PRACTICAL & CONTROVERSIAL ISSUES IN DIAGNOSIS, GRADING AND STAGING OF UROTHELIAL CARCINOMA: AN APPROACH

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SCOPE OF DISCUSSION

(ISSUES I GRAPPLE WITH EVERYDAY IN UROLOGIC PATHOLOGY PERTAINING TO "DIAGNOSIS, GRADING AND STAGING OF UROTHELIAL CARCINOMA")

DIAGNOSIS:
A. All that is papillary in the bladder is not papillary urothelial neoplasia:
   i. Diagnostic pitfalls: papillary hyperplasia, nephrogenic adenoma, fibroepithelial polyp and papillary/polyoid cystitis
   ii. Artifacts: pseudopapillary folds and avulsed urothelium
B. All urothelial proliferations that expand the lamina propria are not invasive carcinoma:
   i. Diagnostic pitfalls: florid von Brunn nests and pseudocarcinomatous hyperplasia
C. Formes frustes of papillary urothelial neoplasia:
   i. Papillary hyperplasia
   ii. Dysplasia/CIS with early papillary formations
   iii. Truncated papillae of treated urothelial neoplasia
   iv. Bulbous inverted prolongations without surface papillary component
D. Pitfalls in the diagnosis of flat lesions:
   i. Radiation and chemotherapy related atypia
   ii. Reactive atypia
   iii. Human polyoma virus

GRADING:
   i. Grading of inverted lesions
   ii. Grading of "cusp" lesions - those boarding between papillary urothelial neoplasm of low malignant potential (PUNLMP) vs. low-grade, and low-grade vs. high grade
   iii. Does "low-grade" invasive urothelial carcinoma of the bladder exist?

STAGING:
   o Recognition of early invasion
   o Isolated superficial and "muscularis propria-like" muscle bundles involved by carcinoma in transurethral resection (TURBT) specimens
I. DIAGNOSIS

A. All that is papillary in the bladder is not papillary urothelial neoplasia
   i. Diagnostic pitfalls: papillary hyperplasia, nephrogenic adenoma, fibroepithelial polyp and papillary/polypoid cystitis

The diagnosis of papillary neoplasia in the urinary bladder is based on the fundamental observation of a papillary stalk. The presence of normal appearing urothelium on a stalk indicates urothelial papilloma, the presence of hyperplastic urothelium on a stalk indicates papillary urothelial neoplasm of low malignant potential (PUNLMP), the presence of cytologically atypical urothelium, depending on the extent of atypia, defines papillary urothelial carcinoma, either low-grade or high-grade. Not every lesion with a papillary architecture, however, represents urothelial neoplasia. Diagnostic pitfalls include papillary hyperplasia, nephrogenic adenoma, fibroepithelial polyp and papillary/polypoid cystitis. The stalk of urothelial neoplasia typically consists of a narrow-necked, thin extension of the lamina propria with distinct delicate blood vessels and usually with minimal inflammation or stromal cellularity. Variations include presence of stromal edema and xanthomatous inflammation, although in most cases the stalk has an overall uniformity in its width. The discussion which will follow later on "tumor-like lesions and benign histologic mimickers of bladder carcinoma" will elucidate diagnostic discriminators with pitfalls. Papillary hyperplasia consists of undulating cytologically unremarkable urothelium arranged in narrow mucosal folds of varying heights with associated increased vascularity at the base of the papillary folds; branching is unusual. The lining in nephrogenic adenoma is cuboidal to columnar in nature in contrast to the multilayered urothelium in urothelial neoplasia. Fibroepithelial polyps usually have a greater stromal to epithelial ratio and multiple finger-like and polypoid projections of cellular, edematous or inflamed stroma. Papillary polypoid cystitis is a reaction to inflammatory insult and represents an outward luminal prolongation of an inflamed and edematous lamina propria with neovascularity such as seen in granulation tissue.

   ii. Artifacts: Pseudopapillary folds and strips of avulsed benign urothelium mimicking bladder neoplasia.

Small and superficial biopsies and lack of optimal orientation may result in difficulty in the distinction of artifactual papillary structures from true neoplasms. The distinction is facilitated by identifying detached pieces of urothelium without neovascularity and folded urothelium resulting in pseudopapillary proliferations (without true vascular cores). The lining epithelium is usually normal, reactive, hyperplastic or associated with von Brunn nests. Key in these settings is the correlation with the cystoscopic impression of whether a papillary lesion or mass was present. This problem is particularly exaggerated in small renal pelvic and ureteral biopsies where extreme caution must be exercised, as an over diagnosis may result in a radical nephroureterectomy.

B. All urothelial proliferations that expand the lamina propria are not invasive carcinoma
   Diagnostic pitfalls: florid von Brunn nests and pseudocarcinomatous hyperplasia

Reactive proliferative changes of the urothelium resulting in invaginated nests of cytologically benign urothelium in the lamina propria may mimic urothelial carcinoma, particularly the nested variant. Lesions are typically 5 mm or less in diameter but may occasionally be larger appearing as mucosal blebs. The nests have variable continuity with the surface urothelium and have smooth and regular round contours. Overall the lesion has a lobular configuration with sharp stromal-epithelial interface. Inflammation may be minimal and stromal reaction is absent. Association with cystitis cystica and cystitis glandularis is a helpful feature. If there is any reservation, correlation with cystoscopy is essential. The clinical presence of a mass lesion and/or an absence of an appreciable base of the lesion should raise concern for malignancy.
Patients with prior radiation and/or chemotherapy may develop florid benign urothelial proliferations within the lamina propria with a pseudoinfiltrative growth pattern which may have severe overlap with invasive carcinoma. A subset of patients may have no prior therapy but an underlying systemic ischemic etiology such as atrial fibrillation or diabetes. Clinically the lesions present with hematuria and small polyloid lesions. The urothelial proliferation is characterized by small to intermediate sized nests with round or sharp-edged borders and prominent cytoplasmic eosinophilia imparting a "squamoid" appearance. The key to the recognition of a pseudoneoplastic lesion is the appreciation of background ischemic/radiation changes, such as cytoplasmic ballooning and vacuolation with smudged nuclear chromatin and karyorrhectic cellular debris in the overlying urothelium, and other stromal changes such as vascular ectasia and edema, stromal hemorrhage and hemosiderin deposition and stromal fibrosis with atypical fibroblasts. The diagnostic hallmark is epithelium that characteristically encircles or wraps around blood vessels with intravascular fibrin deposits. The pseudoinfiltrative epithelium has atypia in the range of reactive atypia; lacking features of high-grade neoplasia. The presence of muscularis propria involvement should caution against the diagnosis of pseudocarcinomatous hyperplasia.

C. Formes frustes of papillary urothelial neoplasia

*Histologic manifestations: papillary hyperplasia, dysplasia/CIS with early papillary formations, truncated papillae of treated urothelial neoplasia and bulbous inverted prolongations without surface papillary component*

Papillary urothelial hyperplasia is a putative precursor lesion to low-grade papillary urothelial neoplasms. Typically it is discovered on routine follow-up cystoscopy for papillary urothelial neoplasms and less frequently in the workup for microhematuria or urinary obstructive symptoms. In most cases at cystoscopy, a focal elevated lesion is identified that is variably described as “bleb-like,” “papillary,” “raised,” “sessile,” or “frondular”. Studies with sufficient follow-up and large number of cases that permit evaluation of the true clinical significance of de novo papillary hyperplasia are lacking. Many cases are diagnosed in cases with known urothelial neoplasia and if this history is not present, it may be reasonable to suggest that patients should be followed up for at least 1-3 years or more closely if symptoms persist. If papillary hyperplasia is diagnosed in a patient with urothelial neoplasia, it most likely indicates recurrence of papillary neoplasia and warrants continued close follow-up. Some experts prefer to designate these early lesions as urothelial neoplasia (rather than hyperplasia), even in the absence of a true fibrovascular cores. Recent molecular studies demonstrate that at least a subset of papillary hyperplasia represents pre-cancerous lesions of the bladder that subsequently progress to papillary bladder cancer.

Much more infrequently, the overlying urothelium in a lesion has an architectural pattern of papillary hyperplasia and demonstrates distinct cytologic atypia seen in the range of dysplasia to carcinoma in situ (CIS). It is conceivable that, at least in some cases, CIS or dysplasia may evolve into a papillary lesion, with further progression to low-grade or high-grade papillary cancer. In such cases, I usually correlate the histology with the cystoscopic impression. If a papillary lesion was clinically noted, I diagnose the case as urothelial carcinoma and grade the lesion based on the degree of atypia. If a distinct papillary lesion was not recognized, it is reasonable to use diagnostic terminology such as “CIS with early papillary formation” or “dysplasia with early papillary formation”.

Mitomycin C, BCG and thiotepa therapy destroys the tips of the papillae of papillary urothelial carcinoma because they act as surface abrasives. In patients who are undergoing surveillance cystoscopy, the earliest forms of papillary urothelial neoplasm may be biopsied due to early intervention. This may result in "truncated papillae" or early papillary carcinoma i.e., dysplastic or CIS urothelium with tenting of urothelium and neovascularity at the base of the lesion.
Correlation with cystoscopy is, again, essential. Sometimes patients on surveillance will have broad bulbous inverted prolongations into the lamina propria without an obvious surface component. The clue to the diagnosis of neoplasia includes the presence of increased vascularity between the bulbous prolongations suggesting the presence of a papillary architecture which is not fully manifest.

D. Pitfalls in the diagnosis of flat lesions

Radiation and chemotherapy related atypia, reactive atypia and human polyoma virus

Inflammatory atypia and non-pleomorphic forms of CIS share in common the presence of enlarged nuclei with prominent nucleoli. Attention to other nuclear characteristics of neoplasia is necessary to diagnose CIS in such settings. The presence of acute or significant chronic inflammation, particularly in an intrarethelial location, warrants caution in the interpretation of dysplasia or CIS, although inflammatory atypia may coexist with dysplasia or CIS.

Upon cursory examination, post-therapy related changes could easily be mistaken for intrarethelial neoplasia. The regenerative and reactive changes imparted by therapy to some extent vary upon the type of therapy. Intravesical BCG therapy is the most common therapy for CIS and pathologic alterations include denudation and ulceration of the urothelium, non-caseating granulomas, lamina propria edema and inflammation and regenerative atypia (enlarged uniform nuclei with even chromatin distribution and prominent nucleoli). The urothelium may be entirely normal. Thiotepa and mitomycin C induce atypical changes primarily on the surface umbrella cells (large cells with multinucleation, smudged nuclear chromatin and cytoplasmic vacuolation). In some patients, a range of findings from reactive/regenerative urothelium, normal urothelium and CIS may be present concurrently in biopsies. Systemic cyclophosphamide therapy may result in large multinucleated urothelial cells with smudged to vacuolated nuclear chromatin, identical to changes seen with radiation. Other changes include hemorrhagic cystitis, encrusted cystitis and reactivation of polyoma virus infection. Bladder cancer following cyclophosphamide treatment has been reported. Radiation may induce full thickness atypia closely mimicking CIS; however, there are often multinucleated cells with bizarre nuclei not typical of intrarethelial neoplasia. The cytoplasm in radiation atypia is often prominent and may show degenerative changes with vacuolization. It is sometimes intensely eosinophilic. Other associated changes may be identified in the bladder wall, including atypical fibroblasts and radiation vasculopathy; pseudocarcinomatous epithelial proliferation, mimicking invasive carcinoma, may be seen.

Although pleomorphism in a flat intrarethelial lesion is diagnostic of CIS, the major exceptions include reactive atypia in umbrella cells, usually secondary to therapy or stones and human polyomavirus-associated cytopathic effects. Infection of immunocompromised patients with the human polyomavirus, usually a non-pathogenic virus, results in large homogeneous inclusions in enlarged nuclei of urothelial cells, or “decoy cells”. The findings are more frequently seen in urine cytology but may rarely be found on biopsy; in both specimens, malignancy (CIS) is mimicked.

II. GRADING

i. Basic paradigm of grading urothelial neoplasia

The 7th edition of the AJCC Staging Manual, the 3rd series AFIP Fascicle, major practice protocols including the CAP checklists and the 2004 W.H.O. Blue Book advocate using the W.H.O. (2004)/ISUP classification system. For practical purposes, a similar basic paradigm may be used to grade papillary, flat and endophytic lesions of the bladder. The presence of normal appearing urothelium (seven layers or less without cytologic atypia) in the context of a flat lesion represent normal urothelium, in a papillary lesion represents urothelial papilloma and in an inverted lesion represented inverted papilloma. Hyperplastic urothelium (multiple layers
with increased cell density and without cytologic atypia) represents papillary hyperplasia in a flat lesion, PUNLMP in a papillary lesion and inverted PUNLMP in an inverted lesion. The presence of distinct nuclear atypia including nuclear border irregularities, irregularities of nuclear chromatin, loss of polarity and occasional prominent nucleoli in a flat lesion represent dysplasia, in a papillary lesion represent low-grade papillary urothelial carcinoma and in an inverted lesion represent inverted low-grade urothelial carcinoma. The presence of high-grade nuclear atypia, including prominent nucleoli, multiple nucleoli, nucleolar pleomorphism, markedly irregular chromatin, atypical mitoses towards the surface and nuclear pleomorphism in a flat lesion represent urothelial carcinoma in situ, in a papillary lesion represent high-grade papillary urothelial carcinoma and in an inverted lesion represent inverted high-grade urothelial carcinoma.

