Changes produced in the urothelium by traditional and newer therapeutic procedures for bladder cancer

A Lopez-Beltran, R J Luque, R Mazzucchelli, M Scarpelli, R Montironi

A handful of traditional and newer therapeutic procedures, such as chemotherapy, immunotherapy, radiotherapy, photodynamic and laser treatment, and gene therapy, are used to treat epithelial malignancies of bladder origin. These treatment modalities, used either intravesically or systemically, produce morphological changes in the urothelial mucosa that can be mistaken for carcinoma. The pathologist must be able to separate toxic and drug-related alterations from tumour-related changes. The clinical history is usually invaluable in this assessment.

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ladder cancer has proved to be a great challenge to pathologists and urologists. Bladder cancer is a paradigm of malignancy. Some cancers are of low malignancy potential, whereas others are highly aggressive, making early diagnosis and appropriate treatment crucial. In most cases, transurethral resection of the bladder (TURB) is the primary mode both of treatment and diagnosis. Persistent high grade tumours confined to the urothelial mucosa may require further treatment to prevent recurrence or possible progression. Further treatment is usually in the form of intravesical therapy or immunotherapy. Alternative therapeutic approaches, such as gene therapy, have been adopted in recent times. Although superficial bladder cancer is managed conservatively, muscle invasive bladder cancer is usually treated with radical cystectomy or radical radiotherapy. Systemic chemotherapy has been added to surgical and radiotherapy in an attempt to improve cure rates.

"There are few data on the morphological changes induced in the normal mucosa and the associated neoplasia in relation to the different therapeutic approaches."

A host of information is available on the morphological appearance of untreated bladder cancer, both in its pre-invasive and invasive phases. The literature dealing with the treatment of bladder cancer offers little information about the tissue and cellular changes related to the various types of treatment. Most studies document the efficacy of treatment on the basis of the endoscopic appearance of the bladder, not always confirmed by histology. There are few data on the morphological changes induced in the normal mucosa and the associated neoplasia in relation to the different therapeutic approaches. Here, we aim to review the morphological changes induced in the bladder by a series of traditional and innovative therapeutic procedures used to treat bladder cancer, including the alterations induced by cyclophosphamide, a systemic chemotherapeutic agent used to treat lesions other than bladder cancer. In particular, the following groups of topical and systemic therapeutic procedures together with the morphological changes induced by them are reviewed:

- Chemotherapy.
- Immunotherapy.
- Radiotherapy.
- Photodynamic and laser treatment.
- Gene therapy.

CHEMOTHERAPY

Several chemotherapeutic agents, used either intravesically or systemically, produce urothelial changes. Some of them can be mistaken for carcinoma.

Intravesical chemotherapy

The fundamental purpose of treatment with intravesical chemotherapy is threefold:

1. eradication of existing disease,
2. prevention of recurrence,
3. prevention of tumour progression.

Common indications for intravesical chemotherapy include multiple primary tumours, frequent tumour recurrence, stage T1 grade 3 tumours, post-resection positive urine cytology, and carcinoma in situ (CIS). Several intravesical chemotherapeutic agents are used.

Triethylenethiophosphoramide (thiotepa) and mitomycin C

Thiotepa, an alkylating agent, is the oldest of the intravesical chemotherapeutic agents still actively used today. Its mechanism of action involves the formation of covalent bonds between DNA, RNA, nucleic acids, and protein. The result is the inhibition of nucleic acid synthesis. In addition to this effect, thiotepa reduces cell adherence, with a direct cytotoxic effect.

Mitomycin C, an antitumour antibiotic, can induce interstrand and intrastrand crosslinks in many types of DNA, depending on the base composition of the DNA. It has been shown to

Abbreviations: BCG, Bacillus Calmette-Guérin; CIS, carcinoma in situ; IFN, interferon; IL, interleukin; TURB, transurethral resection of the bladder

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and carcinoma in situ have been found in von Brunn's nests. Denudation makes recurrences difficult to detect cystoscopically, as surface abrasives to destroy the tips of papillary fronds, progression, but they do not eradicate cancer. Apparently, they isolated cases after long term topical treatment (table 2).

