55 Important pathologic parameters in reporting urothelial carcinoma of the bladder

Jesse McKenney MD

2011 Annual Meeting – Las Vegas, NV

AMERICAN SOCIETY FOR CLINICAL PATHOLOGY
33 W. Monroe, Ste. 1600
Chicago, IL 60603

55 Important pathologic parameters in reporting urothelial carcinoma of the bladder

The purpose would be to cover the classification of papillary urothelial tumors, diagnostic criteria for carcinoma in situ, important subtypes of urothelial carcinoma, the diagnosis of invasion, and the pathologic staging of bladder cancer. The CAP guidelines for reporting these specimens would be covered. Also good for residents.

- Learn the variation in lamina propria and muscularis propria microanatomy to improve the staging of urothelial carcinoma in biopsy specimens.
- Learn the recommended WHO/ISUP criteria for classifying non-invasive papillary urothelial neoplasms.
- Recognize invasive urothelial carcinoma subtypes that have significance for clinical therapy.

FACULTY:

Jesse McKenney MD

Practicing Pathologists
Surgical Pathology
Surgical Pathology (GI, GU, Etc.)
2.0 CME/CMLE Credits

Accreditation Statement: The American Society for Clinical Pathology (ASCP) is accredited by the Accreditation Council for Continuing Medical Education to provide continuing medical education (CME) for physicians. This activity has been planned and implemented in accordance with the Essential Areas and Policies of the Accreditation Council for Continuing Medical Education (ACCME).

Credit Designation: The ASCP designates this enduring material for a maximum of *2 AMA PRA Category 1 Credits*™. Physicians should only claim credit commensurate with the extent of their participation in the activity. ASCP continuing education activities are accepted by California, Florida, and many other states for relicensure of clinical laboratory personnel. ASCP designates these activities for the indicated number of Continuing Medical Laboratory Education (CMLE) credit hours. ASCP CMLE credit hours are acceptable to meet the continuing education requirements for the ASCP Board of Registry Certification Maintenance Program. All ASCP CMLE programs are conducted at intermediate to advanced levels of learning. Continuing medical education (CME) activities offered by ASCP are acceptable for the American Board of Pathology's Maintenance of Certification Program.

Important Pathologic Parameters for Reporting Urothelial Carcinoma of the Urinary Bladder

American Society for Clinical Pathology

2011 Annual Meeting

Thursday, October 20th

4:00-5:50 PM

Las Vegas, NV

Jesse K. McKenney, MD

Director of Urologic Pathology

Associate Professor, Departments of Pathology and Urology

Stanford University Medical Center

300 Pasteur Dr, L235

Stanford, CA 94305

jmck@stanford.edu

Faculty/Author/Speaker Disclosure

The faculty/speaker for this live session, Jesse K. Mckenney, has no relevant financial relationships with commercial interests to disclose.

WHO (2004)/ISUP CLASSIFICATION OF BLADDER TUMORS

NORMAL

Normal

HYPERPLASIA

Flat Hyperplasia

FLAT LESIONS WITH AYTPIA

Reactive (Inflammatory) Atypia

Atypia of Unknown Significance

Dysplasia (Low-grade Intraurothelial Neoplasia)

Carcinoma In Situ (High-grade Intraurothelial Neoplasia)

PAPILLARY NEOPLASMS

Inverted Urothelial Papilloma

Urothelial Papilloma

Papillary Urothelial Neoplasm of Low Malignant Potential (PUNLMP)

Papillary Urothelial Carcinoma, Low grade

Papillary Urothelial Carcinoma, High grade

PART I: FLAT UROTHELIAL LESIONS

Normal Urothelium: Urothelium (previously referred to as transitional epithelium), is the dominant type of epithelium lining the urinary bladder, ureters, and renal pelvis. It is a unique multilayered epithelium, which typically contains longitudinal nuclear grooves in some of the cells, that matures to form very large surface cells known as "umbrella cells." Umbrella cells may have nuclear atypia, which should not be misconstrued to be dysplastic. The urothelial cells typically have a somewhat linear organization streaming upward, perpendicular to the basement membrane.

There has been overuse of the diagnosis "mild dysplasia" for flat lesions with normal cytology, and a minimally disordered architectural pattern; vagaries of staining and fixation may also impart hyperchromasia to benign nuclei. Flat lesions with benign cytology and minimal disorder should not be designated as mild dysplasia but rather as normal.

Flat Urothelial Hyperplasia: Flat urothelial hyperplasia historically has been defined as urothelium greater than seven cells layers thick. In practice, counting the number of urothelial cell layers is not reproducible, as urothelial cells do not line up in precise rows.

Under the WHO 2004, flat urothelial hyperplasia is defined as a markedly thickened mucosa without cytological atypia and with normal polarity. Rather than requiring a specific number of cell layers, marked thickening at low power magnification is needed to diagnose flat hyperplasia. This lesion may be seen in the flat mucosa adjacent to low-grade papillary urothelial lesions. When seen by itself, there is no data suggesting that it has premalignant potential.

In my opinion, hyperplastic lesions with a papillary architecture that were previously described as "papillary urothelial hyperplasia" should be assigned a papillary diagnosis (such as PUNLMP) under the WHO 2004. Data from clinical follow-up studies of lesions diagnosed as "papillary hyperplasia", many of which show progression/recurrence to low grade papillary neoplasms, seem to support this approach (Readal N, Epstein JI. Papillary urothelial hyperplasia: relationship to urothelial neoplasms. Pathology. 2010;42(4):360-3. PubMed PMID: 20438409).

Flat Lesions with Atypia

Background: The diagnosis of flat urothelial lesions has been complicated by a lack of uniformity because of the use of multiple classification systems, often with marked differences in histologic criteria. The history of the concept of

intraepithelial neoplasia in the urinary bladder and the evolution of the WHO/ISUP classification have been previously reviewed in detail.[15] The WHO 2004 classification of flat urothelial lesions with atypia[5] now provides well-defined histologic criteria for stratifying lesions within the full spectrum of cytologic atypia and provides a means to compare studies between institutions, a practice that could not be undertaken previously. The diagnostic categories include reactive atypia, atypia of unknown significance, dysplasia (low-grade intraurothelial neoplasia), and carcinoma in situ (high-grade intraurothelial neoplasia).

Diagnostic Approach: Evaluation of cold cup random biopsies, which are routinely obtained in patients with non-invasive papillary tumors on surveillance, involves attention to several histologic parameters. It is the constellation of the presence or absence of some of these parameters that help to arrive at the appropriate diagnosis for a given case.

Histologic parameters useful in the evaluation of flat lesions with atypia

- **Nuclear size**
- **Nuclear chromatin distribution**
- **Polarity**
- **Nuclear borders including notches**
- Thickness of urothelium
- Cytoplasmic clearing
- Nuclear crowding
- Nucleoli
- Mitoses
- Accompanying inflammation

Reactive Atypia:[4,5] Urothelium with benign reactive changes (reactive atypia) consists of nuclear abnormalities occurring in acutely or chronically inflamed urothelium. In reactive atypia, nuclei are uniformly enlarged (nuclei are typically less than 3-4 times the size of a stromal lymphocyte), with small, central prominent nucleoli. Mitotic figures may be frequent, but the chromatin distribution is homogeneous without significant hyperchromasia. A history of instrumentation, stones, or therapy is often present. In the absence of appreciable nuclear hyperchromasia, pleomorphism, and irregularity in the chromatin pattern, the lesion should not be considered neoplastic. If denudation

is present, the residual lining cells should be small basal cells lacking significant nuclear atypia.

Atypia of Unknown Significance:[4,5] In some cases it is difficult to differentiate between reactive and neoplastic atypia. There may be a greater degree of pleomorphism and/or hyperchromasia, out of proportion to the extent of the inflammation, such that dysplasia cannot be ruled out with certainty. These cases should be designated as "atypia of unknown significance" so that the patients may be followed more closely and re-evaluated once the inflammation subsides.

Dysplasia (Low-grade Intraurothelial Neoplasia):[4-8,21,24] In the ISUP/WHO system, dysplastic urothelium was defined as "appreciable cytologic and architectural changes felt to be preneoplastic, yet falling short of the diagnostic threshold for transitional cell carcinoma in situ (CIS)." The category of urothelial dysplasia is very controversial for several reasons that include 1) problems with interobserver reproducibility,[3] 2) the lack of a uniform definition, 3) confusing reports in the historical literature which often combine moderate and severe dysplasia (the latter currently regarded as CIS), and 4) no current treatment implication. Because of these issues, the natural history of bladder dysplasia in humans is poorly understood. The major problem is lack of histologic criteria and poor reproducibility amongst experts. For this reason, I personally do NOT diagnosis urothelial dysplasia. When I am faced with a flat urothelial lesion showing a level of atypia that does not reach my threshold for carcinoma in situ, but beyond which I can accept as definitively benign reactive changes, I use the diagnostic category "urothelial atypia of unknown significance". This ensures clinical follow-up care without designating the lesion as definitively neoplastic. In a recent clinical outcome study of cases diagnosed as "urothelial dysplasia" that was presented in abstract form, there was not a significant progression of disease. This suggests that many flat lesions with cytological atypia that are diagnosed as "dysplasia" may not be neoplastic in nature.

Most importantly, one should establish their minimal threshold for CIS (see below), and distinguish this from other flat lesions with atypia. This identifies the lesion (i.e. CIS) with definite biologic and therapeutic importance. Flat lesions with a benign cytology and minimal disorder should be considered within the spectrum of "normal."[4]

The diagnostic term "dysplasia" should not be used for markedly atypical cells involving only partial thickness of the urothelium; these should be regarded as carcinoma in-situ. It had been proposed previously that bladder intraepithelial lesions, like intraepithelial lesions of the cervix, be graded on the basis of level of involvement of atypical cells (i.e., mild dysplasia for lesions showing atypia confined to the lower one-third, moderate dysplasia for atypia up to the middle-third and so on).[6] Evidence from morphological observations in the animal model system of Wistar rats exposed to N-butyl-N (4 hydroxybutyl) nitrosamine

emphasizes the pitfall in defining urothelial dysplasia through criteria and terminology employed for cervical carcinoma.[7] The bladders were studied at regular intervals during the development of carcinoma after exposure to the above carcinogen. In the controlled environment of carcinogenesis, atypia progressed quantitatively and qualitatively (the urothelium did not develop progressive changes starting at the basal layer as seen in the uterine cervix), stressing the importance of cytological features of individual cells rather than histologic pattern (i.e. the level of atypia). The collective experience of urologic pathologists supports a similar progression in humans, as many patterns of carcinoma in situ involving only partial thickness of the urothelium may be associated with underlying invasion.