**ii. Grading of inverted lesions**

Urothelial neoplasms exhibit two patterns of endophytic or inverted growth: (a) broad front verrucous carcinoma-like growth and (b) inverted papilloma-like growth. There is no accepted and consistently used nomenclature for inverted lesions with inverted papilloma-like architecture, although as outlined above and in the table below, the classification used for exophytic tumors may be extrapolated for inverted lesions. In tumors with inverted papilloma-like growth, the basic configuration is like inverted papilloma, that is, endophytic, expansile growth with or without peripheral palisading of basaloid cells. In contrast to inverted papilloma, inverted urothelial carcinomas usually have a variable proportion of exophytic component; exceptionally cases solely consist of urothelial carcinoma with inverted features. Within inverted carcinoma, the ramifying cords and trabeculae are less uniform, sometimes solid without polarity and typically consist of large well-circumscribed rounded nests as opposed to anastomosing trabeculae. If the endophytic component is markedly thickened but otherwise cytologically and architecturally unremarkable and the exophytic component is similar, the designation of inverted urothelial neoplasm of low malignant potential is appropriate, although this category does not presently exist in the ISUP/WHO classification. As with other urothelial neoplasms, if there is cytologic atypia, depending on the grade, the tumors would be designated as inverted (i.e., not with invasion) urothelial carcinoma, low grade or high grade. As in broad-front verrucous carcinoma-like growth, inverted papilloma-like destructive invasion may be present in tumors with endophytic growth. In such a case, diagnostic terminology such as urothelial carcinoma with endophytic growth, high grade, with invasion into lamina propria may be used.

**Table: Analogy in nomenclature for inverted lesions**

<table>
<thead>
<tr>
<th>Flat Lesions</th>
<th>Papillary Lesions*</th>
<th>Predominant or Exclusive Inverted Lesions*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Papilloma</td>
<td>Inverted papilloma</td>
</tr>
<tr>
<td>Urothelial hyperplasia</td>
<td>Papillary neoplasm of low malignant potential</td>
<td>Inverted papillary neoplasm of low malignant potential</td>
</tr>
<tr>
<td>Urothelial dysplasia</td>
<td>Papillary urothelial carcinoma, low grade, non-invasive</td>
<td>Inverted papillary urothelial carcinoma, low grade, non-invasive</td>
</tr>
<tr>
<td>Urothelial CIS</td>
<td>Papillary urothelial carcinoma, high grade, non-invasive</td>
<td>Inverted papillary urothelial carcinoma, high grade, non-invasive</td>
</tr>
<tr>
<td></td>
<td>Papillary urothelial carcinoma, high grade, invasive</td>
<td>Inverted papillary urothelial carcinoma, high grade, invasive</td>
</tr>
</tbody>
</table>

From Epstein JI, Reuter VE, Amin MB. Biopsy Interpretation of Bladder, 2nd Ed.  
Lesions may be both exophytic and endophytic
iii. Grading of "cusp" lesions - those boarding between papillary urothelial neoplasm of low malignant potential (PUNLMP) vs. low-grade, and low-grade vs. high-grade

Histologic grading provides powerful prognostic information in non invasive papillary tumors and is an exercise which is relatively easy to implement in practice based on above proposed paradigm.

The table below provides information on risk for recurrence and progression between the different categories. Grading shows significant differences in progression between different grades and in recurrence rates between papilloma, PUNLMP and the carcinomas (low grade and high grade); i.e., once a tumor is designated as a carcinoma, the recurrence rates are similar but there are differences in progression rates. In grading papillary lesions, it is important to evaluate thin sections and areas where the fibrovascular cores are cut perpendicular to the long axis of the papillae. Evaluation of tangential sections and fused areas could result in "overgrading". As with many neoplasms, there may be a range of cytologic atypia and by convention, urothelial carcinoma is graded by the highest degree of abnormality noted. If the higher grade area is obvious, applying the grading scheme is not problematic. However, since a spectrum of atypia may be present, difficulty may be encountered in cusp lesions, those bordering between different categories. In such cases, I use the following approach as guiding principles in my personal practice. The distinction between papilloma and PUNLMP or PUNLMP or low-grade is not always therapeutically critical although there are prognostic differences between the categories but not at the individual patient level. In problematic cases in this area, I tend to rely on my first impression. Recognition of high-grade papillary neoplasia has prognostic significance and likely therapeutic implications at the individual patient level. If there is a dilemma in assigning a tumor between a high or low grade carcinoma, it is helpful to have knowledge of prior history of bladder cancer, specifically, grade of papillary lesion, presence or absence of dysplasia or CIS and history of prior therapy. If the patient has a history of a high-grade papillary urothelial carcinoma, this diagnosis should be favored. Other factors that influence my grading in difficult lesions include information on size of the lesion, frequency of recurrence, multifocality and cytology findings; large size, short span to recurrence or multiple recurrences, multifocality with at least one lesion being high-grade and positive urine cytology favoring high-grade lesion. These are factors often considered by urologists for administrating intravesicle therapy, even in low grade tumors. I do not use biomarkers such as CK20 or p53 to grade papillary lesions of the bladder.

<table>
<thead>
<tr>
<th>Author</th>
<th>Papilloma</th>
<th>PUNLMP</th>
<th>Low Grade</th>
<th>High Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Progression</td>
<td>Recurrence</td>
<td>Progression</td>
<td>Recurrence</td>
</tr>
<tr>
<td>Magi-Galuzzi</td>
<td>0</td>
<td>18%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>McKenney</td>
<td>0</td>
<td>14%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cheng</td>
<td>0</td>
<td>10%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Herr</td>
<td>0</td>
<td>31%</td>
<td>0</td>
<td>52%</td>
</tr>
<tr>
<td>Oosterhius</td>
<td>0</td>
<td>14%</td>
<td>3%</td>
<td>32%</td>
</tr>
<tr>
<td>Samaratunga</td>
<td>0</td>
<td>-</td>
<td>7%</td>
<td>-</td>
</tr>
<tr>
<td>Fuji</td>
<td>-</td>
<td>-</td>
<td>0</td>
<td>60%</td>
</tr>
<tr>
<td>Campbell</td>
<td>-</td>
<td>-</td>
<td>0</td>
<td>25%</td>
</tr>
<tr>
<td>Desai</td>
<td>-</td>
<td>-</td>
<td>0</td>
<td>33%</td>
</tr>
<tr>
<td>Holmang</td>
<td>-</td>
<td>-</td>
<td>0</td>
<td>35%</td>
</tr>
<tr>
<td>Ramos</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>62%</td>
</tr>
<tr>
<td>Pitch</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>47%</td>
</tr>
<tr>
<td>Alsheikh</td>
<td>-</td>
<td>-</td>
<td>0</td>
<td>25%</td>
</tr>
<tr>
<td>Yin</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>17%</td>
</tr>
<tr>
<td>Johns Hopkins</td>
<td>0</td>
<td>9%</td>
<td>3%</td>
<td>42%</td>
</tr>
<tr>
<td>Chin Chen</td>
<td>-</td>
<td>-</td>
<td>2%</td>
<td>18%</td>
</tr>
<tr>
<td>Literature Range</td>
<td>0</td>
<td>9-31%</td>
<td>0-7%</td>
<td>17-62%</td>
</tr>
</tbody>
</table>
iv. Does "low-grade" invasive urothelial carcinoma of the bladder exist?

A very small subset of bladder cancer may have low grade nuclear features. Although assigning a grade to these tumors is controversial, most data suggest that once a tumor is invasive, pathologic stage is the key prognostic factor and grade has minimal impact on outcome, if any. Nested variant, microcystic variant, etc. may have low nuclear grade and the former, particularly, is associated with a higher pathologic stage and disease progression in spite of its low-grade nuclear atypia. For all practical purposes, once I diagnose a case as invasive carcinoma, I assign the tumor as a high grade.

STAGING:

i. Recognition of early invasion

Pathologic stage is the most powerful prognostic indicator in urothelial carcinoma and is a major defining parameter in the management of this disease. The TNM staging system defines pT1 tumors as those invading into the lamina propria but not the muscularis propria. Detection of early or subtle invasion may be difficult but is important as contemporary series have shown clear differences in progression rates between pTa and pT1 tumors. Tumors with involvement of the muscularis propria and beyond (pT2–4) are usually managed with definitive surgical therapy (i.e., cystectomy) or radiation therapy with or without neoadjuvant or adjuvant therapy. The criteria for invasion into lamina propria by urothelial carcinoma and the pitfalls in the diagnosis of lamina propria invasion by urothelial carcinoma are outlined in tables below.

Recent studies have shown that second review of transurethral resection specimens show significant differences in interpretation which, in one study, was sufficient to alter management in up to one third of cases. Diagnostic difficulty persists even between experts, and is higher in cases with crush and cautery artifact.

<table>
<thead>
<tr>
<th>Table: Criteria for diagnosis of invasion into lamina propria by urothelial carcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Histologic grade</strong></td>
</tr>
<tr>
<td>Invasion seen much more frequently, although not exclusively, in high-grade [WHO(2003)/ISUP classification] lesions.</td>
</tr>
<tr>
<td><strong>Invading epithelium</strong></td>
</tr>
<tr>
<td>Irregularly shaped nests</td>
</tr>
<tr>
<td>Single-cell infiltration</td>
</tr>
<tr>
<td>Irregular or absent basement membrane</td>
</tr>
<tr>
<td>Tentacular finger-like projections</td>
</tr>
<tr>
<td>Invasive component with higher nuclear grade or more cytoplasm (or morphologically different) than overlying noninvasive component (paradoxical differentiation)</td>
</tr>
<tr>
<td><strong>Stromal response</strong></td>
</tr>
<tr>
<td>Desmoplasia or sclerosis</td>
</tr>
<tr>
<td>Retraction artifact</td>
</tr>
<tr>
<td>Inflammation</td>
</tr>
<tr>
<td>Myxoid stroma</td>
</tr>
<tr>
<td>Pseudosarcomatous stroma</td>
</tr>
<tr>
<td>Absent stromal response</td>
</tr>
</tbody>
</table>

From Epstein JI, Reuter VE, Amin MB. Biopsy Interpretation of Bladder, 2nd Ed.
Table: Pitfalls in the diagnosis of lamina propria invasion by urothelial carcinoma

<table>
<thead>
<tr>
<th>Pitfall</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tangential sectioning</td>
</tr>
<tr>
<td>Thermal artifact</td>
</tr>
<tr>
<td>Obscuring inflammation</td>
</tr>
<tr>
<td>Carcinoma in situ involving von Brunn nests</td>
</tr>
<tr>
<td>Invasion into muscle, indeterminate for type of muscle (muscularis mucosae vs. muscularis propria)</td>
</tr>
<tr>
<td>Invasion into adipose tissue within lamina propria (overdiagnosis of extravesical invasion)</td>
</tr>
<tr>
<td>Deceptively bland urothelial carcinomas</td>
</tr>
<tr>
<td>- Microcystic variant of urothelial carcinoma (invasive)</td>
</tr>
<tr>
<td>- Broad front or inverted growth, raising the question of invasion</td>
</tr>
<tr>
<td>- Nested variant of urothelial carcinoma (invasive)</td>
</tr>
<tr>
<td>Overdiagnosis of vascular invasion</td>
</tr>
</tbody>
</table>

From Epstein JI, Reuter VE, Amin MB. Biopsy Interpretation of Bladder, 2nd Ed.

ii. Isolated superficial “muscularis propria-like” muscle bundles involved by carcinoma in transurethral resection (TURBT) specimens

There is greater recent knowledge regarding the histoanatomy of the bladder that has direct implications on staging of bladder cancer (see table below). The recognition and distinction of muscle bundles (muscularis mucosae vs. propria) is critical, as the latter is the landmark for designating a tumor as pT2, the crossroads for more aggressive intervention. Adipose tissue is relatively commonly present in the bladder wall and involvement by tumor does not designate extravesical extension. It is consistently present in the muscularis propria and more commonly present in the deep lamina propria than in the superficial lamina propria, where it is rare. Recent studies have identified variations in the muscularis mucosae muscle and include hyperplastic patterns with relatively haphazardly oriented muscle bundles with jagged outlines. Seen very focally, but in up to 45% of cystectomy specimens, one may encounter muscle bundles that have a round smooth contour that are distinct from the more superficially located muscularis mucosae muscle and from the deeper muscularis propria muscle. Distinction of these muscle bundles, from muscularis propria in limited samples and without orientation, would be impossible. Muscularis propria muscle bundles are generally very tight aggregates of several discernable large bundles of similar size and shape, arranged both horizontally and vertically, whereas these larger caliber superficially situated round muscle bundles in the lamina propria were typically single and isolated bundles. Our unpublished data with this type of muscle, distinct from the typical muscularis propria, show moderate to strong positivity in 85% of these muscle bundles for smoothelin and suggest that most of these isolated muscle bundles are aberrant muscularis propria muscle bundles in the lamina propria; although it is conceivable that a few of these muscles represent a unique form of hyperplastic muscularis mucosae. The staging implication for this type of muscle is that caution should be exercised in designating a tumor as a pT2 tumor if carcinoma involves only isolated muscle bundles with a smooth round caliber or those which are situated very close to the urothelium. Factors to consider while assigning a stage include the size of the lesion, extent of invasive carcinoma, and presence or absence of other more typical muscle bundles of muscularis propria that are distinct from those that are involved. A possible approach for such cases, which are rare in clinical practice, is to recommend restaging by performing a repeat TURBT.
## Table: Potential impact of histologic variations in bladder cancer on staging

