Classification and Grading of Noninvasive and Invasive Neoplasms of the Urothelium

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The classification and grading of the noninvasive, intraepithelial neoplasms of the urothelium are based on the morphological pattern of growth, i.e., papillary and flat (and endophytic), and on their degree of architectural and cytologic abnormalities. Recent advances in the morphological, molecular and quantitative evaluation of these lesions have contributed to the refinement of the current classification and grading schemes. However, some controversies on the precise criteria and terminology, especially when the papillary lesions are concerned, are still present. (Anal Quant Cytol Histol 2012;34: 111–119)

Keywords: inverted neoplasms, inverted papilloma, urothelial carcinoma, urothelial neoplasms, urothelium.

The noninvasive urothelial lesions and neoplasms can be flat (planophytic), papillary (exophytic) and inverted (endophytic), depending on their growth pattern relationship with the surface of the surrounding urothelial mucosa.1 Extensive clinical-pathologic studies have been published related to the first two growth patterns. The clinical significance of the third has been dealt with only at a very limited extent.

Flat (Planophytic) Lesions and Neoplasms

The 2004 World Health Organization (WHO) classification of the flat lesions includes flat hyperplasia, dysplasia and carcinoma in situ (CIS). In addition, this classification also lists reactive atypia, secondary to inflammation, and atypia of unknown significance.
Flat Urothelial Hyperplasia

Urothelial hyperplasia is characterized by markedly thickened urothelium with an increase in the number of cell layers, usually 10 or more. The cells do not show cytologic abnormalities, although slight nuclear enlargement may be present focally. Morphologic evidence of maturation from base to surface is evident. Mitoses are absent. It has been described in association with inflammatory disorders as well as adjacent to low-grade papillary tumors. Molecular analyses have shown that this lesion may be clonally related to the papillary tumors in bladder cancer patients and suggest a role in the pathogenesis of low-grade papillary urothelial carcinoma (LG PUC).

Urothelial Dysplasia

Urothelial dysplasia is characterized by architectural distortion and a variable degree of atypia. The thickness is usually normal, and cytologic changes are restricted to the intermediate and basal cells. The nuclei are irregularly enlarged with loss of polarity and pleomorphism. Mitotic activity is scant, usually involving only the basal and intermediate cell layers. Overall the features are those of a neoplastic atypia but fall short of the criteria for CIS. There is some evidence, largely genetic, that dysplasia shares some abnormalities with CIS and therefore likely represents a precursor lesion. One study indicates a 19% risk of developing cancer with a mean follow-up of 4.9 years.

Urothelial CIS

CIS is characterized by architectural disorder and nuclear pleomorphism (Figure 1). There is loss of nuclear polarity, and the cells show a high degree of atypia. Mitoses are generally frequent and may be seen at any level of the epithelium. Since the histological criteria for distinguishing severe dysplasia from CIS are unreliable, it is recommended to combine them into a single category, i.e., CIS. The development of invasion is seen in 20–30% of the cases.

CIS with microinvasion (CISmic) of the urinary bladder is defined by invasion into the lamina propria to a depth of 5 mm from the basement membrane, or, according to Lopez-Beltran et al, should not exceed 20 cells in the subepithelial connective tissue. CISmic is a clinically relevant lesion. Thirty-four percent of totally embedded cystectomy specimens that contain extensive CIS, i.e., involving at least 25% of the bladder, are found to contain microinvasion. A total of 5.8% have lymph node metastases and die of disease; for this reason the clinical use of the term is not recommended, thus communicating to the urologist a high grade T1 cancer.

Urothelial Denudation

Urothelial CIS is often associated with prominent cellular discohesion. The presence of extensive denudation in urothelial biopsies has been shown to be associated with a risk of CIS on either prior or subsequent bladder biopsies. Tissues obtained by a hot wire loop may also show urothelial denudation, even in those patients with low risk for subsequent CIS, and is likely due to thermal effects. In contrast, denuded biopsy samples obtained by cold cup biopsy in most cases are related to CIS. Levi et al showed that in patients with denuded bladder biopsy, CIS was diagnosed in 31% within 24 months, in 75% of patients with a history of CIS and 29% without a history of CIS.