Carcinoma In Situ (High-grade Intraurothelial Neoplasia):[4,5,10] Carcinoma in situ (CIS) is a flat lesion of the urothelium that is a documented precursor of invasive cancer in some cases. The lesion is characterized by the presence of cells with large, irregular, hyperchromatic nuclei that may be either present in the entire thickness of the epithelium or only a part of it. The WHO 2004/ISUP classification expands the previous definition of carcinoma in situ; there need not be full thickness cytologic atypia and an umbrella cell layer may still be present (i.e.- CIS encompasses lesions which in the past were designated as severe dysplasia or marked atypia). The urothelium may be denuded, reflecting the discohesive nature of the cells; it may be diminished in thickness, or even hyperplastic. There may be alteration or complete loss of polarity, marked crowding, pleomorphism, and frequent mitoses; the lamina propria is frequently hypervascular and inflamed. The nuclear anaplasia is generally obvious, although a spectrum of cytologic atypia may exist. Additionally, there are varied cytologic and architectural patterns of CIS.[9]

Morphologic expressions of CIS

Large cell CIS with pleomorphism

Large cell CIS without pleomorphism

Small cell CIS*

Clinging CIS

Cancerization of normal urothelium

- Pagetoid CIS
- Undermining/Overriding

* Does not imply neuroendocrine differentiation

Although the spectrum of morphologic patterns is described below to aid in the recognition of CIS, they should not be included in pathology reports, as there is no known prognostic significance to subtyping. We simply use the WHO 2004 diagnostic term "urothelial carcinoma in situ" because these additional terms would probably only serve to confuse treating urologists. The most easily recognized pattern of CIS is large cell CIS with pleomorphism. The neoplastic cells show considerable loss of polarity and nucleomegaly with marked variation in nuclear size and shape and obvious nuclear hyperchromasia; the cells retain abundant eosinophilic cytoplasm. The neoplastic cells in other examples of CIS may be rather monomorphic (large cell CIS without pleomorphism). These lesions may mimic reactive urothelial atypia because of the uniformity of the cells with conspicuous eosinophilic cytoplasm; however, they have marked nucleomegaly and the clumped, irregular chromatin distribution characteristic of CIS. Some authors have suggested that comparison to stromal lymphocytes may aid in the assessment of nuclear size, particularly in monomorphic cases, as the nuclei of CIS are often 3-6 times the size of stromal lymphocytes. When one identifies urothelial nuclei that are greater than 5-6 times the size of a lymphocyte (excluding umbrella cells), CIS should be strongly considered. Small cell CIS has nuclear features identical to large cell CIS, but there is very scant cytoplasm. Although the cells appear smaller because of decreased cytoplasm, the nuclei are markedly enlarged with nuclear chromatin abnormalities. It should be emphasized that this is simply a descriptive term and does not imply neuroendocrine differentiation. Clinging CIS is characterized by a partially denuded urothelium with a patchy, usually single layer of residual urothelial cells meeting the morphologic criteria for CIS.

CIS may also show cancerization of adjacent normal urothelium in two patterns: 1) pagetoid spread with the presence of clusters or isolated single cells with features of CIS within normal urothelium and, 2) undermining or overriding of the normal urothelium by CIS of any pattern. Additionally, CIS may show cell dropout secondary to necrosis forming small, gland-like spaces.

Adjunctive Immunohistochemistry in Flat Lesions

Although studies have shown distinct, contrasting patterns of immunoreactivity in morphologically straightforward cases of CIS, reactive atypia, and normal urothelium as shown below, the utility of the panel in morphologically ambiguous cases or dysplasia (with regard to predicting the biologic significance) has not yet been studied.[11,12] Morphology should remain the gold standard for diagnosis, as unexpected immunophenotypes are occasionally encountered in obvious CIS or benign lesions.

Prototypical Immunoprofiles for CIS, Reactive Atypia, and Normal Urothelium[11,12]

	CK 20	p53	CD44
CIS	**Cytoplasmic staining in full thickness (81%)**	**Strong nuclear reactivity in full thickness (57%)**	**Non-reactive; loss of normal basal staining**
Reactive atypia	**Reactive only in umbrella cells**	**Non-reactive***	**Diffuse membranous staining in full thickness**
Normal urothelium	**Reactive only in umbrella cells**	**Non-reactive***	**Membranous staining in basal cell layer only**

* Weak, background staining is frequently identified

Problems and Pitfalls in the Diagnosis of Flat Lesions with Atypia

(i) Innate vagaries of normal urothelium and histologic sectioning: The thickness of the normal urothelium varies with the state of distention of the bladder (2 to 4 cell layers when dilated and 5 to 7 layers when contracted). If the sections are thick, the urothelium may appear hyperchromatic and this artifact compounded with tangential sectioning may result in changes felt to represent dysplasia. The urothelium of the renal pelvis, urethra and the bladder neck is usually composed of slightly larger cells, which have diminished cytoplasmic clearing and hence may be interpreted as dysplasia.

(ii) Inflammatory atypia: The presence of acute or significant chronic inflammation, particularly in an intraurothelial location warrants caution in the interpretation of dysplasia or carcinoma in situ, although it must be borne in mind that inflammatory atypia may coexist with dysplasia or in situ carcinoma.

(iii) Therapy associated atypia: Upon cursory examination the changes could easily be mistaken for intraepithelial neoplasia, but the presence of abundant cytoplasm, nuclear chromatin degeneration, multinucleation, prominent nucleoli and involvement of mainly the superficial cells are key features to associate the changes with chemotherapy or radiation.

(iv) Extensive denudation: Trauma due to instrumentation, prior therapy, and carcinoma in situ are the main conditions associated with denudation. The

presence of a few atypical single cells clinging to the surface is sometimes the only sign of carcinoma in situ ("denuding cystitis") – deeper sections through the block may be helpful. In the absence of atypical cells, the findings of extensive denudation, particularly that associated with neovascularity and chronic inflammation in the lamina propria must be included in the report and correlation with urine cytology findings may be suggested.

(v) Truncated papillae of treated papillary carcinoma: Mitomycin C and thiotepa therapy destroys the tips of the papillae of papillary transitional carcinoma because they act as surface abrasives. When these truncated papillae are seen in areas associated with denudation and inflammation they may be mistaken for carcinoma in situ or dysplastic changes.[13]

(vi) Carcinoma in situ involving von Brunn nests – overdiagnosis of invasion:

Carcinoma in situ can involve von Brunn nests resulting in the presence of neoplastic cells within the superficial lamina propria. In the presence of inflammation, the basement membrane may be obscured and distorted, simulating invasion.

(vii) Carcinoma in situ with microinvasion – underdiagnosis of invasion:

A histologically subtle, but clinically potentially ominous situation arises when carcinoma in situ is associated with microinvasion. The urologist is most often unsuspecting of invasive disease on the basis of cystoscopic evaluation and the pathologist may fail to recognize single cell invasion or small clusters of invasion that may be camouflaged by background inflammation. Desmoplasia or retraction artifacts are useful in recognizing invasion. [9,9A]

(viii) Polyoma virus infection: Infection of immunocompromised patients with the human polyoma virus, which is usually a non-pathogenic virus, results in large homogeneous inclusions in enlarged nuclei of urothelial cells. The findings are more frequently seen in urine cytology but may rarely be seen in biopsies, in both specimens malignancy (carcinoma in situ) is mimicked.[16]

Selected References for Flat Urothelial Lesions

1. Taylor DC, Bhagavan BS, Larsen MP, Cox JA, Epstein JI. Papillary urothelial hyperplasia. A precursor to papillary neoplasms. *Am J Surg Pathol* 1996;20;1481-88.
2. Oberman EC, Junker K, Stoehr R, Dietmaier W, Zaak d, Schubert GE, Hofstaedter F, Knuechel R, Hartmann A. Frequent genetic alterations in flat urothelial hyperplasias and comcomitant papillary bladder cancer as detected by CGH, LOH, and FISH analyses. *J Pathol* 2003;199:50-7.

3. Amin MB, Grignon DJ, Eble JN, Reuter VE, Ro JY, Srigley JR, Weiss MA, Young RH: Intraepithelial lesions of the urothelium: An interobserver reproducibility study with proposed terminology and histologic criteria. *Mod Pathol* 1997;10:69A,
4. Epstein JI, Amin MB, Reuter VR, Mostofi FK: The World Health Organization/International Society of Urological Pathology consensus classification of urothelial (transitional cell) neoplasms of the urinary bladder. *Am J Surg Pathol* 1998;22:1435-1448.
5. Sauter G, Algaba F, Amin MB, Busch C et al: Non-invasive urothelial tumours. In: Epstein JI, Eble JN, Sesterhenn I, Sauter G (eds): World Health Organization Classification of Tumours Pathology and Genetics: Tumours of the Urinary System and Male Genital Organs. IARC Press, Lyon, 2004; pp 110.
6. Ayala AG, Ro JY: Premalignant lesions of urothelium and transitional cell tumors, in Young R.H. (ed): *Contemporary Issues in Surgical Pathology: Pathology of the Urinary Bladder.* Churchill Livingstone, New York, NY, 65-101, 1989.
7. Murphy WM, Soloway MS (eds): Developing carcinoma (dysplasia) of the urinary bladder, in Sommers SC, Rosen PP (eds): *Pathology Annual*, 17. Norwalk, CT, Appleton-Century-Crofts, 197-217, 1982.
8. Amin MB, Murphy WM, Reuter VE, et al: Controversies in the pathology of transitional cell carcinoma of the urinary bladder, in Rosen PP, Fechner RE (eds): Reviews in Pathology, Chicago, IL, ASCP Press, 1-38, 1996.
9. McKenney JK, Gomez JA, Desai S, Lee MW, Amin MB: Morphologic expressions of urothelial carcinomas in situ: a detailed evaluation of its histologic patterns with emphasis on carcinoma in situ with microinvasion. *Am J Surg Pathol* 25:356-362, 2001.
9A. Jiminez RE, Keane TE, Hardy H, Amin MB. pT1 urothelial carcinoma of the bladder: criteria for diagnosis, pitfalls, and clinical implications. *Adv Anat Pathol* 2000;7:13-25.
10. Amin MB, Young RH: Intraepithelial lesions of the urinary bladder with a discussion of the histogenesis of urothelial neoplasia. *Semin Diag Pathol* 14:84-97, 1997.
11. McKenney JK, Desai S, Cohen C, Amin MB. Discriminatory immunohistochemical staining of urothelial carcinoma in situ and non-neoplastic urothelium. An analysis of cytokeratin 20, p53, and CD44 antigens. *Am J Surg Pathol 2001*;25:1074-78.
12. Harnden P, Eardley I, Joyce AD et al. Cytokeratin 20 as an objective marker of urothelial dysplasia. *Br J Urol* 1996;78:870-5.
13. Murphy WM, Soloway MS, Finebaum PJ: Pathologic changes associated with topical chemotherapy for superficial bladder cancer. *J Urol* 1980;126:461-465.
14. Amin MB, Gomez JA, Young RH: Urothelial transitional cell carcinoma with endophytic growth patterns. A discussion of patterns of invasion

and problems associated with carcinoma-in-situ. *J Urol* 1976;116:575-580.
15. Amin MB, McKenney JK: An approach to the diagnosis of flat intraepithelial lesions of the urinary bladder using the World Health Organization/International Society of Urological Pathology consensus classification system. *Adv Anat Pathol* 2002;9:222-232.
16. Koss LG: "Diagnostic cytology and its histopathologic basis," 4th Edition. Philadelphia: JB Lippincott, 1992.
17. Zuk RJ, Rogers HS, Martin JE, et al: Clinicopathological importance of primary dysplasia of bladder. *J Clin Pathol* 1988;41:1277-1280.
18. Murphy WM, Miller AW: Bladder cancer, in Javadpour NE (ed): Baltimore, MD, William & Wilkins, 100-122, 1984.
19. Smith G, Elton RA, Beynon LL: Prognostic significance of biopsy results of normal-looking mucosa in cases of superficial bladder cancer. *Br J Urol* 1983;55:665-669.
20. Althausen AF, Prout GRJ, Dal JJ: Noninvasive papillary carcinoma of the bladder associated with carcinoma-in-situ. *J Urol* 1976;116:575-580.
21. Cheng L, Cheville JC, Neumann RM, Bostwick DG: Flat intraepithelial lesions of the urinary bladder. *Cancer* 2000;88:625-631.
22. Amin MB. Urothelial dysplasia. *In:* World Health Organization Classification of Tumours. Pathology and Genetic Tumors of the Urinary System and Male Genital Organs. Editors: Eble JN, Sauter G, Epstein J, Sesterhenn I; IARC Press, Lyon, 2004; pages 111-112.
23. Lamm DL: Carcinoma in situ. *Urol Clin North Am* 19:499-508, 1992.
24. Murphy WM, Soloway MS: Urothelial dysplasia. *J Urol* 1982;127:849-854.
25. Weinstein RS, Miller AW, Pauli BU: Carcinoma in situ: Comments on the pathobiology of a paradox. *Urol Clin of North America* vol 7, No. 3, 1980.
26. Orozco RE, Martin AA, Murphy WM: Carcinoma in situ of the urinary bladder: Clues to host involvement in human carcinogenesis. *Cancer* 1994;76115-122.
27. Mostofi FK, Sesterhenn IA. Pathology of epithelial tumors and carcinoma in situ of bladder: Bladder cancer. *Progress Clin Bio Res* 1984;162A:55-74.