<table>
<thead>
<tr>
<th>Histoanatomic structures</th>
<th>Implications</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Types of muscle</strong></td>
<td></td>
</tr>
<tr>
<td><em>Typical MM</em></td>
<td>Involvement implies at least pT1 disease</td>
</tr>
<tr>
<td>Thin slender muscle bundles frequently associated with vascular plexus</td>
<td></td>
</tr>
<tr>
<td><em>Typical MP</em></td>
<td>Involvement implies at least pT2 disease</td>
</tr>
<tr>
<td>Compact round muscle bundles of overall similar size occurring in groups both horizontally and vertically</td>
<td></td>
</tr>
<tr>
<td><strong>MM variations</strong></td>
<td></td>
</tr>
<tr>
<td><em>Conventional non-hyperplastic</em></td>
<td>Involvement implies at least pT1 disease</td>
</tr>
<tr>
<td><em>Hyperplastic haphazard</em></td>
<td></td>
</tr>
<tr>
<td>Thickened muscle bundles with irregular outlines</td>
<td></td>
</tr>
<tr>
<td><em>Aberrant MP-like muscle in LP - isolated or superficial muscle bundles with smooth outlines and distinct from MP muscle</em></td>
<td>Since significance of involvement is uncertain, caution in interpreting superficial or isolated MP-like muscle by limited amount of cancer</td>
</tr>
<tr>
<td><strong>MM distribution</strong></td>
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<td>MM not reliable histologic landmark for substaging pT1 disease</td>
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<td><em>Trigone - attenuated lamina propria and MP is very superficial and smaller caliber towards the surface</em></td>
<td>Caution in interpreting inverted and low grade tumors in trigone</td>
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<td><em>Ureteral insertion - ureteral MP superficial and merges with bladder MP</em></td>
<td>Caution in interpreting MP invasion in tumors involving trigone</td>
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<td><em>Dome - hyperplastic muscle most common</em></td>
<td>Caution as cross sectioning, abundance or prominence may result in overlap features with MM muscle</td>
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<td><em>Does not always follow MM</em></td>
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<td><em>May not be present as a single distinct layer</em></td>
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<td><strong>Adipose tissue</strong></td>
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<td><em>Infrequent in LP</em></td>
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<td><em>More common in deep LP</em></td>
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<td><em>Always present in MP</em></td>
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<td><em>More common in dome</em></td>
<td>Involvement of sizable clusters of adipose tissue from a tumor in the dome without orientation or muscle would suggest at least deep LP invasion</td>
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<td><em>Poor demarcation between MP fat and perivesicle fat</em></td>
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SELECTED REFERENCES

DIAGNOSIS


GRADING


STAGING


Urothelial carcinoma is a heterogeneous tumor with great propensity for divergent differentiation resulting in an urothelial carcinoma admixed with other morphologically recognizable components. [1] The most common mixed differentiation is squamous followed by glandular. [1-5] The whole spectrum of bladder carcinoma variants may be seen in variable proportion from an otherwise typical urothelial carcinoma, but most importantly, these variants may be relevant in clinical practice since they may simulate benign lesions, secondary tumors or necessitate a more complex therapeutic approach beyond surgery.[1, 6] Recent studies suggest an increasing likelihood of lymph node metastases associated with the finding of pathologic variants in the primary tumor.[7] Main pathologic variants of urothelial bladder carcinoma according to their suggested clinical and pathologic significance, follows:

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**Misdiagnosis as choriocarcinoma either primary/secondary**
- Urothelial carcinoma with syncytiotrophoblastic giant cells
  
  Choriocarcinoma either primary or secondary

**Misdiagnosis as myofibroblastic proliferation**
- Sarcomatoid urothelial carcinoma (carcinosarcoma)
  
  Inflammatory myofibroblastic tumor (inflammatory pseudotumor)

**Misdiagnosis as squamous cell carcinoma or adenocarcinoma either primary or secondary**
- Urothelial carcinoma with squamous and/or glandular differentiation
  
  Squamous cell carcinoma, adenocarcinoma

**Misdiagnosis as other tumor types or lesions**
- Urothelial carcinoma, lipoid-cell variant
  
  Carcinosarcoma/sarcomatoid carcinoma of heterologous type
- Urothelial carcinoma with pseudosarcomatous stroma
  
  Sarcomatoid carcinoma, myxoma, Chordoma
- Urothelial carcinoma with myxoid stroma
- Urothelial carcinoma with stromal osseous or cartilaginous metaplasia
  
  Carcinosarcoma/sarcomatoid carcinoma heterologous type
- Urothelial carcinoma with osteoclast-type giant cells
  
  Reactive granulomatous lesion, metastasis
- Osteoclast type Undifferentiated Carcinoma
- Urothelial carcinoma with prominent lymphoid infiltrate
- Rare urothelial carcinomas with discohesive, prostate-like or endometrioid-like morphologies
  
  Lobular carcinoma of breast, endometrial carcinoma or Gleason 3+3 prostate cancer

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**UROTHELIAL CARCINOMA WITH DECEPTIVELY BENIGN APPEARANCE**

**Nested variant of urothelial carcinoma**

The nested variant of urothelial carcinoma is an aggressive neoplasm with less than 50 reported cases.[1, 8-10] There is a male predominance, and 70% of patients died 4-40 months after diagnosis, despite therapy.[9, 11] This pattern of urothelial carcinoma was first described as a tumor with a “deceptively benign” appearance that closely resembles Brunn’s nests infiltrating the lamina propria.[8, 12-14] Nuclei generally show little or no atypia, but the invariable presence of an infiltrative growth should facilitate accurate diagnosis. There is high p53 accumulation and tumor cell proliferation; findings that result useful in some cases. [1] In spite of the deceptively benign appearance of the tumor, recently, it has been proposed to grade this as high grade to assist the urologist in selecting the appropriated therapy.[1] A report based on 30 urothelial carcinoma cases with pure or predominant nested morphology [9] found patient’s age ranged from 41 to 83 years (average, 63 years) with a male-female ratio of 2.3:1. The architectural pattern ranged from a predominantly disorderly proliferation of discrete, small, variably sized nests to focal areas demonstrating confluent nests,
cordlike growth, and cystitis cystica-like areas to tubular growth pattern. The deep tumor-stroma interface was invariably jagged and infiltrative. Despite overall bland cytology, tumor nests demonstrated focal random cytologic atypia (90%) and focal high-grade cytologic atypia centered within the base of the tumor (40%). The tumor stroma ranged from having minimal stromal response to focally desmoplastic and myxoid. A component of usual urothelial carcinoma was present in 63% of cases. [9]

The nested component demonstrated an immunophenotype identical to usual urothelial carcinoma, with CK7, CK20, p63, and CK903 expression in 93%, 68%, 92%, and 92% of cases, respectively. At resection, 65% demonstrated invasion into perivesical fat, and 17% into adjacent organ(s). When compared with pure high-grade urothelial carcinoma, nested urothelial carcinoma was associated with muscle invasion at transurethral resection (31% vs. 70%), extravesical disease at cystectomy (33% vs. 83%), and metastatic disease (19% vs. 67%). [9]

Follow-up was available on 29 patients (97%) with a median of 12 months (range, 1-31 months); 3 (10%) died of disease, 16 (55%) were alive with persistent or recurrent disease, and 10 (34%) were alive without disease. Response to neoadjuvant chemotherapy was observed in 2 (13%) of 15 patients. Wasco et al [9] concluded that nested urothelial carcinoma seen either in pure form or with a component of usual UC had poor outcomes.

Urothelial carcinoma with endophytic growth (inverted papilloma-like carcinoma)

The recent interest in urothelial carcinoma with endophytic growth relays on the fact that may be misdiagnosed as the benign inverted papilloma.[1, 15-18] This is because inverted papilloma-like carcinoma may show variable-to- minimal cytologic and architectural abnormalities, but invariably has higher mitotic count and cell proliferation as seen by ki67, high p53 accumulation, aberrant cytokeratin 20 expression, and frequent polysomies at chromosome 3, 9 and 17.[19] This profile has been found very useful in differential diagnosis with inverted papilloma.[18, 19] In some instances, both inverted papilloma and inverted papilloma-like carcinoma are intimately mixed.[18] Other important feature in papillary tumours with endophytic growth is that they invade lamina propria with a pushing border.[20] Unless this pattern is accompanied by true destructive stromal invasion the likelihood of metastasis is minimal because the basement membrane is not truly breached.[15]

UROTHELIAL CARCINOMA WITH FEATURES SUGGESTING SECONDARY INVOLVEMENT TO THE BLADDER

Micropapillary urothelial carcinoma

This variant of urothelial carcinoma was initially described because it resembles papillary serous carcinoma of the ovary. [1, 21] This morphology has been further recognized in other organs such lung, colon, breast, renal pelvis or salivary glands.[1, 22] A recent report based on 100 consecutive cases treated by cystectomy, showed a mean age of the patients was 64.7 years, with a male: female ratio of 10:1. [22]Stage at the time of presentation was Ta in 5 patients, carcinoma in situ in 4 patients, T1 in 35 patients, T2 in 26 patients, T3 in 7 patients, T4 in 6 patients; N+ in 9 patients, and M+ in 8 patients. 5-year and 10-year overall survival rates were 51% and 24%, respectively. Intravesical bacillus Calmette-Guerin therapy was attempted in 27 of 44 patients with non-muscle-invasive disease; 67% (18 patients) developed disease progression (>/=cT2), including 22% who developed metastatic disease.[22] Of 55 patients undergoing radical cystectomy for surgically resectable disease (<|=cT4a), 23 received neoadjuvant chemotherapy and 32 were treated with initial cystectomy, with no significant difference noted in stage distribution between the 2 groups. For the 23
patients treated with neoadjuvant chemotherapy, the median overall survival was 43.2 months with 32% of patients still alive at 5 years. [22] For the 32 patients treated with initial cystectomy, 71% remained alive at 5 years. It was concluded that micropapillary bladder cancer is associated with a poor prognosis and that intravesical therapy appears to be ineffective in this disease and patients with surgically resectable disease should be offered early radical cystectomy. [22] Increased lymph node positivity was also associated with the presence of micropapillary carcinoma in a recent study. [7] [23]

At histology, the micropapillary component is found in association with non-invasive papillary or invasive urothelial carcinoma in 80% of reported cases. [1] Twenty-five percent of cases show glandular differentiation, and some authors consider it as a variant of adenocarcinoma. Vascular and lymphatic invasion is common, and most cases show invasion into muscularis propria or deeper, often with metastases at time of diagnosis. [24] Immunohistochemical studies show immunorreactivity for epithelial markers similar to conventional urothelial carcinoma in all and CA125 staining in 43% of cases. [25] The presence of a surface micropapillary component in bladder biopsy specimens is a useful clue for further investigation and an indication for deeper biopsies in order to determine the level of invasion. In addition to stage, the prognosis seems to be related to the proportion and location of the micropapillary component. [10, 25] Cases with a moderate or extensive micropapillary component are at high risk of having an advanced stage at presentation. Cases with <10% micropapillary component have a high chance of detection at an early stage.