Similar to those in flat lesions, denudation in papillary urothelial lesions may have some important implications. The majority of papillary urothelial lesions associated with prominent urothelial denudation are high grade.

Flat and/or papillary urothelial neoplasms with epithelial denudation may occur with either prominent cautery artifact or in anatomically confined areas, suggesting both iatrogenic and mechanical contributing factors. Thus, it is recommended that the presence of extensive or com-

Figure 1 Urothelial CIS.
plete urothelial denudation in a benign bladder biopsy report should be specified by the pathologist. Given its association with CIS, denuded urothelium, particularly when present extensively or complete, in cold cup biopsies, with no recent intravesical therapy, and in patients with risk for CIS, should provide the urologists an option to repeat the bladder biopsy.

**Reactive Changes**

Therapeutic procedures to bladder cancer, such as chemotherapy, immunotherapy, radiotherapy, photodynamic and laser treatment, and gene therapy, may produce morphological changes in the urothelial mucosa that can be mistaken for carcinoma. Radiation or chemotherapy cystitis can show epithelial proliferations that may be confused with invasive urothelial carcinomas even after the therapy was remotely performed. Histological features associated with radiation or chemotherapy cystitis include epithelial proliferation that mimicked invasive cancer within the lamina propria, mild to moderate nuclear pleomorphism, hemorrhage, fibrin deposition and fibrin thrombi, fibrosis, acute and chronic inflammation, hemosiderin deposition, edema, vascular congestion, and thickened vessels and vascular changes associated with radiation injury, respectively. The presence of mitosis is relatively rare, compared to flat dysplasia and CIS.

Pseudocarcinomatous epithelial hyperplasia related to localized ischemia or injury to the urothelium also occurs in patients with no prior history of radiation or chemotherapy. Most of these patients have potential etiology for the ischemia or injury, such as cardiac and/or vascular diseases.

Viral infection–related cellular changes, for example polyoma (BK) virus–type cellular changes, can mimic urothelial carcinoma.

Thus, it is important for the submitting urologist to note (in specimen requisition form) any prior therapy procedures performed and other bladder conditions, as some reactive changes can mimic or alter bladder neoplasm morphology, which may cause confusion for pathologists.

**Papillary (Exophytic) Lesions and Neoplasms**

The lesions and neoplasms of this group grow exophytically into the lumen of the urinary system with a papillary configuration (Figure 2). According to the 2004 WHO classification, this group includes urothelial papilloma, papillary urothelial hyperplasia, papillary urothelial neoplasm of low malignant potential (PUNLMP), low-grade papillary carcinoma and high-grade papillary carcinoma.

Urothelial papilloma is characterized by a few fine papillary fronds lined by normal urothelium and shows a very low recurrence rate. Papillary urothelial hyperplasia is defined as undulating urothelium arranged into thin mucosal papillary folds of varying heights occurring in a noninflamed setting. The lack of cytologic atypia and fibrovascular cores characterize this lesion. Papillary urothelial hyperplasia appears to be a precursor lesion to papillary urothelial neoplasms, predominantly lower grade lesions.

PUNLMP, low-grade papillary carcinoma and high-grade papillary carcinoma show a morphological spectrum with similarities with flat hyperplasia, dysplasia and CIS, respectively.

**PUNLMP**

PUNLMP largely, though not completely, corresponds to part of the grade 1 papillary carcinoma of the 1973 WHO system. The lesion consists of delicate papillae with little or no fusion. The covering urothelium shows minimal architectural irregularity. Nuclei lack significant nuclear hyperchromasia or pleomorphism, compared to flat dysplasia and CIS. The chromatin is fine and nucleoli are inconspicuous. Mitoses are infrequent and basally located. This tumor has a significantly lower rate of
recurrence than either low- or high-grade papillary carcinomas and a very low rate of grade and stage progression. In a review of published studies, mean tumor recurrence rate was reported to be 36% and stage progression rate, 3.7%.4,23

