IATROGENIC PATHOLOGY OF THE URINARY BLADDER

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Short Title: Iatrogenic pathology Urinary Bladder

Key Words: Bladder, flat lesions, urothelium, Chemotherapy, atypia, Immunotherapy, Radiation therapy.

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Conflicts of interest: none

This is the author's manuscript of the article published in final edited form as:

Lopez-Beltran, A., Montironi, R., Raspollini, M. R., Cheng, L., & Netto, G. J. (2018). Iatrogenic pathology of the urinary bladder. Seminars in Diagnostic Pathology. https://doi.org/10.1053/j.semdp.2018.03.001

ABSTRACT

Intravesical immunotherapy, chemotherapy, and neoadjuvant systemic chemotherapy are among the most frequent therapeutic procedures to treat malignancies of the urinary bladder. These treatment modalities produce reactive morphologic changes in the urothelium that can mimic urothelial carcinoma in situ, urothelial dysplasia or true invasive urothelial neoplasia. Mitomycin C used after transurethral resection of bladder tumor to reduce recurrences, BCG intravesical immunotherapy to treat high risk non-muscle invasive bladder cancer and urothelial carcinoma in situ, and platinum-based systemic chemotherapy to improve post-cystectomy disease-specific survival some of the causes of therapy related atypia in urinary bladder. In addition, a number of systemic drugs in use to treat other systemic diseases, such as cyclophosphamide used to treat certain auto-immune disorders or hematologic malignancies, or the anesthetics ketamine increasingly used as illegal recreational drug, may produce similarly relevant atypical changes in the urothelium, and therefore, need to be differentiated from intraepithelial neoplasia. Immunohistochemical approach to reactive urothelium from CIS using CK20, p53, and CD44 may also be of utility in the pos-therapy scenario.

INTRODUCTION

About 70% of newly diagnosed urothelial bladder cancer cases are classified as non-muscle-invasive (NMIBC) which includes Ta (non-invasive) and T1 (subepithelial connective tissue invasion) tumors and carcinoma in situ (CIS).[1] Urothelial carcinomas of the bladder are frequently treated by a combination of surgery, intravesical immunotherapy, chemotherapy or radiation-therapy using specific pathology-based protocols, mostly grade and stage.[2-86] For instance, intravesical Mitomycin C to reduce tumor's recurrences or intravesical BCG (Bacillus Calmette-Guerin) immunotherapy will follow the diagnosis of high grade non-muscle invasive bladder tumor in addition to transurethral resection of the bladder tumor. According to current guidelines, these patients receive frequent cystoscopies with mapping biopsies to evaluate therapeutic efficacy or to diagnose potential urothelial alterations.[2] The level of cellular atypia may simulate intraepithelial neoplasia, or may represent areas of true residual neoplastic disease.[18] The pathologist should keep in mind that pos-therapeutic diagnosis of residual and recurrent

bladder cancer is a great challenge. [2,12] Alternative therapeutic approaches, such as gene therapy or different forms of immunotherapy alone or in different combinations with chemotherapy have been recently adopted although remain as experimental procedures.[2-12]

Neoadjuvant systemic platinum-based chemotherapy is increasingly applied in most institutions before surgery in an attempt to improve cancer specific survival.[42-47] However, there is limited data on tissue and cellular changes related to this therapy, both in the tumor itself and the non-neoplastic urothelium. [2-20]

The aim of this paper is to review the morphologic urothelial changes induced by traditional and novel therapeutic procedures in use to treat bladder urothelial cancer and to provide morphologic and immunohistochemical clues that maybe useful in resolving difficult differential diagnoses that could be encountered in such settings. Changes induced by systemic chemotherapy in the urothelium are also presented. The following intravesical, local and systemic therapeutic procedures are reviewed:

- Chemotherapy
- Immunotherapy
- Radiation Therapy
- Surgery
- Photodynamic and laser therapy
- Gene therapy
- Other therapy related alterations

CHEMOTHERAPY

Chemotherapeutic agents frequently used either intravesically or systemically, may produce urothelial reactive atypical changes that occasionally might be mistaken for urothelial carcinoma in situ.[2-32]

INTRAVESICAL CHEMOTHERAPY

Intravesical therapy is applied in order to eradicate existing disease, to prevent tumor recurrences, and to prevent tumor progression.[6, 12-16] Nowadays, the main goal for intravesical chemotherapy is to limit the number of recurrences after TUR of bladder tumor, most frequently by bladder instillations of mitomycin C after resection. [6, 12-16]

Mitomycin C

Mitomycin C is currently the most frequent intravesical chemotherapeutic agent in use. It is an antitumor antibiotic which induces inter and intra-strand cross links in DNA molecules. It has been shown to degrade DNA and inhibit its synthesis, thus making it effective during the late G1 and S phases of the cell cycle. [6, 12-16] Table 1

Mitomycin C produces histologic and cytologic alterations in normal urothelium including cellular exfoliation, denudation, and atypical changes in the superficial umbrella cells.[2-16] Such cells become large, vacuolated and often multinucleated with small nucleoli. The cellular alterations are not specific and may also be caused by chronic irritation due to inflammation, catheterization, and instillation of saline solutions. These atypical cells can persist in cytologic specimens for a variable period after discontinuation of therapy. Less significant abnormalities may be seen in the deeper layers of the urothelium. Limited data suggest that immunohistochemical expression of p53, CD44, and CK20 in this setting is similar to what is seen in reactive urothelium. Furthermore, AMACR is frequently negative in reactive atypia in the post-therapy setting thus suggesting a role of AMACR in combination with p53, CD44 and CK20 in differentiating reactive atypia vs. CIS after therapy.[59-,61]

A hemorrhagic and necro-inflammatory process similar to chemical cystitis follows intravesical administration of mitomycin C. There is often a histiocytic response that extends deep into the bladder wall with isolated single and clustered macrophages. Mitomycin C may also initiate an eosinophilic cystitis. Fibrosis with scarring and bladder wall calcification has been documented in rare cases after long-term topical therapy.[2-16] Fig 1

In case of persistent papillary neoplasia, Mitomycin C suppresses tumor growth and limit progression, but does not eradicate cancer. It is thought to act as surface abrasive to destroy the tips of papillary fronds, resulting in stubby papillae lined by neoplastic cells or fully denuded papillae. Urothelial denudation makes recurrences difficult to detect cystoscopically and document histologically but urothelial dysplasia and carcinoma *in situ* have been found in von Brunn's nests in case of residual disease.[12-16]

Other topical agents

A range of other intravesical chemotherapeutic agents remain useful in selected patients. Thiotepa (triethylenethiophosphoramide), an alkylating agent, is the oldest of the intravesical chemotherapeutic agents still in use. Its mechanism of action involves the formation of covalent bonds between DNA /RNA and proteins which thus results in nucleic acid synthesis inhibition. Thiotepa also reduces cell adherence with a direct cytotoxic effect.[14] Thiotepa and Mitomycin C produce identical histologic and cytologic alterations in the normal urothelium and the bladder wall.[12-16]

Doxorubicin (Adriamycin), epirubicin, valrubicin, ethoglucid (epodyl), cisplatin and mitoxantrone are known to cause alterations in the bladder mucosa.[2-16] The frequency varies from agent to agent. For example, there is a 21 to 25% incidence of epirubicin and doxorubicin-induced cystitis, respectively. Cystitis ranging from 3-56% has been seen with ethoglucid. Gemcitabine, a pyrimidine analog with a broad spectrum of antitumor activity is gaining activity as second line intravesical chemotherapy agent.[2-16]

The full morphologic description of the changes in the urothelium and in the lesions being treated specific to these agents is not available, but limited data from our experience suggest pathologic alterations similar to the changes described for mitomycin C and Thiotepa.

