
Mitotic-high	5.53	1.81-16.86	.0027
Mitotic-low	3.13	1.18-8.30	.022
Mitotic-inert	Reference		
Tumor grade			
High-grade	1.76	0.78-3.97	.17
Low-grade	Reference		
BCG status			
BCG	0.29	0.14-0.59	.00063
No BCG	Reference		

NOTE. Four multivariate models are presented here, including various combinations of mitotic group, tumor grade, and BCG treatment. All models also included patient age and sex ($P > .1$ for these in all models). The multivariate model including mitotic group, tumor grade, and BCG status shows that only mitotic group and BCG status were significantly associated with recurrence. n = 124.

system, high-grade tumors are distinguished from low-grade tumors based on severity of cytologic atypia and degree of architectural disorder [2]. Although mitotic figures are mentioned in the criteria—specifically that mitotic figures are numerous in high-grade tumors and may or may not be present in low-grade tumors—precise guidance in the use of mitotic counts is not part of the current grading schema. The contemporary 2-tier system was developed to improve on the suboptimal interobserver reproducibility of the older 3-tier system. Although this change improved diagnostic reproducibility, it did so only marginally [7], and the reproducibility of the 2-tier system remains suboptimal. The consequences of this irreproducibility are not trivial. Patients with high-grade tumors are typically treated with intravesical BCG and become candidates for early cystectomy if the tumor recurs frequently [5], whereas patients with low-grade tumors are not treated with BCG and rarely become candidates for early cystectomy [7]. Given these marked differences in treatment, any improvement to our grading schema could improve the quality and consistency of the care bladder cancer patients receive.

We have shown that measures of mitotic activity predict recurrence of NI-PUC. Prior studies have shown similar results using various methods, including mitotic counts, Ki-67 labeling index, and complex gene signatures of the cell cycle activation [8-19]. However, we have uniquely shown that mitotic group adds predictive information beyond that provided by the contemporary 2-tier system. Specifically, in the current study, only mitotic activity remained statistically significant in multivariate models including grade and measures of mitotic activity. These results by no means imply that the current 2-tier system should be abandoned and replaced by mitotic group. NI-PUC is a biologically diverse disease, with tumors differing dramatically in histomorphology and molecular alterations [13,15,26]. Any grading system must take this diversity into account. Thus, we believe that mitotic activity has greatest value as an adjunct to the 2-tier system. That is, we believe that measures of mitotic activity are valuable in ambiguous cases of NI-PUC, which are not clearly high-grade or low-grade. These cases are common. Given its independent power to predict tumor recurrence, mitotic activity could be a rational marker to decide tumor grade in such equivocal cases. A potential approach to including mitotic group in tumor grade is presented in Table 6. Mitotic group also has the advantage that it is inexpensive and accessible compared with molecular tests and immunohistochemistry. This approach could thus be useful to pathologists around throughout the world, including those in resource-poor environments.

In the present study, mitotic group also predicted recurrence in tumors considered low grade on the 2-tier system, suggesting that mitotic counts could help to stratify recurrence risk in such patients. Indeed, low-grade cases comprised most of mitotic-inert and mitotic-low tumors, and these mitotic groups largely served to split the low-grade tumors into groups

Table 4 Risk of recurrence, as shown by frailty models using all occurrences of NI-PUCs (first-occurrence and recurrent tumors)

Variables in model	Hazard ratio (recurrence)	Confidence interval (95%)	P
BCG Status in model alone			
BCG	0.63	0.30-1.31	.21
No BCG	Reference		
Mitotic group + BCG			
Mitotic group			
Mitotic-high	9.08	3.58-22.98	<.0001
Mitotic-low	2.86	1.17-7.01	.021
Mitotic-inert	Reference		
BCG status			
BCG	0.40	0.19-0.83	.014
No BCG	Reference		
Tumor grade + BCG			
Tumor grade			
High-grade	3.53	1.76-7.06	.00037
Low-grade	Reference		
BCG status			
BCG	0.45	0.22-0.92	.030
No BCG	Reference		
Mitotic group + tumor grade + BCG			
Mitotic group			
Mitotic-high	7.08	2.25-22.31	.00083
Mitotic-low	2.79	1.14-6.87	.025
Mitotic-inert	Reference		
Tumor grade			
High-grade	1.40	0.56-3.52	.47
Low-grade	Reference		
BCG status			
BCG	0.39	0.19-0.81	.012
No BCG	Reference		

NOTE. Four multivariate models are presented here, including variably mitotic group, tumor grade, and BCG status as covariates. All models also included patient age and sex ($P > .1$ for these in all models). The multivariate model including mitotic group, tumor grade, and BCG status shows that only mitotic group and BCG status were significantly associated with recurrence. n = 199.

