Carcinoma in situ of the Urinary Bladder: Review of Clinicopathologic Characteristics with an Emphasis on Aspects Related to Molecular Diagnostic Techniques and Prognosis

Nalan Nese, MD; Ruta Gupta, MD; Matthew H. T. Bui, MD, PhD; and Mahul B. Amin, MD; Los Angeles, California

Key Words
Carcinoma in situ, urinary bladder, molecular mechanisms, prognostic factors

Abstract
Carcinoma in situ (CIS) of the urinary bladder is defined as a flat lesion comprising of cytologically malignant cells which may involve either full or partial thickness of the urothelium. De novo CIS constitutes less than 3% of all urothelial neoplasms; however, CIS detected concurrently or secondarily during follow-up of urothelial carcinoma constitutes 45% and 90%, respectively, of bladder cancer. CIS is noted predominantly in male smokers in the sixth or seventh decade. Patients may present with dysuria, nocturia, and urinary frequency and urgency with microscopic hematuria. Cystoscopic findings may range from unremarkable to erythema or edema. Urine cytology is an important diagnostic tool. Cellular anaplasia, loss of polarity, discohesion, nuclear enlargement, hyperchromasia, pleomorphism, and atypical mitoses are the histopathologic hallmarks of CIS. Extensive denudation of the urothelium, monomorphic appearance of the neoplastic cells, inflammatory atypia, radiation induced nuclear smudging, multinucleation, and pagetoid spread of CIS may cause diagnostic difficulties. Together with clinical and morphologic correlation, immunostaining with CK 20, p53 (full thickness), and CD44 (absence of staining) may help accurately diagnose CIS. Fluorescent in situ hybridization analysis of voided urine for amplification of chromosomes 3, 7, and 17 and deletion of 9p has high sensitivity and specificity for diagnosing CIS in surveillance cases. Several other molecular markers, such as NMP 22 and BTA, are under evaluation or used variably in clinical pathology. Intravesical bacillus Calmette-Guerin (BCG) instillation is considered the preferred treatment, with radical cystectomy being offered to refractory cases. Chemotherapy, interferon, and photodynamic therapy are other modalities that can be considered in BCG-refractory cases. Multifocality, involvement of prostatic urethra, and response to BCG
Carcinoma in situ of the urinary bladder is defined as a flat lesion of the urothelium characterized by presence of cytologically malignant cells involving either the entire thickness of the urothelium or a part of it.\(^1\)\(^,\)\(^2\) It has been documented to be a precursor of invasive cancer.\(^1\) Carcinoma in situ (CIS) is clinically considered an ominous lesion that is difficult to identify and survey because of its occult and multicentric nature.\(^1\)\(^,\)\(^2\) It is an important prognostic factor in patients with bladder carcinoma and has significant treatment implications.\(^5\)\(^,\)\(^6\) However, it is frequently underdiagnosed.\(^3\)

This article encompasses the salient clinicopathologic features of CIS while emphasizing the prognostic implications and recent advances in the understanding of CIS.

In 1952, Melicow\(^7\) and Melicow and Hollowell\(^8\) were the first to describe CIS in grossly normal vesical mucosa and to document its multifocal nature. Koss\(^9\) was the first to recognize the importance of urine cytology in diagnosing CIS in mucosa that could appear relatively unremarkable on cystoscopy. Subsequent mapping studies of cystectomy specimens and surveillance biopsies showed that apparently grossly unremarkable vesical mucosa often exhibited a spectrum of nuclear abnormalities culminating into CIS, invasive carcinoma, or both.\(^10\)\(^,\)\(^11\)

More recently, the focus has shifted to discovering distinct genetic changes that can accurately predict responsiveness to therapeutic modalities, thus decreasing the mortality and morbidity associated with CIS. Identification of mutations in p53, and occasionally deletions of 9p21 involving the tumor suppressor gene p16INK4a, has led to the development of a 2-pathway model.\(^12\)\(^,\)\(^13\) It is thus proposed that the pathway initiated by p53 mutation leads to CIS, with further loss of chromosome 9 leading to invasion. However, the pathway initiated through deletion of tumor suppressor genes on 9p and 9q leads to superficial papillary urothelial tumors.\(^14\)

Discovery of molecular markers, such as NMP-22, ImmunoCyt/uCyt, telomerase, Urovysion, and BLCA4, which may help in early diagnosis of CIS from urine samples, has generated additional clinical interest in molecular mechanisms of CIS.\(^15\)\(^,\)\(^16\)

