UPDATE ON THE 4TH EDITION OF THE WORLD HEALTH ORGANIZATION
CLASSIFICATION OF UROTHELIAL TUMOURS*

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The content of the handout is part of a manuscript in European Urology (in press)
The 4th edition of the World Health Organization classification of tumours of the urothelial tract provides a contemporary review of the morphology of urothelial neoplasms, emphasizing its unique ability to exhibit divergent differentiation, its multiple morphologic variants and its diverse genomic landscape. It is becoming clearer how both morphology and genotype may be exploited to select therapy and, in the case of the latter, clinical protocols are in place to take advantage of activated molecular pathways in specific tumors. What follows is not a comprehensive summary of the entire WHO narrative but rather a selected summary of new or evolving concepts or entities. Mesenchymal, neuroendocrine and other types of non-urothelial lesions are beyond the scope of this summary.

**Grading of urothelial tumors**

Grading of urothelial tumors has particular importance in non-invasive disease, specifically papillary neoplasms. While a small percentage invasive carcinomas are low grade, usually limited to the lamina propria, over 95% of invasive tumors are high grade. Exceptions exist, a good example being the nested variant of urothelial carcinoma that, despite its deceptively bland cytornorphology, may present as locally advanced disease and is associated with poor outcome. Non-invasive tumors can be divided into two categories, either papillary or flat. Carcinoma devoid of papillary structures is called carcinoma in situ and is by definition high grade. Importantly, flat urothelium can exhibit a wide spectrum of atypia, from reactive to preneoplastic to frankly malignant. Papillary tumors are also quite varied, ranging from reactive proliferations and papilloma, to papillary urothelial proliferation of low malignant potential (PUNLMP), to low and high grade papillary carcinoma. Interobserver variability, even among experienced pathologist is high, despite many decades of efforts to come up with pathologic classifications that best reflect clinical behavior. As in 2004, the 2016 WHO continues to recommend the application of the grading classification first put forth by the International Society of Urological Pathology (ISUP) in 1997 (Table 1). In fact this classification continues to be endorsed by ISUP as well as all major contemporary pathology textbooks and guidelines, including the United States Armed Forces Institute of Pathology (AFIP) Atlas of Tumor Pathology, Fourth Series fascicle on tumors of the kidney, bladder, and related urinary structures, the latest editions of the American Joint Committee on Cancer (AJCC) Cancer Staging Manual and the International Collaboration on Cancer Reporting. Multiple studies have been published comparing this classification to others, particularly the 1973 WHO classification, in terms of reproducibility and clinical impact. Results have been mixed but mostly positive. It is recommended that this classification be adopted worldwide due to its inherent advantages, including:

- Uniform terminology and definitions based on the level of cytological and architectural abnormalities (order and disorder) as well as establishment of detailed criteria for various preneoplastic conditions and tumor grades.
- Definition of a group of lesions (high grade) with a high risk of progression that may be candidates for adjuvant therapy.
- Elimination of ambiguity in diagnostic categories in the 1973 WHO system (grade 1-2, grade 2-3)
- Inclusion of a category of papillary neoplasm (PUNLMP) that is not associated with invasion at the time of diagnosis and has a negligible risk of progression, although the potential for recurrence requires clinical surveillance

Admittedly, controversy remains, reasons for which are multifactorial but mainly due to the fact that the clinical risk of recurrence and progression are not determined solely by growth pattern and grade but also by other factors such as size, multifocality, time to recurrence, and prior intravesical therapy. In addition, we must accept the fact that grading is largely subjective and that,
in the future, ancillary studies, either immunohistochemical or molecular assays, will lead to enhanced reproducibility and better correlation with clinical outcome.

The term Urothelial Proliferation of Uncertain Malignant Potential (UPUMP) has been introduced, supplanting the term "hyperplasia. It describes a thickened urothelium with minimal or no cytological atypia and no true papillary fronds although undulations are common. They may be seen de novo and in this setting the clinical relevance is unknown. More frequently they are seen in patients with a history of prior carcinoma or adjacent to papillary lesions. It is likely that most represent lateral extension ("shoulder lesion") of a papillary neoplasm, an assumption that is supported by its high incidence of chromosome 9 deletions and lesser but significant incidence of FGFR3 abnormalities.

