Classification and grading of the non-invasive urothelial neoplasms: recent advances and controversies

R Montironi, A Lopez-Beltran, R Mazzucchelli, D G Bostwick

The classification and grading of the non-invasive, intraepithelial neoplasms of the urothelium are based on the morphological pattern of growth—that is, papillary or flat (and endophytic)—and on their degree of architectural and cytological abnormalities. Recent advances in the morphological, molecular, and quantitative evaluation of these lesions have contributed to the refinement of the current classification and grading schemes. However, some controversies on the precise criteria and terminology, especially when the papillary lesions are concerned, are still present.

Early clinical observations regarding the biology of the “at risk” field suggested that sites of urothelial preneoplastic changes could follow several distinct clinical courses (fig 1). It is possible that areas of dysplasia remain simply dysplastic. Alternatively, the urinary epithelium can progress either to superficial bladder neoplasm, characterised by recurrence but rare life threatening progression, or along the path towards invasion, with its well recognised risk of mortality. Evidence in support of these disparate pathways comes from the low progression rate of most superficial bladder tumours, coupled with that fact that many invasive neoplasms present as such initially.

From the morphological point of view, two basic pathways are identified on the basis of the pattern of growth of the intraepithelial lesions (papillary and flat), the behaviour of these lesions being related to the degree of architectural and cytological alteration of the urothelium. Several classification and grading schemes (including revisions and refinements) of urothelial non-invasive or intraepithelial lesions have been reported in the literature.

“The morphological criteria useful for classification and grading have been continuously refined and updated”

The grading of papillary urothelial carcinoma is based on the worst grade present. G1 carcinoma consists of a urothelium more than seven cell layers thick containing cells that display minimal to slight nuclear enlargement, normal or slightly distorted architecture, and rare or absent mitotic figures. In contrast, G2 carcinoma displays greater nuclear pleomorphism, coarsely clumped chromatin, and some disruption of the normal architecture. Malmstrom et al subdivided G2 into G2A and G2B in 1987. Morphologically, G1 and G2A are similar to urothelial flat dysplasia (see below). Grade 3 carcinoma displays the most extreme nuclear abnormalities, similar to those seen in carcinoma in situ (CIS). Cellular anaplasia, characteristic of grade 3 carcinoma, is defined as increased cellularity, nuclear crowding, disturbance of cellular polarity, absence of differentiation from the base to the mucosal surface, nuclear pleomorphism, irregularity in the size of the cells, variation in nuclear shape and chromatin pattern, increased number of mitotic figures, and the occasional presence of neoplastic giant cells.

Recent efforts to grade...
urothelial carcinoma using image analysis based on nuclear morphometry, silver staining nucleolar organiser regions, diagnostic decision support systems (Bayesian belief networks), and other markers have also been successful, but are not used routinely. This has led to a high degree of reproducibility and accuracy in the classification and grading of papillary neoplasms.

WHO/ISUP 1998 and WHO 1999 classifications

The WHO/International Society of Urological Pathology (ISUP) consensus classification of 1998 (WHO/ISUP 1998) distinguishes between papilloma, papillary urothelial neoplasm of low malignant potential (PUNLMP), and low and high grade carcinoma. The term “papillary urothelial neoplasm of low malignant potential” was introduced to replace WHO 1973 G1 carcinoma in recognition of the low probability of recurrence or progression of this neoplasm, especially after complete removal, and the preference not to label these patients with the term “cancer”. The WHO classification introduced in 1999 (WHO 1999) is almost identical to the WHO/ISUP classification, the difference being that the WHO 1999 scheme subdivides the low and high grade spectrum into three grades (grades I, II, and III).


The topic of the best contemporary classification of the papillary neoplasia was debated at the Ancona international consultation on the diagnosis of non invasive urothelial neoplasms (11–12 May 2001, Ancona, Italy). The discussion was basically around the WHO 1973 scheme versus the WHO/ISUP 1998 and WHO 1999 classifications. In particular, the following problems were considered:

- Are detailed morphological criteria available for the WHO 1973 scheme and for the WHO/ISUP 1998 and WHO 1999 classifications?
- Reproducibility: is reproducibility better with the WHO/ISUP 1998 and WHO 1999 classifications?
- G1 papillary carcinoma (1973 scheme): is this a misnomer for a lesion that does not have the morphological and clinical features of cancer? Should the term “papillary neoplasm with low malignancy potential” be used to avoid the term carcinoma?
- Translation from the WHO 1973 scheme to the WHO/ISUP 1998 and 1999 WHO classifications: is it feasible and easy?
- How much clinical and prognostic information is available in favour of the WHO 1973 scheme and how much in favour of the WHO/ISUP 1998 and WHO 1999 classifications?