PART 2: PAPILLARY UROTHELIAL NEOPLASMS

Background: Although many different classification systems have been proposed for papillary bladder neoplasms over the past 30 years, the International Society of Urologic Pathologists (ISUP) and the World Health Organization (WHO) have developed a consensus derived system that has become the accepted standard by urologic pathologists and urologists worldwide.[1] The current WHO 2004 system is essentially identical to the WHO/ISUP (1998) system,[2] which is a modified version of the scheme proposed by Malmström et al. in 1987.[3]

Although the WHO 1973 system had been used widely, the diagnostic criteria were not well-defined, leading to many diagnoses that "bridged" different categories (e.g.- Transitional cell carcinoma, Grade II-III). In addition, the use of the term "urothelial papilloma" had been expanded by some authors to include a spectrum of low-grade lesions (including lesions termed "Grade I carcinomas" under the WHO 1973 system) in order to avoid a diagnosis of carcinoma for a lesion that seemed to pose little immediate threat to patients' health. The WHO and ISUP felt a new consensus classification was needed to resolve these major issues and insure that a unified terminology was in place to better study patient outcome and treatment modalities.[4,5]

The diagnostic categories provided for papillary urothelial neoplasms include inverted papilloma, urothelial papilloma, papillary urothelial neoplasm of low malignant potential, low-grade papillary urothelial carcinoma, and high-grade papillary urothelial carcinoma. The histologic criteria for each of these diagnoses are discussed individually below and summarized in Table 2, followed by outcome data in Table 3.

Inverted Urothelial Papilloma:[1,6-8] Although not strictly a papillary lesion, inverted papilloma is described here because it shares certain features with exophytic urothelial papilloma. The histology of inverted papillomas has been well described, but it typically consists of complex interanastomosing cords of urothelium within the lamina propria. The amount of intervening stroma is variable. There is usually a well-circumscribed border at the base, and a characteristic palisading of basaloid cells at the periphery of the nests/cords. Centrally within the nests/cords, the neoplastic cells often have a spindled appearance or rarely, show non-keratinizing squamous metaplasia. Mitotic figures are rare or absent. Rarely, cases are hybrid with different areas of the lesion resemble exophytic urothelial papillomas and inverted urothelial papillomas; these lesions are generally classified as papillomas with both exophytic and inverted features. By definition, inverted papillomas lack nuclear pleomorphism, nuclear hyperchromasia, or significant mitotic activity; however, scattered cells with multinucleation or degenerative type atypia have been

described and do not seem to alter the benign clinical course. Central cystic change (cystitis cystica-like or colloid cyst pattern) has also been described. When prominent, this cystitis cystica-like pattern of inverted papilloma may have a glandular appearance at low-power magnification.

The differential diagnosis is limited. Inverted papillomas usually show the anastomosing growth pattern with peripheral basaloid cells that is distinct from florid cystitis cystica which is characterized by well-delineated, round nests of normal appearing urothelium; however, this distinction may be somewhat arbitrary in occasional cases. Endophytic patterns of urothelial carcinoma may also closely resemble inverted papillomas,[9] but the invaginated cords are typically broader with greater variation in size including transition to solid areas. In addition, carcinomas typically have a greater degree of cytologic atypia, do not demonstrate the homogeneous basaloid appearance of inverted papillomas, and often have a prominent exophytic component that does not resemble a urothelial papilloma (as discussed below). Any true stromal invasion (discussed under patterns of invasion) would disqualify a lesion from being classified as inverted papilloma.

When diagnosed by these strict criteria and completely excised, inverted papillomas have a very low risk of recurrence (less than 1%). The controversy in the literature (conflicting reports) regarding the prognosis of inverted papillomas and their association with carcinoma is likely due to endophytic patterns of urothelial carcinoma being classified as inverted papilloma in the older literature.

Urothelial Papilloma:[1,10-12] Under the WHO 2004 classification, very restrictive criteria are employed for the diagnosis of urothelial papilloma. “Urothelial papilloma” without qualifiers refers to the exophytic variant of papilloma, defined as a discrete papillary growth with a central fibrovascular core lined by urothelium of normal thickness and normal cytology. The low-power papillary architecture is a relatively simple branching pattern without fusion. The umbrella cell layer is often prominent and may show prominent vacuolization, nuclear enlargement, or cytoplasmic eosinophilia. Some unusual features that have been reported in papillomas include dilation of lymphatic spaces within the papillae, gland-in-gland patterns, and foamy histiocytes within the papillae. This is a rare, benign condition typically occurring as a small, isolated growth, commonly, but not exclusively seen in younger patients.

The outcome review in Table 2 refers to urothelial papillomas as defined above; we have selected the reported series that utilize the restrictive WHO 2004 criteria. Although the term “papilloma” has been used in the literature for a variety of papillary neoplasms with variable degrees of architectural and cytologic atypia (including lesions currently classified as papillary urothelial neoplasms of low malignant potential), the data from those studies was not included.

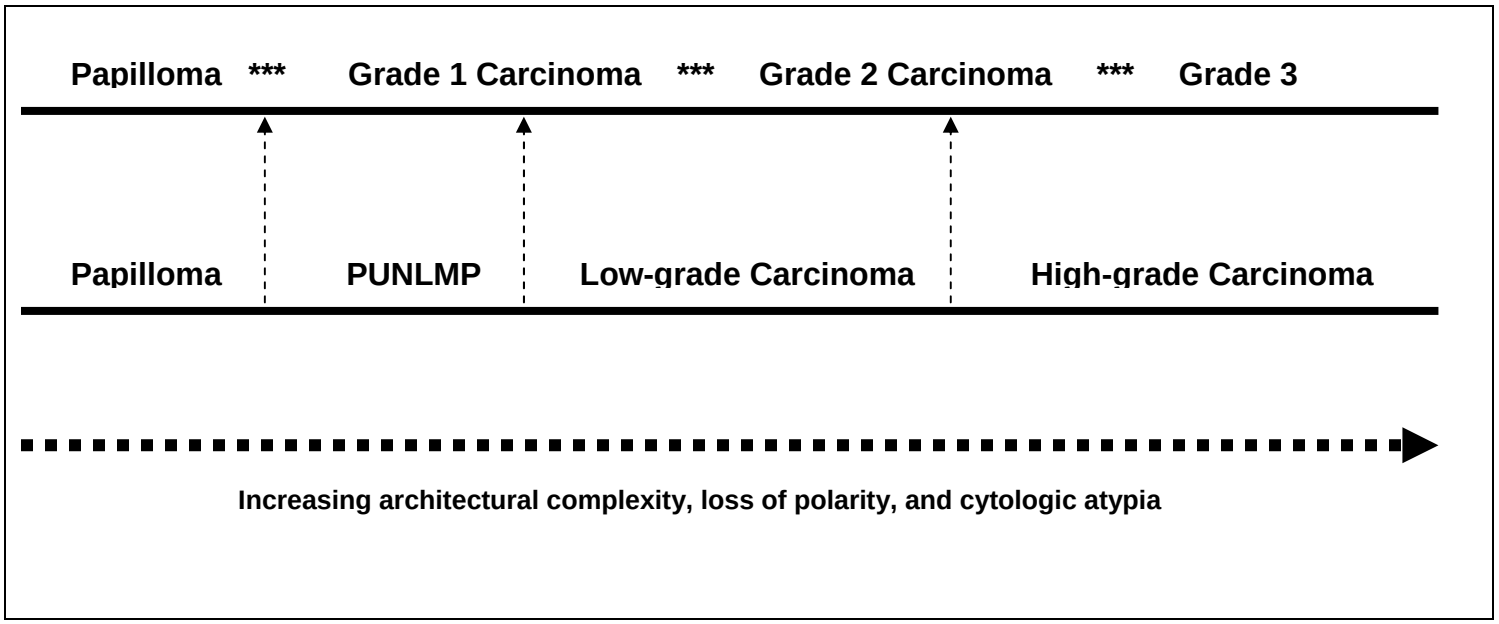
Papillary Urothelial Neoplasm of Low Malignant Potential (PUNLMP):[1,13-20] These tumors resemble urothelial papillomas, but generally have a markedly thickened (hyperplastic) urothelial lining. By definition, the nuclei may be slightly enlarged, but there is minimal to absent cytologic atypia. There is also normal polarity of the urothelial cells with an orderly, predominantly linear arrangement perpendicular to the basement membrane. Mitotic figures are infrequent in papillary urothelial neoplasms of low malignant potential, and usually limited to the basal layer. The umbrella cell layer is frequently maintained. Like papillomas, the papillae of PUNLMP generally have a simple branching pattern with discrete, slender papillae. These patients are at an increased risk of developing recurrent or new papillary lesions that occasionally are of higher grade and may progress. The diagnosis of PUNLMP should be carefully reconsidered in the presence of stromal invasion because that finding would be highly unusual.

Papillary Urothelial Carcinoma, Low-Grade:[1,13,15-20] Low-grade papillary urothelial carcinomas are characterized by an overall orderly appearance but with easily recognizable variation of architectural and or cytologic features seen at scanning magnification. Variation of polarity (loss of perpendicular arrangement to the basement membrane) and nuclear size, shape, and chromatin texture comprise the minimal criteria for the diagnosis of low-grade carcinoma. Mitotic figures are infrequent and usually seen in the lower half; but may be seen at any level of the urothelium. Tangential sections near the base of the urothelium may be misleading and result in sheets of immature urothelium with frequent mitotic activity. A spectrum of cytologic and architectural abnormalities may exist within a single lesion, stressing the importance of examining the entire lesion and noting the highest grade of abnormality.

Papillary Urothelial Carcinoma, High-Grade:[17-20] High-grade carcinomas are characterized by a complex, disordered architecture and moderate to marked cytologic atypia. Although the low-power papillary architecture is frequently complex with obvious anastomosis of adjacent papillae creating fused, confluent formations, the definitional feature for a diagnosis of high-grade carcinoma is the cytology of the neoplastic cells. Cytologically, there is a spectrum of pleomorphism ranging from moderate to marked, but obvious nuclear membrane irregularity and irregular, clumped chromatin represent the minimal diagnostic criteria. The individual neoplastic cells are often more rounded than in lower grade lesions and have a loss of polarity in relation to the basement membrane (random, non-perpendicular arrangement within the urothelium). Mitotic figures, including atypical forms, are frequently seen. In tumors with variable histology, the tumor should be graded according to the highest grade. High-grade papillary urothelial carcinomas have a much higher risk of progression than low-grade lesions. These tumors also have a high risk of association with invasive disease at the time of diagnosis. Paralleling the high-grade cytologic atypia within these lesions, the surrounding flat urothelial mucosa may also demonstrate urothelial carcinoma in-situ.

COMPARISON OF WHO 2004/ISUP CLASSIFICATION TO WHO 1973

Since many pathologists have practiced under the WHO 1973 system, it should be emphasized that the WHO 2004 diagnostic categories are not directly translatable into the WHO 1973 system. Although the criteria for papilloma are identical, as shown in the schematic below, the PUNLMP category is more restrictive than the 1973 Grade I carcinoma category and the diagnostic threshold for high-grade carcinoma has been lowered.



CLINICAL RELEVANCE OF WHO/ISUP CLASSIFICATION

Emerging clinical follow-up data suggest that the WHO 2004/ISUP diagnostic category of papillary urothelial neoplasm of low malignant potential (PUNLMP) identifies a clinically and biologically distinct lesion within the spectrum of papillary urothelial neoplasia. Table 3 compares the recurrence rates, grade progression, stage progression and survival rates between tumors in the different categories of non-invasive papillary urothelial neoplasms of the bladder.

