Histologic Grading of Noninvasive Papillary Urothelial Tumors

Validation of the 1998 WHO/ISUP System by Immunophenotyping and Follow-up

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Key Words: Bladder; Papillary urothelial tumor; Cytokeratin 20; Ki-67; p53; World Health Organization/International Society of Urological Pathology classification

DOI: 10.1309/0KATYHQBD5XHQB8J

Abstract

Cytokeratin (CK) 20, Ki-67, and p53 were applied to 84 noninvasive papillary urothelial tumors graded by the 1973 World Health Organization (WHO) and 1998 WHO/International Society of Urological Pathology (ISUP) systems. In the WHO/ISUP classification, all benign lesions showed normal CK20 staining and all carcinomas showed abnormal staining. The Ki-67 index was significantly different between benign and malignant lesions (P < .05) and between low- and high-grade carcinomas (P < .001). p53 was negative in all benign lesions, with a significant difference between low- and high-grade carcinomas (P < .001). Tumor recurrence was significantly different between low- and high-grade carcinomas (no recurrences among the papillary urothelial neoplasms of low malignant potential). By the 1973 WHO classification, normal CK20 staining was present both in benign lesions and in carcinomas. Ki-67 staining did not distinguish between grade 2 and grade 3 carcinomas (P > .05), and there was no difference in p53 staining in grades 1 and 2 carcinomas (P > .05). Recurrences were not different between grades 1, 2, and 3 carcinomas. All biologic markers studied and tumor recurrences were significantly different among papillary lesions classified by the WHO/ISUP system but not by the 1973 WHO system, validating the predictive value of the WHO/ISUP system and providing objective markers for the grading of papillary urothelial tumors.

Tumor stage is the best predictor of clinical outcome in urothelial neoplasms; however, the prognosis of patients with noninvasive papillary urothelial tumors is influenced largely by the pathologic grade of the initial tumor. Grading systems proposed for papillary carcinomas of the bladder include 2-, 3-, 4-, and 5-tiered systems. Complex systems can provide the most data but might be less reproducible and difficult to implement in routine practice. Simple systems with fewer categories are the easiest to learn and use but convey less information. The best grading system should be not only easy to apply but also able to divide tumors into groups with different biologic characteristics that correlate with different clinical outcomes.

A number of classification systems for the grading of papillary urothelial neoplasms of the bladder have been introduced, but despite the absence of detailed defining criteria, the 1973 World Health Organization (WHO) classification has remained widely used. In 1998, the WHO/International Society of Urological Pathology (ISUP) consensus classification was developed in an effort to reach a universally acceptable system. Noninvasive papillary urothelial tumors essentially were divided into 4 groups, namely, papilloma, papillary urothelial neoplasm of low malignant potential (LMP), low-grade carcinoma (LGC), and high-grade (HGC) carcinoma (Table 1). However, several authors have argued that the prognostic value of this classification is limited, reproducibility is worse than the widely used 1973 WHO system, and the new entity, LMP, showed increased risk of local recurrence, progression, and death due to the tumor.

One method used by pathologists to test the validity of a classification system is to compare tumor recurrence and progression or survival of patients between the different...
prognostic groups. Papillary urothelial tumors tend to recur. Some tumors recur within a short period, and others recur after a long period. Recurrences of papilloma have been reported in as many as 50% of cases if follow-up is long enough. As such, long-term follow-up for recurrences does not differentiate different prognostic groups. It would be predictable for high-grade tumors mostly to recur early and low-grade tumors to recur late. In the present study, we adopted this premise and set the cutoff at 36 months for comparison of recurrent rates between the different tumor grades.

In normal urothelium, the expression of cytokeratin (CK) 20 is related to differentiation and is limited to superficial and occasional intermediate cells. Abnormalities of urothelial differentiation are accompanied by loss of this restriction so that the immunexpression of CK20 is seen in deeper cell layers or in all layers.12,13 Tumors with normal CK20 staining have shown no recurrences, whereas those with abnormal staining developed recurrences,14 suggesting that changes in CK20 expression might be useful for predicting behavior.15

Ki-67 is a nuclear protein that is present during the G1, S, G2, and M phases of cycling cells. Previous studies have demonstrated a significant correlation between the Ki-67 index and tumor grade and stage of urinary bladder cancer.16-19

Mutations of the p53 gene have been found in a wide variety of malignant neoplasms, including urothelial carcinomas,17-22 and have been shown to be an independent predictor of survival, progression, and development of metastasis in bladder cancer.23

In the present study, we correlated the immunexpression of these biologic markers with recurrence of noninvasive papillary urothelial tumors graded by the 1973 WHO and the 1998 WHO/ISUP systems to identify which of the systems was a better predictor of tumor behavior and whether immunophenotyping can aid grading.