Systemic chemotherapy

Several agents are systemically administered to treat different neoplastic and non-neoplastic disorders. Among these agents, cyclophosphamide is known to severely affect the

urothelium. Other agents as ketamine are increasingly used as illegal recreational drug may produce ketamine cystitis with severe changes in the urothelium with potential to mimic of urothelial CIS. [17-34] Neoadjuvant systemic platinum-based chemotherapy is increasingly applied before cystectomy in attempt to improve cancer specific survival. The pathologists is increasingly asked to evaluate the efficacy of such treatment and recognize the reactive lesions that could be associated . Table 2

Cyclophosphamide

Cyclophosphamide is an alkylating agent used to treat a variety of malignancies including lymphoproliferative disorders, as well as diseases such as systemic lupus erythematosus, rheumatoid arthritis, organ transplantation, or nephrotic syndrome.[17-34] Active metabolites, acrolein and phosphoramide concentrate in the urine and thus be in contact with the urothelium. The drug is a toxic to the urinary bladder that increases the risk for urinary bladder cancer for years after therapy.[17-34] Cyclophosphamide causes cell division arrest that results in large, bi-or-multinucleated cells often with bizarre nuclei resembling changes of radiation injury mimicking intraepithelial neoplasia. There is marked but variable cellular and nuclear enlargement. Nuclei are usually hyperchromatic, often eccentric with slightly irregular outlines. Nuclear pyknosis is a common late effect that results in loss of chromatin texture. Chromatin may be coarse but is usually evenly distributed. Nucleoli are single or double and are occasionally large and distorted with irregular and sharp edges. These architectural and cellular abnormalities of the urothelial cells may be mistaken for malignancy.[17-34]

Systemic cyclophosphamide therapy typically induces hemorrhagic cystitis.[17-34] Histology also include vascular ectasia, severe edema, and hemorrhage of the lamina propria, usually associated with necrosis of the epithelial lining and mucosal ulceration covered with fibrinopurulent exudate. In our experience, immunohistochemical expression of p53 and Ki67 is low, and CK20 is expressed in superficial cells of the urothelium, therefore supporting the

reactive nature of the pathologic changes. Systemic cyclophosphamide therapy may induce reactivation of polyomavirus infection, a change that mimics dysplasia or CIS. [17-34]

Late fibrosis of the lamina propria and the muscularis propria develops in about 25% of cases examined at autopsy.[30-34] Bladder wall calcification may also be seen.[28] Hemorrhagic cystitis may rarely occur also in busulfan treated patients. Busulfan is an alkylating antineoplastic drug in use to treat chronic myeloid leukemia. [17-34]

The evidence that cyclophosphamide increases bladder cancer risk is primary based on a series of case reports, but it seems that the risk increases with cumulative total dose of cyclophosphamide.[17-34] Bladder cancer occurs, most commonly, several years after treatment of lymphoproliferative or myeloproliferative disorders, mainly multiple myeloma, Hodgkin's disease and in patients receiving cyclophosphamide after organ transplantation.[17-34] Urothelial carcinoma is the most common form of cancer, although squamous cell carcinoma, adenocarcinoma, undifferentiated carcinoma, sarcomatoid carcinomas (carcinosarcoma) and even sarcomas have been observed.. [17-34]

Ketamine

Ketamine cystitis shares a number of histopathological features with interstitial cystitis (bladder pain syndrome) including urothelial ulceration, inflammation, and variable degree of bladder wall fibrosis at later stage.[9] However, the degrees of bladder wall inflammation and fibrosis are more severe in ketamine cystitis thus resulting in contracted bladder and upper urinary tract damage with hydronephrosis, vesicoureteral reflux, and recurrent urinary tract infection.

In these cases, the urothelium may be diffusely denuded and ulcerated with prominent granulation tissue, congested vessels, lymphocyte infiltration and variable number of eosinophils in the lamina propria. Ketamine related reactive urothelial changes may mimic carcinoma in situ.[10] Interestingly, most of these cases were negative

for cytokeratin 20 but had high p53 immunoreactivity and moderate to high levels of Ki67 reactivity, a fact to keep in mind in the differential with CIS..

Different mechanisms to develop ketamine cystitis have been proposed.[11] These include: (1) direct effect of ketamine or its metabolites on bladder wall interstitial tissues; (2) microvascular changes in the bladder and possibly kidney by ketamine and/or its metabolites; (3) indirect effect of ketamine by causing an autoimmune reaction against the urothelium and submucosa, due to circulating ketamine or urinary ketamine and its metabolites; and (4) bacterial infection

Neoadjuvant systemic chemotherapy

Neoadjuvant platinum-based chemotherapy is now part of the management of patient with high risk bladder cancer prior to cystectomy in cT2-T4cN0M0 tumors. Neoadjuvant chemotherapy may result in significant tumor response and thus necessitate careful macroscopic assessment for residual tumor.[43-47] At cystectomy patients that received neoadjuvant chemotherapy are often down staged to be pT0, a finding which is associated with improved survival.[43-47] Improved survival following neoadjuvant chemotherapy has also been studied for variant histologic types with similar results. [46] A "tumor regression grade" by comparing the tumor in the TURBT with residual tumor in the cystectomy following neoadjuvant chemotherapy has been developed. The grade was based on the amount of residual tumor with respect to the size of the TUR of bladder tumor site scar. [23] In this system, 3 grades were assigned: TRG1 – no identifiable residual tumor (complete response), TRG2 – residual tumor occupying < 50% of the area of fibrosis and TRG3 – residual tumor overgrowing or occupying ≥ 50% of the fibrotic area. The TRG correlated significantly with overall survival. Fig 2 Wang et al [24], more recently, compared cystectomy findings in patients with TUR of bladder tumor alone compared with TUR of bladder tumor with neoadjuvant chemotherapy and found significant histologic overlap between the two groups. The

only morphologic features that were significantly different between the two were the presence of hyalinization of the wall and fewer foreign body giant cells in the neoadjuvant chemotherapy patients. They also noted more frequent lymph node changes, in particular hyalinization in patients receiving neoadjuvant chemotherapy. Associated xanthoma may also be seen in samples of the bladder, mainly after neoadjuvant chemotherapy. [63] Rarely, we have seen lymphatic vessels dilatation of variable intensity in cystectomies from patients receiving neoadjuvant chemotherapy. Fig 3

Some patients treated by neoadjuvant systemic chemotherapy present residual areas of urothelial CIS or papillary NMIBC (cTa, cT1) with emerging data suggesting that they carry no prognostic differences when compared with pT0 cases. [47]

A limited number of studies had reported tissue biomarkers with the potential of being predictors of response to systemic neoadjuvant chemotherapy. Of particular interest is Bcl-2 overexpression in chemotherapy-naive primary bladder cancer which is related to poor chemotherapy response and therefore, might assist to select likely non-responders to platinum-base chemotherapy. Also, the combination of GDPD3 and SPRED1 resulted as independent predictor of neoadjuvant chemotherapy response status in muscle-invasive bladder cancer. [48-49,51]