A full consensus on which classification should be used by practising pathologists and followed by urologists and oncologists was not reached. Drs Bostwick and Mikuz represented the majority opinion—for example:

- The WHO 1973 classification for papillary urothelial neoplasms was still superior to all existing alternatives (including WHO/ISUP 1998 and WHO 1999), although some refinement of diagnostic criteria would be useful.
- Some pathologists may prefer to report additional, synonymous classifications in other schemes, but this is discouraged owing to variations and difficulties in translation.
The lack of rules for the distinction of G1 from G2 tumours, particularly, they affirmed that:

- Genetic changes that commonly occur in advanced stage papillary neoplasia are as follows:

  - **Recent advances**

    Recent reports on the classification and grading schemes of papillary neoplasia are as follows:
    
    - Reproducibility with the WHO/ISUP 1998 and WHO 1999 classifications is worse than with the WHO 1973 scheme.
    - The fibroblast growth factor receptor 3 (FGFR3) mutation is among the earliest events in urothelial carcinogenesis—this mutation is already present in a very high proportion of urothelial papillomas. A comparable proportion of mutations is found in papillary urothelial neoplasm of low malignancy potential of the urinary bladder.
    - Genetic changes that commonly occur in advanced stage bladder cancer are frequently found in papillary urothelial neoplasm of low malignancy potential of the urinary bladder.

    - Samaratunga et al. investigated the risk of progression for the WHO/ISUP 1998 and WHO 1973 classifications. They observed progression in 8% of patients with PUNLMP and in 11% of patients with G1 papillary carcinoma (WHO 1973). The same group claim an advantage of the WHO/ISUP 1998 system over the WHO 1973 system in that the WHO/ISUP 1998 classification recognised a larger proportion of cancers with a poor prognosis.

    - Oosterhuis et al. found that the prognostic value of the WHO/ISUP 1998 classification is limited, thus questioning the clinical role of this new system in comparison with conventional grading systems.

    - The WHO 1973 standard for classification and grading of bladder tumours is robust, clinically confirmed, widely used, time tested, and reasonably reproducible method for pathological reporting, and is recommended with minor modifications.

**INTRAEPITHELIAL FLAT LESIONS**

The most recently revised classification of flat (and endophytic) lesions was published by López-Beltrán et al. This included epithelial abnormalities (reactive urothelial atypia and flat urothelial hyperplasia), presumedpreneoplastic lesions and conditions (keratinising squamous and glandular metaplasia, and malignancy associated cellular changes), in addition to preneoplastic (dysplasia) and non- invasive (CIS) lesions. Each of these lesions was defined with strict morphological criteria to provide more accurate information to urologists in managing patients. Particular attention was paid to the definition and importance of dysplasia and CIS.

In general, the morphology of dysplasia shows cohesive cells with umbrella cells usually present and characterised by mild nuclear/nucleolar changes that focally include irregular nuclear crowding, and slight hyperchromasia. Anisonucleosis is usually present, and there may be an increased number of cell layers. Nucleoli may be prominent and mitotic figures, when present, are generally basally located. Most cellular abnormalities in dysplasia are restricted to the basal and intermediate layers. Nuclear and architectural features are considered most useful in distinguishing between reactive atypia and dysplasia. Cytokeratin 20 and high molecular weight cytokeratin immunoreactivity might be objective markers of dysplasia in selected cases. To use the term dysplasia without a qualifier is preferable to describe the morphological spectrum of dysplastic lesions of the urothelium.

The morphological diagnosis of CIS requires the presence of severe cytological atypia (nuclear anaplasia); full thickness change is not essential, although it is usually present. Interobserver agreement with CIS is high. The cells of CIS may form a layer that is only one cell thick, of normal thickness (up to seven cells), or the thickness of hyperplasia (greater than seven cells). Prominent disorganisation of cells is characteristic, with loss of polarity and cohesiveness. Superficial (umbrella) cells may be present except in areas of full thickness abnormality. The tumour cells tend to be large and pleomorphic, with moderate to abundant cytoplasm, although they are sometimes small with a high nucleus to cytoplasmic ratio. The chromatin tends to be coarse and clumped. Morphometrically, the cells display increased nuclear area, nuclear perimeter, and maximum nuclear diameter. Nucleoli are usually large and prominent in at least some of the cells, and may be multiple. Mitotic figures are also seen in the uppermost layers of the urothelium, and may be atypical.

**Controversies**

There are many urothelial “benign” epithelial abnormalities, the identification of which is not always straightforward because of the lack of precise morphological criteria. Some of them represent benign lesions, the morphological identification and interpretation of which do not pose a particular
problem from the diagnostic point of view. Others are either part of the morphological spectrum of non-papillary neoplasia or reactive lesions, the histological appearance of which cannot be easily distinguished from that of dysplasia. In such situations morphometrical, immunohistochemical, and molecular techniques should be of great help. For instance, McKenna et al showed that the abnormal expression of cytokertatin 20 (increased), p53 (increased), and CD44 (decreased) in urothelial CIS and the increased expression of CD44 in reactive atypia allows more confident distinction of non-neoplastic urothelial atypia from urothelial CIS.45 "There are many urothelial benign epithelial abnormalities, the identification of which is not always straightforward because of the lack of precise morphological criteria"

The use of the term atypia (urothelial atypia) without further specification to encompass reactive atypia is discouraged by Lopez-Beltran et al.46 Reactive urothelial atypia is more appropriate. The introduction of the term “atypia of unknown significance” does not add value in practice. In those rare cases of reactive urothelial atypia in which the pathologist is unsure of whether the changes are reactive or true dysplastic, a conservative approach with repeated cystoscopy and biopsy, after the inflammation has subsided, is indicated.