### Materials and Methods

Biopsy specimens from 84 patients with a first noninvasive papillary urothelial tumor (pTa) of the bladder between January 1, 1995, and May 30, 2000, were retrieved from the files of the Division of Anatomical Pathology, Hunter Area Pathology Service, Newcastle, Australia. These cases all were diagnosed previously according to the 1973 WHO classification. Cases with invasion (either lamina propria or muscularis propria) or concurrent carcinoma in situ were not included, nor were adenocarcinomas or other variants of urothelial neoplasms. The cases in which the WHO grade straddled 2 grades were assigned to the higher grade. All histologic slides were reviewed and classified independently by both of us, without knowledge of previous grading results or clinical outcome, according to the 1998 WHO/ISUP classification of urothelial neoplasms (Table 1). When our grading disagreed, we reexamined the slides and reached agreement.
consensus. Treatment of the primary tumor consisted of transurethral resection in all cases. Cases treated with other methods were not included.

Follow-up

Patient records were examined for follow-up information, which included cystoscopy and biopsy findings and urine cytologic findings. All patients had a minimum of 36 months of follow-up. The study endpoint was recurrence before 36 months from diagnosis of the primary tumor. Cases with recurrence within 4 months of the primary diagnosis were excluded because of the possibility of residual primary tumor rather than true recurrence. Recurrences were graded according to the 1998 WHO/ISUP classification system. Statistical analysis of follow-up data was performed by using the Fisher exact test.

Immunohistologic Examination

All cases were stained for CK20 (dilution 1:25; DAKO, Copenhagen, Denmark), Ki-67 (dilution 1:50; BioGenex, San Ramon, CA), and p53 (dilution 1:200; BioGenex). A standard streptavidin-biotin complex method was used.24

Briefly, representative 5-µm sections from each case were deparaffinized and rehydrated in graded alcohols. All sections were subjected to microwave superheating antigen retrieval at 120°C, except for p53, which was subjected to retrieval at 98°C, in a 10-mmol/L concentration of citrate buffer, pH 6.0, for 10 minutes. Endogenous peroxidase was blocked with 3% hydrogen peroxide in methanol for 5 minutes, followed by incubation in 5% normal goat serum for 20 minutes. Phosphate-buffered saline was used in all washing steps. All incubations were performed at 22°C, except for the primary antibodies, which were incubated at 4°C. Primary antibodies were applied to sections overnight, followed by incubation with goat antimouse antibody (dilution 1:200; DAKO) for 30 minutes and incubation with streptavidin horseradish peroxidase (dilution 1:500; DAKO) for another 30 minutes. The sections were developed in diaminobenzidine (Sigma, St Louis, MO) for 5 minutes and counterstained lightly in Mayer hematoxylin.

Negative control studies were performed by replacing primary antibodies with phosphate-buffered saline. Nonlesional bladder urothelium and normal colon tissue samples were used as positive control samples for CK20 and Ki-67. A breast cancer tissue sample was used as a positive control sample for p53.

Evaluation of Immunostains

The pattern of CK20 expression was recorded as normal when staining was restricted to superficial cells or in single, scattered intermediate cells. Staining was regarded as abnormal when immunoexpression extended to deeper layers as clusters of more than 3 positively stained cells or when there was diffuse staining of the urothelium. For assessment of Ki-67 and p53, 500 tumor cells from the most immunoreactive regions of the section were counted, and the percentage of positive cells was recorded as an index. Cases showing fewer than 5% positive cells were considered negative. Areas with false-positive staining from diathermy were avoided. Statistical analysis of the staining results was performed by using the Tukey-Kramer multiple comparisons test.

Results

The 84 papillary urothelial tumors originally diagnosed by the 1973 WHO and regraded by the 1998 WHO/ISUP systems are shown in Table 2. Examples of each grade according to the 2 systems are shown in Image 1.

Follow-up

Recurrences within 36 months from the diagnosis of primary tumors occurred in 2 (17%) of 12 LMP cases, 24 (45%) of 53 LGCs, and 14 (74%) of 19 HGCs. The differences between LMP cases and LGCs and between LGCs and HGCs were significant (P < .05). Recurrences for tumors in the 1973 WHO system were as follows: papilloma, 0 (0%); grade 1, 13 (41%) of 32; grade 2, 25 (54%) of 46; and grade 3, 2 (67%) of 3; the differences were not significant.