Table 5 Risk of recurrence, including only low-grade, first-occurrence NI-PUC

Variables in model	Hazard ratio (recurrence)	Confidence interval (95%)	P
Mitotic group + BCG			
Mitotic group			
Mitotic-high	5.30	1.52-18.42	.0087
Mitotic-low	3.01	1.11-8.14	.030
Mitotic-inert	Reference		
BCG status			
BCG	0.40	0.12-1.36	.14
No BCG	Reference		

NOTE. Cox proportional hazards model included mitotic group and BCG status. All models also included patient age and sex ($P > .1$ for these in all models). n = 78.

Table 6 Suggested incorporation of mitotic counts into contemporary 2-tiered grading system

Tumor grade, initial impression	Mitotic group	Impact of mitotic group on tumor grade
Ambiguous	High	High grade is best diagnosis.
	Low	High grade or low grade, depending on other features of tumor.
	Inert	Low grade is best diagnosis.
Low	High	Inconsistent—reconsider grade and diagnose low grade only if other features strongly support the diagnosis; add a comment the tumor is more likely to recur than typical low-grade carcinoma.
	Low	Supportive, provided additional features keep closely with low grade.
	Inert	Strongly supportive
High	High	Strongly supportive
	Low	Supportive, provided additional features keep closely with high grade.
	Inert	Inconsistent, reconsider grade and diagnose high grade only if other features of tumor strongly support the diagnosis.

NOTE. *Initial impression* refers to the pathologist's histomorphologic impression of a tumor's grade, based on WHO criteria. *Mitotic group* is the 3-tier mitotic groups described above. *Impact of mitotic group on tumor grade* indicates a suggested best course of action in response to mitotic group.

at low risk and high risk of recurrence. These finding keeps with earlier studies, which showed that mitotic activity can predict recurrence of low-grade NI-PUC [17,19].

Perhaps most importantly, in the present study, mitotic activity was associated with stage progression. However, our study included only 5 patients who progressed, a number insufficient to compare predictive value of tumor grade to mitotic activity. This is a common challenge in studying NI-PUC, as only a small fraction of cases progress to muscle invasion or metastasis. Nonetheless, these findings keep closely with the biology of NI-PUC, specifically knowledge about the importance of cell cycle activation on behavior of the disease. As such, the findings indicate that mitotic activity has value in supporting a diagnosis of high-grade NI-PUC, particularly as a risk factor for progression to muscle invasion.

A weakness to the approach we propose is potential diagnostic reproducibility. Mitotic index is used to risk-stratify tumors in many organ systems, most notably breast cancer [27]. Despite widespread use, mitotic counts in invasive breast cancer are only moderately reproducible, although composite grade, specifically the Nottingham score, is more reproducible [27]. In addition, the cutoffs used in this study are low and thus could be problematic. However, we analyzed our data using multiple different cutoffs (Supplementary Table S1) and consistently found substantial differences in clinical behavior among different mitotic groups. The optimal cutoffs for mitotic index are thus unclear but we believe achievable. We further believe that the cutoffs presented in this study are reasonable and could be practically applied in the clinical setting.

5. Conclusion

In conclusion, we have shown that mitotic group is associated with recurrence, and possibly stage progression, in NI-PUC and could thus be a useful histologic feature in tumor grading. Incorporation of mitotic figures into current grading criteria stands to improve the prognostic value of tumor grading.

The work described here was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. The study was retrospective and posed no direct risk to subjects. Thus, requirement for signed consent was waved per the Human Subjects Protection Office at Penn State College of Medicine (institutional review board).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.humpath.2018.10.008.

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