Recent advances
The concept of malignancy associated change was introduced by Lopez-Beltran and colleagues47 to encompass those epithelial abnormalities that are present in urinary bladders harbouring preneoplastic and neoplastic lesions, and that are not detectable by routine light microscopic examination.48–50 A few recent studies showed that 50% of histologically normal urothelium samples adjacent to superficial urothelial carcinoma show genetic anomalies on chromosome 9, similar to the anomalies found in the coexistent carcinoma.34–36,38–42 These genetic alterations suggest a neoplastic potential for this flat urothelial lesion,43 and that additional preneoplastic conditions/lesions not recognisable by light microscopy may be clinically relevant, as shown in the prostate.44–45

Urothelial flat hyperplasia consists of an increase in the number of cell layers, usually 10 or more (but at least more than seven). There are few or no important cytological abnormalities, although slight nuclear enlargement may be focally present.46,47 Cytologically, this is similar to papillary hyperplasia. Hyperplasia may occasionally be associated with dysplasia or carcinoma in situ in the adjacent mucosa. A recent report found that 71% of cases of urothelial hyperplasia had the same chromosome 9 deletions seen in coexistent low grade papillary carcinoma.44 In contrast, 17p13 deletion was found in 8% of urothelial hyperplasia and low grade carcinoma.35 Urothelial hyperplasia has been considered the source of papillary neoplasia.32–44

Malignancy associated change and urothelial flat hyperplasia are considered to be the earliest detectable steps in the development of bladder cancer.5

MOLECULAR AND GENETIC BASIS OF CLASSIFICATION AND GRADING
Loss of heterozygosity (LOH) on chromosome 9 is an early event in the generation of superficial bladder cancer, such as G1 and at least part of the spectrum of G2. On the other hand, mutation of p53 represents an early genetic alteration in CIS (and G3). Later on, during progressive growth, p53 also contributes to the carcinogenesis of superficial bladder tumours, whereas LOH on 9p and 9q is involved in the development of CIS to T1–3 tumours. The final progression of superficial carcinomas and CIS seems to require another large series of genetic aberrations (fig 2).47 The exact sequence of these alterations has not yet been elucidated, even though it is correlated with the morphological classification and grading schemes.

APPENDIX
The following histological criteria are followed at the Institute of Pathological Anatomy and Histopathology, University of Ancona, to define histologically normal urothelium48–50:

• Multilayered (for example, five to seven layers) epithelium with two distinctive types of cells: cells of the superficial layer and the underlying intermediate and basal cells.
• The cells comprising all but the superficial layer are small and uniform in size, with well defined borders and amphiphilic cytoplasm rich in glycogen. The nuclei are regularly arranged. In particular, they are evenly distributed and polarised towards the surface, and show very finely granular chromatin which accentuates the regularly contoured nuclear borders. Nucleoli are not apparent in tissue fixed in formalin. It is unusual to see mitoses.
• Cells that are much larger than the underlying elements and are often binucleated or multinucleated compose the superficial layer of urothelium. Each cell covers several smaller cells of the immediately underlying layer. The nuclei are large and often show the presence of nucleoli.

ACKNOWLEDGEMENTS
This research has been partly supported by grants from the University of Ancona and the Italian Ministry of University and Scientific Research.

Appendix continued

Acknowledgements
The following histological criteria are followed at the Institute of Pathological Anatomy and Histopathology, University of Ancona, to define histologically normal urothelium:

• Multilayered (for example, five to seven layers) epithelium with two distinctive types of cells: cells of the superficial layer and the underlying intermediate and basal cells.
• The cells comprising all but the superficial layer are small and uniform in size, with well defined borders and amphiphilic cytoplasm rich in glycogen. The nuclei are regularly arranged. In particular, they are evenly distributed and polarised towards the surface, and show very finely granular chromatin which accentuates the regularly contoured nuclear borders. Nucleoli are not apparent in tissue fixed in formalin. It is unusual to see mitoses.
• Cells that are much larger than the underlying elements and are often binucleated or multinucleated compose the superficial layer of urothelium. Each cell covers several smaller cells of the immediately underlying layer. The nuclei are large and often show the presence of nucleoli.

ACKNOWLEDGEMENTS
This research has been partly supported by grants from the University of Ancona and the Italian Ministry of University and Scientific Research.

Authors’ affiliations
R Montironi, R Mazzucchelli, Institute of Pathological Anatomy and Histopathology, Ancona University School of Medicine, I-60020 Torrette, Ancona, Italy
A Lopez-Beltran, Department of Pathology, Reina Sofia University Hospital and Faculty of Medicine, 14071 Cordoba, Spain
DG Bostwick, Bostwick Laboratories, Richmond, and University of Virginia, Charlottesville, VA 23294 Virginia, USA

REFERENCES
Non-invasive urothelial neoplasms