TABLE 2: HISTOLOGIC FEATURES OF UROTHELIAL PAPILLARY LESIONS

	Papilloma	Papillary neoplasm of low malignant potential	Low-grade papillary carcinoma	High-grade papillary carcinoma
Architecture				
Papillae	Delicate	Delicate. Occasional fused	Fused, branching, and delicate	Fused, branching and delicate
Organization of cells	Identical to normal	Polarity identical to normal. Any thickness Cohesive	Predominantly ordered, yet minimal crowding and minimal loss of polarity. Any thickness. Cohesive	Predominantly disordered with frequent loss of polarity. Any thickness. Often discohesive
Cytology				
Nuclear size	Identical to normal	May be uniformly enlarged	Enlarged with variation in size	Enlarged with variation in size
Nuclear shape	Identical to normal	Elongated, round-oval, uniform	Round-oval. Slight variation in shape and contour	Moderate-marked pleomorphism
Nuclear chromatin	Fine	Fine	Mild variation within and between cells	Moderate-marked variation both within and between cells with hyperchromasia
Nucleoli	Absent	Absent to inconspicuous	Usually inconspicuous*	Multiple prominent nucleoli may be present
Mitoses	Absent	Rare, basal	Occasionally at any level	Usually frequent, at any level
Umbrella cells	Uniformly present	Present	Usually present	May be absent

* If present, small and regular and not accompanied by other features of high-grade carcinoma

TABLE 3

	Papilloma	Papillary neoplasm of low malignant potential	Low-grade papillary carcinoma	High-grade papillary carcinoma
Recurrence	0-8.8%	25-35%	48-77%	55%
Grade progression	0-8.8%	11%	7%	Not applicable
Stage progression	0%**	0-4%	2-12%	27%
Survival	100%	93-100%*	82-96%	80-90%

*4% of PUNLMPs in one series developed invasive disease (mean interval 13.3 years) and 3% died of bladder cancer, suggesting that long-term follow-up is clearly needed for patients with these tumors

**One reported patient progressed to invasive carcinoma, but the case was complicated by immunosuppressive therapy for a renal transplant[12]

Selected References for Papillary Urothelial Neoplasms

1) Sauter G, Algaba F, Amin MB, Busch C et al: Non-invasive urothelial tumours. In: Epstein JI, Eble JN, Sesterhenn I, Sauter G (eds): World Health Organization Classification of Tumours Pathology and Genetics: Tumours of the Urinary System and Male Genital Organs. IARC Press, Lyon, 2004; pp 110.
2) Epstein JI, Amin MB, Reuter VE, Mostofi FK: The World Health Organization/International Society of Urological Pathology consensus classification of urothelial (transitional cell) neoplasms of the urinary bladder. *Am J Surg Pathol* 22:1435-1448, 1998.
3) Malmstrom P-U, Busch C, Norlen BJ. Recurrence, progression, and survival in bladder cancer: a retrospective analysis of 232 patients with 5-year follow-up. *Scand J Urol Nephrol* 1987;21:185-195.
4) Eble JN, Young RH: Benign and low-grade papillary lesions of the urinary bladder: a review of the papilloma-papillary carcinoma controversy and a report of 5 typical papillomas. *Sem Diagn Pathol* 6:351-371, 1989.
5) Amin MB, Murphy WM, Reuter VE, et al: Controversies in the pathology of transitional cell carcinoma of the urinary bladder. In: Rosen PP, Fechner RE (eds): Reviews in Pathology, Chicago IL, ASCP Press, 1996, pp.1-38.
6) Kunze E, Schauer A, Schmitt M. Histology and histogenesis of two different types of inverted urothelial papillomas. *Cancer* 1983;51:348-358.
7) Demeester LJ, Farrow GM, Utz DC. Inverted papilloma of the urinary bladder. *Cancer* 1975;36:505-513.
8) Broussard JN, Tan PH, Epstein JI. Atypia in inverted urothelial papillomas:

pathology and prognostic significance. *Hum Pathol* 2004;35:1499-1504.
9) Amin MB, Gomez JA, Young RH. Urothelial transitional cell carcinoma with endophytic growth patterns: a discussion of patterns of invasion and problems associated with assessment of invasion in 18 cases. *Am J Surg Pathol* 1997;21:1057-1068
10) Cheng L, Darson M, Cheville JC, Neumann RM, Zincke H, Nehru A, Bostwick DG: Urothelial papilloma of the bladder. Clinical and biologic implications. *Cancer* 86:2098-2101, 1999.
11) Magi-Galluzzi C, Epstein JI. Urothelial papilloma of the bladder. A review of 34 de novo cases. *Am J Surg Pathol* 2004;28:1615-1620.
12) McKenney, Amin MB, Young RH. Urothelial (transitional cell) papilloma of the urinary bladder: a clinicopathologic study of 26 cases. *Mod Pathol* 2003;16:623-9.
13) Holmang S, Hedelin H, Anderstrom C, Holmberg E, Busch C, Johansson S: Recurrence and progression in low-grade papillary urothelial tumors. *J Urol* 1999;162:702-707.
14) Cheng L, Neumann RM, Bostwick DG: Papillary urothelial neoplasms of low malignant potential. Clinical and biologic implications. *Cancer* 1999;86:2102-2108.
15) Pich A, Chiusa L, Formiconi A, Galliano D, Bortolin P, Navone R. Biologic differences between noninvasive papillary urothelial neoplasms of low malignat potential and low-grade (grade 1) papillary carcinoma of the bladder. *Am J Surg Pathol* 2001;25:1528-1533.
16) Alsheikh A, Mohamedali Z, Jones E, Masterson J, Gilks CB. Comparison of the WHO/ISUP classification and cytokeratin 20 erxpression in predicting the behavior of low-grade papillary urothelial tumors. *Mod Pathol* 2001;14:267-272.
17) Desai S, Lim SD, Jimenez RE, Chun T, Keane TE, McKenney JK, Zavala-Pompa A, Cohen C, Young RH, Amin MB: Relationship of cytokeratin 20 and CD44 protein expression with WHO/ISUP grade in pTa and pTI papillary urothelial neoplasia. *Mod Pathol* 2000;13:1315-1323.
18) Holmang S, Andius P, Hedelin H, Wester K, Busch C, Johansson SL: Stage progression in Ta papillary urothelial tumors: relationship to grade, immunohistochemical expression of tumor markers, mitotic frequency and DNA ploidy. *J Urol* 2001;165:1124-1128.
19) Oosterhuis JW, Schapers RF, Janssen-Heijnen ML, Paulwels RP, Newling DW, ten Kate F. Histological grading of papillary urothelial carcinoma of the bladder: prognostic value of the 1998 WHO/ISUP classification system and comparison with conventional grading systems. *J Clin Pathol* 2002;55:900-905.
20) Samaratunga H, Makarov DV, Epstein JI. Comparison of WHO/ISUP and WHO classification of noninvasive papillary urothelial neoplasms for risk of progression. *Urology* 2002;60:315-319.

PART 3: INVASIVE UROTHELIAL CARCINOMA: STAGING ISSUES

Outline

1. Anatomy of the Bladder Wall
    A. Recent descriptions of variable urinary bladder musculature with staging implications
2. Staging
    A. Patterns of Invasion
    B. Muscle Invasion (Muscularis Mucosae versus Propria)
    C. Pitfalls

1. Anatomy of the Bladder Wall

Classic Lamina Propria:
The lamina propria is defined as the region of the bladder below the basement membrane of the urothelium, but superficial to the muscularis propria. It consists predominantly of very hypocellular, loosely collagenized stroma. Rare, scattered multinucleated stromal cells are common in the lamina propria. In addition, prominent medium-sized blood vessels are commonly present and may be associated with small wispy fascicles of smooth muscle (typical muscularis mucosae). The muscularis mucosa is often incomplete, consisting of irregular, isolated fascicles. Muscularis mucosa is occasionally hyperplastic with thickened muscle fibers, but the individual muscle fibers are haphazardly arranged in different directions with a jagged outline. Normal adipose tissue has also been described within the lamina propria, and does not signify extravesical tissue. The thickness of the lamina propria greatly varies, but is usually much thinner in the trigone and bladder neck.

Classic Muscularis Propria:
The muscularis propria or “detrusor” muscle consists of large, thick, compact bundles of smooth muscle with variable amounts of interspersed collagen. Compared to the muscularis mucosae, the muscularis propria contains larger, better developed fascicles of smooth muscle organized into compact bundles.

Because of variation in thickness of the lamina propria, the muscularis propria may be surprisingly superficial in some biopsies of the trigone. Adipose tissue is commonly identified in the muscularis propria.

Adventitia and Perivesical Adipose Tissue:
These tissues are deep to the muscularis propria and consist of loose fibroconnective and adipose tissue; however, they are not sampled by routine bladder biopsy.

Recently Described Variability in Bladder Musculature:
Two recent studies have carefully examined the microscopic anatomy of the bladder musculature with special attention to variation by anatomic region (i.e. dome versus trigone, etc). Patterns of muscle distribution that do not precisely fit the classic descriptions of lamina propria and muscularis propria were described, and included:

1. Large compact bundles of smooth muscle scattered individually within the lamina propria (commonly in the dome). These bundles have smooth round contours unlike classic hypertrophic muscularis mucosae (Figure 1B).
2. The superficial border of the muscularis propria may not be distinct. There can be dispersion of individual smooth muscle bundles extending upward into the lamina propria from the dense compact bundles of the muscularis propria (Figure 1C). In the trigone, these dispersed muscle bundles are usually smaller caliber, while they are larger caliber in the dome.

Immunohistochemistry in bladder musculature:
Immunostains for smooth muscle actin may occasionally help delineate the shape/contour of muscle bundles, or help identify the presence of muscle trapped within confluent invasive tumor. A few studies have examined immunohistochemical differences between muscularis mucosae and muscularis propria, but none have had sufficient specificity for routine practice. Smoothelin is a marker of contractile muscle that has potential utility in this setting. Classic muscularis propria stains strongly and diffusely with smoothelin, while classic muscularis mucosae show absent to weak, patchy reactivity; however studies have now reported varying results. At present, pathologic staging should be based on morphology.

References for Microscopic Anatomy of Bladder Wall

Dixon JD, Gosling JA. Histology and fine structure of the muscularis mucosae of the human bladder. *J Anat* 1983;136:265-71.