### Epidemiology and Risk Factors

De novo CIS (primary CIS) constitutes less than 1% to 3% of all urothelial neoplasms. On the other hand, 45% to 65% of invasive urothelial carcinoma and 7% to 15% of papillary urothelial carcinoma are accompanied by CIS (concurrent CIS).\(^4\) CIS may also be detected during follow-up (secondary CIS) in 90% of cases with urothelial neoplasms.\(^17\)\(^,\)\(^18\) Tobacco smoking seems to be the most important risk factor in all countries, followed by analgesic abuse and occupational exposure to arylamine compound analogues, to risk factors for all urinary bladder malignancies. Long-term exposure to cyclophosphamide and pelvic irradiation may also act as predisposing factors.\(^1\)\(^,\)\(^9\)

### Genetic changes

Karyotypic analysis, comparative genomic hybridization, and other molecular data have identified several genetic changes that may predispose patients to CIS or progression of CIS to invasive urothelial carcinoma. The most important are deletion/mutation of p53 gene, also known as ‘guardian of the genome,’ located on 17p13.1.\(^10\)\(^,\)\(^21\) The other changes include loss of...
tumor suppressor genes, such as cyclin-dependent kinase inhibitor (CDKN2/p16), and deletion of 9q.\textsuperscript{20,22} Figure 1 shows the impact of these molecular changes on development and progression of CIS.

p53 is essential for cell-cycle arrest to allow for DNA repair after environmental damages.\textsuperscript{21} It acts as a molecular policeman by causing cell apoptosis with genetic aberrations. Mutations in p53 play a pivotal role in the initiation of CIS, because it allows survival of genetically abnormal cells and induces genetic instability.\textsuperscript{21}

Deletions in tumor suppressor genes such as p16INK4 and RB are responsible for unregulated clonal proliferation of these genetically abnormal cells and for absence of senescence in this population. Accumulation of other genetic molecular alterations secondary to loss of p53 can allow the neoplastic cells to overcome immunity, induce angiogenesis, and lead to invasive urothelial carcinoma (Figure 1).\textsuperscript{20–22} Targeted therapy directed at any of the steps in this model offers the promise to improve the outcome.

Clinical Features
CIS generally occurs in the sixth or seventh decade of life, and is 3 times more common in men than women. Patients may be asymptomatic, but irritative voiding symptoms, including frequency, urgency, and dysuria, are particularly associated with CIS in the absence of infection. Hematuria, if present, is typically microscopic.\textsuperscript{22}

On standard white light cystoscopy, areas with CIS may appear normal or erythematous and granular. Zaak et al.\textsuperscript{23} observed that standard white light cystoscopy may miss nearly 53% of CIS cases. Thus, multiple cold cup biopsies from vesical mucosa that may seem relatively normal are recommended in the presence of persistent unexplained bladder symptoms. Several authors have also shown the benefit of using 1414 5-ALA–induced fluorescence endoscopy based on the principal of preferential accumulation of photosensitive porphyrins in neoplastic tissue or hexaminoacetyl porphyrin fluorescence cystoscopy to detect CIS.\textsuperscript{23,24} These methods may improve the sensitivity for cystoscopic sampling of CIS up to 95% to 68%.\textsuperscript{24}

Pathology

Gross Features
CIS is almost completely a microscopic diagnosis as the appearance of the vesical mucosa may exhibit a range of nonspecific macroscopic features, such as unremarkable, erythema, edema, or erosion.

Cytology
Cytology is a useful diagnostic tool and has a high sensitivity (approaching 77%) for high-grade neoplasms of the urinary bladder, such as CIS, in which the cells are discohesive.\textsuperscript{25} The presence of severely atypical epithelial cells in urine in conjunction with normal cystoscopy is suggestive of CIS.\textsuperscript{26} Reactive atypia may prove to be a challenging differential diagnosis on cytology.\textsuperscript{26}

Histopathologic Features
Unequivocal severe cytologic atypia analogous to that expected in any high-grade malignancy is essential for diagnosing CIS.\textsuperscript{1–3,17,28} Notably, these changes may involve either the full or partial thickness of the urothelium, and the umbrella cells may be present occasionally.\textsuperscript{13} The urothelium may be hyperplastic or denuded secondary to the discohesive nature of the neoplastic cells or artifactually because of hot wire loop biopsy techniques, if these are
Loss of polarity leading to nuclear overcrowding may be evident at low magnification. The cytoplasm may be eosinophilic or amphophilic, and the nuclear cytoplasmic (N/C) ratio may not always be altered. The nuclei are generally enlarged, pleomorphic, hyperchromatic, with irregular notched nuclear contours and have coarse chromatin and prominent nucleoli. Atypical mitoses may be present even in the more superficial layers. CIS can be multifocal and may show various patterns (Table 1; Fig. 2). Although awareness of these patterns is essential for accurate diagnosis, they do not carry any prognostic significance.