A recent pathology report based on 13 cases of invasive micropapillary carcinoma [23] showed the micropapillary component varied from 50-100% of the tumor specimen with 5 cases showing pure micropapillary carcinoma. The architectural pattern of the tumor varied from solid expansile nests with slender papillae within tissue retraction spaces to pseudoglandular growth with prominent ring-like structures (2 cases, 15%), and invasive micropapillary carcinoma with squamous differentiation (2 cases, 15%); a streaking solid architectural pattern of micropapillary carcinoma was additionally present in 2 cases (15%). The individual tumor cells had abundant eosinophilic cytoplasm and nuclei with prominent nucleoli and irregular distribution of chromatin, and frequent mitotic figures. Most neoplastic cells had nuclei of low to intermediate nuclear grade with occasional nuclear pleomorphism. Eight mixed cases had concurrent conventional high-grade urothelial carcinoma with squamous or glandular differentiation in 3 and 1 cases, respectively. All patients had advanced stage cancer (>pT2); and 8 (62%) had lymph node metastases. [23] Some limitations still exists concerning the diagnostic reproducibility in invasive micropapillary carcinoma. [28]

Immunohistochemical staining demonstrated that both micropapillary and associated conventional urothelial carcinoma were positive for MUC1 and 2; CK 7, PTEN, p53, and ki67; Her2Neu, Uroplakin, CK 20, 34ßE12, CA125 and p16 were positive in 4, 10, 8, 7, 3 and 3 cases, respectively. On follow up, 11 of the patients died of disease from 2 to 14 months. It was concluded that invasive micropapillary variant of urothelial carcinoma is an aggressive variant associated with poor prognosis that presents at an advanced clinical stage. The immunophenotype of invasive micropapillary carcinoma supports urothelial origin; the immunorreactivity to Her2Neu and PTEN might be relevant in terms of future targeting therapy. [23]

**Plasmacytoid urothelial carcinoma**

This is an unusual variant in which the tumour cells show eosinophilic cytoplasm and eccentric nuclei producing a plasmacytoid appearance, thus suggesting
multiple myeloma/lymphoma.[1, 6, 10, 26] The epithelial nature of the malignancy is confirmed by positive immunohistochemistry of cytokeratin and negativity for lymphoma/plasmacytoma markers. This tumour is an aggressive neoplasm with stage-related outcome and frequent lymph node positivity at diagnosis. [27] A recent report based on 11 cases [27] showed that the plasmacytoid component varied from 30-100% of the tumor specimen; in 8 cases the plasmacytoid component composed greater than 50% of the tumor. The architectural pattern of the tumor varied from solid expansile nests with noncohesive cells to mixed solid and alveolar growth; a streaking discohesive architecture was additionally present in 2 cases (18%). Rarely, a myxoid pattern can be present. Histologically, the individual tumor cells had an eccentrically placed nucleus and abundant eosinophilic cytoplasm reminiscent of plasma cells. Most neoplastic cells had nuclei of low to intermediate nuclear grade with occasional nuclear pleomorphism. [27] Seven of 9 mixed cases had concurrent conventional high-grade urothelial carcinoma, and the remaining 2 cases presented features of nested or micropapillary urothelial carcinoma. All patients had advanced stage cancer (>pT3); and 8 (73%) had lymph node metastasis. [27]

Immunohistochemical staining demonstrated that both plasmacytoid and associated conventional urothelial carcinoma were positive for CKs7, 20 and AE1/AE3, and epithelial membrane antigen; CD138 was positive in 3 cases. On follow up 9 of patients died of disease from 2 to 11 months and 2 patients were alive with disease at 8 and 16 months. Lopez-Beltran et al [27] concluded that plasmacytoid variant of urothelial carcinoma is an aggressive variant associated with poor prognosis that presents at an advanced stage.

The differential diagnostic considerations include lymphoma (plasmacytoid type) and multiple myeloma. Identification of an epithelial component by immunohistochemistry confirms the diagnosis. Immunohistochemistry using CD45 (leucocyte comon antigen) or cytokeratins is useful. [27] In limited samples, it may be misdiagnosed as chronic cystitis or plasmacytoma, a pitfall further compounded by CD138 (marker of plasma cells) expression is some cases. [27]

**Clear cell (glycogen-rich) urothelial carcinoma**

Up to two-thirds of cases of urothelial carcinoma have foci of clear cell change resulting from abundant glycogen, but the glycogen-rich clear cell “variant” of urothelial carcinoma, recently described,[30]appears to represent the extreme end of the morphologic spectrum, consisting predominantly or exclusively of cells with abundant clear cytoplasm that stains for CK 7, thus confirming urothelial origin.[30, 31] Recognition of this pattern avoids confusion with clear cell adenocarcinoma of the bladder and metastatic clear cell carcinoma of the kidney and other sites. Recent data relates this morphology with lymph node metastases.[7]

**Large cell undifferentiated carcinoma**

This is a poorly defined category that contains large cell type carcinoma that cannot be otherwise classified.[1, 6, 33, 35] They are extremely rare in the urinary tract, aggressive, and of high stage at presentation including frequent lymph node metastases.[7] One reported case showed increased α-fetoprotein production in patient’s serum and by immunohistochemistry in the tumour cells.[32] Some may contain abundant epithelial tumour giant cells resembling giant cell carcinoma of the lung (so called pleomorphic giant cell carcinoma of the bladder);[1, 6, 33] this is very infrequent and aggressive variant with poor prognosis, similar to that associated with giant cell carcinoma in the lung or prostate. The giant cells display cytokeratin immunoreactivity. We believe this should be considered in the spectrum of the large cell undifferentiated carcinoma of the bladder instead of a different pathologic variant until more experience
is made available. [1] The spectrum of neuroendocrine carcinomas of the urinary bladder may include rare examples of large cell neuroendocrine carcinomas, similar to the more common counterpart in the lung. The experience with these cases suggests they are at advanced stage at presentation. [34, 35]

**UROTHELIAL CARCINOMA WITH FEATURES SUGGESTING BENIGN PROLIFERATIVE LESIONS**

**Microcystic urothelial carcinoma**
This pathologic variant is characterized by the formation of cysts ranging from microscopic up to 1-2 cm in diameter. [13, 36, 37] The cysts or small tubules may be empty or contain necrotic debris or mucin. This variant of cancer may be confused with benign proliferations such as florid polypoid cystitis cystica and glandularis, nephrogenic adenoma and rarely with adenocarcinoma. [13, 36]

**UROTHELIAL CARCINOMA WITH A COMPLEX THERAPEUTIC APPROACH**

**Lymphoepithelioma-like urothelial carcinoma**
This tumour resembles lymphoepithelioma of the nasopharynx. [38, 39] The male-to-female ratio is 3:1, and occurs in late adulthood (range: 52-81 years; mean: 69 years). Most patients present with hematuria. [39-41] The tumour is solitary and usually involves the dome, posterior wall, or trigone, often with a sessile growth pattern. At histology, it may be pure or mixed with typical urothelial carcinoma. [42] The epithelial tumour cells show poorly defined cytoplasmic borders imparting a characteristic syncytial appearance. The background consists of a prominent lymphoid stroma that includes T- and B-lymphocytes, plasma cells, histiocytes, and occasional neutrophils or eosinophils. [39] Epstein-Barr virus infection has not been identified in lymphoepithelioma-like carcinoma of the bladder. [43] This tumour has been found to be responsive to chemotherapy when it is encountered in its pure form.

**Small cell carcinoma**
A recent molecular study indicated that small cell carcinoma and urothelial carcinoma are derived from the same clonal population and we consider small cell carcinoma of the urinary bladder as a variant of urothelial carcinoma with a dismal prognosis. [44, 45] In a recent series of 64 cases of small cell carcinoma of the urinary bladder, the mean age at diagnosis was 66 years and the male: female ratio was 3.3:1; 88% presented with hematuria. [44, 46, 47] All the patients except one had muscle-invasive disease at presentation. Thirty-eight patients (59%) underwent cystectomy and 66% of patients had lymph node metastasis at the time of cystectomy with regional lymph nodes, bone, liver and lung being the most common locations. Forty four cases (68%) cases consisted of small cell carcinoma with other histological types (urothelial carcinoma, 35 cases; adenocarcinoma, 4 cases; sarcomatoid urothelial carcinoma, 2 cases; and 3 cases with both adenocarcinoma and urothelial carcinoma). [44, 47] No clinico-pathologic parameters studied correlated with survival. No significant survival difference was found between patients who underwent cystectomy and those who did not receive cystectomy. Patients with organ-confined cancers had marginally better survival than those with non organ-confined cancer (P=0.06). [44] Overall, 1-year, 18-month, 3-year, and 5-year cancer-specific survivals were 56%, 41%, 23%, and 16%, respectively. The prognosis of small cell carcinoma of the urinary bladder remains poor irrespective of therapy. Hypercalcemia or hypophosphatemia, and ectopic secretion of ACTH have also been reported as part of the paraneoplastic syndrome associated with primary small cell carcinoma of the bladder. [46] On histological examination, they
mimic its pulmonary counterpart. The immunohistochemical profile reveals neuroendocrine markers at variable frequency, but the diagnosis of small cell carcinoma can be made on morphologic grounds alone, even if neuroendocrine differentiation cannot be demonstrated. The recent finding of c-kit and epidermal growth factor receptor expression by immunohistochemistry opens new possibilities for targeted therapy in small cell carcinoma of the bladder.[46, 48, 49]

**UROTHELIAL CARCINOMA THAT CAN BE MISDIAGNOSIS AS CHORIOCARCINOMA EITHER PRIMARY OR SECONDARY**

Urothelial carcinoma with syncitiotrophoblastic giant cells

Up to 12% of cases of urothelial carcinoma show syncitiotrophoblastic giant cells producing substantial amounts of β-human chorionic gonadotropin (HCG). [50-53]Secretion of HCG into the serum may be associated with a poor response to radiation therapy.[53] The most important differential diagnostic consideration is choriocarcinoma; most but not all cases previously reported as primary choriocarcinoma of the bladder represent urothelial carcinoma with syncytiotrophoblasts rather than pure choriocarcinoma; a lesion that is very unusual in the bladder.[1] One reported primary choriocarcinoma of the bladder that occurred in a 19 year-old man showed high copy number of the isochromosome 12p supporting germ cell differentiation.[54]

**UROTHELIAL CARCINOMA WHICH CAN BE MISDIAGNOSIS AS MYOFIBROBLASTIC PROLIFERATION OR SARCOMA**

Sarcomatoid urothelial carcinoma (carcinosarcoma, spindle cell carcinoma)

The term sarcomatoid variant of urothelial carcinoma should be used for all biphasic malignant neoplasms exhibiting morphologic and/or immunohistochemical evidence of epithelial and mesenchymal differentiation (with the presence or absence of heterologous elements acknowledged in the pathology report).[55] Some patients have previous history of radiation or cyclophosphamide therapy.[56] The most frequent presenting signs and symptoms are hematuria, dysuria, nicturia, acute urinary retention, and lower abdominal pain.[56] The mean age is 66 years (range, 50-77 years). Pathological stage is the best predictor of survival in sarcomatoid carcinoma.[56] The tumours are often polypoid with large intraluminal masses. Microscopically, sarcomatoid carcinoma is composed of a urothelial, glandular or small cell component showing variable degrees of differentiation; carcinoma in situ is present in 30% of cases and occasionally is the only apparent epithelial component.[56] A small subset of sarcomatoid carcinoma of the bladder and the renal pelvis may have a prominent myxoid stroma, a finding that misguide the pathologist into inflammatory pseudotumour (inflammatory myofibroblastic tumour) but this is characteristically positive for anaplastic lymphoma kinase stains unlike sarcomatoid carcinoma which is negative.[56-61] The most common heterologous element in a large series is osteosarcoma followed by chondrosarcoma, rhabdomyosarcoma, leiomyosarcoma, liposarcoma, or angiosarcoma, but multiple types may be present.[56] By immunohistochemistry, epithelial elements react with cytokeratins, whereas stromal elements react with specific markers corresponding to the type of mesenchymal differentiation. Recent molecular evidences strongly argue for a monoclonal origin of both components in sarcomatoid carcinoma. [62]

**MISDIAGNOSIS AS SQUAMOUS CELL CARCINOMA OR ADENOCARCINOMA EITHER PRIMARY OR SECONDARY**
Urothelial carcinoma with squamous and/or glandular differentiation (mixed differentiation carcinoma)

Squamous differentiation, defined by the presence of intercellular bridges or keratinisation, occurs in 21% of urothelial carcinomas of the bladder.[1, 3, 6, 56] Its frequency increases with grade and stage.[3] The proportion of the squamous component varies considerably, with some cases having urothelial carcinoma in situ as the only urothelial component. These cases may have a less favorable response to therapy (surgical, radiation or systemic chemotherapy) than pure urothelial carcinoma. Of 91 patients with metastatic carcinoma, 83% with mixed adenocarcinoma and 46% with mixed squamous cell carcinoma experienced disease progression despite intense chemotherapy, whereas progression occurred in <30% of patients with pure urothelial carcinoma.[4, 5] Low-grade urothelial carcinoma with focal squamous differentiation also has a higher recurrence rate. Tumors with any identifiable urothelial element are classified as urothelial carcinoma with squamous differentiation.[3] Rare cases of squamous differentiation in the bladder with basaloid or clear cell features have been recognized.[3] The morphologic patterns of keratinizing and non-keratinizing squamous differentiation in a recent study by Lopez-Beltran et al.[3] were: i) wide nests and cords showing diskeratosis, intercellular bridges and corneous pearls; ii) small foci frequently overcome under conventional evaluation showing intercellular bridges but not diskeratosis or corneous pearls; iii) additional features suggestive of koilocytosis; iv) occasional but focally prominent intracytoplasmic lumina in nests of Squamous differentiation; and v) rare cases had rather clear cells forming nests of variable size well demarcated from concomitant urothelial carcinoma.

Glandular differentiation is less common than squamous differentiation and may be present in about 6% of urothelial carcinomas of the bladder.[2] Glandular differentiation is defined as the presence of true glandular spaces within the tumor. These may be tubular or enteric glands with mucin secretion. A colloid-mucinous pattern characterized by nests of cells “floating” in extracellular mucin, occasionally with signet ring cells, may be present. Cytoplasmic mucin-containing cells are present in 14-63% of typical urothelial carcinoma and are not considered to represent glandular differentiation.[1, 6] In rare cases, the glandular component has the morphology of hepatoid or clear cell adenocarcinoma.[63] A tumour with mixed glandular and urothelial differentiation is best classified as urothelial carcinoma with glandular differentiation.

A recent report by Scosyrev et al.[10] concluded that mixed histological features (presence of squamous and/or glandular differentiation) affect survival benefit from neoadjuvant platinum-based combination chemotherapy in patients with locally advanced bladder cancer, that this finding does not confer resistance to MVAC and in fact may be an indication for the use of neoadjuvant chemotherapy before radical cystectomy.