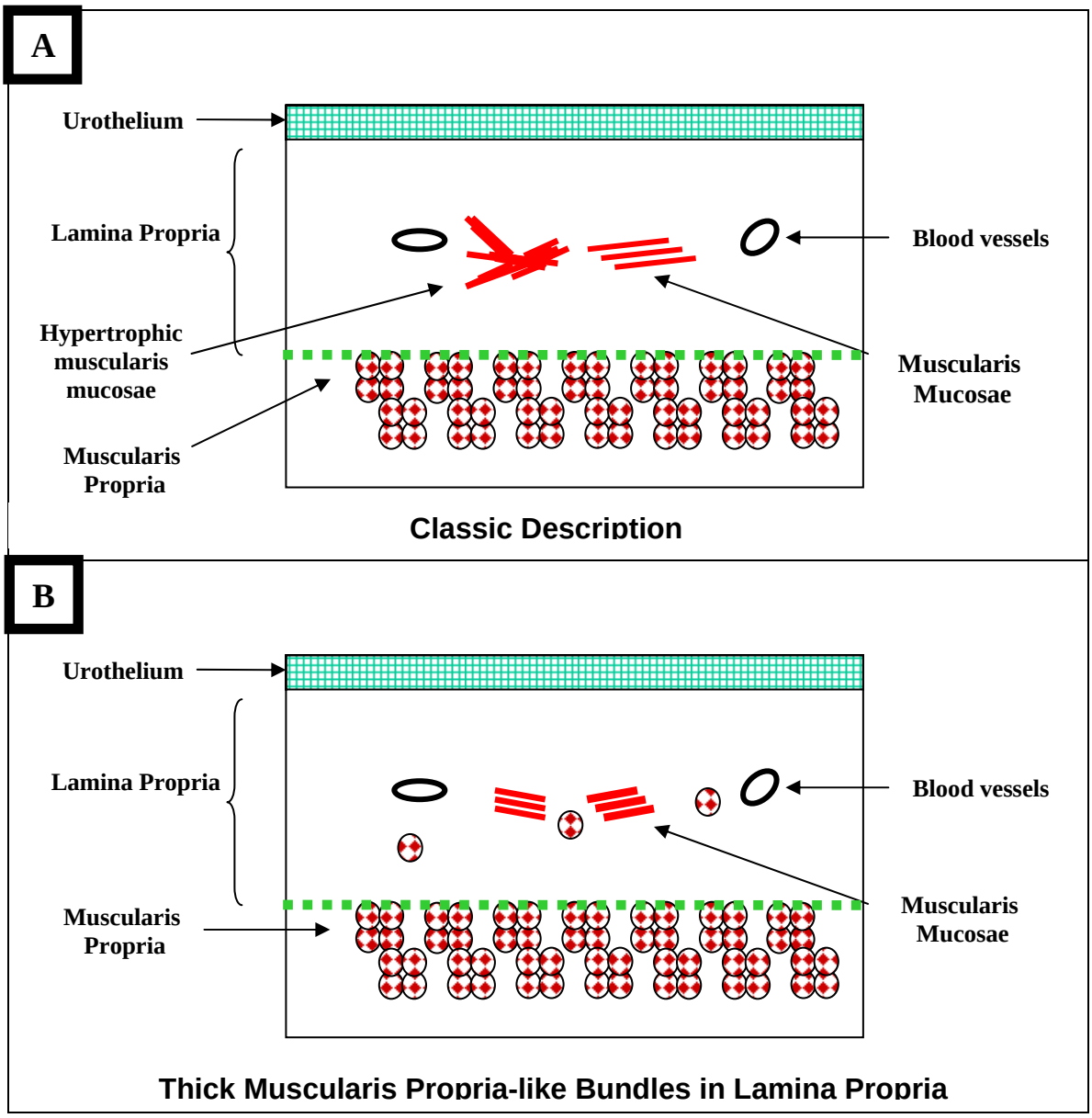
Ro JY, Ayala AG, El-Naggar A. Muscularis mucosa of urinary bladder-importance for staging and treatment. *Am J Surg Pathol* 1987;11:668-73.

Paner GP, Ro JY, Wojcik EM et al. Further characterization of the muscle layers and lamina propria of the urinary bladder by systematic histologic mapping. Implications for pathologic staging of invasive urothelial carcinoma. *Am J Surg Pathol* 2007;31:1420-1429.
Vakar-Lopez F, Shen SS, Zhang S, et al. Muscularis mucosae of the urinary bladder revisited with emphasis on its hyperplastic patterns: a study of a large series of cystectomy specimens. *Ann Diag Pathol* 2007;11:395-401.
Lapetino S, Paner GP, Venkataraman G et al. Diagnostic utility of antibody to smoothelin in the distinction of muscularis mucosa from muscularis propria of the urinary bladder. Mod Pathol 2008; 21:165A (751).
Philip AT, Amin MB, Tamboli T, et al. Intravesical adipose tissue: a quantitative study of its presence and location with implications for therapy and prognosis. Am J Surg Pathol 2000;24:1286-1290.

Smoothelin Immunohistochemistry

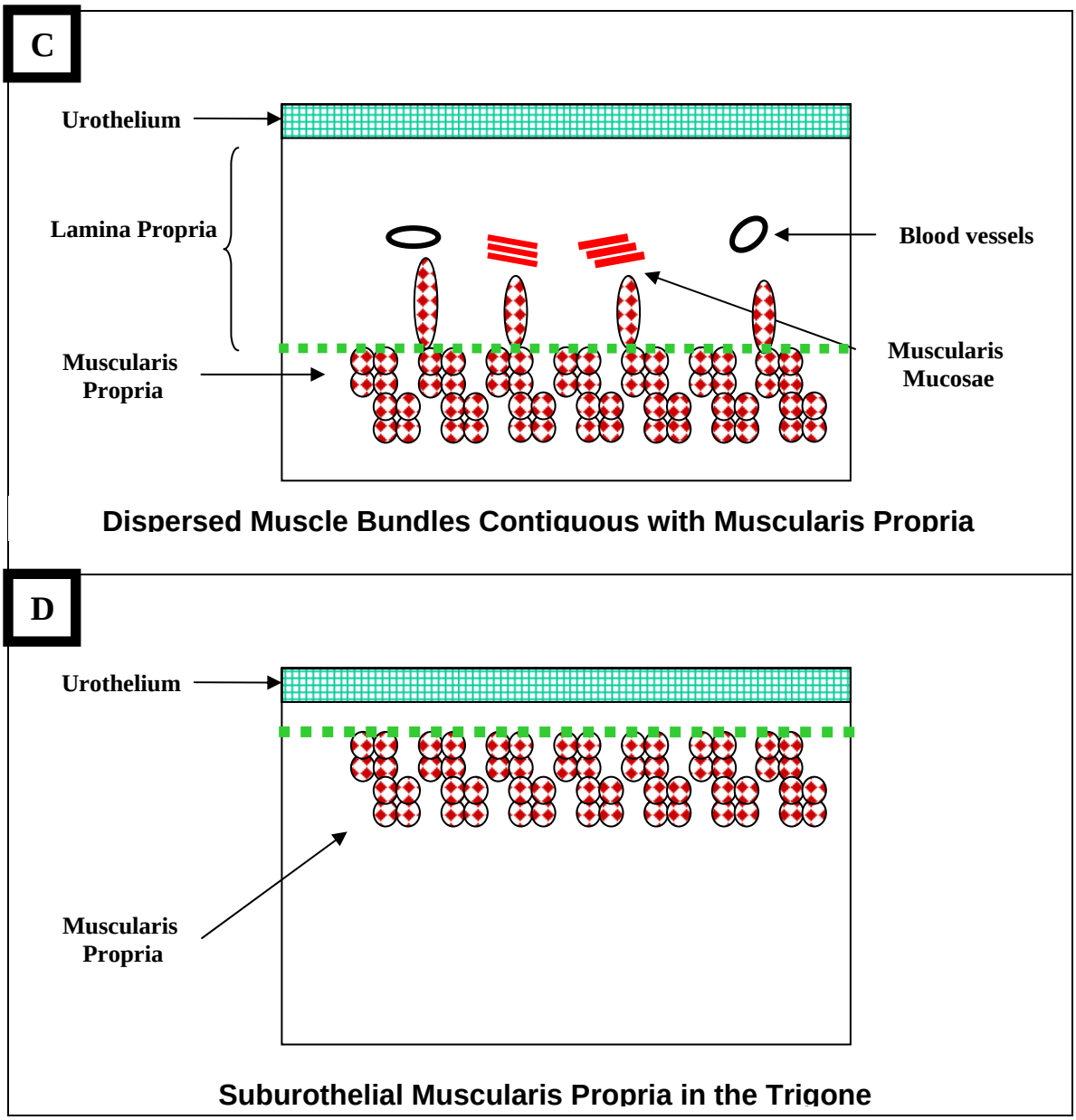
Bovio IM, Al-Quran SZ, Rosser CJ, Algood CB, Drew PA, Allan RW. Smoothelin immunohistochemistry is a useful adjunct for assessing muscularis propria invasion in bladder carcinoma. Histopathology 2010;56(7):951-6.
Council L, Hameed O. Differential expression of immunohistochemical markers in bladder smooth muscle and myofibroblasts, and the potential utility of desmin, smoothelin, and vimentin in staging of bladder carcinoma. Mod Pathol 2009;22(5):639-50.
Hansel DE, Paner GP, Nese N, Amin MB. Limited smoothelin expression within the muscularis mucosae: validation in bladder diverticula. Hum Pathol 2011 [Epub ahead of print].
Lindh C, Nilsson R, Lindstrom ML, Lundin L, Elmberger G. Detection of smoothelin expression in the urinary bladder is strongly dependent on pretreatment conditions: a critical analysis with possible consequences for cancer staging. Virchows Arch 2011;458(6):665-70.
Miyamoto H, Sharma RB, Illei PB, Epstein JI. Pitfalls in the use of smoothelin to identify muscularis propria invasion by urothelial carcinoma. Am J Surg Pathol 2010;34(3):418-22.
Paner GP, Brown JG, Lapetino S, Nese N, Gupta R, Shen SS, Hansel DE, Amin MB. Diagnostic use of antibody to smoothelin in the recognition of muscularis propria in transurethral resection of urinary bladder tumor (TURBT) specimens. Am J Surg Pathol 2010;34(6):792-9.
Paner GP, Shen SS, Lapetino S, Venkataraman G, Barkan GA, Quek ML, Ro JY, Amin MB. Diagnostic utility of antibody to smoothelin in the distinction of muscularis propria from muscularis mucosae of the urinary bladder: a potential ancillary tool in the pathologic staging of invasive urothelial carcinoma. Am J Surg Pathol 2009;33(1):91-8.

Figure 1: Representation of Bladder Wall Microscopic Anatomy



A. Classic lamina propria: Thin wispy fascicles of smooth muscle are present in the lamina propria, associated with medium caliber thin walled blood vessels. When hypertrophic, the fascicles are thickened, but they have a haphazard, jagged arrangement.

B. Muscularis propria-like bundles in the lamina propria: Individual compact bundles of smooth muscle, similar to the individual bundles of the muscularis propria, are present scattered within the lamina propria.



C. Dispersed smooth muscle contiguous with muscularis propria (dispersed boundary): In contrast to the usual sharp interface between the lamina propria and muscularis propria, the interface may be irregular with dispersion of muscle bundles upward into the lamina propria.

D. Suburothelial muscularis propria in the trigone: Within the trigone, the muscularis propria may be very superficial with little intervening lamina propria.

2. Staging

a. Patterns of Lamina Propria Invasion: The earliest recognizable invasion into the lamina propria has two main morphologic patterns: 1) small clusters and individual neoplastic cells in the stroma, usually with surrounding retraction artifact, and 2) irregular, jagged projections of epithelium extending from the overlying urothelial carcinoma (papillary carcinoma or CIS). The neoplastic cells are high-grade in the vast majority of cases and may show increased cytoplasmic eosinophilia when compared to the overlying non-invasive tumor (i.e. paradoxical maturation). There may be no stromal response, stromal retraction, peritumoral edema and inflammation, stromal myxoid change, and/or stromal desmoplasia.

b. Muscularis Propria Invasion: Muscularis propria invasion is defined by carcinomas invading between thick bundles of compact smooth muscle. There is a spectrum of morphologic patterns and stromal reactions identical to those seen in lamina propria invasion. It should be emphasized that some deeply invasive tumors do not have a stromal response, but extend between the fascicles of the muscularis propria in a "melting" pattern.

Most centers follow the AJCC cancer staging guidelines as follows:

pT0: No evidence of primary tumor
pTa: Non-invasive papillary carcinoma
pTis: Carcinoma in situ
pT1: Tumor invades subepithelial connective tissue (**lamina propria**)
----
pT2:Tumor invades muscularis propria
pT2a: inner half
pT2b: outer half
pT3: Tumor invades perivesical tissue
pT3a: microscopically
pT3b: macroscopically
pT4: Tumor invades any of the following: prostate, uterus, vagina, pelvic wall, abdominal wall

Adipose tissue is commonly present within the muscularis propria and does not signify a pT3 tumor. It is recommended that bladder biopsy evaluation be restricted to pT2 disease; pT3 and pT4 carcinomas can only be diagnosed at cystectomy. The most important distinction for papillary neoplasms on biopsy evaluation is pT1 vs. pT2 disease. Urologists usually favor conservative management with intravesical treatments for high-grade pTa and any pT1 disease. The diagnosis of pT2 carcinoma is generally the threshold for surgical management (cystectomy) or radiation therapy. The histologic parameters and

reporting discussed in this section should allow the urologist to easily assign a T stage to the patient, and to select the appropriate treatment plan.