Table 2

<table>
<thead>
<tr>
<th>1998 WHO/ISUP</th>
<th>Papilloma</th>
<th>Low Malignant Potential</th>
<th>Low-Grade</th>
<th>High-Grade</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Papilloma</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>3 (4)</td>
</tr>
<tr>
<td>Grade 1</td>
<td>0</td>
<td>8</td>
<td>21</td>
<td>3</td>
<td>32 (38)</td>
</tr>
<tr>
<td>Grade 2</td>
<td>0</td>
<td>3</td>
<td>30</td>
<td>13</td>
<td>46 (55)</td>
</tr>
<tr>
<td>Grade 3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>3 (4)</td>
</tr>
<tr>
<td>Total</td>
<td>0</td>
<td>12 (14)</td>
<td>53 (63)</td>
<td>19 (23)</td>
<td>84 (100)</td>
</tr>
</tbody>
</table>

ISUP, International Society of Urological Pathology; WHO, World Health Organization.
* Numbers in parentheses are percentages.
Immunohistologic Findings

Except for 1 LMP case that did not stain at all for CK20, all LMP cases showed normal CK20 immunoreactivity. Three LGCs failed to stain or stained weakly, but all remaining carcinomas displayed abnormal staining with stronger and more diffuse staining in HGC than in LGC. About two thirds of LGCs (34/53 [64%]) and all HGCs (19/19 [100%]) showed a diffuse staining pattern Image 2i.

The remaining LGCs displayed focal staining in the deep epithelium. In contrast, a normal staining pattern was seen in 7 (22%) of 32 grade 1 carcinomas, 3 (7%) of 46 grade 2 carcinomas, and 0 (0%) of 3 grade 3 carcinomas.

The Ki-67 proliferative index was less than 10% in LMP cases (range, 0%-10%; mean, 5.67%). LGCs had a significantly higher proliferative index than LMP cases (range, 5%-60%; mean, 22.76%; \( P < .001 \)) with 70% showing an index...
of more than 10%, mostly 15% to 40%. The Ki-67 indices of the HGCs were significantly higher than those of the LGCs, with 13 (68%) of 19 having an index of more than 40% [Image 3] and [Figure 1].

p53 was not immunoexpressed in LMP cases. Only 5 cases of LGC showed a p53 index of more than 10%, and in 42 (79%) of 53 cases, the p53 index was less than 5% and deemed negative. All but 2 cases of HGC immunoexpressed p53, with 14 (74%) of 19 with an index of more than 10%; the difference between LGCs and HGCs was significant \( (P < .001) \) [Figure 2]. There was no significant difference between LGCs and LMP cases \( (P > .05) \) [Figure 2].

**Discussion**

The 1998 WHO/ISUP classification provided refined criteria for papillary urothelial tumors and carcinoma in situ and introduced the new categories of LMP, LGC, and HGC. However, translation between this classification and the widely used 1973 WHO classification has been applied in several different ways, resulting in confusion regarding prognostication and treatment.

It has been stated that the 1973 WHO grade 1, 2, and 3 carcinoma should be replaced by the 1998 WHO/ISUP papillary urothelial neoplasm of low malignant potential, low grade urothelial carcinoma, and high grade urothelial carcinoma, respectively.9,11 In a widely used textbook, it was stated “tumors previously called papillary carcinoma, grade 1, in the present WHO classification are now called papillary neoplasms of LMP.”25 Others have advocated that grade 1 comprises LMP and LGC, and grades 2 and 3 are combined into HGC.26 We concur with William Murphy’s concept that grade 1 consists of LMP and LGC, grade 2 of LGC and HGC,
and grade 3 of HGC (W.M. Murphy, verbal communication, October 2000).

The main advantage of the 1998 WHO/ISUP classification is that detailed histologic criteria have been delineated for each entity, but the prognostic relevance of the various grades remains to be proven. As in a recent study, we did not use the 1999 WHO classification because this “system is not in widespread use and not familiar to most pathologists.”

We have not assessed the reproducibility of the WHO/ISUP grading system, although we experienced some difficulty differentiating LMP from LGC. We found that nuclear clustering is an important feature to differentiate LMP from LGC. Nuclei are spaced evenly in the epithelium of LMP when viewed with the low-power objective (4x). In carcinomas, architectural variation and cytologic features are distinguishing hallmarks. In addition, even in LGCs, nuclear clustering is a distinctive feature at scanning magnification (4x objective).

The short-term recurrence rate among our cases was significantly different among LMP cases, LGCs, and HGCs, providing support that these WHO/ISUP groups are biologically different. Although long-term follow-up for LMP cases has shown higher recurrence in other studies, the majority of the recurrences reported were of similar histologic grade and might represent multifocality rather than true recurrences. One study reported that patients with LMP had a high risk of local recurrence, progression, and even death due to bladder tumors. In that study, simple substitution of grade 1 carcinoma for LMP was used, and that substitution might have accounted for some of the recurrences and progression in the latter.