Bladder wall calcification has been documented in association with necrosis of the epithelial lining and mucosal ulceration. Fibrosis of the lamina propria and the muscularis propria is present in 25% of cases examined at necropsy. Haemorrhagic cystitis can be caused by systemic cyclophosphamide treatment and it appears to be dose independent. The histological changes include vascular ectasia with severe oedema and haemorrhage of the lamina propria. Fibrosis of the lamina propria and the muscularis propria is present in 25% of cases examined at necropsy. Bladder wall calcification has been seen in occasional cases. The histological changes include vascular ectasia with severe oedema and haemorrhage of the lamina propria. Fibrosis of the lamina propria and the muscularis propria is present in 25% of cases examined at necropsy. Bladder wall calcification has been seen in occasional cases.
and in patients receiving cyclophosphamide after organ transplantation.\textsuperscript{643, 21} The risk of bladder cancer associated with cyclophosphamide is apparently increased in patients with a history of cystitis. Urothelial carcinoma is the most common form of cancer, although squamous cell carcinoma, adenocarcinoma, undifferentiated carcinoma, and even sarcomas have been observed. Sarcomatoid carcinomas (carcinosarcoma) have also been reported in a few patients after prolonged administration.\textsuperscript{23, 21}

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<th>Table 2</th>
<th>Pathological alterations associated with intravesical chemotherapy (thiotepa and mitomycin C)</th>
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<tbody>
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<td>- Denudation of the surface urothelium</td>
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<td>- “Atypical” changes in the superficial umbrella cells</td>
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<td>- Large cells with nuclear enlargement, multination, and small nuclei on cytological examination (see also table 8)</td>
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<td>- Eosinophilic cystitis (rare)</td>
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<td>- Haemorrhagic cystitis (rare)</td>
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<td>- Encrusted cystitis (rare)</td>
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<th>Table 3</th>
<th>Pathological alterations associated with systemic cyclophosphamide treatment</th>
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<tr>
<td>- Large, binucleated and multinucleated urothelial cells often with large bizarre nuclei resembling changes of radiation injury</td>
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<td>- Bladder cancer following cyclophosphamide treatment (uncommon)</td>
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Figure 1  (A) Early histological changes in the urothelium after mitomycin C treatment. The superficial umbrella cells are large, vacuolated, and binucleated (original magnification, ×400; haematoxylin and eosin stained). (B) The effect of treatment with thiotepa. The changes are more pronounced than in (A) (see also text) (original magnification, ×400; haematoxylin and eosin stained). (C) The effect of treatment with cyclophosphamide. Large cells with large bizarre nuclei are easily identifiable (original magnification, ×250; haematoxylin and eosin stained). (D) The effect of treatment with BCG. Residual urothelial carcinoma. Denudation of the urothelium is present (upper right corner) (original magnification, ×400; haematoxylin and eosin stained). (E) The effect of treatment with interferon α. Oedema of the lamina propria, or subepithelial connective tissue, and perivascular collections of inflammatory cells are present (original magnification, ×400; haematoxylin and eosin stained). (F) Acute radiation cystitis. Partial detachment of the urothelium from the lamina propria where a mild inflammatory infiltrate is recognisable (original magnification, ×250; haematoxylin and eosin stained). (G) Acute radiation cystitis. Oedema of the lamina propria with atypical looking endothelial and stromal cells (original magnification, ×250; haematoxylin and eosin stained). (H) Atypical looking stromal cells (“radiation fibroblasts”) similar to those seen in giant cell cystitis (original magnification, ×400; haematoxylin and eosin stained). (I) Coagulation necrosis after laser treatment (original magnification, ×100; haematoxylin and eosin stained). (J) Postoperative spindle cell nodule (original magnification, ×250; haematoxylin and eosin stained). (K) Acute radiation cystitis after topical mitomycin C treatment. Cluster of atypical looking cells with degenerative features (original magnification, ×400; Papanicolaou stain). (L) Urinary cytology after systemic cyclophosphamide treatment. Atypical looking cell with enlarged, eccentrically located, and extremely hyperchromatic nucleus (original magnification, ×400; Papanicolaou stain). (N) Urinary cytology after systemic cyclophosphamide treatment. Atypical looking cell in a patient with reactivation of polyomavirus infection (original magnification, ×400; Papanicolaou stain). (O) Urinary bladder cytology after external beam radiation. Degenerated urothelial cells and extensive background debris with histiocytes (original magnification, ×250; Papanicolaou stain). (P) Atypical epithelial cells in the urinary cytology after BCG treatment in a patient with urothelial carcinoma in situ (original magnification, ×400; Papanicolaou stain).
In patients with muscle invasive bladder cancer, systemic (neoadjuvant) chemotherapy, in which cyclophosphamide may be given in combination with other agents, has been added to locoregional treatment in an attempt to downstage the primary tumour and reduce micrometastases and, in some instances, as a radiosensitiser. The morphological changes are basically characterised by tumour cell necrosis. A similar effect can be seen following peri-operative chemotherapy.