Urothelial dysplasia is defined as a flat lesion with appreciable cytological and architectural abnormalities that are believed to be preneoplastic but fall short of the criteria required for urothelial carcinoma in situ. It is rarely described de novo and for this reason it is poorly studied. More importantly, it is the most difficult category to define morphologically due to significant interobserver variability and the total absence of large clinical studies documenting its relationship to the development of subsequent CIS. In patients with a prior history of urothelial carcinoma, this diagnosis is particularly challenging and fraught with variability in interpretation, given the changes induced by prior instrumentation, biopsy site changes and intravesical therapy. It is of no surprise that urologists rarely alter management based on this diagnosis alone.

**Invasive urothelial carcinoma with divergent differentiation:**
By definition, urothelial carcinoma with divergent differentiation refers to tumors arising within the urothelial tract in which some percentage of "usual type" urothelial carcinoma is present along with other morphologies (Figure 1)(Table 2). Urothelial carcinoma has long been known to have a remarkable propensity for divergent differentiation which is seen most commonly in association with high grade and locally advanced disease. The incidence of divergent differentiation in cystectomy specimens is as high as 33%. Its presence is associated with established predictors of aggressive behavior and, while some studies have found an association with adverse outcome on univariate analysis, the effect does not remain significant on multivariable analysis. The amount of divergent histology present does not seem to have a bearing on outcome, although limited data is available to this effect. However it is recommended that pathologists report the percentage of divergent histologies in the pathology report.

Common morphologic manifestations of divergent differentiation include along squamous, glandular, small cell and even trophoblastic lines. Squamous cell carcinoma is defined by the presence of intercellular bridges or keratinization and may be present in up to 40% of invasive urothelial carcinomas. It is virtually never associated with HPV infection, with the rare exception of some cases with a basoid morphology. Interestingly, recent genomic data has described a basal/squamous-like molecular subtype that has squamoid morphology and immunophenotype, is associated with poor survival and poor response to systemic therapy. Glandular neoplasms constitute the second most common form of divergent differentiation, seen in up to 18% of invasive tumors and defined by the presence of gland formation. These tumors commonly have enteric features and, in isolation, can be easily confused with colonic adenocarcinoma. These tumors can express an identical immunophenotype such that site of origin is best determined clinically. In this setting as well as in others where the tumor is composed exclusively of a variant morphology, pathologists are encouraged to include a comment in the pathology report stating "we would accept as primary at this site if direct extension or a metastasis..."
form another organ can be ruled out clinically”. Some tumors are associated with extravasated mucin (mucinous), with or without signet ring cells. Rare tumors exhibit trophoblastic differentiation (syncytiotrophoblasts) with HCG production and some may even have an endodermal sinus which expresses αFP.

Many other morphologic manifestations of divergent differentiation may be encountered including nested, micropapillary, small cell, etc. When present in a pure form, these are considered VARIANTS of urothelial carcinoma. As previously mentioned their presence in a tumor with mixed histology is of questionable clinical importance when compared to urothelial carcinomas of equal stage and grade, although some exceptions exist, such as small cell carcinoma and possibly micropapillary carcinoma.

**Invasive variants of urothelial carcinoma:**
The variants of urothelial carcinoma are listed in Table 2. This discussion will highlight only either novel entities or novel concepts within selected variants.

The morphologic types of glandular neoplasms arising in the urothelial tract include enteric and mucinous types. The enteric type is morphologically identical to its colonic counterpart with which it can be easily confused. The mucinous type is characterized by the presence of abundant extravasated mucin within which free floating neoplastic cells, including signet ring cells, are floating (Figure 2). Contemporary thinking suggests that signet ring cell carcinoma, which by definition is not associated with any extravasated mucin, should not be included in this variant. Experience has taught us that tumors previously classified as such were either of the mucinous type or consisted of tumors with a variable number of signet ring cells as well as a significant number of cells with plasmacytoid features. In fact, plasmacytoid cells almost always predominate. These facts and recent molecular studies suggest that such tumors fit best in the plasmacytoid variant category (see below).