Given the recent studies on bladder musculature, what should be regarded as muscularis propria for staging purposes in surgical specimens today?

Until outcome studies are performed that evaluate the involvement of these varying patterns of smooth muscle by urothelial carcinoma, we are left trying to develop a practical approach. I try to reserve "muscularis propria invasion" for carcinomas that I would interpret as definitively extending beyond the boundary of continuous compact, aggregated smooth muscle bundles (shown in Figure 1 by the dashed green line). Obviously, this may be difficult in biopsies given the new musculature descriptions, but I personally try to apply the following guidelines when staging in TURBT specimens.

1. *Individual compact bundles of smooth muscle with a sharp round contour should not be classified as muscularis propria when separated by stroma.* Their scattered nature, their location in otherwise typical lamina propria, their continuity with otherwise typical muscularis mucosae, and the presence of deeper aggregated bundles of obvious muscularis propria in the same section should help in the distinction from true muscularis propria.
2. Dispersed thick muscle bundles *separated by stroma* with extension upward from the muscularis propria, should not be classified as definitive muscularis propria.
3. Confluent invasive carcinoma entrapping scant muscle fibers in which the muscle architecture or the associated surrounding structures (e.g. vessels and stroma) cannot be seen because of a sheet-like growth of carcinoma should be interpreted cautiously. In these instances, a descriptive diagnosis is usually best.
4. The low power architecture and association with other structures may aid in the interpretation of difficult cases, especially if definite muscularis propria is present in the same tissue fragment.

In equivocal cases, restaging biopsies should be performed, and I utilize the sign-out template below. If the features are equivocal because of extensive confluent carcinoma, I would also comment on the volume/extent of invasive tumor.

Urinary bladder, trigone, transurethral resection of bladder tumor:
-- Invasive urothelial carcinoma (see comment regarding stage)

Comment: This invasive urothelial carcinoma involves at least the lamina propria. There is infiltration of smooth muscle, but the histologic features do not allow distinction of muscularis mucosae from muscularis propria in this biopsy specimen.

C. Pitfalls in the Diagnosis of Invasive Urothelial Carcinoma

Adipose tissue in lamina propria and muscularis propria: As discussed above, adipose tissue is commonly found in the lamina propria and muscularis propria and should not be taken as evidence of invasion into perivesical adipose tissue in biopsy specimens.

Involvement of muscularis mucosae: The classic pattern of hyperplastic muscularis mucosae has a jagged irregular contour, and is associated with other elements of the lamina propria such as prominent blood vessels and loose stroma.

Urothelial carcinoma in situ involving von Brunn nests: The smooth, round contour and lobular configuration of von Brunn nests (despite colonization by high-grade urothelial cells) are the most useful features for the distinction from invasive carcinoma. The presence of small, irregular clusters, single cells, or retraction artifact would favor stromal invasion.

Colonization of prostatic glands: At the bladder base, the muscularis propria becomes continuous with the fibromuscular stroma of the prostate. If prostatic tissue is biopsied, it may closely mimic a glandular pattern of invasion into the muscularis propria. The secretory-type prostatic epithelium lining the glands aids in this distinction. If the glands become colonized by urothelial neoplasia, this distinction becomes very difficult because of the presence of epithelium within dense muscle. The smooth, round contours of the epithelium, the lack of a stromal response, the identification of adjacent prostate glands, and the location of the biopsy (bladder base/prostatic urethra) should all suggest the possibility of the colonization of prostatic glands by urothelial neoplasia, and argue against invasion.

Non-invasive endophytic growth patterns: Described below.

Over-diagnosis of vascular invasion: Retraction artifact around nests of infiltrating urothelial carcinoma is very common. True vascular invasion in the lamina propria, in my experience, is frequently over-diagnosed. We require tumor emboli in an obvious vascular space (with smooth muscle evident in the vascular wall) or immunohistochemical confirmation to render a diagnosis of angiolymphatic invasion.

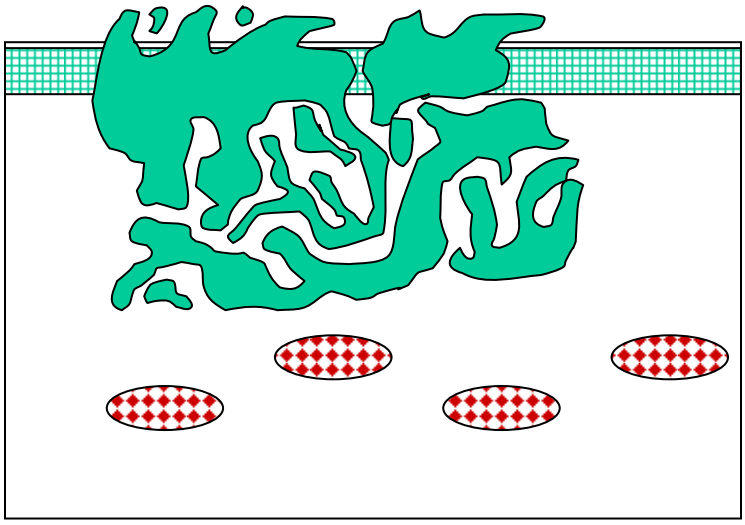
Under-diagnosis of subtle variant patterns of urothelial carcinoma: See selected variants.

Associated myofibroblastic proliferation: Florid myofibroblastic proliferations resembling post-operative spindle cell nodule/pseudosarcomatous myofibroblastic proliferation may be associated with invasive carcinoma and can potentially mimic muscle.

The loose arrangement of the myofibroblasts and the usual intervening blue tinged stroma should allow distinction in most cases. True smooth muscle bundles have a much more compact arrangement without intervening stroma.

Endophytic/inverted urothelial neoplasms:
Endophytic growth of a urothelial carcinoma (represented in the schematic below) is not defined as stromal invasion despite the extension deep into the lamina propria. It is characterized by invaginated, inter-anastomosing cords of urothelium with a relatively smooth, pushing border. It is usually associated with an overlying papillary urothelial neoplasm. In this setting, invasion is only diagnosed when there is also a focus fitting the histologic criteria for lamina propria or muscularis propria invasion as discussed previously.

Endophytic (Inverted) Urothelial Neoplasia:
a Non-invasive Pattern of Growth



This pattern of urothelial neoplasia is infrequent and has not received a great deal of attention in textbooks. It should be emphasized that the entire spectrum of urothelial neoplasia can grow in this endophytic pattern. We recommend a diagnostic approach identical to that used for papillary neoplasms (i.e.- the same diagnostic categories and the same cytologic and architectural criteria [WHO 2004/ISUP]). These tumors should also be staged as otherwise typical urothelial neoplasms, keeping in mind that *the endophytic component, on its own merit, is defined as non-invasive*.

Endophytic (Inverted) Urothelial Neoplams*

-- Inverted papilloma

-- Urothelial neoplasm of low malignant potential, endophytic pattern
-- Urothelial carcinoma, low-grade, endophytc pattern
-- Urothelial carcinoma, high-grade, endophytc pattern

* With the exception of inverted papilloma, these tumors are staged as 1) non-invasive, 2) with lamina propria invasion, or 3) with invasion of the muscularis propria depending on other associated histologic growth patterns of growth. (Amin MB, Gomez JA, Young RH. Urothelial transitional cell carcinoma with endophytic growth patterns: a discussion of patterns of invasion and problems associated with assessment of invasion in 18 cases. *Am J Surg Pathol* 1997;21:1057-1068.)

Appendix

Grading and Substaging of Invasive Urothelial Carcinoma

Studies suggest that stage is the most important prognostic factor in invasive urothelial carcinoma, independent of grade. This is supported by additional studies documenting the capacity of deeply invasive, cytologically bland carcinomas to produce metastases and cause patient mortality. Some authors have suggested the utility of substaging lamina propria invasion based on depth measurements or relation to the muscularis mucosae. This is difficult in practice because bladder biopsies are frequently sectioned in a tangential plane making orientation difficult to assess. Because of these difficulties and lack of an accepted reproducible method, substaging has not been adopted under the current systems of staging and classification (WHO 2004; 7th edition AJCC).

Sign-out Approach: Reporting

A simple diagnostic template is provided below that includes the relevant diagnostic findings needed for patient management. The use of the term “muscle invasion” should be avoided because it does not distinguish between muscularis propria and muscularis mucosae.

Invasive urothelial carcinoma

a) with lamina propria invasion; muscularis propria is present, but not involved/ not present
b) with muscularis propria invasion

*alternative differentiation (e.g. squamous) or variant subtype should also be included when relevant

PART 4: SELECTED VARIANTS OF UROTHELIAL CARCINOMA

Primary invasive urothelial carcinomas of the urinary bladder have a broad morphologic spectrum. Numerous variant types have been described and most present with high grade, high stage disease. This syllabus reviews selected variant types that either have major clinical relevance or present significant diagnostic difficulty on light microscopic evaluation.

1) PLASMACYTOID CARCINOMA

Rare invasive urothelial carcinomas have cytologic features closely mimicking plasma cells with round cytoplasmic borders, abundant eosinophilic cytoplasm, and an eccentrically placed hyperchromatic nucleus. The neoplastic cells are usually discohesive and set in a loose, myxoid stroma. Many reported cases also have features of conventional invasive urothelial carcinoma at least focally. Small biopsies occasionally show only the plasmacytoid pattern and may closely mimic a plasmacytoma or lymphoma. These carcinomas have been reported under the names “plasmacytoid”, “lymphoma-like”, and “carcinoma mimicking lobular carcinoma”. All three of these carcinomas share frequent loss of E-cadherin.

Immunohistochemistry may be required in this setting, and demonstration of cytokeratin reactivity confirms the diagnosis of carcinoma. Carcinomas may express CD138, so this marker should not be used in isolation as evidence of a plasma cell neoplasm; however, plasmacytoid carcinomas do not express MUM1. Cytokeratin positive tumors may require additional work-up for distinction between primary urothelial and metastatic carcinomas. Metastatic carcinomas (e.g., breast or gastric adenocarcinomas) are not typically plasmacytoid and, in general, have a signet-ring or non-specific appearance. One series of urothelial carcinomas has been reported with discohesive, non-plasmacytoid single cells that may be indistinguishable from a diffuse gastric or lobular breast carcinoma, and we have seen similar carcinomas. In that setting, immunoreactivity for CK20 is characteristic of a bladder or gastric primary. In difficult cases or in women with a known breast primary, immunostains for gross cystic disease fluid protein 15 (GCDFP-15), estrogen receptor, and progesterone receptor may help add evidence of a breast primary. In our experience, p63 expression is commonly lost in the plasmacytoid component, so this should not exclude the possibility of a bladder primary. Close clinical correlation may be essential in establishing the site of origin.

We have recently noted an unusual clinical aspect to these plasmacytoid carcinomas that has not been emphasized. They often have intra-abdominal spread along serosal surfaces, a behavior that is distinct from typical urothelial carcinoma. Some may also recur with carcinomatous effusions.

2) MICROPAPILLARY CARCINOMA

In 1994, Amin et al. described invasive micropapillary carcinoma (IMPC) as a morphologic variant of urothelial carcinoma that closely resembles ovarian serous carcinoma. It is well-recognized as a unique urothelial carcinoma variant that typically presents with high stage disease including lymph node metastases. In our personal experience, at least focal areas with a micropapillary pattern are seen at cystectomy in 3-5% of invasive urothelial carcinomas (unpublished data), and the reported range is 0.6-8.2%.