Immunotherapy

Intravesical BCG

Ideally, intravesical treatment should eradicate residual disease and prevent tumour recurrence, thus ultimately averting the serious consequences of muscle invasion and metastasis. The immunotherapeutic agent Bacillus Calmette-Guérin (BCG) offers high rates of early and durable complete response. However, although several studies have demonstrated a decrease in disease progression, there are no long term studies that provide conclusive evidence of a survival advantage to BCG. BCG is a pleiotropic immune stimulator oriented toward cellular immunity. In particular, BCG has been shown to activate macrophages, natural killer cells, B cells, and various T cells (CD4+, CD8+, and γδ T cells) in vitro and in vivo. The analysis of cytokine production from human urine during BCG treatment has shown that BCG can stimulate the expression of interleukins (IL-1, IL-2, IL-4, IL-6, IL-10, and IL-12), tumour necrosis factor α, granulocyte-macrophage colony stimulating factor, the antiangiogenic chemokine IP-10, and interferon γ (IFN-γ). Of these, IFN-γ appears to be a crucial mediator of the anti-mycobacterial infection response. Although the exact mechanism of BCG action in bladder cancer remains incompletely understood, BCG antitumour efficacy appears to depend on a cell mediated T-helper cell immune response.

The pathological changes associated with BCG treatment are similar to those seen in tuberculous cystitis. This includes acute and chronic inflammation surrounding non-caseating granulomas. BCG may also produce a pattern of reactive epithelial atypia in association with denudation and ulceration of the urothelium (table 4; fig 1D).

Immunotherapeutic agents other than BCG

Several immunotherapeutic agents other than BCG have been investigated for the prophylaxis of superficial bladder cancer, including recombinant IFN-α. IFNs are known to have antiviral and direct antiproliferative activity, and to inhibit angiogenesis, regulate differentiation, activate immune effector cells, induce cytokine production, and enhance tumour associated antigen expression. The precise role of recombinant IFN-α in the treatment of superficial bladder cancer is still under investigation.

Bladder cancer cells express large numbers of the IFN-α receptor, and greater receptor densities are found in high grade lesions. The indirect antitumour effects of IFNs are probably mediated via the stimulation of a cellular immune response. Intravesical recombinant IFN-α increases the cytokotoxic activity of T cells and natural killer cells by increasing the infiltration of these cells into the bladder wall, and this improved immune cell activity persists for three to six months. This increases the susceptibility of urothelial carcinoma cells to attack from cytokotic T cells and directly inhibits the proliferation of tumour cells.