IMMUNOTHERAPY

Intravesical BCG

Intravesical immunotherapy is used to eradicate residual CIS after TUR and prevent tumor recurrence. BCG offers high rates of durable complete response. [39-42] BCG intravesical instillations have been shown to activate macrophages, NK cells, B cells, and various T cells (CD4+, CD8+) both in vitro and in vivo. BCG treatment shows that BCG can stimulate the urinary expression of different interleukins (IL-1, 2, 4, 6, 8, 10 and 12), TNF-α, GMCSF, IP-10 and IFN-γ. In particular, IFN-γ seems to be a critical mediator of anti-mycobacterial infection.[8,52]

Antitumor efficacy of BCG appears to depend on a cell-mediated T helper cells immune response. BCG binds to bladder wall in areas showing a reduced protective glycosaminoglycan layer, such as those involved by CIS and other "injured" sites, thereby exerting a somewhat anti-tumor-specific adhesion.[52] Macrophages, dendritic cells, and even tumor cells internalize and process BCG, thus presenting it to other host cells which activates a Major Histocompatibility Complex class II linked cascade of a local immune response. Table 3

Pathologic changes associated with BCG therapy include acute and chronic inflammation surrounding non-caseating epithelioid granulomas. Typically, the superficial lamina propria contains round or ovoid small granulomas composed of epithelioid histiocytes and lymphocytes. Occasionally, the granulomas are poorly formed and one may see a few multinucleate giant epithelioid histiocytes admixed with lymphocytes. BCG therapy also produces a pattern of reactive atypia in urothelium associated with denudation, ulceration and the posterior healing. Fig 4 Acid-fast stains only rarely reveal the presence of mycobacteria. [2,8, 39-42] Immunohistochemical expression of p53, CD44, and CK20 is similar to what is seen in reactive urothelium; AMACR is frequently negative in post-therapeutic reactive atypia. [59-61]

Rare examples of severe local or systemic complications associated to BCG therapy have been described in patients, usually referred to as "BCG-itis", severe enough to deserve anti-tuberculous therapy. The role of the pathologists in this particular scenario is to diagnose the lesion by finding epithelioid granulomas that can be caseating.

Immunotherapy agents other than BCG

A number of immunotherapeutic agents have been investigated for the prophylaxis of superficial bladder cancer, including recombinant interferon-α.[4] Interferons are known to have antiviral and direct anti-proliferative activity, regulate differentiation, activate immune effector cells, inhibit angiogenesis, induce cytokine production, and enhance tumor associated antigen expression.[4,52]

Bladder cancer cells express high levels of interferon-α receptor with higher receptor densities in high grade carcinomas. Intravesical recombinant interferon-α increases the cytotoxic activity of T lymphocytes and NK cells by increasing infiltration of these cells into the bladder wall which improves immune cell activity for 3 to 6 months. [52] Therefore, urothelial carcinoma cells are susceptible to the attack from cytotoxic T lymphocytes which also directly inhibits proliferation of tumor cells.[4] Most of these treatments currently remain experimental.

Pathologic changes related to these agents are non-specific. They include edema of the lamina propria and perivascular collections of inflammatory cells, mainly lymphocytes, neutrophils and eosinophils.

Intravesical vaccinia virus has also been investigated as immunotherapy for bladder cancer.[5,8] The limited number of reported cases has shown significant mucosal and submucosal inflammatory infiltration, characterized by lymphocytes, eosinophils, plasma cells, and dendritic cells. Nuclear features that suggest viral effect may also be present in some cells.

Novel anti PD-L1 associated targeted immunotherapy is emerging in personalized therapy with bladder cancer showing important reduction on tumor burden. Data on the morphologic effect of anti-PDL1 on the treated urothelium is currently lackinge.[37,38]

RADIATION THERAPY

Twenty-percent of bladder cancer patients present with muscle invasive disease at time of diagnosis and are subsequently treated by radical cystectomy or Cystoprostatectomy with or without preoperative neoadjuvant chemotherapy.[2-12] Definitive radiation therapy has been used for muscle invasive bladder cancer with evidence supporting that patients can maintain a functional bladder and achieve durable local control without a compromise in survival. In the past few decades, radiation therapy has been used in patients who either refused or were not suitable for radical cystectomy, or in cases using bladder preservation protocols. Urothelial CIS and low grade disease are less responsive to radiation than high grade tumors. [2-12] Table 4

Significant alterations may be seen in the bladder wall as a result of radiation therapy applied to other pelvic diseases like prostate, gynecologic or rectal cancers. The mechanism by which such effect occur remains poorly understood, but most probably is the result of damage incurred to the blood supply. Fig 5

Pathologic analysis shows variable morphologic alterations in the bladder mucosa as result of radiation. Main changes include acute and chronic radiation cystitis with mucosal ulceration and denudation and late fibrosis with bladder contracture. [53-59] Radiation therapy results in cellular and nuclear enlargement with large nuclei, frequent multinucleation and vacuolization with low nuclear-cytoplasmic ratio. The morphology of these degenerative nuclear features is usually recognized by the practicing pathologist as radiotherapy related and degenerative in nature. A reactive, tumor-like epithelial proliferation with nodules of squamoid epithelium pushing into the lamina propria without evidence of true infiltrative growth, associated with hemorrhage, fibrin deposits, fibrinoid vascular changes and multinucleated stromal cells is frequently seen at time of histologic evaluation.[53-59] An extreme form of this tumor-like reactive lesion has been reported as pseudo-carcinomatous hyperplasia, a rare lesion initially believed to be radiation related, but that may also be seen in patients without previous history of radiation. [58,59] Pseudo-carcinomatous hyperplasia consists of proliferative, pseudo-infiltrative urothelial nests within the stroma. This late phase of radiation injury usually occurs months or years following ionizing radiation. A characteristic background of radiation therapy– related changes with dilated thrombosed vessels, edema, reactive-appearing endothelial and stromal cells, and hemorrhage, can provide clues to the diagnosis, but known previous history of radiation therapy is essential. [58-59] The main differential diagnoses of pseudo-carcinomatous hyperplasia include invasive urothelial carcinoma and the nested variant of urothelial carcinoma; morphologic features, such as the presence or absence of background therapy-related changes and the architecture and

cytologic atypia of the nests can help distinguish pseudo-carcinomatous hyperplasia and carcinoma.[58,59,62] No specific immunoprofile has been described to differentiate pseudo-carcinomatous hyperplasia and other radiation-therapy related atypia of the urothelium from carcinoma but some authors have suggested that TERT promoter mutation analysis might be used to differentiate pseudosarcomatous hyperplasia from its malignant mimickers (urothelial carcinoma including nested carcinoma).[54]

Available data suggests that immunohistochemical expression of p53, CD44, and CK20 is similar to what is seen in reactive urothelium. [59-61] Table 5. α-Methylacyl-CoA racemase, a tumor-associated marker that is expressed in a subset of high-grade urothelial carcinomas has been recently investigated as differential biomarker between reactive atypia and urothelial carcinoma in situ. [60,61] In one large study, AMACR was able to detect only 50% of CIS in a pos-therapy setting, but benign and reactive urothelium (with and without a history of therapy) showed negative AMACR staining in all the cases (100%). The use of AMACR might have some value in differentiating reactive atypia vs. CIS after radiation therapy. [60,61]

Tissue other than urothelium is hemorrhagic with deposits of fibrin and mesenchymal cells often large and multinucleated (giant cell cystitis). Extensive scarring of the bladder wall is common. An important long-term effect of radiation therapy is de novo radiation-induced urinary bladder cancer, commonly an urothelial or squamous cell carcinoma. Rare examples of sarcomatoid carcinoma (or carcinosarcoma) or true sarcoma of the urinary bladder have been reported. [52-59]

Brachytherapy, a form of local radiation therapy used for prostate cancer may rarely produce atypical changes in the urethra that might be misdiagnosed as malignancy. The morphology of this alteration is similar to what is seen in the bladder urothelium with cellular and nuclear enlargement and streaking nucleoli, but the nuclear-to-cytoplasmic ratio remains low. Some squamoid cellular appearance may also be present. Degenerative nuclear features are

less commonly seen at the stage in which diagnostic biopsy is performed. We have seen rare lesions with pseudo-papillary architecture in the urethra suggesting urothelial neoplasia, however the clinical and morphologic background assists in recognizing the benign nature of such radiation therapy related reactive lesion.