MISDIAGNOSIS AS OTHER TUMOR TYPES

Lipid-cell urothelial carcinoma

The lipoid cell variant is a rare neoplasm defined by the World Health Organization (1999, 2004) as a type of urothelial carcinoma that exhibits transition to cells resembling signet ring lipoblasts [1, 6]. It frequently presents with gross hematuria.[64]

A recent report based on 27 cases [64] showed that the lipid-cell component varied from 10-50% of the tumor specimen; in 11 cases the lipid cell component composed greater than 30% of the tumor. The architectural pattern of the tumor varied
from solid expansile to infiltrative nests. The large epithelial tumor cells had an eccentrically placed nucleus and abundant vacuolated cytoplasm resembling signet-ring lipoblasts. Mucin stains were negative in all cases. Typical features of high grade conventional urothelial carcinoma were present in all cases, with micropapillary or plasmacytoid carcinoma in 2 and 1 cases, respectively; extensive squamous or glandular differentiation was present in two additional cases. Most neoplastic cells had nuclei of intermediate nuclear grade with occasional nuclear pleomorphism. [64]

Immunohistochemical staining demonstrated that the lipid cell component was positive for CKs 7, 20, CAM 5.2, high molecular weight (34BE12) and AE1/AE3, epithelial membrane antigen and thrombomodulin; vimentin and S100 protein were negative. The LOH analysis was performed on 8 cases using 4 polymorphic microsatellite markers (D9S171, D9S177, IFNA and TP 53); LOH at least in one marker was present in 6 cases. The LOH results were the same for lipid variant and conventional urothelial carcinoma. Electron microscopy analysis based on two cases, supported lipid content in tumor cells. [64]

Pathologic stage at diagnosis was Ta (n=1), T1 (=2), T2, at least (n=7), T3a (n=4), T3b (n=8), and T4a (n=5). Sixteen of patients died of disease from 16 to 58 months (mean, 33 mo) and 8 patients were alive with disease at 8 to 25 months (mean, 22 mo). Three additional patients died of other causes at 6 to 15 months (mean, 10 mo). It was concluded that lipid cell urothelial bladder carcinoma is typically associated with advanced stage high grade urothelial carcinoma, where the prognosis is poor; and is clonally related to the concurrent conventional urothelial carcinoma. In limited samples, it may be misdiagnosed as liposarcoma, sarcomatoid carcinoma (carcinosarcoma) with a liposarcomatous component or signet-ring cell carcinoma. [64]

Urothelial carcinoma with stromal reactions (pseudosarcomatous stroma; stromal osseous or cartilaginous metaplasia; osteoclast-type giant cells; prominent lymphoid infiltrate)

Infiltrating urothelial carcinoma may be associated with a variety of stromal reactions, which are occasionally pronounced. The finding of a pseudosarcomatous stroma raises serious concern about sarcomatoid carcinoma or true sarcoma of the bladder.[65] Tumor-associated stromal osseous and/or chondroid metaplasia is present in some cases of urothelial carcinoma and its metastases, and this should be differentiated from osteosarcoma, chondrosarcoma or heterologous type of sarcomatoid carcinoma.[66] The presence of osteoclast-like giant cells in cases of invasive high-grade urothelial carcinoma seems not to be related to tumor prognosis.[67] In fact, ‘Giant cell tumors’ or ‘osteoclastoma-like giant cell tumors’ of the pancreas, gall bladder, liver, breast, salivary gland, thyroid, skin, lung, intestines, larynx and female genital tract have been reported. [67] Less than 20 cases of a similar spectrum have been reported in the bladder, most as case reports, with one series of 6 cases. The tumors are composed of mononuclear cells (frequently positive for epithelial markers), osteoclast-like giant cells (CD68-, CD51-, CD54-positive) and recognizable usual urothelial neoplasia (carcinoma in situ, papillary or invasive carcinoma) in varying proportions.[67]

An inflammatory cell response in the stroma adjacent to the invasive tumors is relatively common. [69] This response usually takes the form of a lymphocytic infiltrate with a variable admixture of plasma cells. Generally, this cellular reaction is mild to moderate, but occasionally it may be dense. [69]

Other morphological variants of urothelial carcinoma

Baldwin et al. described a series of 10 cases of urothelial carcinoma with a striking discohesive growth pattern which show morphological features that mimicked
infiltrating lobular carcinoma of the breast and diffuse carcinoma of the stomach.[70]
The mean age was 67 years at presentation. All cases had advanced stage at time of
diagnosis. This pattern is important to recognize in order to avoid misdiagnosis of
metastatic lobular carcinoma of the breast or diffuse carcinoma of the stomach,
especially in small biopsies; most authors currently believe this represent a form of
plasmacytoid carcinoma [70] Gleason 3 prostate cancer with tubular pattern of growth
which may be seen is the main differential consideration concerning bladder cancer with
small tubules formation.[71] A rare case of urothelial carcinoma simulating
endometrioid carcinoma of the endometrium has been reported.[1] Urothelial carcinoma
with chordoid features is a recently described entity based on 12 invasive urothelial
carcinomas with a unique chordoid morphology characterized by prominent cellular
cording and associated myxoid stromal matrix, a pattern closely resembling
extraskeletal myxoid chondrosarcoma.[68] The percentage of tumor with a chordoid
appearance ranged from 5% to 95% (mean: 39%; median: 25%). No conventional
sarcomatous differentiation, no intracytoplasmic mucin, and no glandular formation
were present in any case. All 12 cases had foci of typical urothelial carcinoma present at
least focally and a gradual transition to the chordoid pattern was commonly seen.[68]
Rarely, bladder carcinomas may exhibit rhabdoid features.

Other important observation when dealing with urothelial carcinoma is that
occasionally tumors with divergent differentiation show multiple histologic patterns
within the same tumor such as sarcomatoid, smallcell, micropapillary, squamous and
glandular differentiation, and virtually all variants described above could be present. [1]
When multiple histologies are encountered, it is recommended to report the relative
percentage of each of the different components.

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The following table lists the most common tumor-like lesions of the urinary bladder:

Epithelial abnormalities:
- Von Brunn’s nests
- Cystitis glandularis
- Cystitis cystica
- Squamous metaplasia
- Nephrogenic adenoma
- Papillary hyperplasia

Non-specific cystitis
- Papillary-polypoid cystitis

Specific cystitis
- Follicular cystitis
- Interstitial cystitis
- Eosinophilic cystitis
- Giant cell cystitis
- Radiation cystitis
- Incrusted cystitis
- Emphysematous cystitis
- Granulomatous cystitis

Malakoplakia

Polyps:
- Fibroepithelial
- Other

Amyloidosis

Müllerian lesions including endometriosis, endocervicosis, and müllerianosis

Postoperative spindle cell nodule

Inflammatory pseudotumor

Ectopic prostate

Paraganglionic tissue

From all the above, I will focus on the ones that more often may cause problems in differential diagnosis with malignant tumors involving the urinary bladder, mostly related to transitional cell carcinoma.
CYSTITIS GLANDULARIS

This lesion evolves and imperceptibly merges with Von Brunn’s nests, a common epithelial abnormality of the bladder. When these nests become cystic with central lumen formation and polarization of the inner most cells, the term cystitis glandularis is used. Cystitis glandularis is a relatively common histologic finding, most frequently seen in the trigone as seen in Von Brunn’s nests. It is divided into:

a) Usual type
b) Intestinal type

On cystoscopic examination, these lesions may be seen as a nodular or polypoid growth, especially in cases of cystitis glandularis of intestinal type, mimicking on occasion a malignant neoplasm. On microscopic examination, cystitis glandularis, of the usual type, is composed of an inner layer of columnar to cuboidal cells surrounded by an outer layer/s of transitional cells. Similar epithelium may be present on the surface. Cystitis glandularis of the intestinal type is less frequent (incidence of 0.1-0.9%) and more commonly seen in patients with extrophy and pelvic lipomatosis. The glands are lined by mucinous epithelium including goblet cells, closely resembling colonic epithelium. It may be very florid and associated with mucin extravasation. The stroma may show mild edema or hyalinization and inflammatory infiltrate. Young & Bostwick reported 6 of such lesions that caused serious problems of differential diagnosis with mucinous adenocarcinoma of the bladder. Five out of the 6 patients had a gross abnormality, thought to be consistent with a neoplasm, in the region of the trigone or bladder neck under cystoscopy. On microscopic examination, numerous glands were present in the lamina propria, some had the appearance of cystitis glandularis of the usual type; however, most of them were lined by tall columnar epithelial cells, including goblet cells without surrounding transitional type cells. This intestinal type epithelium was also seen lining the surface of the bladder. On closer examination, the glands had basally located nuclei without any significant degree of cytologic atypia. Paneth cells could also be found. Extravasated basophilic mucin was present in the stroma in all, and in four cases this finding was conspicuous, however, no intestinal epithelium was seen floating in the extracellular mucin.

The differential diagnosis includes:

**Bladder adenocarcinoma.** This is frequently of intestinal type. The distinction between these two entities is based on architecture and cytology. The benign process is characterized by an orderly distribution of glands lacking cytologic atypia and absence of mucinous cells free floating in mucinous pools.

**Urachal adenocarcinoma.** This type of adenocarcinoma may be low grade, paucicellular, and show extensive mucin extravasation, and thus, it may be difficult to make the diagnosis of malignancy in a small specimen. It is important to remember that urachal lesions are typically located in the anterior aspect of the bladder dome while cystitis glandularis is located in the trigone or bladder neck.
**Secondarily involvement by large bowel colloid carcinoma.** The distribution of disease is helpful as the bulk of the tumor is present in the serosa and muscularis propria with much less involvement of the lamina propria and mucosa and the degree of cytologic atypia is marked at least focally. Patients also have clinical symptoms and signs related to the main tumor.

**Endocervicosis.** Benign müllerian lesion composed of glands lined by endocervical type epithelium, some of which may rupture resulting in mucin extravasation. However, the latter if present is very focal and associated with histiocytic and fibroblastic inflammatory response. Furthermore, endocervicosis is centered in the muscularis propria.

Finally, it should be noted that there is controversy regarding the malignant potential of cystitis glandularis of intestinal type. Over the years it has been stated that diffuse intestinal metaplasia is associated with an increased risk of bladder carcinoma, as intestinal metaplasia often coexists with adenocarcinoma. However, Corica and colleagues collected a series of 53 patients with intestinal metaplasia and long follow-up (approximately 14 years). They concluded that intestinal metaplasia was not a strong risk factor for adenocarcinoma of the bladder.

**NEPHROGENIC ADENOMA**

This is a rare benign lesion of the urothelium, first described by Davis in 1949, but characterized in detail by Friedman and Kuhlenbeck in 1950. The term nephrogenic adenoma (NA) was coined because of the morphologic similarity of the lesion to the distal nephron. Predisposing factors include genitourinary trauma, surgery, mechanical irritation, chronic inflammation, and renal calculi. Nephrogenic adenoma has also rarely described in bowel conduits. The lesion also has been noted in immunosuppressed patients, especially those with a transplanted kidney. Some authors prefer the term nephrogenic metaplasia, as this lesion was initially thought to arise from a metaplastic process. More recently, it has been shown that at least a subset of NAs that develop in patients with a kidney transplant derive from renal tubular cells that implant in the bladder mucosa as NAs in female recipients of transplants from male donors and male recipients of transplants from female donors showed the same sex-chromosome status as the donor kidney, but not the same sex-chromosome status as the recipient’s surrounding bladder tissue. The rationale behind these findings is that in renal diseases and hypoxic conditions, including immunosuppression, there is an increased detachment of viable renal tubular cells that secondarily seed, implant, and grow in the urothelial mucosa. However, the origin of NA in non-immunocompromised patients is still unclear, as there is some type of injury related to most NAs, and consequently, these lesions could also have a similar pathogenesis.

The lesion occurs mainly in adults, where it is more common in males, but the reverse pertains to children. Approximately 80% of the lesions occur in the bladder, with the remainder involving the urethra (15%) or ureter (5%) and, rarely, the renal pelvis. In most cases, it is an incidental finding, but in 1/3 of the cases, the lesions are sizable and 10% are 4 cm or even larger. In those cases, it may be potentially confused with a malignant tumor, most commonly a low-grade papillary transitional cell carcinoma.