**Nested variant of urothelial carcinoma** is characterized by cytologically bland tumor cells infiltrating as disorderly arranged, discrete or confluent small nests or tubules. A large nested variant of urothelial carcinoma has been recently described which is composed of equally bland tumor cells. The importance of identifying this variant cannot be overstated since it can mimic benign urothelial proliferations, particularly in superficial transurethral resections and cold cup biopsies but characteristically present as locally advanced tumors and are associated with poor clinical outcome. The traditional grading scheme for urothelial carcinomas does not apply to these deceptively bland variants. While the microcystic variant of urothelial carcinoma is considered a distinct entity, some examples can have nests and tubules of neoplastic cells as well. Importantly, what they also share is a deceptively bland cytologic appearance that can mimic benign conditions such as cystitis glandularis.

The micropapillary variant of urothelial carcinoma has been well documented in the literature. Morphologically, it is defined as small nests and aggregates of tumor cells within lacunae. Multiple small nests without vascular cores are characteristic of this entity. The nuclei are markedly atypical and oriented to the periphery of the cell cluster. Cytoplasmic vacuoles with distortion of the nuclear contour are common. These tumors are commonly associated with lymphovascular invasion, present at a high pathological stage and exhibit aggressive clinical behavior. Despite the early literature advocating early cystectomy in all cases, it remains controversial whether these tumors should be treated differently form other high grade, locally advanced bladder tumors, particularly as it pertains to early cystectomy or neoadjuvant therapy. Whether clinical outcome is related to the morphology per se or to the stage at presentation is unclear, as is whether the proportion of the
micropapillary component influences outcome. At the molecular level, overexpression or amplification of ERBB2 is more common in this variant than in conventional urothelial carcinoma (Figure 1)\textsuperscript{52-54}.

Plasmacytoid urothelial carcinoma was described several decades ago but recent data have defined the morphologic spectrum, clinical behavior and genotype in a more comprehensive manner\textsuperscript{55-61}. This is a rare tumor characterized by the presence of mononuclear tumor cells with plasmacytoid, lymphoid or even rhabdoid features. Very commonly the tumor will exhibit a variable percentage of cells with cytoplasmic vacuoles, imparting an appearance of signet ring cells, with or without intracellular mucin but never associated with extracellular mucin (Figure 3). In fact virtually every case of signet ring cell carcinoma of the urinary bladder that has been described in the literature would now be placed into this category of tumor, assuming absence of extracellular mucin. Of all the variants, this one is most likely to be encountered in its pure form although it can also be seen in association with usual urothelial carcinoma or other variants. It is invariably diagnosed in a locally advanced stage and is associated with a dismal outcome. At the molecular level, these tumors are characterized by the presence of truncating mutations of CDH-1 and loss of e-cadherin expression (Figure 3)\textsuperscript{60}. However, it retains a similar “trunk” mutational profile as usual urothelial carcinomas, a feature that can help in establishing site of origin.

\textit{Tumors arising along the genitourinary tract but not of urothelial origin}

As previously described, the morphologic plasticity seen in urothelial carcinoma is very broad, and includes tumors with clear cell features\textsuperscript{62-66}. However, there are a series of tumors, predominantly encountered in women, which appear to arise from Müllerian precursors present within the bladder wall or adjacent soft tissues, commonly endometriosis but rarely Müllerianosis\textsuperscript{62}. Clear cell carcinoma predominates but occasional cases of endometrioid type carcinoma have been described, these only in women. Clear cell carcinomas are characterized by the usual tubulocystic, papillary or diffuse growth patterns (Figure 4). Hobnail cells are common, as are basophilic or eosinophilic secretions. While some case may be confused with nephrogenic adenoma, the level of nuclear enlargement and hyperchromasia present in clear cell carcinoma should lead to the proper diagnosis. As might be expected, this tumor is immunoreactive for PAX8, HNFß1, CA-125 and p53, similar to its ovarian counterparts. The endometrioid variant is usually PAX8 and p53 negative but positive for ER and PR.