SURGERY

The urinary bladder may show a variety of surgery-related changes. These can be divided into three groups: pathological changes associated with transurethral resection of the bladder, suture granuloma and related lesions, and pathological changes associated with bladder augmentations and intestinal conduits.[75] Table 6

Pathological changes associated with TUR

Transurethral resection of the bladder wall is followed by an intense necro-inflammatory reaction followed by non-specific granulomatous reaction, but also by formation of necrotizing palisading granuloma resembling rheumatoid nodule (postsurgical necrobiotic granuloma) and foreign body-type granuloma eventually resulting in fibrous scarring with occasional dystrophic calcification and the presence of xanthoma cells at a later stage.[3]

A relevant tumor-like lesion with potential of being misdiagnosed as malignancy is the postoperative spindle cell nodule (PSCN), an uncommon lesion in the lower genitourinary tract that develops within 3 months of a surgical procedure. Fig 6. Most bladder cases follow transurethral resection. Macroscopically, PSCN appears a friable occasionally polypoid mass. Microscopically, it consists of intersecting fascicles of spindle cells which often display conspicuous numbers of mitotic figures, but none are atypical.[83,87-89] The proliferating cells have the cytologic characteristics of Myofibroblasts. PSCN belong to the spectrum of myofibroblastic proliferations that may be seen along the urinary tract. There is usually a delicate network of small blood vessels, mild to moderate edema, acute and chronic inflammation, small foci of hemorrhage and variable extent of myxoid change. Cellular atypia may be present, but the cells do not exhibit marked cytologic abnormalities. History of a recent operation is the major

diagnostic clue and points toward and exuberant reactive proliferation. [83,87-89] Outcome is invariably benign but lesions may rarely recur. PSCN may be mistaken as leiomyosarcoma, Kaposi's sarcoma or other sarcomas. PSCN displays cytokeratin immunoreactivity in about 80% of cases, whereas this is an uncommon finding in leiomyosarcoma of the bladder. By immunohistochemistry, the spindle cells react with vimentin, desmin and actin. In about 50% of cases the cells react with the antibodies to the anaplastic lymphoma kinase (ALK-1) which may support the diagnosis.[83,87-89] Fig 7

Suture granuloma

Suture granuloma may rarely produce a tumor-like mass in or adjacent to the bladder in response to silk sutures introduced at the time of herniorhaphy or other surgical procedure.[90-93] The process most frequently occurs in the bladder wall or perivesical tissues. Bladder neoplasm was the clinical impression in most of reported cases, and the patients presented with variable urinary symptoms, including hematuria, frequency, and dysuria. Microscopically, there is a predominantly histiocytic reaction to suture with foreign body-giant cells and varying degrees of fibrosis and chronic inflammation. [90-93]

Bladder augmentation and intestinal conduits

Orthotopic neo-bladder with ileal segments is the elective technique in the reconstruction of the urinary passages following radical cystectomy. [94-98] Pathological changes in the intestinal mucosa have practical importance because the histological modifications may alter the functional characteristics of the mucosa through metaplastic and reactive changes secondary to prolonged contact with urine. Rarely, the alteration may lead to secondary malignancies. Most patients had developed adenocarcinoma, high grade urothelial carcinoma, undifferentiated sarcoma or small cell undifferentiated carcinoma. [94-98] Fig 8 Examples of adenomatous polyp and Adenomatoid tumor of the ileal conduit are on record.[95,98]

PHOTODYNAMIC AND LASER THERAPY

Photodynamic therapy

Photodynamic therapy based on local or systemic administration of photosensitizers (hematoporphyrin derivatives) is a form of focal therapy applied to bladder cancer that can achieve a high initial complete response rate, especially in CIS treatment, but the observed generalized cutaneous photosensitivity that appears in some patients makes the procedure of limited applicability. [65-71]Severe local irradiative symptoms that may persist for months are frequent, and some patients may develop bladder contracture. When administered systemically, these photosensitizers accumulate in tumor and only to some extent if any in normal tissue. They are then activated by light leading to tumor necrosis while preserving non-neoplastic tissues. The photosensitizer also accumulates in the stroma and the vessel wall suggesting ischemia as an additional mechanism of action. Therapy response is typically noted one or two days after treatment.[65-71] Table 7

Pathology features show coagulation necrosis sometimes with hemorrhagic necrosis sharply demarcated from the non-neoplastic tissue. [65-71] Early morphologic changes observed include intravascular coagulation and adjacent tumor cell necrosis. Adjacent normal, non-neoplastic tissue may show moderate to severe edema but necrosis is rare. Other findings include rare spindle cell artifact of urothelial cells and dystrophic calcification. [65-71]

Laser therapy

The neodymiumYAG laser is most commonly. Flexible fibers can usually be inserted through standards cystoscopes, or through cystoscopic equipment modified for use with laser fibers. [72-74] Lasers are mostly in use to treat patients with recurrent low grade Ta tumors as tissue is no longer available for histological evaluation.[72-74] One advantage of the laser is that it allows for transmural coagulative necrosis without perforation and extravasation. The l necrotic area is sharply delimited from surrounding tissue. The endothelial cells in the tissue adjacent to cancer tissues may acquire atypical appearance, and the pathologists should avoid misdiagnosis these cells as residual cancer. [72-74]

GENE THERAPY

Gene therapy and tumor vaccine studies have been shown to be effective in animals by increasing the sensitivity of bladder cancer cells to chemotherapeutic agents.[84-86] This finding support the combined regimen of gene therapy with intravesical chemotherapy as a potential tool for bladder cancer treatment. However, currently this procedure is considered experimental. [5,84-86]

Table 8

Very few studies addressed the cytopathic effects of gene therapy in urothelial tumors. Variable degree of necrosis, more frequent in high grade tumors is the hallmark. Nuclear changes include loss of chromatin details and nucleoli in the earlier stages. In the late stages, the nuclei shrink, become pyknotic, and acquire a spindled morphology instead of being round to ovoid, and dead cells show dark, dense, pyknotic or comma-shaped nucleus with no nuclear detail. Bizarre hyperchromatic nuclei may be focally present.[5,84-86]

The urothelium is rarely affected by necrosis, but contains an intense chronic inflammatory infiltrate of mostly B lymphocytes. Some lymphocytic infiltration may be seen at the tumor/normal urothelium interface or inside the tumor itself. Macrophages are abundant within tumor with higher frequency in areas of necrosis. Injection sites with hemorrhagic foci and foreign body-type giant cell reaction may be identifiable. [5,84-86]

Data on the histological changes resulting from methods of gene therapy other than tumor suppressor gene therapy (e.g., pro-drug activation, immuno-modulatory, and anti-angiogenesis) are not available.