On microscopic examination, NAs may show tubular or tubulocystic, papillary, and, much less frequently, solid growth. The tubular pattern is the most common, present in 96% of cases in the
largest review of NAs (80 cases). The tubules frequently grow in a band-like pattern with a sharp demarcation from the underlying stroma. The tubules vary in size and shape, and although the majority are small and contain either basophilic or eosinophilic secretions, on rare occasions the tubules may be solid. An appreciable basement membrane may be seen surrounding some of the tubules. The cysts are frequently admixed with the tubules but in most cases are not really conspicuous. The papillary pattern has an exophytic growth in most cases, but it can be endophytic. The papillae do not branch and it is very rare to see this component in the absence of tubules. The final and least common pattern is solid, and when present typically represents a minor component of the lesion. Of note, the tubules of NA may be intermixed with muscle fibers of the muscularis mucosa in the bladder, ureter, or, more often, with the muscle fibers present in the wall of the prostatic urethra. In these cases, the specimens are more often obtained by transurethral resection. The cells that line the tubules, cysts, and papillae are cuboidal to columnar to flattened with scant eosinophilic to slightly clear and granular cytoplasm. Hobnail cells are typically present, most often lining the cysts, but are rarely conspicuous. Cells lining tiny tubules have a compressed nucleus with a single vacuole containing basophilic material resembling signet-ring cells. The nuclei are round to oval with minimal cytologic atypia. Nucleoli are inconspicuous and mitoses rare (<1/10 high power fields). In the largest study of 80 NAs, mitotic activity was only seen in 5% of the lesions. The term “atypical NA,” introduced by Cheng and colleagues, identifies lesions characterized by severe cytologic atypia, including nuclear enlargement, nuclear hyperchromasia, and enlarged nucleoli. One of these features, prominent nucleolus, was also observed at least focally in 16 NAs in the largest published series, and in 14 out of 26 NAs involving the prostatic urethra in another series. The stroma associated with NA is focally edematous and contains variable amounts of inflammatory cells. Stromal calcification may be seen. It is not unusual that NA is seen in association with other reactive benign lesions of the bladder, including cystitis glandularis, polypoid cystitis or squamous metaplasia. A recently described variant of NA is the so-called ‘fibromyxoid nephrogenic adenoma,’ where there is a predominance of spindle cells within a fibromyxoid background. However, in all these cases, the classic tubular pattern is also present. It is always important to be aware of this rare morphology of nephrogenic adenoma and particularly when there is any history that may have elicited this peculiar process diligent search for the more characteristic patterns of this lesion.

The immunohistochemical profile of NA is characterized by diffuse positivity for wide spectrum keratins, CK 7, EMA, vimentin, and PAX2 (an antigen found during nephrogenesis) and PAX8. Different types of lectins are expressed, as seen in renal tubular epithelium. There is frequent staining for CA-125 as well as focal positivity for CEA, CK 20, CD10, and, in some cases, for RCC antibody. Kidney-specific cadherin (KSP-CAD) is a recently characterized calcium-dependent cell adhesion molecule that appears to be kidney-specific in its distribution, with expression localized primarily in the distal nephron. We have stained 10 NAs and all were negative for this antibody. Aquaporin 1 (marker of the proximal and descending thin limb of Henle’s loop renal tubular cells) has been shown to be positive in one study of NAs in immunosuppressed patients. However, in our experience, most NAs in patients without known immunosuppression are negative for aquaporin 1, whether by immunohistochemistry or immunofluorescence; thus, it is still unclear whether the origin of these lesions is related to the proximal nephron, at least in non-immunocompromised patients. Nephrogenic adenoma does not show the typical membranous/luminal staining for uroplakin as seen in transitional epithelium,
and the immunohistochemical profile just described raises questions about the metaplastic nature of NA overall. It is important to be aware that P50 4S (AMACR), which is an enzyme normally present in the mitochondria of renal tubular epithelium and hepatocytes but absent or minimally expressed in benign prostatic glands and urothelium, is expressed in NAs, including those involving the prostatic urethra. NAs are negative for p63 and may not express 34βE12 increasing further the confusion with prostatic adenocarcinoma. It is noteworthy that the finding of AMACR and 23βE12 in renal tubules would support the hypothesis that NA may have a renal origin. Finally, one recent study has reported focal weak positivity for PSA and PSAP in some cases. This overlapping profile highlights the importance of clinicopathologic correlation and careful histologic examination, and demonstrates the importance of using a panel of antibodies in differentiating these tumors through immunohistochemistry. Recently, S100 has been shown to be helpful in this differential diagnosis. A strong and distinct cytoplasmic or nucleocytoplasmic staining of S100A1 was found in 17 out of 18 cases of nephrogenic adenoma (94%), but never in prostatic adenocarcinoma. p53 and Ki-67 expression are typically negative in NAs. Ploidy analyses have shown that these lesions are typically diploid, while cytogenetic studies have shown that NA cells are characterized by monosomy 9.

The differential diagnosis includes:

**Clear cell carcinoma (CCC).** This is probably the most common and most difficult problem in differential diagnosis with NA and may arise in biopsy specimens where there is only a small amount of sampled lesion. Clear cell carcinoma is more frequently seen in older women. Patients with these tumors frequently present with hematuria or other clinical symptoms, and the tumors often form a visible mass. On microscopic examination, the histologic patterns of CCC overlap with those seen in NA, although a solid growth is more common. The papillae tend to branch and they may contain hyalinized fibrovascular cores, as seen in CCCs of the female genital tract. Clear cell carcinoma and NA also share the presence of clear and hobnail cells. However, the degree of cytologic atypia and mitotic activity seen in CCC is by far more conspicuous than in NA. Clear cell carcinoma may have a subtle appearance focally; however, the presence of any degree of cytologic atypia or mitotic activity should alert any pathologist, who should be very cautious in making a benign diagnosis. Furthermore, CCC frequently shows areas of hemorrhage and necrosis and infiltrates deep into the vesical wall. Both lesions may arise in association with a diverticulum, especially when located in the urethra. It has been postulated by some investigators that NA may be the precursor lesion of CCC; however, this theory has never been proven. Immunohistochemical stains are not helpful in this differential diagnosis, as the lesions show an overlapping immunohistochemical profile, including positive staining for low-molecular weight keratins, CK7, CK20, PAX2, and PAX8 and negative staining for estrogen and progesterone receptors, PSA, and PSAP.

**Bladder cancer with prominent nested or tubular growth patterns.** Helpful features in this distinction include more than one cell layer lining the tubules in cases of bladder cancer (typical of urothelium but not NA); the presence of more appreciable cytologic atypia; and the absence of other patterns typically seen in NA, as well as the invasion into the muscularis propria seen in some carcinoma cases. Immunohistochemically, the nested variant of urothelial carcinoma shares some features with high-risk conventional urothelial carcinomas, such as high proliferation index, and with rare exceptions, this feature contrasts with the absence of Ki-67 expression in
most NAs. Nevertheless, p53 immunoreactivity is not frequently seen and cannot be used in this
differential diagnosis. Of interest, recent studies have revealed that NA shows the same
chromosomal aberrations as superficial bladder cancer, though to a smaller extent.

**Prostate adenocarcinoma** may enter into the differential diagnosis in those cases where NA
involves the prostatic urethra as mentioned earlier. Tubules of NA may show a pseudoinfiltrative
growth, intercalating between muscle fibers. Furthermore, the cells lining the tubules may show
nuclei with prominent nucleolus, and mucinous secretions may be present in the lumens of the
tubules. In these cases, it is important to keep in mind the possibility of NA before making the
diagnosis of prostate cancer. Immunohistochemical stains for p63, 34\(\beta\)E12 and P504S are not
helpful in this differential diagnosis in many instances, and it seems that compared to bladder
NAs, urethral NAs express AMARC more often. However, PSA and PSAP are helpful in most
cases, as prostatic adenocarcinoma typically shows diffuse and strong positivity, in contrast to
the weak and focal positivity seen in some NAs. Most importantly, the tubular pattern is
associated with other typical architectural patterns of NA, the cytologic atypia is frequently of
degenerative type, and no mitotic figures are found. Finally, the presence of acute or chronic
inflammatory cells is not a feature of prostate cancer, whereas it is typically present in NAs.

**Signet ring cell carcinoma of the bladder or metastatic to the bladder** very rarely may enter
into the differential diagnosis, especially when the most prominent pattern is that of very small
tubules containing basophilic secretion. This growth is always accompanied by larger tubules
and neither shows diffuse involvement of the bladder wall nor the cytologic atypia seen in signet
ring cell carcinoma.

Nephrogenic adenoma may recur, and the recurrence rate ranges from 28 to almost 90%, but
there is no proof that NA may undergo malignant transformation.

**POLYPOID/PAPILLARY CYSTITIS**

These lesions are the result of inflammation and edema in the lamina propria. The term polypoid
is used for bulbous, broad-based lesions and the term papillary for lesions with a finger-like
appearance. The former is by far more common. Polypoid cystitis is more frequently seen in
men and it is present in up to 80% of patients with indwelling catheter. Vesical fistulae, whether
resulting from appendicitis, Crohn disease, diverticulitis or colorectal cancer, are also frequently
associated with polypoid cystitis. However, in about 50% of patients, obvious signs of
extravesical disease may be initially absent making the diagnosis more difficult. Most lesions are
microscopic in size, but in up to 33% may reach 5 mm in largest dimension. On *cystoscopy*, the
appearance of the lesions not infrequently suggests the possibility of bladder carcinoma. On
*microscopic examination*, the main problem is to distinguish polypoid/papillary cystitis from
papillary urothelial carcinoma, but on low-power examination in contrast to papillary transitional
cell carcinoma, the fronds are much broader and branching is absent or minimal. There is
prominent edema associated with inflammatory and metaplastic changes such as squamous
metaplasia. On high-power examination, although the urothelium is hyperplastic, it is not by far
to the degree seen in papillary transitional cell carcinoma and umbrella cells are more commonly
found in these benign urothelial lesions. Finally, polypoid cystitis does not have cytologic atypia,
other than that associated with inflammation.
REACTIVE ATYPIA INCLUDING RADIATION INDUCED ATYPIA

These are lesions that may cause problems in differential diagnosis on clinical examination but mainly under the microscope. Patients obviously have a previous history of radiation, not necessarily for bladder cancer, but for prostate or cervical cancer, in some instances forgotten. Cystoscopy may show small papillary lesions, slight mucosal irregularities or be non-specific. On microscopic examination, the earlier changes appear after 3 to 6 weeks and include acute cystitis and desquamation of the urothelial cells associated with hyperemia. Later, there is epithelial hyperplasia producing a pseudoinfiltrative appearance with some nests being jagged and others having rounded contours. Budding off single cells may also be seen, and the nests may show squamous differentiation, all those features raising the suspicion of a malignant process. These cells show mild to severe cytologic atypia with nuclear hyperchromasia, however, nucleoli are inconspicuous, the nuclear/cytoplasmic ratio is preserved or increased in most cases and mitotic figures are typically absent, features that are not characteristic of a malignant process. Other helpful features are the finding of scattered enlarged fibroblasts in the lamina propria associated with nuclear hyperchromasia, hemorrhage and or fibrin deposition, edema, inflammatory cells and ectatic vessels, some of them showing myointimal thickening and eccentric accumulation of amorphous pink material, all these features being highly suggestive of a reactive lesion. The immunohistochemical profile of radiation induced atypia, even when marked, typically shows CK20-p53-CD44+ immunoprofile, while a CK20+p53+CD44- profile is characteristic of carcinoma in situ (the most important differential diagnosis), with CK20 being the most reliable marker. As with all immunohistochemical stain interpretation, very close correlation with morphology is essential due to varying immunoreactivity in a given case.

MULLERIAN LESIONS

a) ENDOMETRIOSIS

The bladder is the most common organ involved by endometriosis in the urinary tract. One percent of women with endometriosis have urinary bladder involvement and it typically occurs in women of reproductive age. Up to 50% have a history of pelvic surgery. Rarely vesical endometriosis has been reported in men with prostate carcinoma treated with estrogen therapy. Female patients most commonly complain of frequency, dysuria, and hematuria, and in 75% of cases these symptoms have catamenial exacerbations. A suprapelvic mass is palpable in 50% of the patients. Secondary and significant complications include obstruction of the ureteric orifices with secondary hydronephrosis or vesicocolic fistula. On cystoscopic examination, as many of 90% women have bladder abnormalities most commonly involving the posterior wall or trigone. The urologist typically describes an elevated, congested and edematous area in the mucosa overlying a translucent, blue-black or red brown area of discoloration. On rare occasion, the appearance may mimic cystitis glandularis or an unusual gross morphology of bladder cancer. On gross examination, it may form a mass involving the urinary bladder wall most often
secondary to muscle hypertrophy or fibrosis associated with endometriosis. If there is no mass, a hemorrhagic punctate may be observed if the glands are cystically dilated secondary to catamenial changes. On microscopic examination, it is composed of endometriotic glands and stroma. The endometrial glands are typically lined by cuboidal cells with eosinophilic cytoplasm and pseudostratified nuclei that may show mitotic activity depending of the phase of the cycle. The stroma may contain foamy histiocytes with some chronic inflammatory cells. The endometriotic stroma may be focally absent around some of the glands and in postmenopausal women that are not on estrogen replacement the diagnosis of endometriosis may be justify in the absence of endometrial stroma, as the latter becomes atrophic. In such instances, the glands still retain their endometriotic appearance. In some cases the endometrial stroma may be replaced by elastotic stroma similar to that seen in radial scars of the breast. The surface urothelium is not replaced by endometrioid-type epithelium as it may happen with endocervicosis. In the urinary bladder, malignant tumors have arisen in association with endometriosis, most commonly endometrioid and clear cell carcinomas. In such cases, the glands have typically a more complex architecture showing crowding, back to back, and cribriforming, there is more prominent cytologic atypia and a marked desmoplastic response in contrast to the endometrial stroma seen in endometriosis.

b) ENDOCERVICOSIS

It is well-known that non-neoplastic and neoplastic proliferations with müllerian differentiation may arise from the pelvic peritoneum (secondary müllerian system). Most of them show endometrioid differentiation (endometriosis and endometrioid tumors) or serous differentiation (endosalpingiosis and extraovarian serous tumors); lesions with endocervical-type differentiation are less common. Endocervicosis is composed of benign endocervical mucinous-type epithelium involving pelvic peritoneum, pelvic lymph nodes, urinary bladder, outer wall of uterine cervix and paracervical connective tissue, and vagina. Patients are typically reproductive age females that complain of suprapubic pain, dysuria, frequency, and hematuria with catamenial exacerbation of the symptoms. Some patients have a history of cesarian section. On cystoscopy, a mural mass (up to 5 cm) is frequently seen in the posterior wall or bladder dome covered by intact mucosa.