\textit{Tumors arising in a bladder diverticulum}

Epithelial neoplasms have been reported in up to 14% of bladder diverticula, comprising approximately 1% of bladder neoplasms\textsuperscript{67-69}. Based on the unique clinical scenario and anatomy of diverticula, it is an important topic that was not covered in the prior edition. The majority of tumors arise in acquired diverticula whose wall is composed of urothelium and lamina propria only; by definition no muscularis propria is present, except in the bladder wall immediately adjacent to the diverticulum (diverticular os). As such, pathologic staging of these tumors is different from those that arise within the bladder since pT2 disease does not exist. Up to 50% of cases are non invasive, either papillary or flat. Of those tumors that are invasive, most are of usual urothelial type while the rest may exhibit a variant morphology or mixed histologic features (divergent differentiation). Similar to vesical primaries, pathological stage is the most important prognostic factor.

\textit{Genomics of urothelial carcinoma}

Studies have suggested that invasive urothelial tumors develop along at least two molecular pathways, via either high grade papillary tumors or CIS. Molecular alterations differ markedly between low and high grade tumors and those that are invasive from those that are not. Since it is
likely that tumors develop from areas of premalignant urothelial cells, it is not surprising that multifocal and metachronous tumors show common as well as novel, uniquely acquired mutations 70-72. Copy number abnormalities, loss of heterozygosity and increased genomic instability have been associated with increasing tumor grade and stage. Multiple tumor suppressor genes and well as oncogenes have been described in invasive urothelial carcinoma, but often it is difficult to determine if these are required for cancer development 73, 74. Recurrent mutations in genes such as TP53, FGFR3, PIK3CA, RB1 and HRAS with TP53 and FGFR3 being the most common, together with promoter mutations of TERT 75-77. While TERT mutations are present in up to 79% of bladder neoplasms, they have no association with clinical outcome. However, its presence can be of great diagnostic utility, given the relative rarity of this mutation in other tumors which may have overlapping histology. Next generation sequencing efforts have demonstrated that the mutational landscape of urothelial tumors are quite complex with over 300 mutations, over 200 copy number alterations and more than 20 rearrangements per tumor 71, 78-80. Overall, only lung cancer has been shown to harbor a higher rate mutations, although most are certainly passenger mutations with no functional consequence 81.

The most frequently altered pathways in bladder cancer include the PI3K/AKT/mTOR pathway 59, 82-84 FGFR3/RAF/RAS pathway, TP53/RB1 pathway, immune response checkpoint modulators 85, 86 and chromatin regulating and remodelling genes 87-89. In general, mutations along a given pathway are mutually exclusive. Some of the components of these pathways are altered in low risk while others are characteristic of high risk disease. For example, FGFR3 mutations are seen in up to 80% of papillary non-invasive and low grade carcinomas. While these mutations have been associated with a higher risk of recurrence they are not associated with disease progression 90, 91. Mutations in chromatin remodelling and histone modifying genes have been described in up to 89% of muscularis propria invasive bladder tumors 79, 92. As novel therapeutic agents are developed that target these pathways, improvements in therapy are certainly to come. In addition, there is emerging data that immune modulating agents may have a promising role in the management of advanced urothelial carcinoma.

The discovery of the molecular pathways involved in urothelial cancer recurrence and progression has allowed for the identification of potential prognostic and predictive markers 93-95. It has also permitted the development of novel non-invasive detection and surveillance strategies as well as revealed potential therapeutic targets 96-101. However the absence of multi-institutional randomized prospective trials has delayed the validation of these prognostic and predictive markers for routine clinical use. The good news is that a significant number of these trials have been and will be launched in the near future which will certainly alter the way we identify, risk-assess and treat these tumors.
### Table 1.
**WHO Classification of Tumours:**
**Tumours of the Urothelial Tract**
**Differences between the 3rd and 4th editions**

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<th>Non-invasive urothelial lesions</th>
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<td><strong>Third edition:</strong></td>
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<td><em>Non-invasive urothelial lesions</em></td>
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<td>Urothelial carcinoma in situ</td>
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<td>Urothelial proliferation of uncertain malignant potential (hyperplasia)</td>
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<td>Urothelial dysplasia</td>
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Table 2.

WHO Classification of Tumours:
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Differences between the 3rd and 4th editions

Invasive urothelial tumors

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<tr>
<td>Infiltrating urothelial carcinoma</td>
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<td>with trophoblastic differentiation</td>
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<td>Undifferentiated</td>
<td>Clear cell</td>
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<td>Tumours of Müllerian type</td>
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<td>Tumors arising in a bladder diverticulum</td>
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References:


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