OTHER THERAPY RELATED ALTERATIONS

Chemical cystitis is nowadays a rarity. Most cases reported are related to intravesical instillations of ether introduced into the bladder to dissolve a catheter balloon that resists mechanical deflation. Formalin intravesical instillation has been used to control bleeding in cases of hemorrhagic cystitis but is no longer in use. Bonney blue may be instilled in the bladder during gynecologic surgery, but failure to dilute the concentrate

to 0.5% working solution produces a severe chemical cystitis. Turpentine ingestion, excreted through the kidney creates ether-type injury, including edema, hemorrhagic necrosis of the bladder wall and the urothelium, and neutrophilic infiltration.

IMMUNOHISTOCHEMISTRY IN ASSESSING THERAPY RELATED UROTHELIAL ATYPIA VS. UROTHELIAL CARCINOMA *IN SITU*

Distinction of reactive atypia from CIS may be particularly challenging in patients previously treated using BCG. A helpful tip is to know that when CIS recurs, the morphology and immunohistochemical profile remains similar to untreated CIS.[60,61,99]

Immunohistochemical features of urothelial CIS include aberrant CK20 with expression seen at different levels of the urothelium, overexpression of p53 with "intense" nuclear staining though all cellular layers and negative staining by CD44 (isolated basal cells may be occasionally positive with CD44 in CIS). By contrary, atypical reactive urothelium shows normal CK20 immunostaining limited to the superficial cell layers in urothelium. In contrast, variably full-thickness positive membranous CD44 staining is typical of reactive urothelium, whereas p53 nuclear accumulation is undetectable or weak in the basal and parabasal cells of reactive urothelium.[60,61] Occasionally, in the post-therapy setting we can see unexpected "paradoxical" markers' expression, mainly in cases of previous radiotherapy. Most relevant pitfalls seen in post-therapy urothelial atypia include occasional aberrant CK20 expression seen at different levels of the urothelium instead of being limited to superficial umbrella cells. If this is the case, a patchy staining is seen with a pattern like "islands" of positivity though all cell layers separated of "islands" completely negative or showing only umbrella cell positivity. CD44 immunostaining is more variable and ranges from completely negative (positive only in basal cells) to full urothelial layers positivity. p53 nuclear accumulation is also variable mainly due to differences in available antibodies. In some cases, one can see a pattern of staining in other cell layers in addition to basal and parabasal cells, when this is the case, typically most "positive-looking" cells are

weak and this should be considered as a negative staining pattern and therefore may support reactive urothelium in the appropriate clinical and morphology context. These limitations are relevant to daily practice of pathology. [60,61, 99]

There is limited data on utility of other markers in the setting of therapy related atypia of the urothelium. This includes UroVysion probe or Cyclin D3 by FISH analysis, and the immunohistochemical expression of p16, AMACR, HER2, or Lewis(y) antigen. All of them are claimed to be negative in reactive atypia and positive in CIS in variable proportions. Currently, it is recommended to consider these markers experimental pending validation studies.

CONCLUSIONS

Intravesical and systemic therapeutic agents, as well as other therapeutic procedures including mitomycin C, cyclophosphamide, BCG, platinum-based chemotherapy agents, radiotherapy, photodynamic and laser treatment, and gene therapy, produce a host of changes and alterations in the urothelium and bladder wall, some of them may mimic carcinoma. The diagnosis of these lesions relies on H&E based pathologic evaluation, and therefore, pathologists must be aware of the altered morphology secondary to therapy. Knowledge of previous history is of great relevance in clinical practice. Immunohistochemistry has a role as an adjunct to morphology, in differentiating reactive changes from CIS. A panel of p53, CK20 and CD44 is recommended to provide useful information but the pathologist needs to be aware of their limitations and potential pitfalls. A conservative approach with repeat cystoscopy and biopsy after the inflammation has resolved is suggested in equivocal cases.

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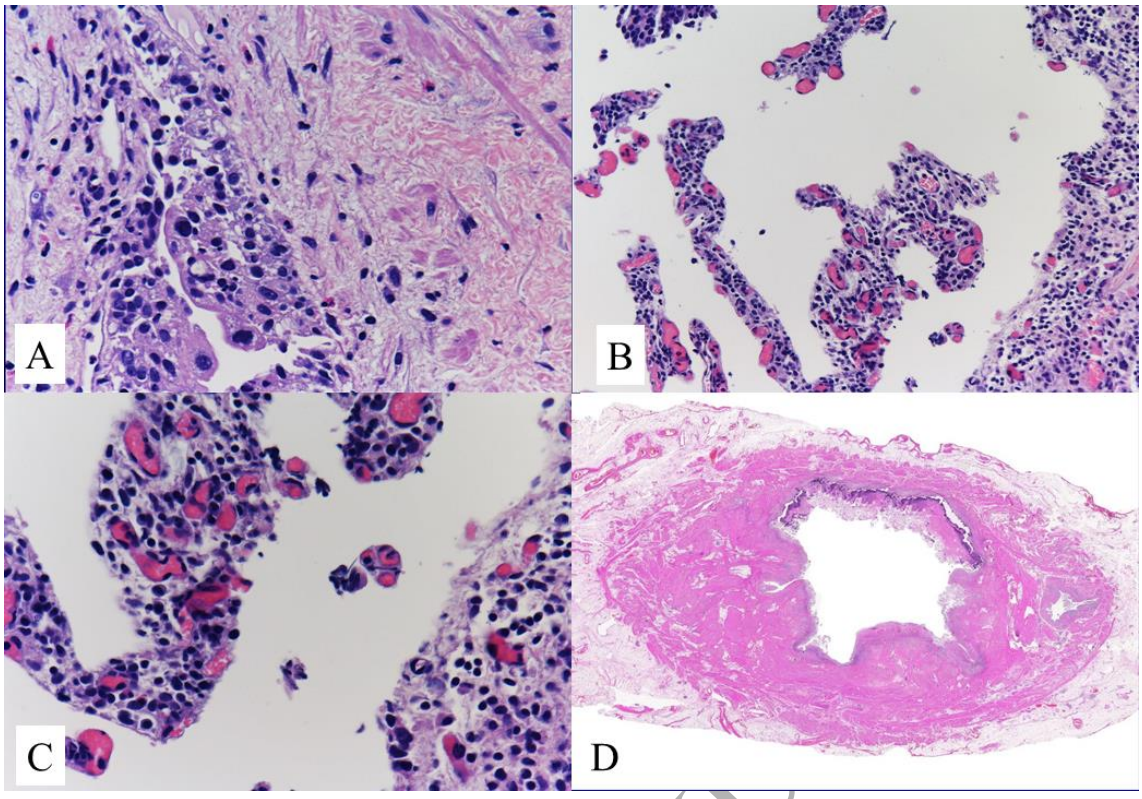

Fig 1. Mitomycin C therapy related changes. (A) Intraepithelial atypia. (B and C) Denuding papillae. (D) Encrusted cystitis.