Establishing a minimal threshold for histologic classification of a given urothelial carcinoma as micropapillary is the most significant problem in the pathologic diagnosis of IMPC. One of the histologic hallmarks of IMPC is the presence of stromal retraction spaces surrounding the nests of carcinoma cells. Unfortunately, this feature is not entirely specific as many invasive urothelial carcinomas of no special type share this feature. The cellular nests of IMPC are typically quite small, but there is a wide spectrum of nest sizes that may be encountered in urothelial carcinomas, making the establishment of definitive criteria based entirely on size problematic. In a recent interobserver reproducibility study by our group, a set of restrictive criteria were proposed that allowed for a greater degree of agreement. These restrictive criteria identify a group of more prototypical IMPCs that include cases with small tumor cell nests within stromal retraction spaces, which are often back-to-back and often contain multiple tumor cell nests within each single retraction space. Other secondary features that are frequently seen include epithelial ring forms, intracytoplasmic vacuolization, elongated epithelial nests (i.e. micropapillae), and peripherally-oriented nuclei. Microscopic features suggesting that a urothelial carcinoma should not be classified as micropapillary include large nest size (generally greater than 5 or more nuclei across the narrowest width of the nest) and large branching aggregates or epithelial confluence of the tumor cell aggregates.

Non-invasive patterns of micropapillary carcinoma are also seen and are characterized by thin elongated filiform processes of neoplastic cells arising from the main papillary stalk. These “micropapillae” in the non-invasive component typically have a longer length than width. Studies have not fully addressed the prognostic significance in non-invasive micropapillary tumors.

Several studies have evaluated the potential utility of adjunctive immunohistochemical markers in the diagnosis of IMPC. A “reverse polarity” pattern of immunoreactivity with MUC1, whereby the outer stroma-facing (basal) cell surfaces stain, has been previously reported as typical of IMPC. Similarly, immunohistochemical expression of CA125 and Her2Neu have been reported as distinctive for IMPC; however, we have previously reported that these markers are not entirely specific for IMPC when compared specifically to invasive

urothelial carcinomas with prominent stromal retraction [specificity: MUC1 (37%), CA125 (87%), and Her2Neu (92%)]. As such, these markers lack reliable diagnostic utility in challenging cases, especially since it is our experience that invasive urothelial carcinoma with associated stromal retraction is much more common than IMPC. At present, we would recommend that the diagnosis of IMPC should be based on histologic features.

Micropapillary carcinomas typically present at high-stage with invasion into the muscularis propria, obvious vascular invasion, and lymph node metastases. For micropapillary carcinoma of the bladder, cystoscopic re-evaluation with additional biopsy is usually suggested if no muscularis propria invasion is identified at the initial staging. In fact, a recent clinical publication is advocating cystectomy for micropapillary carcinoma of the urinary bladder even in the absence of muscularis propria invasion. For this reason, we would suggest the use of the restrictive criteria proposed by Sangoi et al. until further outcome studies evaluate other less pronounced histologic patterns. This would avoid effectively changing the standard of care for a set of carcinomas without significant supporting data.

In cases presenting with metastatic disease, the most problematic pathologic issue is establishing the anatomic site of origin since many parenchymal organs may give rise to carcinomas with an identical micropapillary appearance. In general, carcinomas with a micropapillary pattern, including those of the bladder, maintain the expected immunophenotypic findings of their site of origin.

3. “DECEPTIVELY BLAND” CARCINOMA

Reports of cytologically bland urothelial carcinomas showing deep invasion of the bladder wall and associated lymph node metastases have been described under different names including nested variant, microcystic variant, and “deceptively bland” urothelial carcinoma. Individual cases may have mixed features or may be associated with a more typical papillary urothelial neoplasm, but three main histologic patterns closely mimicking a benign process are generally recognized as described below. By definition, these tumors do not show the high-grade nuclear features typical of invasive urothelial carcinoma, at least in the more superficial component.

Nested Pattern

The nested pattern is the most frequently reported subtle pattern of urothelial carcinoma. It may closely mimic von Brunn nests and is characterized by small, well-delineated, confluent nests of urothelial cells infiltrating the lamina propria and/or muscularis propria. It has been reported in association with papillary urothelial tumors and urothelial carcinoma in-situ, but in most cases the nested pattern of carcinoma has no other component. There is often only subtle nuclear

enlargement with mild variation in nuclear size and occasional nucleoli. The neoplastic cells do not show the typical high-grade features (marked hyperchromasia, obvious pleomorphism, mitotic activity) of invasive urothelial carcinoma. Despite the bland appearance of this neoplasm, deep muscle invasion, metastases, and tumor related death have all been reported with the nested variant of carcinoma. The main reason for recognizing these tumors is to avoid misinterpretation as a benign mimic because stage-for-stage they appear to behave as aggressively as conventional invasive urothelial carcinoma despite the low-grade appearance. No studies have looked specifically at the potential for different responses to treatment.

The main differential diagnosis is von Brunn nests. The best distinguishing feature is the extension of von Brunn nests to a uniform level within the lamina propria creating a sharp, linear border at the base that contrasts with the irregular, infiltrative base of nested carcinoma. Other subtle features of invasion may be seen in nested carcinomas such as small clusters or individual neoplastic cells with surrounding retraction artifact. The overall architectural arrangement of the neoplastic cells may also have a subtle difference when compared to von Brunn nests, but these features are not as definitive. The presence of small, irregularly sized, unevenly distributed nests creating confluent, branching patterns should serve as a clue to carefully consider the possibility of a nested carcinoma, as von Brunn nests are often clustered, evenly spaced, and round. In the ureter and renal pelvis, von Brunn nests may have smaller more irregular nests, but they still have a lobular or linear arrangement with a sharp border at the base. Invasion of the muscularis propria, despite the bland nuclear features, is diagnostic of carcinoma and is the most definitive distinguishing feature. Unfortunately, the distinction of nested carcinoma from a mimic such as von Brunn nests may not be possible in superficial biopsies when some of the subtle clues are not present, particularly when complicated by extensive cautery artifact. In difficult cases, correlation with the clinical impression of the urologist may suggest the presence of a more aggressive lesion.

Nephrogenic adenoma may also enter the differential diagnosis as it sometimes has a more irregular border and deeper location than von Brunn nests. The admixed tubular and papillary architecture is very characteristic of nephrogenic adenoma. In addition, the tubules and papillae of nephrogenic adenoma are lined by a single cell layer of cuboidal, columnar, or flattened cells, as opposed to the typically stratified urothelial layer or solid nests of nested carcinomas.

Finally, inverted papillomas might be considered because of their irregular, anastomosing pattern of growth within the lamina propria. Inverted papillomas, however, generally have a more complex anastomosing or trabecular architecture with little intervening stroma. As with von Brunn nests, they have a sharp border at the base. In addition, the individual nests have a distinct basal palisading of cells around the periphery and also frequently show spindling of the

lesional cells centrally. The subtle low-power architectural differences distinguishing these lesions are summarized in schematic form below.

Small Tubular Pattern

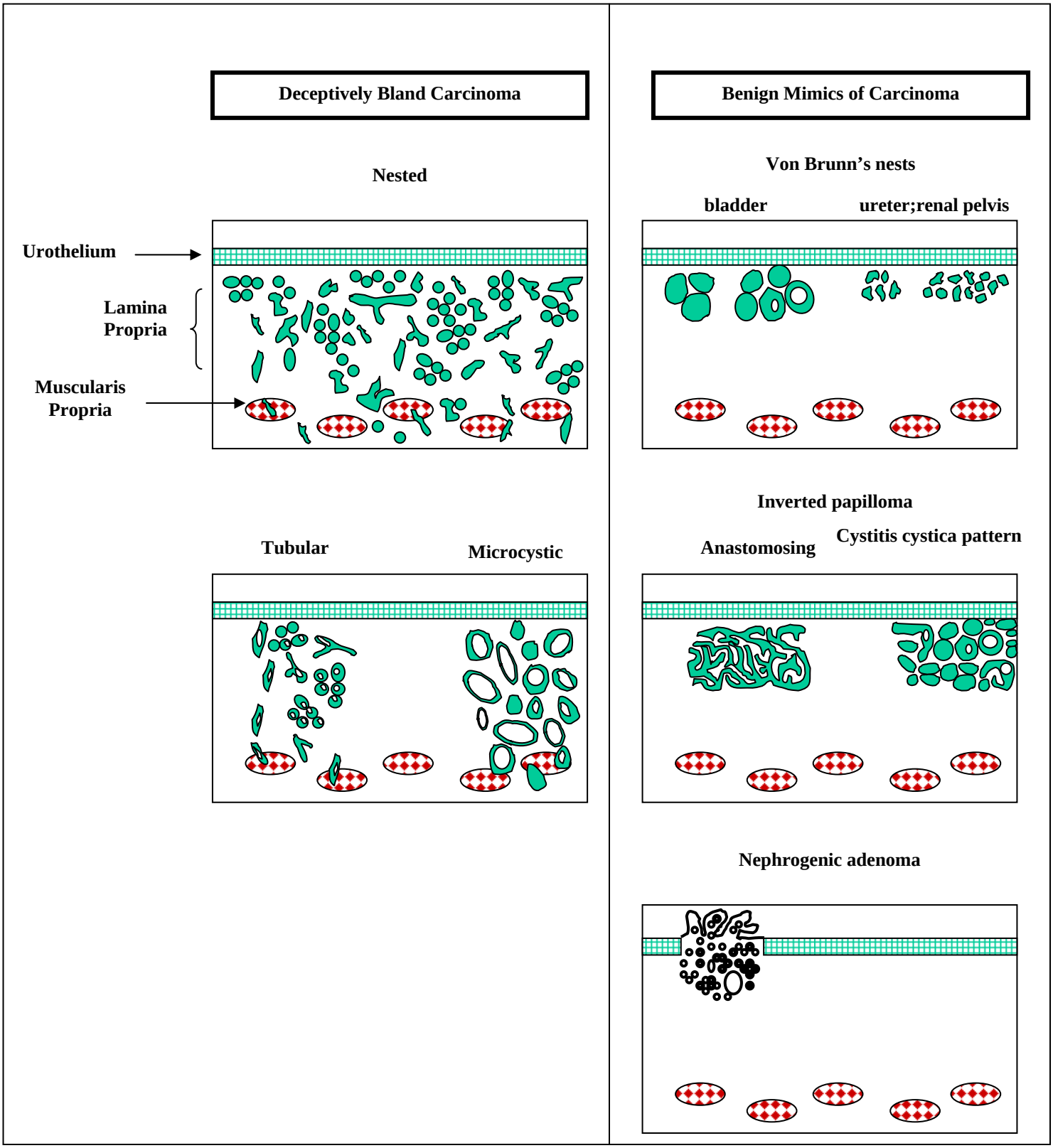
Other examples of deceptively bland carcinoma show the small irregular nests of urothelium typical of nested carcinoma, but with small central lumina. Again, these tumors may be mixed with the nested pattern, and the differentials are the same as discussed above.

Microcystic Pattern

Another reported pattern of deceptively bland invasive urothelial carcinoma has been called “microcystic”. As with the nested pattern, the nests of cytologically bland urothelium infiltrate the lamina propria and/or muscularis propria, but they contain prominent cystic change with central lumina. The lumina are lined by urothelial cells or cells with a more cuboidal appearance due to increased eosinophilic cytoplasm at the luminal surface or mucin. The nests show more variation in size than the nested pattern, and the larger cysts may have an attenuated or denuded lining. Calcification has been reported in the walls of the cysts. In the reported cases, the surrounding stroma may have no morphologic changes or, or rarely, a desmoplastic response.

The most important differential diagnostic considerations include benign mimics such as cystitis cystica glandularis and nephrogenic adenoma, but carcinomas such as clear cell carcinoma and urothelial carcinoma with glandular differentiation might also be considered. As discussed above, this pattern of carcinoma has an infiltrative growth with an uneven, irregular border at the base and irregularly spaced nests of variable size, features that aid in distinction from cystitis cystica. Although nephrogenic adenoma may have large dilated cystic structures and an irregular base, they are lined by a single cuboidal layer of epithelium, in contrast to the stratified urothelium of the microcystic carcinoma. Clear cell carcinoma also frequently shows a tubular pattern, but, by definition, those tumors have marked nuclear pleomorphism and hyperchromasia, features absent in the microcystic pattern. Finally, by strict definition, urothelial carcinoma with glandular differentiation refers to tumors with luminal spaces lined entirely by columnar or cuboidal cells with prominent apical cytoplasm. In contrast, microcystic carcinomas have luminal spaces lined by an admixture of stratified urothelial cells.