The pathological changes associated with IFN based treatment are not specific and are characterised by oedema of the lamina propria and perivascular collections of inflammatory cells, mainly lymphocytes, neutrophils, and eosinophils. Intravesical vaccinia virus is currently under study as immunotherapy for bladder cancer. The limited number of cases has shown a significant mucosal and submucosal inflammatory infiltration, characterised by lymphocytes, eosinophils, plasma cells, and dendritic cells (table 4; fig 1E). The tumour cells show some nuclear features that suggest a viral effect.

Radiotherapy

Approximately 20–25% of patients present with muscle invasive bladder cancer, which is life threatening, and require radical treatment. Definitive radiotherapy has been used for muscle invasive bladder cancer since the early 1900s and there is evidence that patients can achieve durable local control and maintain a functional bladder. However, the standard approach to the management of bladder cancer not suitable for conservative measures is radical cystectomy. In the past few decades, radical radiotherapy has been used in patients who either refused or were not suitable for radical cystectomy. Therefore, there is a limited amount of information on the precise role that radiotherapy plays in the management of bladder cancer.

External beam radiotherapy is rarely appropriate for the treatment of superficial bladder cancer because it can cause considerable local morbidity while displaying limited efficacy. CIS is particularly resistant and low grade disease responds less well than higher grade disease. Some benefit may be derived for patients with stage T1 grade 3 tumours, especially if combined with aggressive TURB and chemotherapy. A high degree of local morbidity is seen in the bladder as a result of radiotherapy for other pelvic diseases. The mechanism by which these effects are accomplished is poorly understood, and it is possible that x rays may eradicate tumours by severely damaging their blood supply.

"A variety of radiotherapy induced abnormalities may be seen in the bladder mucosa"

A variety of abnormalities may be seen in the bladder mucosa. These include acute and chronic radiation cystitis, with mucosal ulceration and late bladder contracture. In particular, radiotherapy results in urothelial cell enlargement, multinucleation, and vacuolisation, although nuclear to cytoplasmic ratios remain low. Enlarged nuclei may have large nucleoli, but degenerative nuclear features are usually present. A reactive, tumour-like epithelial proliferation associated with

Table 4  Pathological alterations associated with intravesical immunotherapy (BCG, interferon α)

- Denudation and ulceration of urothelium
- Non-caseating granulomas
- Reactive epithelial atypia
- Degenerated urothelial cells and extensive background debris with histiocytes and rare multinucleated giant cells on cytological examination
- Lamina propria oedema (interferon α)
- Mild perivascular inflammation with lymphocytes, eosinophils, plasma cells, and dendritic cells (interferon α)
- Persistence of carcinoma in situ (in von Brunn’s nests)

Table 5  Radiation related pathological alterations

- Ulceration of urothelium
- Urothelial cell enlargement, multinucleation, and cytoplasmic vacuolisation, but nuclear to cytoplasmic ratios remain low
- Acute and/or chronic inflammation of the lamina propria
- Nodules of squamous epithelium (reactive)
- Giant cell cystitis
- Bladder wall fibrosis (late stage)
haemorrhage, fibrin deposits, fibroid vascular changes, and multinucleated stromal cells is seen in chronic cases. This late phase of radiation cystitis usually occurs months or years after ionising radiation. Nodules of squamous epithelium push into the lamina propria without evidence of true infiltrative growth. The adjacent tissue is haemorrhagic with deposits of fibrin and, deeper within the stroma, mesenchymal cells are often large and multinucleated (for example, giant cell cystitis). Extensive scarring of the bladder wall is common (table 5; fig 1F,G,H).

An important long term effect of radiotherapy is de novo radiation induced bladder cancer. In general, it is a urothelial carcinoma; occasionally it is a squamous cell neoplasm. Rare examples of sarcomatoid carcinoma (or carcinosarcoma) and sarcoma of the urinary bladder have been reported.