The underlying lamina propria is generally inflamed, edematous, and congested, which is responsible for the erythematous and edematous appearance seen on cystoscopy.

Although this article focuses on urothelial CIS, CIS changes can also be seen after squamous or glandular metaplasia, especially in countries where Schistosoma hematobium infestation is endemic. Mal

### Diagnostic Approach to Urinary Bladder Biopsies and Morphologic Pitfalls

Cold cup random biopsies are obtained routinely for surveillance in patients with urothelial malignancies to detect flat lesions not evident on cystoscopy. Appropriate interpretation of these biopsies may be confounded by tangential sectioning, extensive urothelial denudation, inflammatory atypia, and treatment-related changes, such as radiation-induced multinucleation or cytoplasmic vacuolation, or truncation of papillae secondary to chemotherapy.

These pitfalls can be avoided through close attention to cellular cytoplasmic and nuclear details. Inflammatory and regenerative atypia can cause cellular enlargement and may closely resemble the large cell, nonpleomorphic pattern of CIS. However, the nuclei in reactive atypia show smooth regular nuclear contours with open chromatin and an occasional single large nucleolus in contrast to CIS (Figure 3A). Extensive urothelial denudation may be introduced through instrumentation or before chemotherapy, or may also be seen in clinging type of CIS. Deep sectioning of the tissue block or correlation with urine cytology is warranted to find diagnostic cells. Radiation induces cytoplasmic vacuolation, multinucleation, and smudging of nuclear details. Associated fibroblastic changes and vasculopathy may also be present. Intravesical chemotherapy or other surface ablative agents may lead to nuclear abnormalities in only the superficial cells or cause truncation of residual papillary carcinoma.

Microinvasion is another important diagnostic consideration with prognostic implications (Figure 3B). Zincke et al. reported that nearly 34% of the cystectomy specimens with CIS showed microinvasion when a depth of 5 mm was considered as the cutoff for microinvasion. Later, the depth criterion was modified to 2 mm; however microinvasion may still occur in fewer than 1% to 20% of cases with CIS. Superimposed inflammation or failure to notice single-cell invasion may lead to underdiagnosis of CIS. The urologist may also not suspect an invasive malignancy in the presence of nonpapillary mucosa.

### Table 1 Histologic Patterns of Urothelial Carcinoma in Situ

<table>
<thead>
<tr>
<th>Pattern</th>
<th>Description</th>
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<tbody>
<tr>
<td>Large-cell pleomorphic CIS</td>
<td>Easily recognized because of loss of polarity, nucleomegaly, and nuclear pleomorphism; nuclear/cellular ratio is unaltered because of abundant cytoplasm</td>
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<tr>
<td>Large-cell nonpleomorphic CIS</td>
<td>Often mimics reactive atypia; although nuclei are enlarged and hyperchromatic, the cells are monomorphic with abundant cytoplasm</td>
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<tr>
<td>Small-cell CIS</td>
<td>Enlarged nuclei with chromatin abnormalities; cells appear small because of scant cytoplasm</td>
</tr>
<tr>
<td>Clinging CIS</td>
<td>Denuded urothelium with patchy to single layer of cytologically malignant cells</td>
</tr>
<tr>
<td>Cancerization of the urothelium</td>
<td>Presence of atypical urothelium within otherwise normal-appearing urothelium: pagetoid spread or undermining/overriding</td>
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Abbreviation: CIS, carcinoma in situ.
A diametrically opposite problem of overdiagnosis of invasion is encountered when CIS involves von Brunn's nests (Figure 3C). The smooth rounded contours of von Brunn's nests in the lamina propria may mimic invasion, and the assessment may be further confounded when the usual smooth contours are obscured or distorted by inflammation.