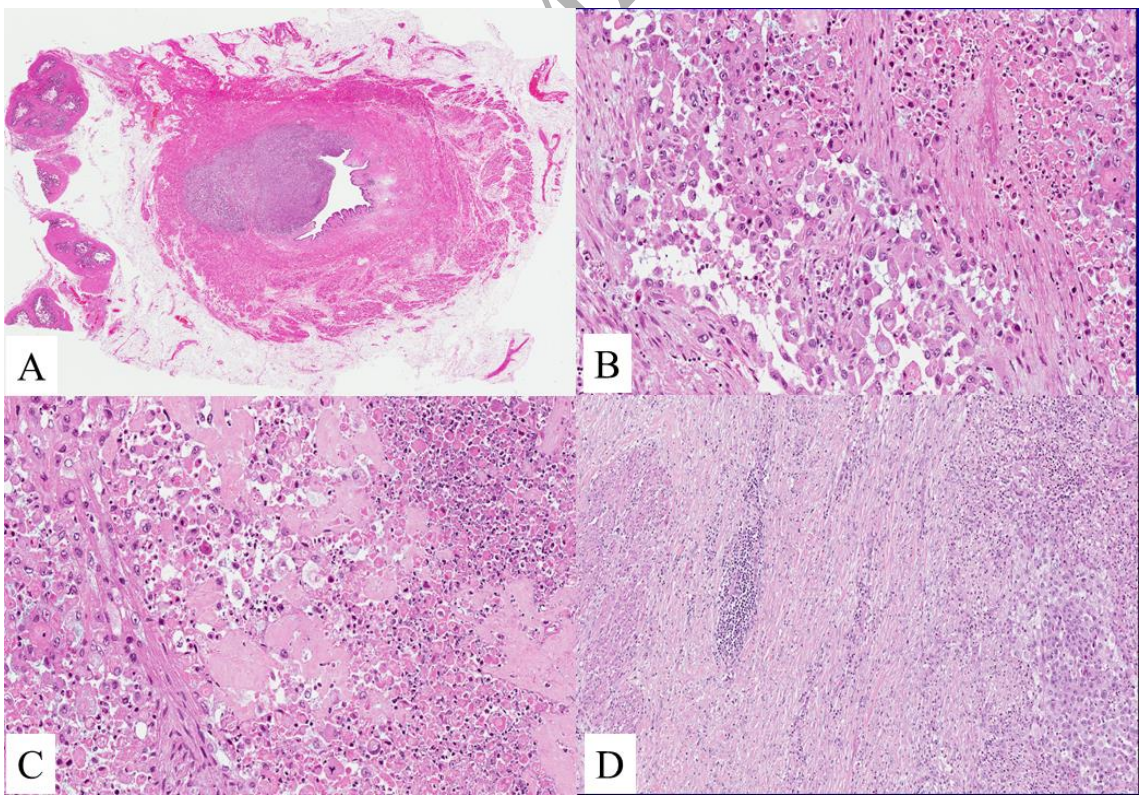

Fig 2. Neoadjuvant chemotherapy before cystectomy. (A) Tumor with low response. (B and C) Variable degree of tumor necrosis. (D) Minor residual tumor.

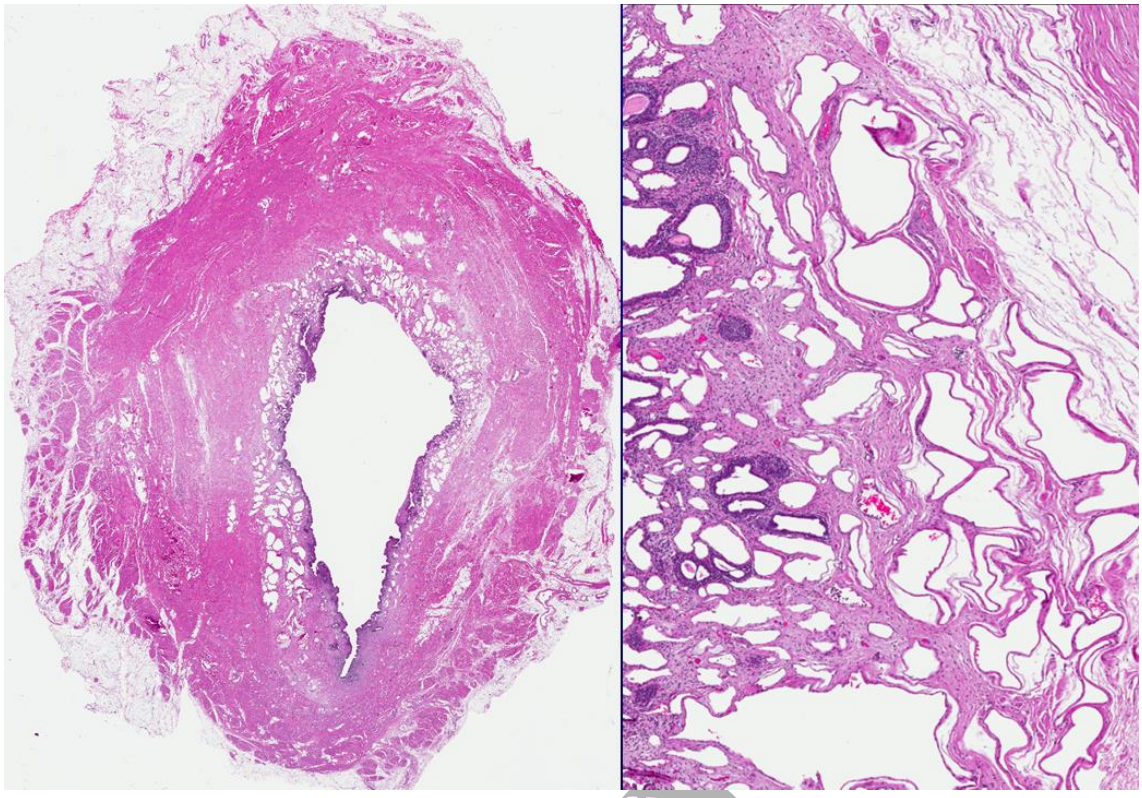

Fig 3. Cystectomy specimen. Rare cases show lymphatic dilatation (A) mostly located at the lamina propria/submucosa (B).

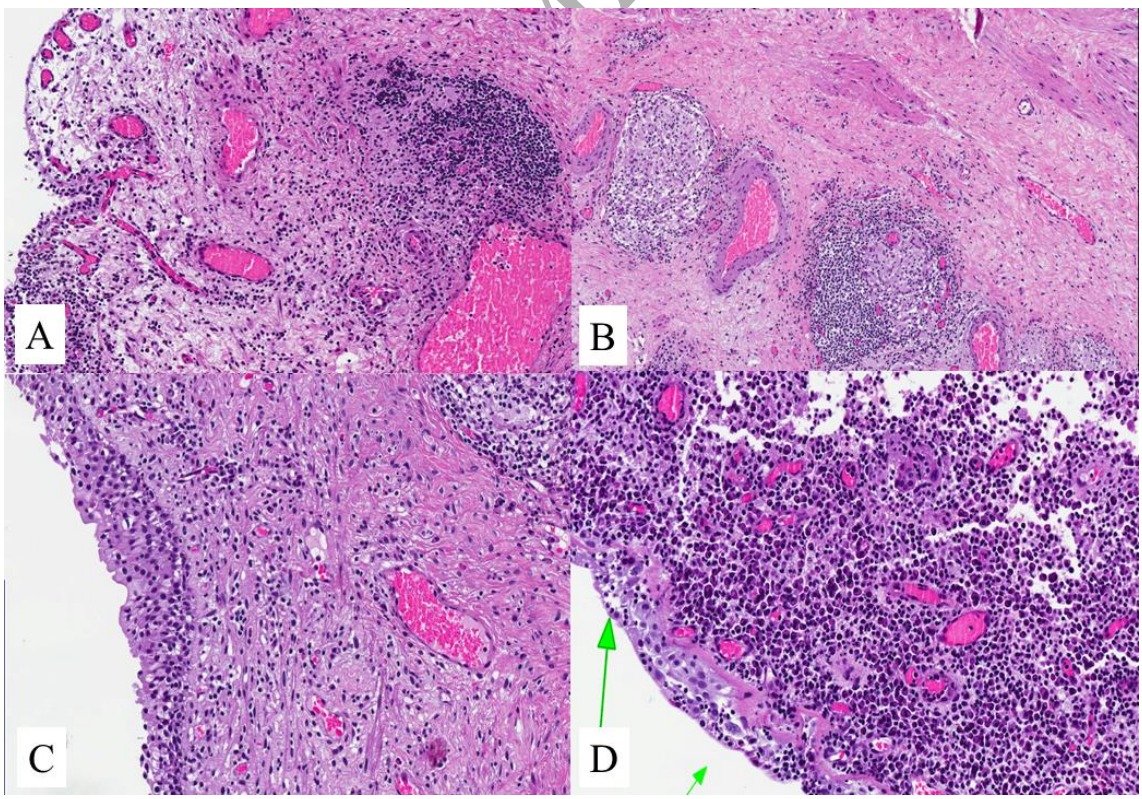

Fig 4. BCG related changes. (A) Partially denuded urothelium. (B) Focal epithelioid granulomas (B). Urothelial reactive atypia ranges from mild (C) to severe as pointed out by the arrows (D).