**Low-Grade Papillary Carcinoma**

The majority of low-grade papillary carcinoma cases would have been considered as grade 1 of 1973 WHO and around 70% of the grade 2 lesions in the 1973 WHO system. WHO grade 1 neoplasms showing slight cytologic atypia and mitoses are diagnosed in the 2004 WHO system as LG PUC. At low magnification there is a generally ordered appearance of the cells within the epithelium. The nuclei tend to be uniformly enlarged but retain the elongated to oval shape of normal urothelial cells. The chromatin remains fine with small nucleoli. Mitoses may be present but are few and remain basally located. These tumors have a significantly higher recurrence rate than that of PUNLMP. They also have a significantly higher rate of stage progression than do PUNLMP but a significantly lower than for high-grade papillary carcinoma. A review of the literature reveals a mean recurrence rate of 50% and mean stage progression rate of 10%.4

**High-Grade Papillary Carcinoma**

High-grade papillary carcinoma (HG PUC) corresponds to grade 3 papillary carcinoma in the 1973 WHO system. It includes around 30% of the 1973 WHO grade 2 lesions and bordering-on-higher-grade lesions, which in many institutions are called WHO grade 2-3. Individual cells are haphazardly arranged within the epithelium and have a generally discohesive nature. Nuclei are hyperchromatic and pleomorphic. The chromatin is dense, irregularly distributed and often clumped. Nucleoli may be single or multiple and are often prominent. Mitoses are generally frequent and may be seen at any level of the epithelium. It is often associated with invasive disease at the time of diagnosis.24-33 These tumors not only have a risk of invasion but have a significant risk of recurrence and progression. The overall progression rate (to invasive carcinoma) ranges from 15-40%.

**Grading Papillary Urothelial Neoplasm with Histologic Heterogeneity**

Grade represents a morphological spectrum, and variation in the degree and distribution of dysplasia within one tumor is well-recognized by pathologists. Admixture of at least two different grades in a papillary urothelial neoplasm is not uncommonly encountered and is reported in 3–43% of tumors.9,34-37 This histologic heterogeneity contributed to the lumping of grades (e.g., G1-G2 or G2-G3) in the older grading systems, and avoidance of these nondefinitive lumped grades was one impetus for modifications toward a more defined grading system. The 1998 International Society of Urological Pathology (ISUP) consensus, cognizant of urothelial tumors with variable histology, suggested that grade should be reported according to the highest grade present in heterogeneous tumors but stressed the need of studies to determine how significant a minor component must be in order to have an impact on overall prognosis.38

Cheng et al36 took into account the heterogeneity in papillary urothelial neoplasm and proposed a modified grading approach by combining the most common and second-most-common 1998 ISUP/WHO grades with corresponding number designations (e.g., PUNLMP = 1, etc.) to come up with a score, in a similar manner to Gleason scoring. The volume of a grade should be >5% to be considered. The combined scores resulted in a scale of grades 2 to 6, wherein the odd numbers 3 and 5, representing mixed grades, accounted for 32% of tumors. Histologic grade based on the worst grade (p = 0.0009), primary (p = 0.0004), secondary (p = 0.001) and combined (p = 0.0001) were all significant in predicting progression. Significant difference in progression-free survival between patients with a combined score of 5 (LG PUC + HG PUC) and those with a score of 6 (pure HG PUC) (p = 0.02) was observed. Of note among mixed tumors with predominant LG PUC, more minor component of HG PUC (21%, score of 5) than PUNLMP (6%, score of 3) was observed, the former by conventional WHO 2004 grading will be designated as HG PUC.

Billis et al34 applied an approach similar to that of Cheng et al36 in 293 bladder tumors but instead used the 1999 WHO grading system and correlated with stage. The study was able to stratify G2 into subgroups (mixed [G1 + G2] and pure [G2 + G2]), which were statistically different when considering stage. In G3 there was also a trend for statistical difference between mixed (G2 + G3) and pure (G3 + G3) tumors. The study, however, provided no information on disease outcome.

Bircan et al35 applied an approach similar to that of Cheng et al36 and Billis et al34 in 87 bladder carcinomas and used 1998 ISUP/WHO, 1973
WHO and 1999 WHO grading systems and correlated with stage. Mixed histology (odd number scores) constituted 18%, 29% and 33% of tumors by 1998 ISUP/WHO, 1973 WHO and 1999 WHO grading systems, respectively. In the 1998 ISUP/WHO system there was significant difference between LG and HG carcinomas (p = 0.000) and between mixed LG and HG (p = 0.011) when correlated to stage. The 1998 ISUP/WHO system positively correlated with stage, but the 1998 combined scoring did not. The 1973 WHO system positively correlated with stage, and there was a weak association between the 1973 combined scoring and stage. The study also did not provide information on disease outcome.