Ancillary Techniques in the Diagnosis of CIS

Immunohistochemistry
Immunohistochemistry can play an adjunctive role to morphologic diagnosis of flat urothelial lesions while establishing a diagnosis of de novo CIS or when unusual morphologic features are present. A panel comprising cytokeratin 20 (CK 20), CD44, and p53 is useful (Figure 4). In a normal urothelium, CK 20 stains only the superficial umbrella cell layer and CD44 stains only the basal and parabasal cell layer whereas nuclear staining for p53 is absent in normal urothelium or has spotty/weak staining in the basal cells. In contrast, full thickness membranous staining of CK 20 and nuclear staining of p53 is observed in cases with CIS, whereas immunostaining for CD44 is absent. Immunostaining patterns in reactive atypia generally parallel those of normal urothelium or may show some amount of p53 immunostaining restricted to only the basal layer. All 3 immunostains in the panel must be used in conjunction with morphologic evaluation, because not all cases of CIS exhibit the pattern described.

Recently, Yin et al. and Mallofre et al. evaluated the Ki67 staining index and found it to be higher in CIS than in dysplasia or reactive atypia. However, the authors have found limited use of this immunostain in their practice. Other immunostains are being evaluated in CIS, such as E-cadherin and p16INK4, which is a negative cell-cycle regulator located on chromosome 9p21. Yin et al. showed strong cytoplasmic and nuclear staining of p16INK4 in CIS and minimal to absent staining in reactive or normal urothelium. Sun and Herrera and Shariat et al. showed efficacy of the adhesion molecule E-cadherin in distinguishing CIS from invasive carcinoma. Therefore, experts propose that loss of E-cadherin may have prognostic significance. The diagnostic efficacy of these immunostains in clinical practice needs further evaluation.

Fluorescent in situ Hybridization
Urothelial neoplasms most commonly show amplifications of chromosome 3, 7, 17, and deletion of 9p21.
which harbors the tumor suppressor gene p16\textsuperscript{INK4}, and can be studied in exfoliated cells found in voided urine. Multitarget, multicolored probes such as those manufactured by Vysis Urovysion can be used to detect numerical abnormalities in chromosomes 3, 7, 17, and 9 (Figure 5). Urine specimens from patients with urothelial carcinoma show either 4 or more cells with chromosomal amplifications or 12 or more cells with deletion of 9p.\textsuperscript{42} Alvarez and Lokeshwar\textsuperscript{42} showed that fluorescent in situ hybridization (FISH) had a sensitivity of 68% to 81% and specificity of 79% to 96% for detecting urothelial neoplasms.

FISH testing has also identified a population of anticipatory-positive patients among those undergoing urothelial carcinoma surveillance. In a recent study by Yoder et al.,\textsuperscript{49} 27% of patients undergoing surveillance had a positive FISH result without cystoscopic or morphologic evidence of disease recurrence. They termed these patients anticipatory-positive, of whom 65% developed recurrence within 29 months.

FISH seems to play an important role in diagnosing cystoscopically silent patients with persistent unexplained urinary symptoms or those with cytologically equivocal results.\textsuperscript{44,45} FISH would also play a role in accurate diagnosis of patients in whom biopsy interpretation is confounded by treatment-related changes or reactive atypia.

Other markers being evaluated with enzyme-linked immunosorbent assay on voided urine samples include NMP 22 and bladder tumor antigen.\textsuperscript{44}

**Treatment**

The treatment modalities depend on the accompanying papillary or invasive urothelial carcinoma. In invasive carcinoma, the extent of invasion determines the management. In a CIS with concurrent noninvasive carcinoma, preferred treatment may vary, with intravesical instillation of bacillus Calmette-Guerin (BCG) the first choice with chemotherapy or cystectomy for refractory disease.