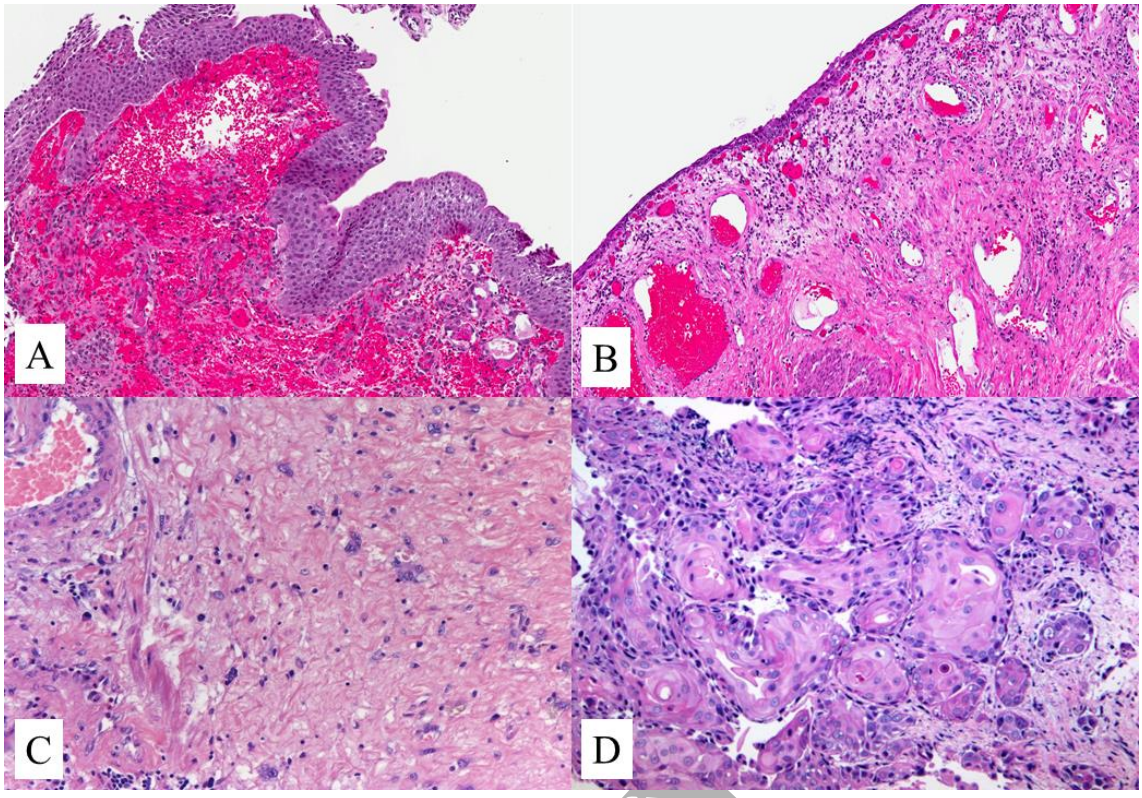

Fig 5. Radiation therapy related changes. (A) Acute radiation with hemorrhage. (B) Chronic radiation changes with fibrosis and vascular wall thickness. (C) Multinucleated fibroblasts seen in the lamina propria/submucosa after radiation therapy. (D) Pseudocarcinomatous hyperplasia seen in the urethra of a patient with previous brachytherapy.

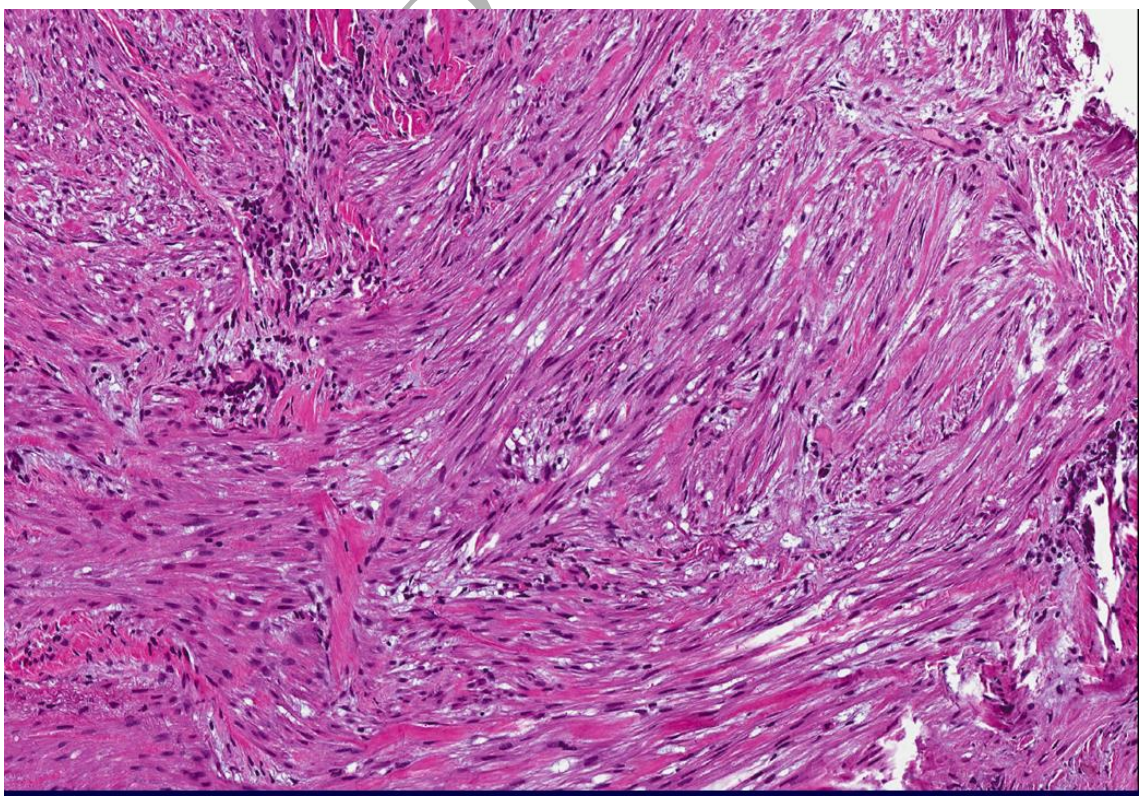

Fig 6. Postoperative spindle cell nodule 4 months after bladder TUR.