Krüger et al 37 examined the prognostic significance of grade, taking into account the presence of tumor heterogeneity in 151 muscle-invasive bladder carcinomas. Using the 1998 ISUP/WHO grading system, 43% of tumors had mixed LG and HG PUC components, which resulted in a 3-tiered grading of LG, mixed and HG PUCs. By Kaplan-Meier analysis both the 1998 ISUP/WHO grading system and the 3-tiered grading failed to reach the level of statistical significance. However, when LGs were combined with mixed versus HGs, this approach allowed stratification of patients showing a significant difference in disease-related survival (p = 0.0404). In multivariate analysis, however, this 2-tiered grading approach did not reach the level of significance.

May et al9 identified 3% in 269 nonmuscle invasive tumors that contained elements of both LG PUC and HG PUC and were able to classify these tumors into LG or HG grades by separating HG PUC based on 5% cut-off for high grade areas. Grade heterogeneity is not uncommonly encountered in papillary urothelial neoplasm. The 2004 WHO (1998 ISUP/WHO) system suggested that grading of heterogeneous tumor should be based on the highest grade present. The current practice is to ignore miniscule areas (<5%) of higher grade present when assigning the overall grade, although it currently remains unknown whether such a small focus of high grade will have a prognostic impact. From the few studies available,9,35,37 there was evidence to suggest that pure HG PUCs have a different biologic behavior or have higher disease progression than do mixed HG and LG tumors. Overall, additional studies with long-term follow-up are needed to firmly establish any prognostic impact or lack thereof, to determine application in grading, and to clearly define the allowable extent or percentage cut-off, if any, of high-grade focus in heterogeneous papillary urothelial neoplasm. More studies from other investigators are needed to determine whether the broader scale summation grading provides advantage in outcome information and observer agreement than the conventional WHO 2004 or WHO 1973 grading systems.

**Inverted (Endophytic) Lesions and Neoplasms**

The lesions and neoplasms of this group are basically characterized by an epithelium that shows a noninfiltrative growth into the subepithelial connective tissue, even though cystoscopically they might show a polypoid appearance. von Brunn’s nests and cystitis cystica and glandularis are the benign prototypes of inverted (endophytic) lesions. von Brunn’s nests refer to small groups of basal-like cells lying in the subepithelial connective tissue and attached to the basal cell layer of the urothelium. Cystitis glandularis is composed of glands in the lamina propria which are lined by cuboidal or columnar cells surrounded by urothelial cells. Cystitis cystica is made of cystically dilated von Brunn’s nests acquiring a luminal space.

Inverted urothelial papilloma, included in the 2004 WHO classification, shows a polypoid appearance and consists of thin anastomosing trabeculae of urothelial cells within the subepithelial connective tissue and covered by a normal or attenuated urothelium.39-41 There is no nuclear pleomorphism, and few mitoses can be seen. Inverted papilloma is associated with a low risk of recurrence (<5%). Cases of synchronous inverted papilloma and papillary carcinoma are known.

Reports of a noninvasive urothelial carcinoma with inverted growth pattern (Figure 3) are found in the literature and mentioned in some recent books.2,42-44 The endophytic growth pattern in this carcinoma has been described either as interanastomosing cords and columns of urothelium, often with a striking resemblance to inverted papilloma (inverted papilloma-like pattern), or as broad, pushing bulbous invaginations into the lamina propria (broad-front pattern) (Table I). A diagnosis of invasion requires the unquestionable presence within the lamina propria of irregularly shaped nests or single cells that may have evoked a desmoplastic or inflammatory response. When a stromal response is absent, irregularity of the contours of the invasive nests, architectural complexity and recognition of single-cell invasion are helpful (Table II).
Morphologic Spectrum of the Noninvasive Urothelial Neoplasms with an Inverted Growth Pattern