Deceptively Bland Carcinoma vs. Benign Mimics: Schematic Architectural Comparison



4. SMALL CELL CARCINOMA

Small cell carcinoma of the urinary bladder is histologically identical to the pulmonary type. It may be pure or admixed with a typical urothelial carcinoma (approximately 50%), squamous cell carcinoma (rare), or adenocarcinoma (rare). The differential diagnosis includes lymphoma, another subtype of poorly differentiated carcinoma, and alveolar rhabdomyosarcoma. Immunoreactivity for cytokeratins and chromogranin or synaptophysin is confirmatory. The distinction from a metastatic small cell carcinoma (when the tumor does not show evidence of an underlying urothelial carcinoma) is based on clinical/radiographic correlation. TTF-1 may be expressed in small cell carcinomas of any anatomic site, and does not indicate a lung origin. As in the lung, these small cell carcinomas are clinically aggressive.

Large cell neuroendocrine carcinomas have also been reported in the urinary bladder and, again, are identical to those occurring in the lung. They differ from small cell carcinoma because of more prominent eosinophilic cytoplasm and recognizable nucleoli. In our experience, large cell areas are commonly admixed with small cell carcinoma in the bladder.

Small cell carcinoma of the urinary bladder is the most commonly missed clinically significant diagnosis when outside cases are re-reviewed for planned surgery at our hospital. The cases are commonly called “poorly differentiated urothelial carcinoma”. It is important to recognize and diagnosis small cell carcinoma when present because it requires a different chemotherapeutic approach than used in urothelial carcinoma.

Selected References for Variants of Urothelial Carcinoma

Plasmacytoid carcinoma

1) Zuckerberg LR, Harris NL, Young RH. Carcinomas of the urinary bladders simulating malignant lymphoma. *Am J Surg Pathol* 1991; 15:569-576.
2) Sahin AS, Myhre, M., Ro JY, et al. Plasmacytoid transitional cell carcinoma. Report of a case with initial presentation mimicking multiple myeloma. *Acta Cytologica* 1991; 35:277-280.
3) Baldwin L, Lee AH, Al-Talib RK, Theaker JM: Transitional cell carcinoma of the bladder mimicking lobular carcinoma of the breast: a discohesive variant of urothelial carcinoma. *Histopathol* 2005;46:50-56.
4) Raspollini MR, Sardi I, Giunti L, Di Lollo S, Baroni G, Stomaci N, Menghetti I, Franchi A. Plasmacytoid urothelial carcinoma of the urinary bladder: clinicopathologic, immunohistochemical, ultrastructural, and molecular analysis of a case series. *Hum Pathol* 2011;42(8):1149-58.
5) Nigwekar P, Tamboli P, Amin MB, Osunkoya AO, Ben-Dor D, Amin MB. Plasmacytoid urothelial carcinoma: detailed analysis of morphology with clinicopathologic correlation in 17 cases. *Am J Surg Pathol* 2009;33(3):417-24.
6) Fritsche HM, Burger M, Denzinger S, Legal W, Goebell PJ, Hartmann A. Plasmacytoid urothelial carcinoma of the bladder: histological and clinical features of 5 cases. *J Urol* 2008;180(5):1923-7.
7) Ro JY, Shen SS, Lee HI, Hong EK, Lee YH, Cho NH, Jung SJ, Choi YJ, Ayala AG. Plasmacytoid transitional cell carcinoma of urinary bladder: a clinicopathologic study of 9 cases. *Am J Surg Pathol* 2008;32(5):752-7.

Deceptively bland urothelial carcinoma

1) Young RH, Zukerberg LR. Microcystic transitional cell carcinomas of the urinary bladder: a report of four cases. *Am J Clin Pathol* 1991; 96:635-639.
2) Murphy WM, Deana DG. The nested variant of transitional cell carcinoma: a neoplasm resembling proliferation of von Brunn's nests. *Mod Pathol* 1992; 5:240-243.
3) Holmang S, Johansson SL. The nested variant of transitional cell carcinoma-a rare neoplasm with poor prognosis. *Scand J Urol Nephrol* 2001 ;35 :102-105.
4) Drew PA, Furman J, Civantos F, et al. The nested variant of transitional cell carcinoma : an aggressive neoplasm with innocuous histology. *Mod Pathol*

1996 ;9 :989-994.
5) Lin O, Cardillo M, Dalbagni G, Linkov I, Hutchinson, Reuter VE. Nested variant of
urothelial carcinoma : a clinicopathologic and immunohistochemical study of 12
cases. *Mod Pathol* 2003;16:1289-1298.
6) Volmar KE, Chan TY, de Marzo AM, et al. Florid von Brunn's nests mimicking
urothelial carcinoma: a morphologic and immunohistochemical comparison to the
nested variant of urothelial carcinoma. *Am J Surg Pathol* 2003;27:1243-1252.
7) Young RH, Oliva E. Transitional carcinomas of the urinary bladder that may be
underdiagnosed: a report of four cases exemplifying the homology between neoplastic and non-neoplastic transitional cell lesions. *Am J Surg Pathol* 1996; 20:1148-1454.
8) Ro JY, Lapham R, Amin MB. Deceptively bland transitional cell carcinoma of the
urinary bladder- further characterization of subtle and diagnostically treacherous
patterns of invasion in urothelial neoplasia. *Adv Anat Pathol* 1997;4:244-251.

Micropapillary carcinoma

1) Amin MB, Ro JY, El-Sharkawy T, Lee KM, Troncoso P, Silva EG, Ordonez NG,
Ayala AG. Micropapillary variant of transitional cell carcinoma of the urinary
bladder. A histologic pattern resembling ovarian papillary serous carcinoma.
Am J Surg Pathol 1994; 18:1224-1232.
2) Johansson SL, Borghede G, Holmang S. Micropapillary bladder carcinoma: a clinicopathological study of 20 cases. *J of Urol* 1999; 161:1798-1802.
3) Alvarado-Cabrero I, Sierra-Santisteban FI, Mantilla-Morales A, Hernadez-Hernandez DM. Micropapillary carcinoma of the urothelial tract: a clinicopathologic study of 38 cases. Annals of Diag Pathol 2005;9:1-5.
4) Kamat, AM, Gee JR, Dinney CPN, et al. The case for early cystectomy in the treatment of nonmuscle invasive micropapillary bladder carcinoma. *J Urol* 2006; 175:881-5.
5) Sangoi AR, Beck AH, Amin MB, Cheng L, Epstein JI, Hansel DE, Iczkowski KA,Lopez-Beltran A, Oliva E, Paner GP, Reuter VE, Ro JY, Shah RB, Shen SS, Tamboli P, McKenney JK. Interobserver reproducibility in the diagnosis of invasive micropapillary carcinoma of the urinary tract among urologic pathologists. *Am J Surg Pathol* 2010;34(9):1367-76.
6) Sangoi AR, Higgins JP, Rouse RV, Schneider AG, McKenney JK.

Immunohistochemical comparison of MUC1, CA125, and Her2Neu in invasive micropapillary carcinoma of the urinary tract and typical invasive urothelial carcinoma with retraction artifact. *Mod Pathol* 2009;22(5):660-7.

7) Edgerton N, Sirintrapun SJ, Munoz M, Chen Z, Osunkoya AO. Micropapillary urothelial carcinoma of the urinary bladder: a clinicopathological analysis of 24 cases. *Int J Urol* 2011;18(1):49-54.

8) Compérat E, Roupret M, Yaxley J, Reynolds J, Varinot J, Ouzaïd I, Cussenot O, Samaratunga H. Micropapillary urothelial carcinoma of the urinary bladder: a clinicopathological analysis of 72 cases. *Pathology* 2010;42(7):650-4.

9) Lopez-Beltran A, Montironi R, Blanca A, Cheng L. Invasive micropapillary urothelial carcinoma of the bladder. *Hum Pathol* 2010;41(8):1159-64.

10) Heudel P, El Karak F, Ismaili N, Droz JP, Flechon A. Micropapillary bladder cancer: a review of Léon Bérard Cancer Center experience. *BMC Urol* 2009;9:5.

11) Lotan TL, Ye H, Melamed J, Wu XR, Shih IeM, Epstein JI. Immunohistochemical panel to identify the primary site of invasive micropapillary carcinoma. *Am J Surg Pathol* 2009;33(7):1037-41.

12) Kamat AM, Dinney CP, Gee JR, Grossman HB, Siefker-Radtke AO, Tamboli P, Detry MA, Robinson TL, Pisters LL. Micropapillary bladder cancer: a review of the University of Texas M. D. Anderson Cancer Center experience with 100 consecutive patients. *Cancer* 2007; 110(1):62-7.

13) Kamat AM, Gee JR, Dinney CP, Grossman HB, Swanson DA, Millikan RE, Detry MA, Robinson TL, Pisters LL. The case for early cystectomy in the treatment of nonmuscle invasive micropapillary bladder carcinoma. *J Urol* 2006;175(3 Pt 1):881-5.

14) Alvarado-Cabrero I, Sierra-Santiesteban FI, Mantilla-Morales A, Hernández-Hernandez DM. Micropapillary carcinoma of the urothelial tract. A clinicopathologic study of 38 cases. *Ann Diagn Pathol* 2005;9(1):1-5.

15) Samaratunga H, Khoo K. Micropapillary variant of urothelial carcinoma of the urinary bladder; a clinicopathological and immunohistochemical study. *Histopathology* 2004;45(1):55-64.

16) Maranchie JK, Bouyounes BT, Zhang PL, O'Donnell MA, Summerhayes IC, DeWolf WC. Clinical and pathological characteristics of micropapillary transitional cell carcinoma: a highly aggressive variant. *J Urol* 2000;163(3):748-51.

17) López JI, Elorriaga K, Imaz I, Bilbao FJ. Micropapillary transitional cell carcinoma of the urinary bladder. *Histopathology* 1999;34(6):561-2.

18) Johansson SL, Borghede G, Holmäng S. Micropapillary bladder carcinoma: a clinicopathological study of 20 cases. *J Urol* 1999;161(6):1798-802.

Small cell carcinoma

1) Mills SE, Wolfe JT, Weiss MA, et al. Small cell undifferentiated carcinoma of urinary bladders: a light microscopic, immunohistochemical and ultrastructural study of 12 cases. *Am J Surg Pathol* 1987;11:606-617.
2) Hailemariam S, Gaspert A, Komminoth P, Tamboli P, Amin M. Primary, pure, large-cell neuroendocrine carcinoma of the urinary bladder. *Modern Pathol* 1998; 11:1016-1020.
3) Paner GP, McKenney JK, Epstein JI, Amin MB. Rhabdomyosarcoma of the urinary bladder in adults: predilection for alveolar morphology with anaplasia and significant morphologic overlap with small cell carcinoma. *Am J Surg Pathol* 2008;32(7):1022-8.
4) Agoff SN, Lamps LW, Philip AT, Amin MB, Schmidt RA, True LD, Folpe AL. Thyroid transcription factor-1 is expressed in extrapulmonary small cell carcinomas but not in other extrapulmonary neuroendocrine tumors. *Mod Pathol* 2000;13(3):238-42.
5) Macedo LT, Ribeiro J, Curigliano G, Fumagalli L, Locatelli M, Carvalheira JB, Quintela A, Bertelli S, De Cobelli O. Multidisciplinary approach in the treatment of patients with small cell bladder carcinoma. *Eur J Surg Oncol* 2011;37(7):558-62.