With the exception of 1 LMP that did not stain, all of our cases of LMP (11/12 [92%]) displayed normal CK20 immunoreactivity. In contrast, all carcinomas that showed stainable CK20, irrespective of low- or high-grade status,
showed abnormal CK20 immunoexpression. A number of studies confirm the usefulness of CK20 immunoexpression as a predictor of recurrence in pTa urothelial tumors, with nonrecurrence rates varying from 45% to 100% in the presence of normal CK20 immunoexpression.\textsuperscript{12-15,28} While 2 of these studies also showed abnormal CK20 staining in a relatively high percentage of LMP cases, as well as normal staining patterns in LGCs and HGCs,\textsuperscript{15,28} they may well reflect the differences in translation from the 1973 WHO to the 1998 WHO/ISUP system.

Another recent study using CK20 and 34\textbeta E12, the latter for basal urothelial cells, found that abnormal immunoexpression patterns for these antigens was a strong predictor of recurrence in low-grade papillary bladder tumors.\textsuperscript{29} In our hands, CK20 proved to be not only a predictor of recurrence but also a useful marker to separate LMP cases from carcinomas, particularly LGCs. Careful optimization of the immunostaining is essential,\textsuperscript{24} and failure to do so might account for some of the contradictory results previously reported. The capricious nature of antigen retention was demonstrated in 3 of our cases of LGC.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Mean Ki-67 index in lesions graded by 1973 World Health Organization (WHO) (A) and 1998 WHO/International Society of Urological Pathology systems (B). Error bars indicate SD. A, *P < .001. †P > .05. B, *P < .001. †P < .001. G1, grade 1 carcinoma; G2, grade 2 carcinoma; G3, grade 3 carcinoma; HGC, high-grade carcinoma; LGC, low-grade carcinoma; LMP, papillary urothelial neoplasm of low malignant potential; N, normal urothelium; Pap, papilloma.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{Mean p53 index in lesions graded by 1973 World Health Organization (WHO) (A) and 1998 WHO/International Society of Urological Pathology systems (B). Error bars indicate SD. A, *P > .05. †P < .001. B, *P < .001. G1, grade 1 carcinoma; G2, grade 2 carcinoma; G3, grade 3 carcinoma; HGC, high-grade carcinoma; LGC, low-grade carcinoma; LMP, papillary urothelial neoplasm of low malignant potential; N, normal urothelium; Pap, papilloma.}
\end{figure}
that failed to stain for CK20 or stained only weakly because of diathermy effect in small biopsy specimens.

Cina et al\(^{19}\) noted that Ki-67 had good discriminatory power between LMP cases and carcinoma, with a Ki-67 index of more than 10% seen only in carcinomas. There also was significant difference between the 2 grades of carcinoma. In a study involving LMP and grade I tumors, Pich et al\(^{22}\) concluded that proliferative activity was the most significant predictor of recurrence. Our study also revealed that LMP, LGC, and HGC showed significantly higher Ki-67 indices, corresponding to increasing tumor grade. However, nearly one third of LGCs had low proliferative indices similar to the LMP cases. The combination of cytokeratin and Ki-67 expression might help to separate such cases,\(^{30}\) and the addition of the Ki-67 index to other factors such as grade, multifocality, and p53 immunoexpression in superficial urothelial tumors has significantly enhanced the predictive accuracy of recurrence and progression.\(^{31}\)

p53 immunoexpression was shown to be a feature of papillary carcinoma, and the staining pattern permitted a statistically significant separation of LGCs and HGCs.\(^{19-23}\) We also found a significantly higher p53 index in HGC than in LGC; the majority of LGCs and all LMP cases did not stain for p53. These findings support the concept that mutation of p53 is a late event in malignant transformation and suggest that p53 can be used to separate LGCs and HGCs. We found that the majority of HGCs (14/19 [74%]) showed a p53 index of more than 10%, which was the highest index found among LGCs.

LMP cases seemed to share many clinical and biologic similarities with papillomas; the only difference was a higher Ki-67 index in LMP cases. The low numbers of cases of both of these lesions precluded statistical analysis, and the question of whether they represent the same entity remains to be answered.

The 1998 WHO/ISUP system identifies clinically and biologically distinct groups within the spectrum of noninvasive papillary urothelial neoplasms, namely, LMP, LGC, and HGC. This classification system seems to be validated by the immunoexpression of recognized biologic markers, which permitted separation of the 3 main grades of noninvasive papillary urothelial tumors. Our data suggest that antibodies against CK20, Ki-67, and p53 can provide additional objective criteria to aid the morphologic grading of noninvasive papillary urothelial tumors, in particular the separation of LMP from LGC, and LGC from HGC.

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References