Morphologically the urothelial neoplasms with an inverted growth pattern (other than inverted urothelial papilloma) show a spectrum of architectural and cytologic features. In comparison with inverted urothelial papilloma, the architectural features favoring a diagnosis of a urothelial neoplasm with an inverted growth pattern include thick columns with irregularity in their width and transition into more solid areas. The characteristic orderly maturation, spindling and peripheral palisading seen in inverted papilloma are generally absent or inconspicuous. The histologic appearance of urothelial neoplasms with a broad-front pattern is the pushing broad-front extension into the lamina propria, akin to cutaneous and mucosal verrucous carcinoma and reminiscent of the growth pattern of the von Brunn’s nests. The downward projection can be so pronounced that the base of the tumor lies on the muscularis propria. There is a wide range in the severity of cytologic atypia, such as nuclear pleomorphism, irregularities of nuclear borders and chromatin distribution, prominent nucleoli and mitotic rate.45

When diagnosing inverted urothelial neoplasms, pathologists have used a variety of terms, including inverted urothelial papilloma, inverted urothelial papilloma with atypia,45 inverted growth pattern of PUNLMP, urothelial carcinoma with an inverted growth pattern, without any further specification to the degree of cellular anaplasia, or even as invasive urothelial carcinoma.45

Similarly to the flat neoplasms, the overall cyto-

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<tr>
<th>Table I</th>
<th>Morphologic, Immunologic and Molecular Genetic Features of Inverted Urothelial Papilloma and Urothelial Carcinoma with Inverted Pattern</th>
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<tr>
<td>Characteristic</td>
<td>Inverted urothelial papilloma</td>
</tr>
<tr>
<td>Surface</td>
<td>Smooth, dome-shaped, usually intact, cytotopically normal</td>
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<tr>
<td>Growth pattern</td>
<td>Endophytic, expansive, sharply delineated, anastomosing cords and trabeculae</td>
</tr>
<tr>
<td>Cytologic features</td>
<td>Orderly polarized cells, some with spindling and palisading at the periphery. No significant atypia, mitoses rare</td>
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<tr>
<td>Immunohistochemistry</td>
<td>Low p53 expression and Ki-67 proliferation index</td>
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<tr>
<td>Molecular analysis</td>
<td>Rare deletions at chromosome 9 or 17, rare FGFR3 mutations, low rate of LOH</td>
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<tr>
<td>Biological potential</td>
<td>Benign, rare recurrences*</td>
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LOH = loss of heterozygosity.
*Rare recurrences related to incomplete excision.

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<th>Table II</th>
<th>Urothelial Carcinoma with Inverted Pattern; Criteria for Invasion</th>
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<tr>
<td>Features</td>
<td>Noninvasive</td>
</tr>
<tr>
<td>Contours of neoplastic nests/cords</td>
<td>Regular</td>
</tr>
<tr>
<td>Size and shape of nests</td>
<td>Similar, rounded edges</td>
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<tr>
<td>Inflammatory and desmoplastic stroma</td>
<td>Absent</td>
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Figure 3  Noninvasive urothelial carcinoma with inverted growth pattern.
logic changes range from hyperplasia to CIS. Similarly to the papillary neoplasms and in agreement with the approach used in two books published very recently, 45,46 three subgroups could be defined in such range, i.e., neoplasms that have the least degree of cytologic atypia, neoplasms with the most severe degrees of cytologic atypia and those that lie in between, i.e., (1) inverted urothelial neoplasm of low malignant potential, (2) low-grade inverted urothelial carcinoma (Figure 3) and (3) high-grade inverted urothelial carcinoma.

**Conclusion**

The evaluation of the urothelial lesions and neoplasms with an inverted or endophytic growth pattern shows a morphologic spectrum similar to that seen in the flat and papillary counterparts (Chart I). However, this is not fully recognized in the current literature, in which, besides inverted urothelial papilloma, only one form of neoplasm is reported, i.e., urothelial carcinoma with an inverted growth pattern. Scant molecular and clinical studies on the urothelial carcinoma with an inverted growth pattern have been published, 43 mainly focusing on the differences with the inverted urothelial papilloma. A classification and terminology consistent with those of the flat and papillary urothelial lesions and neoplasms should be adopted.

**References**


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