Intravesical BCG seems to be the preferred treatment in patients with de novo CIS. Various studies have reported response rates to BCG ranging from 68% to 83%.\textsuperscript{6,17,46} Although no BCG therapy protocol is optimal, 6 weekly instillations followed by 3 weekly maintenance doses at 3 and 6 months for 2 years is the preferred choice and leads to longer disease-free survival.\textsuperscript{5,47–49} Surveillance cystoscopy for disease recurrence is recommended at every 3 months for 2 years after initial diagnosis, followed by every 6 months for 2 to 3 years, and yearly thereafter. The American Urological Association recommends clinical follow-up, including an appropriate patient history, urinalysis, cystoscopy, and urine cytology.\textsuperscript{50} Herr et al.\textsuperscript{51} showed that 95% of patients who did not respond to BCG experienced progression in 5 years versus 19% in the responsive group. Thus, management of patients with CIS whose disease does not respond to BCG presents a therapeutic challenge.
Cystectomy with or without chemotherapy or radiotherapy is suggested for patients refractory to intravesical BCG therapy. Nearly 80% of patients who do not respond to intravesical BCG after 2 courses may develop progressive disease and thus are candidates for cystectomy. However, the optimal timing of cystectomy remains controversial. Chang and Cookson and Herr and Sogani also recommend early cystectomy before progression to muscle-invasive disease. They also suggest that cystectomy is beneficial in these patients because of the multifocal nature of the disease and it also allows for appropriate staging as microinvasion cannot always be completely ruled out on biopsy. Herr and Sogani also showed a survival advantage in patients in whom cystectomy was performed within 2 years of failure of BCG therapy; in contradistinction Neider et al. did not report similar findings.

Chemotherapy with mitomycin-C or doxorubicin may be offered to patients whose disease does not respond to BCG who decline cystectomy. Immunotherapy with α-interferon may also be used in patients’ refractory to BCG. It is generally used to reduce the dosage of the chemotherapeutic agent. Although it has limited usefulness, photodynamic therapy (PDT) has been shown to be effective, especially for patients refractory to BCG or chemotherapy and who refuse cystectomy. Photosensitizing agents, hexaminolevulinate and its more lipophilic ester 5-ALA, are usually administered intravesically.

**Prognostic Factors**

The biologic behavior of CIS is somewhat unpredictable, although the current literature considers it a precursor to invasive carcinoma; the aggregate evidence in the literature suggests that approximately 50% of patients will develop invasive carcinoma within 5 years. Its inscrutable natural history has led Weinstein et al. to refer to carcinoma in situ as carcinoma paradoxicum. Host-tumor interactions and tumor cell properties are among several factors determining the biologic potential. From a clinical perspective, extent of disease (focal, multifocal, or extensive), involvement of prostatic urethra, and response to therapy or time to recurrence are the principal determinants of clinical outcome.

Patients with primary (de novo) CIS are more likely to have no evidence of disease (62% vs. 45%) and are less likely to progress (28% vs. 59%) or die of disease (7% vs. 45%) compared with patients who have CIS with prior or concomitant papillary bladder neoplasia. Cheng et al. reported disease progression in 35% of the cases, of which 20% succumbed to urothelial carcinoma. Davis et al. estimated a 55% 10-year progression-free survival rate for patients with CIS treated with BCG.

Several molecular prognostic markers, such as p53, p21, p16INK4, and E-cadherin, are being evaluated in different studies. Gene expression profiling has identified that urothelial carcinoma with concurrent CIS has a different expression profile compared with urothelial carcinoma without concurrent CIS. However, clinical efficacy of these markers requires further validation in independent studies.

**Summary**

CIS may occur de novo, or concurrent with or secondary to other urothelial carcinomas, and may present several diagnostic and management challenges. Further studies are essential to discover, validate, and evaluate clinically reliable prognostic markers as currently multifocality, involvement of prostatic urethra, and response to therapy are the most important prognostic factors.

**References**


Carcinoma in situ of the Urinary Bladder


CIS of the Bladder

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1. Which of the following statements about the epidemiology and symptoms of carcinoma in situ (CIS) of the bladder is most accurate?
   A. Primary CIS constitutes 30% of all urothelial neoplasms
   B. CIS is detected during follow-up of 90% of urothelial neoplasms
   C. CIS occurs most commonly in the third and fourth decades of life
   D. CIS is more common in women than men

2. All of the following statements about the diagnosis of CIS of the bladder are accurate, except:
   A. Hematuria is usually microscopic
   B. On white light cystoscopy, CIS may appear as normal mucosa
   C. Urine cytology is not useful in the diagnosis of CIS
   D. Multiple cold-cup biopsies should be obtained during cystoscopy, even if the vesical mucosa appears normal

3. All of the following are common findings on pathology and immunohistochemistry evaluations of CIS of the bladder, except:
   A. Large, pleomorphic, hyperchromatic nuclei
   B. Full-thickness staining for CK 20
   C. Nuclear staining of p53
   D. Full-thickness staining for CD44

4. Which of the following is the preferred treatment for patients with de novo CIS of the bladder?
   A. Cystectomy
   B. Intravesical bacillus Calmette-Guerin (BCG) therapy
   C. Doxorubicin
   D. Alpha-interferon

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