	PSCN	LMS	SFT	IMT
CD34	-	-	+	-
SMA	-	+	-	+
Desmin	-	+	-	+
Myogenin	-	-	-	-
ALK-1	-	-	-	+/-
CAM5.2	+/-	-/+	-	-/+
bcl2	-/+	-	+	-+

Fig 7. Immunohistochemistry is very helpful in differential diagnosis of spindle cell proliferations and tumors of the bladder.

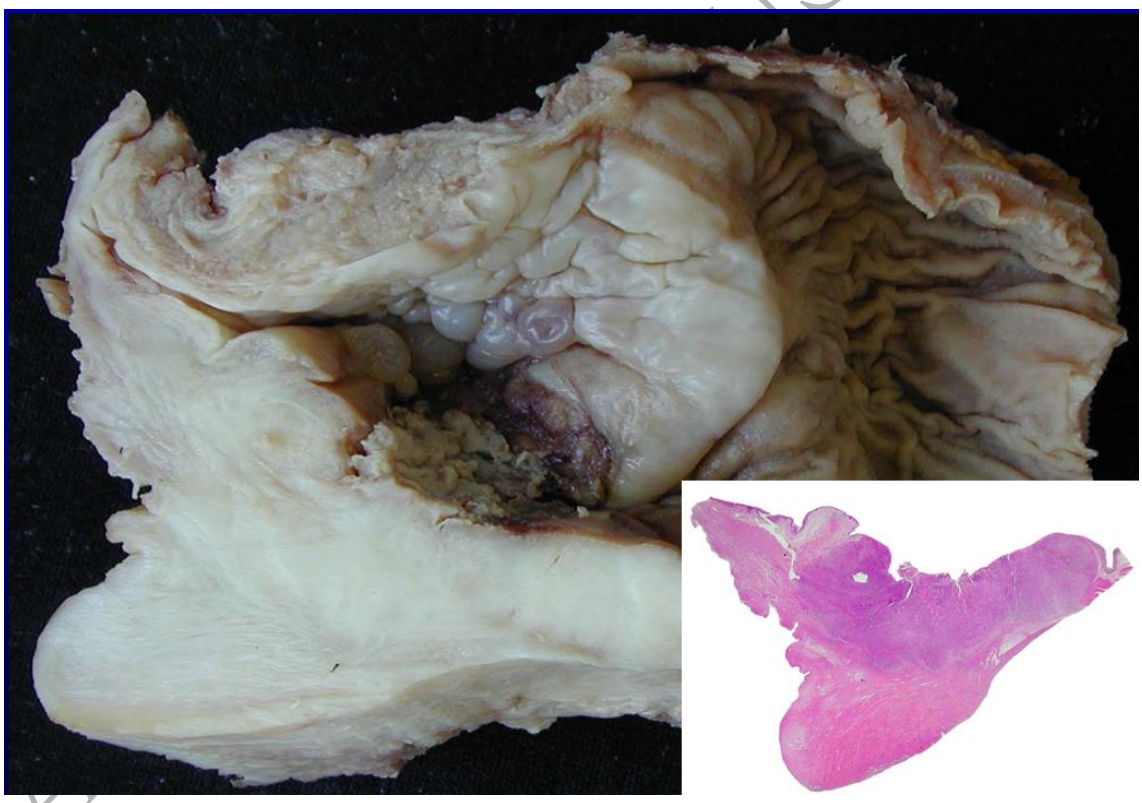

Fig 8. Recurrent urothelial carcinoma after bladder augmentation. Microscopic appearance (in inset).

Table 1. Pathologic features associated with mitomycin C intravesical chemotherapy

-Atypia in the surface umbrella urothelial cells

-Denudation of the surface epithelium

-Less significant abnormalities in the deeper layers of the urothelium

-Denuding papillae of persistent papillary neoplasia

-Associated eosinophilic cystitis (mild-to-moderate; common)

-Low nuclear/cytoplasmic ratio

-Hemorrhagic cystitis (rare)

-Encrusted cystitis (rare)

Table 2. Pathologic features in systemic chemotherapy

Cyclophosphamide

-Atypical form of regeneration

-Necrosis of urothelium of the bladder and upper urinary tract

-Large, bizarre nuclei with coarse chromatin and small to medium sized nucleoli

-Bladder cancer following cyclophosphamide therapy (uncommon)

-Reactivation of polyomavirus infection

-Encrusted cystitis (rare)

-Hemorrhagic cystitis (currently uncommon)

Ketamine

- Reactive urothelial changes that can mimic carcinoma in situ

-Urothelial ulceration (frequent)

-Urothelium diffusely denuded

-Lamina propria with prominent granulation tissue with congested vessels

-Inflammatory cell infiltration infiltrated predominantly by lymphocytes and a variable number of eosinophils

-Upper urinary tract damage and hydronephrosis

-Varying degrees of bladder wall fibrosis (late stage)

-High p53 and Ki67 (frequent).

-Normal CK20

Neoadjuvant chemotherapy

-Hyalinization of the wall

-Urothelium with minor atypia

-Frequent lymph node hyalinization

-Fewer foreign body giant cells

-Persistence of urothelial CIS or NMIBC (frequent)

-Xanthoma (occasional)

-Residual tumor grade: 1) No tumor; 2) Tumor <50%; 3) Tumor >50%.

Table 3. Pathologic features of intravesical immunotherapy (BCG, interferon-α)

-Bladder wall granulomatous inflammation

-Reparative urothelial atypia

-Eosinophilic cystitis (moderate-to-severe, occasional)

-Denudation of urothelium (denuding cystitis) with frequent ulceration

-Lamina propria edema (interferon-α)

-Persistence of carcinoma in situ in von Brunn's nests

-Mild perivascular inflammation with neutrophils, lymphocytes, dendritic reticulum cells, and eosinophils (interferon-α)

Table 4. Radiation induced pathologic features

-Mucosal ulceration

-Denudation of urothelium

-Acute inflammatory reaction in lamina propria with atypical stromal cells

-Overall increase in urothelial cell size with normal nuclear to cytoplasmic ratio

-Bladder wall fibrosis (late stage)

-Blood vessels thrombosis

-Xanthoma (rare)
-Intensified eosinophilic staining of cytoplasm (common)

-Pseudocarcinomatous hyperplasia (uncommon)

Table 5. Immunohistochemical features of reactive urothelium vs. urothelial carcinoma in situ

	Carcinoma in Situ	**Reactive Atypia**	**Normal**
CK20	***Aberrant expression through all cell layers.	Limited to umbrella cells	Limited to umbrella cells
p53	*Positive.	Absent	Absent
CD44	**Absent	Increased reactivity in all cell layers	Limited to basal cells

*Frequently trough all cell layers
** Basal positive cells may be seen, occasionally.
***May be full thickness

Table 6. Surgery related pathologic features

-Non-specific granulomatous reaction

-Postsurgical necrobiotic granuloma

-Xanthoma (rare)

-Postoperative spindle cell nodule

-Suture granuloma

-Fistula

-Recurrent cancer in bladder augmentations and intestinal conduits

Table 7. Pathologic features associated with photodynamic and laser therapy

-Normal tissues range from edema to coagulation necrosis

-Coagulation necrosis

-Hemorrhagic necrosis

-Well demarcated from non-irradiated tissues

-Dystrophic calcification (occasional)

-Vascular endothelium enlargement

-Spindling artifact of urothelial cells

Table 8. Pathologic changes associated to gene therapy

-Marked cytoplasmic vacuolization of tumor cells (early)

-Inflammatory infiltrate mainly of B-cells and macrophages

-Non-neoplastic tissues unaffected

-Apoptosis mediated tumor necrosis