On gross examination, the lesion may have a non-descript appearance or may be seen as a rubbery mass with a spongy cut-surface with the cystic spaces containing clear mucus. On microscopic examination, there is a haphazard proliferation of glands throughout the bladder wall, typically centered in the muscularis propria, which may extent to the lamina propria or bladder serosa. However, the glands do not show crowding or back to back. The glands have irregular sizes and shapes, and are lined by a single layer of columnar cells with abundant pale mucinous cytoplasm (mucicarmine and PAS&D positive) and have a basally located small nucleus as seen in normal endocervical glands. Cells with a goblet-like appearance may be seen with the nucleus eccentrically compressed to the side. Some glands may contain ciliated cells or non-specific cuboidal cells intercalated with the mucinous cells, and in some cases, occasional endometriotic glands may be seen. Cytologic atypia is absent or at most mild and mitotic activity is exceptionally rare. The glands lumens contain mucus frequently admixed with acute inflammatory cells. In most instances, the endocervical-type glands are surrounded by normal or mildly hyperplastic smooth muscle, but they may be surrounded by fibrous or cellular stroma.
resembling endometrial stroma or elastotic stroma (atrophic endometriosis). In most cases, there is also an associated inflammatory infiltrate including foamy histiocytes. The glands may rupture resulting in mucin extravasation and more prominent stromal response. Immunohistochemical stains show apical positivity of the mucinous cells for CA 125 (antigen present in normal müllerian epithelia, including endocervical, endometrial and tubal epithelium); CEA, EMA, cytokeratin, and B72.4 are also positive.

The differential diagnosis includes:

**Cystitis glandularis intestinal type.** The glands tend to be more rounded, although may be cystically dilated and rupture resulting in mucin extravasation as described in endocervicosis. However, the glands are typically located in the lamina propria and do not involve the muscularis propria, they may be associated with a usual component of cystitis glandularis and are not associated with endometrial/elastotic type stroma, indicative of müllerian derivation.

**Urachal remnants.** Although located in the bladder dome, it is typically found in the apex or anterior aspect of the bladder dome and not in the posterior aspect as seen in endocervicosis. Furthermore, in contrast to endocervicosis, it forms a tortuous line indicating the presence of a single canal lined by mucinous epithelium that is surrounded by fibrous tissue which in turn is associated with layers of smooth muscle and finally an outer layer of connective tissue. The lining may also contain goblet cells but it does not have ciliated cells, endometrioid glands or endometrioid-type stroma.

**Primary adenocarcinoma.** Some bladder adenocarcinomas may have a deceptively benign histologic appearance; however the glands are present throughout the wall, with prominent involvement of the surface and lamina propria and not infrequently they have an associated transitional cell component of the tumor on the surface. The glands have angulated ends and elicit a desmoplastic response, the cells have at least focally more prominent cytologic atypia and they almost never contain abundant mucin unless the adenocarcinoma is of the signet-ring cell type, and in those cases there is abundant mucin extravasation.

**Metastatic adenocarcinoma.** Some endocervical adenocarcinomas may secondarily involve the bladder wall and be deceptively benign (adenoma malignum) and in a small biopsy may be difficult to be certain of the nature of the lesion. In cases of secondary involvement by a well-differentiated endocervical adenocarcinoma the patient should have other clinical symptoms and signs suggestive of the cervical origin of the tumor.

A primary adenocarcinoma may develop in a background of endocervicosis. In those cases, one can see transition from benign glands to dysplastic to frankly malignant glands.

c) **MULLERIANOSIS**

This entity is defined as a benign epithelial proliferation composed of an admixture of tubal, endocervical and endometrioid-type müllerian glandular epithelium outside the primary müllerian system. In the urinary bladder, this lesion typically involves the muscularis propria and lamina propria of the posterior wall and may form a tumor-like mass (up to 3 cm). Müllerianosis
is characterized by tubules and cysts with irregular sizes and shapes lined by ciliated cells intercalated with peg cells, next to endometriotic glands with endometrial stroma and/or a component of glands lined by columnar mucinous cells.

The differential diagnosis may include cystitis glandularis, cystitis cystica, and nephrogenic adenoma. As mentioned earlier, cystitis glandularis does not involve the muscularis propria, not cilia but urothelium is present lining some glands and endometriotic stroma is not seen. Nephrogenic adenoma typically shows a tubulocystic pattern, cilia or mucinous cells are not present, and in the bladder proper, NA does not involve the muscularis propria.

One patient reported by Donné and colleagues had symptomatic improvement after GnRH therapy. The authors postulated that the endometriotic component of the lesion became atrophic with tryptolerine, although the size of the lesion remained the same after six months of treatment.

### POSTOPERATIVE SPINDLE CELL NODULE

This is a benign lesion initially described by Proppe, Scully and Rosai in the lower genitourinary tract in 1984. They studied 8 nodular lesions with a fascicular and proliferative appearance that developed between 5 weeks and 3 months after surgery and histologically superficially resembled a spindle cell sarcoma. Two of these cases occurred in the bladder wall. All postoperative spindle cell nodules are typically associated with a previous history of surgery. They may be an incidental finding during routine follow-up or patients may present with hematuria, urinary obstruction or other bladder symptoms. On cystoscopy, they are typically polypoid or nodular with poorly defined margins.

On gross examination, they may be soft or firm with a gray, yellow to tan cut-surface. On microscopic examination, they typically show a homogeneous appearance, being composed of intersecting fascicles with plump spindle cells and a delicate network of small vessels. These lesions often destroy and infiltrate the bladder wall mimicking a malignant tumor. They are typically densely cellular. The spindle cells are admixed with variable amounts of chronic inflammatory cells in the deeper areas of the lesion, while the superficial component is associated with an acute inflammatory infiltrate secondary to surface ulceration. Areas of edema, myxoid change or multiple small foci of hemorrhage are typically seen. At higher magnification, the cells have abundant eosinophilic cytoplasm. The nuclei are oval or spindle throughout the lesion, containing one or two small nucleoli and delicate chromatin, and mitotic activity may be brisk, up to 25/10 HPFs. However, no atypical mitoses or cytologic atypia are seen.

The differential diagnosis includes:

**Leiomyosarcoma.** The distinction may be difficult especially if the leiomyosarcoma is well differentiated as the histologic features may overlap. The clinical history of recent surgery at this site strongly suggests the diagnosis of spindle cell nodule over leiomyosarcoma. In these cases, close follow-up with subsequent biopsies is warranted.

**Kaposi’s sarcoma:** may enter in the differential diagnosis when the hemorrhage forms rows of clusters of red cells within slit-like spaces with the associated finding of hemosiderin-laden
macrophages and chronic inflammatory cells in a background of a spindle cell proliferation. However, there is an inconstancy and irregular distribution of these slit-like spaces in the postoperative spindle cell nodule.

**Benign reactive sarcoma-like processes** are rare in the bladder and the previous history of surgery is typically associated with the postoperative spindle cell nodule.

The cells of the postoperative spindle cell nodule are myofibroblasts by ultrastructure. The lesions have a benign clinical course and conservative treatment is recommended.

**INFLAMMATORY PSEUDOTUMOR**

This is a lesion intimately related to the postoperative spindle cell nodule, an important difference being that inflammatory pseudotumor does not have a previous history of surgery at the site where it develops. First recognized in the lung, it has received different names including pseudosarcomatous fibromyxoid tumor. There is some uncertainty regarding the pathogenesis of this lesion, ranging from reactive to neoplastic, the latter based on cytogenetic studies that have shown evidence for clonal origin. Patients range in age between 30 and 50 years, although it may occur at any age and it appears to be slightly more frequent in women. Gross hematuria is the most common presenting symptom, followed by bladder obstruction. However, some patients may be asymptomatic.

On **gross examination**, the lesions range from pedunculated masses protruding into the bladder cavity to nodules or ulcerating masses that deeply infiltrate the bladder wall. They are solitary with a broad base and may be as large as 37.5 cm. On **microscopic examination**, these lesions may show two different patterns: a) Disorganized, loose fascicles of spindle cells with a noticeable edematous-myxoid background and a prominent vascular network composed of small capillaries (resembling nodular fasciitis), b) Cellular areas with intersecting fascicles of spindle cells and variable amounts of intercellular collagen. Some authors find this pattern more commonly deep in the lesion. On high power examination, the cells are elongated with tapering eosinophilic cytoplasmic processes. Stellate cells and more polygonal cells may also be seen, especially in the more myxoid areas. There is no striking pleomorphism. The nuclei are oval to spindled-shaped with open chromatin and one or multiple apparent nucleoli. There is no marked cytologic atypia and mitotic activity although present (up to 4/10 HPFs) is not as prominent as seen in postoperative spindle cell nodule. There is an inflammatory infiltrate, more commonly composed of eosinophils, lymphocytes and plasma cells. The latter can be quite prominent imparting an appearance reminiscent of a plasma cell granuloma variant of inflammatory pseudotumor. Two worrisome features associated with these lesions are: 1) Frequent wall invasion (may reach the outer aspect of the muscularis propria), and 2) Presence of small areas of necrosis, findings that always raise the possibility of a malignant process. The finding of necrosis at the tumor-detrusor muscle interface is not found in inflammatory pseudotumor but it is indicative of a malignant process.

The **immunohistochemical profile** of the inflammatory pseudotumor is similar to that of the postoperative spindle cell nodule. Both lesions are vimentin positive, frequently positive for keratin, smooth muscle actin, and may be positive for EMA and desmin. They are typically
negative for CK 34betaE12 and CK 5/6, and p63. Recently, it has been shown that the inflammatory pseudotumor is frequently positive for ALK-1. The chromosome 2p23 is the site of the human ALK gene, which codes for anaplastic lymphoma kinase. ALK is a tyrosine kinase receptor and a member of the insulin growth factor receptor superfamily and clonal abnormalities of ALK are typically associated with anaplastic large cell lymphoma. The significance of this finding in inflammatory pseudotumor it is not clear at present time, although it has been shown to be more frequently express in patients under the age of 40 years. Some inflammatory pseudotumors show weak p53 expression. These lesions are typically diploid and by electromicroscopy the lesion has a myofibroblastic lineage.

The differential diagnosis includes:

**Myxoid leiomyosarcoma:** This malignant tumor is typically seen in an older group of patients (mean 50 years). These tumors present as large exophytic tumors or infiltrating masses and are composed of long intersecting fascicles with elongated cells that have eosinophilic cytoplasm and cigar-shaped nuclei. The tumors are hypocellular throughout and the presence of myxoid background is a typical finding, these features overlapping with those seen in inflammatory pseudotumor. By definition, these neoplasms have an infiltrative border, typically show areas of necrosis (of help if present at the tumor-detrusor muscle interface) and are associated at least with moderate cytologic atypia and brisk mitotic activity in most cases especially in the more cellular areas. Associated inflammation and vascularity are not typical features of myxoid leiomyosarcoma but seen in inflammatory pseudotumor. If inflammatory cells are present are typically neutrophils. From the immunohistochemical point of view, these tumors are positive for vimentin, actins, but less frequently for desmin. They are also frequently positive for keratins CK but also negative for 34betaE12 and CK 5/6. Finally, although leiomyosarcomas may be positive for EMA, the frequently is lower than that seen in inflammatory pseudotumor and they are typically ALK negative.