**Photodynamic and laser treatment**

**Photodynamic treatment**

Photodynamic treatment using haematoporphyrin derivatives is a form of treatment applied in bladder cancer. It can achieve a high initial complete response rate, especially against CIS, but generalised cutaneous photosensitivity remains limiting.

Moreover, severe local irritative symptoms persisting for months are not uncommon, in addition to occasional bladder contractures.

It is based on the systemic or local administration of photosensitisers. These substances accumulate in tumour tissue but not, or to some extent only, in normal tissue. When the photosensitiser is activated by light, it produces tumour necrosis, preserving normal structures. The response is noted one or two days after the treatment is applied. On histology, it is characterised by coagulation necrosis, sometimes with haemorrhagic necrosis clearly demarcated from the non-neoplastic tissue (table 6; fig 11). Adjacent non-neoplastic tissues may show morphological changes ranging from moderate to severe oedema, but necrosis is rare.

Other findings include spindle cell artifact of urothelial cells and dystrophic calcification.

The photosensitiser accumulates also in the stroma and in the vessel wall, suggesting tumour ischaemia as a possible mechanism of action. In fact, early morphological changes show intravascular coagulation and adjacent tumour cell necrosis.

**Laser treatment**

Laser treatment has been used to ablate bladder tumours. Lasers are usually reserved for patients with recurrent low grade tumours, because tissue is not usually available for histological evaluation. It is believed that the lack of biopsy tissue in such circumstances does not compromise patient care because these lesions are usually low grade Ta lesions. The neodymium:YAG laser has been most commonly used. Flexible fibres can usually be inserted through standard cystoscopes, or through cystoscopic equipment modified for use with laser fibres.

One advantage of the laser is that it allows for transmural coagulative necrosis without perforation and extravasation (Ross JS. Intravesicle chemotherapy associated atypia in urinary bladder surgical and cytopathology. Presented at the United States and Canadian Academy of Pathology meeting on genitourinary pathology, Washington DC, 1996). The boundary between the necrotic tissue and the surrounding tissue is sharp. The endothelial cells in the tissue adjacent to cancer may acquire an atypical looking appearance. The pathologists should avoid considering these cells as residual cancer.

**Gene therapy**

The discovery that many cancers develop in concert with the loss of function of specific genes, dubbed “tumour suppression genes”, suggests that the replacement of such genes should be therapeutically useful. Studies of bladder cancer have yielded several candidate genes for therapeutic replacement. Among these are the cell cycle related genes Rb, p53, p21/waf1, and p16.

Tumour suppressor gene therapy is well suited for intravesical administration. Gene correcting and tumour vaccination studies have been shown to be effective in animals, in particular by increasing the sensitivity of bladder cancer cells to chemotherapeutic agents. These findings suggest that the combined regimen of gene replacement and chemotherapy may become an efficient and powerful tool for the treatment of bladder cancer.

Very few morphological studies of cytopathological effects of gene therapy have been published. Various degrees of necrosis, more commonly seen in high grade lesions, are present in cancer foci. Nuclear changes include the loss of chromatin detail and nucleoli in the earlier stages. In the late stages, the nuclei shrink, become pyknotic, and acquire a spindled morphology, in contrast to the normal round/ovoid shape. The resulting nucleus, found in dead cells, is dark, dense, pyknotic, and comma shaped with no nuclear detail. Hyperchromatic bizarre nuclei are occasionally seen.

“The combined regimen of gene replacement and chemotherapy may become an efficient and powerful tool for the treatment of bladder cancer”

The normal urothelial mucosa is rarely affected by necrosis, but contains an intense chronic inflammatory infiltrate composed predominantly of B cells. Some lymphocytic infiltration is present at the tumour–normal bladder interface or inside the tumour itself. Macrophages are abundant within tumour

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**Table 6** Pathological alterations associated with photodynamic and laser treatment

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<th>Alteration</th>
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<tr>
<td>Coagulation necrosis, sometimes with haemorrhagic necrosis, clearly demarcated from non-neoplastic tissue</td>
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<tr>
<td>Intravascular coagulation</td>
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<tr>
<td>Moderate to severe oedema of the normal urothelial mucosa.</td>
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<tr>
<td>Necrosis is rare</td>
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<tr>
<td>Spindle cell artifact of urothelial cells</td>
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<td>Dystrophic calcification</td>
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**Table 7** Pathological changes associated with gene therapy

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<th>Change</th>
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<tr>
<td>Various degrees of necrosis in cancer foci with loss of chromatin detail and nucleoli in the earlier phases following treatment</td>
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<tr>
<td>In the late phases, the nuclei shrink, become pyknotic, and acquire a spindled morphology</td>
</tr>
<tr>
<td>Normal urothelial mucosa is rarely affected by necrosis</td>
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<tr>
<td>Intense chronic inflammatory infiltrate composed predominantly of B cells in the lamina propria</td>
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**Table 8** Surgery related pathological lesions

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<th>Lesion</th>
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<tr>
<td>Non-specific granulomatous reaction</td>
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<td>Postoperative necrobiosis granuloma</td>
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<tr>
<td>Xanthogranuloma (rare)</td>
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<td>Postoperative spindle cell nodule</td>
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<td>Suture granuloma</td>
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<td>Development of malignancies in bladder augmentations and intestinal conduits</td>
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Take home messages

- The modalities used for the treatment of epithelial bladder cancer may produce morphological changes in the urothelial mucosa that could be mistaken for carcinoma so that the pathologist must be able to separate toxic and drug related alterations from tumour related changes.

- Intravesical chemotherapy with thiotape and mitomycin C induces cell exfoliation and mucosal denudation, and produces atypical changes in the superficial umbrella cells, which can mimic the changes seen with low grade urothelial tumours.

- Systemic chemotherapy with cyclophosphamide can also cause cytological changes that can be mistaken for malignancy. It can also cause haemorrhagic cystitis and the reactivation of polyomavirus infection, the effects of which can mimic carcinoma.

- Immunotherapy with BCG can induce pathological changes similar to those seen in tuberculous cystitis, including inflammation surrounding non-caseating granulomas, and treatment with interferon also produces inflammatory changes.

- Radiotherapy can induce acute and chronic radiation cystitis (including giant cell cystitis), with mucosal ulceration and late bladder contracture.

- Little is known about the changes induced by gene therapy, although necrosis of tumour tissue and inflammatory changes in the normal mucosa have been documented.

- The changes induced by surgery do not usually pose a diagnostic problem.

- Thus, pathologists must be aware that, following these types of treatment, the clinical usefulness of urinary cytology is reduced.

Data on the morphological changes resulting from methods of gene therapy other than tumour suppressor gene therapy (for example, pro-drug activation, immunomodulatory, and anti-angiogenesis) are not available.

Surgery

The 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 morphological changes associated with bladder augmentations and intestinal conduits (table 7; fig 11, K). Details were reported in a recent review. Usually these changes do not represent a diagnostic problem.

CONCLUSIONS

Topical and systemic therapeutic agents and treatment modalities, such as thiotape and mitomycin C, cyclophosphamide, BCG, radiotherapy, photodynamic and laser treatment, and gene therapy, produce a host of changes and alterations in the bladder, some of them mimicking cancer. Pathologists must be aware that, following these types of treatment, the clinical usefulness of urinary cytology is reduced (fig 1L–P).

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REFERENCES

4 Antonakopoulos GN, Hicks RM, Berry RJ. The subcellular basis of damage to the urinary bladder induced by irradiation. J Pathol 1984;143:103–16.
5 Bostwick DG, Mikuz G. Urothelial papilloma [exophytic] neoplasms. Virchows Arch [In press.]

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