**Sarcomatoid carcinoma:** It occurs in an old population (mean age: 70 years). These tumors have a heterogeneous gross appearance and on microscopic examination they typically have a component of conventional transitional cell carcinoma, carcinoma in situ, squamous cell carcinoma, or small cell carcinoma that merges with the sarcomatoid component. Jones and colleagues reported a series of sarcomatoid carcinomas that were associated with a prominent myxoid background as seen in inflammatory pseudotumor. These tumors characteristically have a gelatinous cut-surface and on microscopic examination display polygonal epithelial cells that alternate with spindled epithelial cells with a fascicular growth pattern. Confluent necrosis as well as deep invasion of the bladder wall is present in most cases. The most characteristic finding is the presence of this myxoid background (Alcian blue positive), which may have a focal or diffuse distribution, associated with a delicate vasculature resembling to some extent granulation tissue. These tumors may also show extensive areas of sclerosis. Cytologically, there is marked atypia, although may be focal, with large cells showing hyperchromatic nuclei and coarse chromatin. The cytologic atypia is accompanied by brisk mitotic activity and presence of atypical mitotic figures. If a small sample of tissue is available and only contains these myxoid areas the differential diagnosis with an inflammatory pseudotumor may be very difficult as these areas typically show bland nuclear features. In fact, one of the cases reported by Jones and colleagues was interpreted by the outside pathologist as
an inflammatory pseudotumor/postoperative spindle cell nodule in the cystectomy specimen of a man, who a few weeks before had been diagnosed with a moderately differentiated transitional cell carcinoma in a TUR specimen. Six months later, the patient presented with a pelvic that revealed a malignant spindle cell tumor. After reviewing the case, the final diagnosis was myxoid and sarcomatoid carcinoma. Helpful features in establishing the diagnosis of carcinoma in these cases include the finding of a conventional carcinomatous component, confluent necrosis and focal marked cytologic atypia. These tumors are usually positive for keratins and EMA, although the sarcomatoid component may be negative or only focally positive. As a consequence the diagnosis is based on morphology and clinical features of the tumor. These neoplasms are positive for vimentin and may express, although infrequently, muscle actins and desmin. Sarcomatoid urothelial carcinoma is positive for keratins including CK 34betaE12 and CK5/6, p63 in about half of the cases but it is ALK negative. Thus in the differential diagnosis of inflammatory pseudotumor, leiomyosarcoma and sarcomatoid carcinoma, a combination of pankeratin, SMA, and ALK positivity favors inflammatory pseudotumor; expression of several cytokeratin and especially CK 34betaE12 and CK 5/6 with p63 favors sarcomatoid carcinoma and SMA positivity with overall absence of other markers favors leiomyosarcoma.

**Inflammatory fibrosarcoma**: This tumor may involve the bladder by extension from an intraabdominal site. In contrast to the inflammatory pseudotumor, the spindle cells have malignant nuclear features and a denser and uniform fascicular growth pattern resembling that of a leiomyosarcoma.

**Malignant fibrous histiocytoma**: These tumors occur rarely in the urinary bladder, they typically show a storiform growth, have an admixture spindle and multinucleated cells associated with lymphocytes. These tumors are typically keratin and EMA negative.

**Postoperative spindle cell nodule**: These are more cellular and have a previous history of surgery as discussed earlier.

Inflammatory pseudotumor may recur but typically does not metastasize. It is important to remember that the diagnosis of inflammatory pseudotumor of the bladder should be made with caution in cases where there is no previous history of surgery especially in older patients.
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Polypoid and papillary cystitis:


**Radiation induced cystitis**


**Endometriosis:**


**Endocervicosis:**

Young RH, Clement PB. Endocervicosis involving the uterine cervix: a report of four cases of a benign process that may be confused with deeply invasive endocervical adenocarcinoma. Int J Gynecol Pathol 2000;19:322-8.

**Mullerianiosis:**


**Postoperative spindle cell nodule inflammatory pseudotumor:**

The Molecular Pathology of Bladder Carcinoma and Future Perspectives.

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ABSTRACT:

Molecular diagnostics applications are now an integral part of the management algorithms of several solid tumors such as breast, colon, and lung. In stark contrast, the current clinical management of urologic malignancies is lagging behind. Clinically robust molecular tests that can identify patients that are more likely to respond to a given targeted agent or even those in need of a more aggressive treatment based on well-validated molecular prognosticators are still lacking. Several promising biomarkers for detection, prognosis, and targeted therapeutics are now under evaluation. The following review discusses some of the candidate biomarkers that may soon make their transition to clinical assays in bladder cancer patients.

Superficial and muscle invasive urothelial carcinoma (URCa) of the bladder display two distinct clinical phenotypes in regards to biologic behavior and prognosis. Increasingly, molecular evidence supporting two divergent pathways of pathogenesis for superficial and invasive disease is accumulating. Superficial URCa is thought to originate from benign urothelium through hyperplasia with only a small contribution (10-15%) to the pool of high grade non-invasive and subsequently invasive URCa. The majority of invasive tumors appear to originate through progression from dysplasia to flat CIS and high grade non invasive URCa where genetic instability lead to accumulation of genetic alterations promoting progression to invasive lesions.

Clinically, a significant proportion of superficial tumors (pTa and pT1) are deemed to recur following TURB with only a minority of cases enduring progression to higher-grade, deeply invasive aggressive tumors.

The superficial URCa pathogenesis pathway is primarily based on alterations in the tyrosine kinase receptor FGFR-3 and H-RAS oncogene. The pathogenic pathway for muscle invasive URCa primarily involves alterations in tumor suppressor genes p53, p16 and Rb.
Figure 1: Divergent molecular pathways of oncogenesis in superficial and muscle invasive urothelial carcinoma of urinary bladder. Genetic alterations are depicted in key stages of disease progression.
As illustrated in figure 1, p53 and Rb alterations are also needed for the progression of a subset of superficial lesions that lead to higher grade muscle invasive URCa. The currently established clinicopathologic prognostic parameters in superficial lesions include: pTNM stage, WHO/ISUP grade, size of tumor, disease multifocality, presence of CIS and frequency and rate of prior recurrences. Prognostic parameters that can accurately predict the subset of superficial tumors that will progress are actively sought in order to identify patients in need for vigilant surveillance and aggressive treatment plan. Equally needed are markers that will improve prognostication in muscle invasive disease given the current poor outcome (60% 10 year overall survival) in this group of patients.

**Molecular Pathways of Oncogenesis in Urothelial Carcinoma:**

Chromosome 9 alterations are the earliest genetic alterations in both pathways of URCa development. These changes are thought to impart the necessary milieu of genetic instability that in turn allows for the accumulation of subsequent genetic defects. Among other common chromosomal gains and deletions, gains of chromosomes 3q, 7p, and 17q and 9p21 deletions (p16 locus) are of special interest given their potential diagnostic value. A multitarget interphase FISH based urine cytogenetic assay was developed based on the above numerical chromosomal alterations. Such test is now commercially available. Initially FDA approved for surveillance of recurrence in previously diagnosed URCa patients, the test subsequently was also approved for screening in high risk patients with hematuria. The multicolor FISH assay appears to enhance the sensitivity of routine urine cytology analysis and can be used in combination with routine cytology as a reflex testing in cases with atypical cytology. A sensitivity range of 69-87% and a specificity range of 89-96% have been reported with the multitarget interphase FISH assay. With
the exception of one study, the multitarget FISH urine assay has been shown to be more sensitive than routine cytology. An additional advantage of urine based FISH testing could be the anticipatory positive category of patients identified by such assay. The latter category refers to patients where FISH assay detects molecular alteration of URCa in urine cells several months prior to cancer detection by cystoscopy or routine cytology. In the study by Yoder et al., two thirds of the 27% of patients categorized as “anticipatory positive” developed URCa that was detected by cystoscopy up to 29 months later. Such encouraging results point to the great potential of molecular testing in early detection and allocation of vigorous/frequent follow-up cystoscopy in at risk patients.

The current detailed understanding of the molecular pathways involved in URCa development and progression has fueled the field of molecular prognostication, theranostics and targeted therapy in bladder cancer. Several recent gene expression studies have highlighted differentially expressed genes that may play a role in predicting recurrence and progression in URCa. Table 1 summarizes the established clinicopathologic and potential molecular prognostic parameters in superficial and muscle invasive urothelial carcinoma of bladder. A series of recent studies have pointed to the potential prognostic value of receptor tyrosine kinase (RTK) markers such as FGFR-3, EGFR and other ERB family members (ERBB2/HER2 and ERBB3) in superficial and invasive bladder cancer disease. Early studies by Sarkis et al. revealed p53 alterations to be a strong independent predictor of disease progression in URCa (superficial, muscle invasive as well as CIS). P53 has also been shown to be predictive of increased sensitivity to chemotherapeutic agents that damage DNA. More recent studies have demonstrated a synergistic role for combining p53 evaluation with other cell cycle control elements such as pRb, p21 and p27, demonstrating the superiority of multi-markers approach.
compared to prior single marker approach for prognostication \cite{31-33,36}. In a recent study by Shariat et al. \cite{22}, superficial URCa patients with TURB demonstrating synchronous immunohistochemical alterations in all four tested p53, p21, pRb and p27 markers were at significantly lower likelihood of sustained disease free survival (DFS) compared to patients with only three markers altered. In turn, those with three altered markers did worse than patients with only two altered markers which in turn had a lower DFS than those with only one alteration as shown in figure 2.

**Figure 2: Synergistic prognostic role of immunohistochemical analysis of four markers (p53,p21,pRb and p27) in superficial URCa. adapted from Shariat et al. \cite{22}.

A similar synergistic prognostic role of multiple molecular markers (p53, pRb and p21) was demonstrated by Chatterjee et al. \cite{20} in patients undergoing cystectomy for invasive URCa using IHC technique. Such multimarker approach of prognostication could soon result in a new standard of care in URCa management once additional multi-institutional, preferably
prospective, trials confirm the above findings. An encouraging such development is the recent report of the bladder consortium multi-institutional trial confirming the role of proliferation index (as measured by Ki67 on IHC) as a prognosticator in URCa\textsuperscript{37}.

The current clinicopathologic based prognostic approach to predicting progression in superficial URCa\textsuperscript{29, 38} is soon to be supplemented by a molecular guided approach based on markers among those listed in table 1.\textsuperscript{5, 39-42, 6, 20, 43-49}
<table>
<thead>
<tr>
<th>Clinicopathologic Prognostic Parameters</th>
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<tbody>
<tr>
<td><strong>Superficial urothelial carcinoma</strong></td>
<td><strong>Muscle invasive urothelial carcinoma</strong></td>
</tr>
<tr>
<td>WHO/ISUP Grade</td>
<td>pTNM</td>
</tr>
<tr>
<td>pT stage</td>
<td>LVI</td>
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<tr>
<td>Presence of associated CIS/Dysplasia</td>
<td>Resistance to neoadjuvant chemotherapy</td>
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<td>Disease duration</td>
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<td>Time to and frequency of recurrences</td>
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<tr>
<td>Multifocality</td>
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<td>Tumor Size (&gt;3 cm)</td>
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<tr>
<td>Failure of prior BCG Rx</td>
<td></td>
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<tr>
<td>Presence of LVI</td>
<td></td>
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<td>Depth of Lamina propria Invasion</td>
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*Emerging Potential Molecular Prognostic Parameters*

<table>
<thead>
<tr>
<th>Superficial URCa</th>
<th>Muscle Invasive URCa</th>
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<tr>
<td><strong>Divergent Histology:</strong></td>
<td></td>
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<tr>
<td>Micropapillary</td>
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<td>Osteoclast rich</td>
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<td>Undifferentiated/Giant cell</td>
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<tr>
<td>Plasmacytoid</td>
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<tr>
<td>Ki 67 proliferation index</td>
<td>Loss of E Cadherin</td>
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<tr>
<td>Ploidy Status</td>
<td>p53 inactivation</td>
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<tr>
<td>Loss of E Cadherin</td>
<td>Alteration of Rb expression</td>
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<tr>
<td>p53 inactivation</td>
<td>Loss of p21 expression</td>
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<tr>
<td>Loss of Rb expression</td>
<td>Alteration of p16 expression</td>
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<tr>
<td>Loss of p21 expression</td>
<td>EGFR overexpression</td>
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<tr>
<td>Loss of p27 expression</td>
<td>HER2 overexpression/amplification</td>
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<tr>
<td>VEGF mRNA overexpression</td>
<td>TSP1 overexpression</td>
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<tr>
<td>HIF 1A overexpression</td>
<td>mTOR</td>
</tr>
<tr>
<td>ERBB3, ERBB4 overexpression (protective)</td>
<td>Phos S6 expression (protective)</td>
</tr>
<tr>
<td>FGFR3 Mutation overexpression (protective)</td>
<td></td>
</tr>
<tr>
<td>TSP1 Overexpression</td>
<td>Epigenetic Alterations:</td>
</tr>
</tbody>
</table>

Epigenetic Alterations:
- RASSF1 promotor hypermethylation
- E Cad promotor hypermethylation
- EDNRB promotor hypermethylation

Table 1: Established clinicopathologic and potential molecular prognostic parameters in superficial and muscle invasive urothelial carcinoma of bladder.

Finally, from a targeted therapy perspective, RTK-HRAS-MAPK (superficial disease) and p53-pRb (muscle-invasive disease) pathogenic pathways as well as angiogenesis pathway of the tumor microenvironment offer tremendous new opportunities for future management of URCa. Phase II trials evaluating the role of tyrosine receptor kinase inhibitors (TKI) targeting EGFR with small molecules such as Gefitinib and Sorafinib or monoclonal antibodies (MoAb) such as Cetuximab are underway. Other Phase II trials are addressing the role of Herceptin (anti HER2 MoAb) and Bevacizumab (anti VEGF MoAb) in URCa. Phase I trials testing the safety of p53 or
Rb intravesical gene therapy are being evaluated. One strategy example is the intravesical introduction of a wild type p53 loaded replication deficient Adenovirus (Ad5CMV-TP53) in an ambitious attempt to compensate for the loss of p53 function in invasive URCa\textsuperscript{50-54}.

In summary, our recent understanding of the complex molecular alterations involved in the development and progression of urologic malignancies is yielding novel diagnostic and prognostic molecular tools and opening the doors for experimental targeted therapies in these prevalent, frequently lethal solid tumors.

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