Fluorescence diagnosis and flat lesions of urothelium: New challenges for pathologists and urologists (Review)

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Abstract. Bladder cancer is a common malignancy. Recurrence rate and progression vary greatly depending on factors such as tumor multiplicity, size, previous recurrence rates, tumor stage, tumor grade and the presence of carcinoma *in situ*. Treatment is expensive, recent studies demonstrated that superficial bladder cancer is a major economic burden. It is necessary to establish new kinds of techniques to improve diagnosis, therapy and follow-up, such as fluorescence diagnosis, without adding significant risk of complications. As urologists have a better sight of bladder lesions with fluorescence diagnosis, pathologists will be asked in the future to evaluate more frequently flat lesions, which up to now would not have been a matter of concern. For several reasons it is very important to have accurate and precise definitions of these flat lesions. First to permit uniform treatment of large groups of patients and second to see in large cohorts the evolution and natural history of several flat lesions, not always well known up to now. The aim of the study was to review the most important flat lesions, to demonstrate the difficulty of classifying several lesions, to introduce to urologists the new problems linked to FD and to suggest new models for accurate analysis. How far can we go in our answer as pathologists and how will it change the patient management?

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1. Introduction

Bladder cancer is the 4th most common malignancy among men in the Western world. Recurrence rate and progression vary greatly depending on factors such as tumor multiplicity, size, previous recurrence rates, tumor stage, tumor grade and the presence of carcinoma *in situ* (Cis) (1). Especially in case of superficial cancer, which concerns 75-85% of bladder cancer patients, treatment is very expensive. Recent studies demonstrated that superficial bladder cancer is a major economic burden (2). These findings illustrate the economic and medical necessity to establish new kinds of techniques to improve diagnosis, therapy and follow-up (3). Fluorescence diagnosis (FD) with 5 aminolevulinic acid (5ALA) has been shown to improve the detection of flat bladder lesions, especially Cis without adding significant risk of complications (4).

Before FD pathologists and urologists focused on Cis and pTa-pT2 tumors on biopsy or resection. As urologists have a better sight of bladder lesions with FD, pathologists will be asked in the future to evaluate more frequently flat lesions, which up to now would not have been a matter of concern. For several reasons it is very important to have accurate and precise definitions of these flat lesions. First to permit uniform treatment of large groups of patients and second to see in large cohorts the evolution and natural history of several flat lesions, not always well known up to now.

The aim of the study was to review the most important flat lesions, to demonstrate the difficulty of classifying several lesions, to sensitize urologists to the new problems linked to FD and to suggest new models for accurate analysis. We evaluated how far pathologists can go and how this will change the patient management.

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The WHO 2003 has considered several lesions as flat intraurothelial lesions (see Table I). Histological parameters to classify the following lesions are as described in Table II.

We describe the major lesions and the lesions difficult to classify and give detailed aspects of the Hexvix examination with the corresponding diagnosis.

2. Normal urothelium

Normal urothelium is composed of three different layers. The most superficial layer is covered by umbrella cells. These might display atypical aspects either in singular cells or in lager sectors. Mild degrees of variation in architecture without cytological atypia can be admitted in urothelium, there are usually no mitosis.

3. Metaplasia

Several types of metaplasia exist and must not be mis-diagnosed.

Squamous metaplasia. The most frequent is squamous metaplasia (SqM), which is common if the area is not extensive, very inflammatory or keratinizing. Parakeratosis, hyperkeratosis and even granular layers might exist in our experience. Nevertheless SqM has been considered as a pre-cancerous lesion by some authors. Guo *et al* showed in a recent study increasing numbers of squamous cell carcinomas after detection of SqM, but the number of cases was restricted (5). Enhanced expression of EGFR in these bladder squamous lesions suggests that EGFR may represent a therapeutic target. Wide-range human papillomavirus DNA signal was occasionally detected (5).

Glandular metaplasia (GM). GM might be seen in the surface of urothelium, as a response to chronic irritation or inflammation, such as neurogenic overactive bladder, bladder extrophy, long-term catheterization or history of calculi. There are still discordant data on the value of GM as a precancerous lesion. Recent data confirm that intestinal metaplasia and cystitis glandularis involve divergent pathways (6). Telomere shortening and analysis of chromosomal abnormalities support the hypothesis of intestinal metaplasia as a precursor lesion and could be a marker in the development of adenocarcinoma of the urinary bladder (7).

Nephrogenic metaplasia (NM). NM is also common with male predominance. It is seen in bladder walls with history of injuries. NM might mimic polypoid hyperplastic lesions, sometimes it is velvet-like. It might be tricky in case of inflammatory sub-mucosa with endothelial or hobnail aspects. These features might be difficult to distinguish from Cis or low grade dysplasia and not experienced pathologist might misinterpret NM as an adenocarcinoma or metastasis of an adenocarcinoma. NM is totally benign and has to be recognized to avoid overtreatment. Recent evidence has demonstrated that nephrogenic adenoma is a true 'nephrogenic' lesion derived from the exfoliated and implanted renal tubular cells in the urinary tract (8,9).

Table I. Flat intraurothelial lesions.

Normal
Hyperplasia
Flat lesions with atypia
Reactive inflammatory type
Dysplasia low-grade intraurothelila lesions
Carcinoma *in situ* (high grade intraurothelial lesions)
Atypia of unknown significance

Table II. Histological parameters.

Thickness of urothelial epithelium
Cellular polarity
Aspects of cytoplasm, especially if clear cytoplasm
Nuclear size, crowding, borders, aspects of chromatin
Presence of nuclei
Mitosis especially if atypical forms
Association with inflammation
Increasing density of small vessels under the lamina propria

4. Flat lesions without atypia

Flat urothelial hyperplasia (FUH). In this kind of settings urothelium is thickened (>7 layers), but lacks cytologic atypia, some degree of nuclear enlargement can be observed. FUH might surround low grade pTa tumors. FUH was mostly seen in cases of follow-up of pTa tumors and generally considered as a minor finding. Several studies could demonstrate genetic abnormalities already present in hyperplasia such as loss of heterozygosity of FGFR-3 gene and deletion of chromosome 9 in 70% of FUH (10-12). This raises the question of whether FUH should be considered as detection of an early premalignant urothelium lesion with low-risk profile (13).

5. Flat lesions with atypia

Reactive atypia (RA). RA is sometimes difficult to classify and not perfectly standardized in classifications. The inflammatory context usually helps to determine the lesions, which might be various. Increasing size of nuclei, with a globally preserved architecture and increasing cellular density are common. Increasing vascularisation has been described in the submucosa (14,15). Several cases of RA with mitosis have been described. Mitosis are in the lower layers of urothelium and never atypical (15). A pitfall might be denuded areas or atrophy of urothelium. Contrary to Cis, no increasing ratio between nucleus and cytoplasm, no hyperchromatic nuclei and no membrane irregularity are observed (16). In case of suspicion of RA, clinical history such as history of previous biopsies is very important to avoid overgrading of lesions (17).

Urothelial atypia with unknown significance. This entity is not very well defined and one should avoid using the term in case of diagnostic difficulties. Usually, inflammatory features are observed, and Cis or dysplasia cannot be totally excluded. Lopez-Beltran discouraged the use of this term, without any value in practice (12). If the pathologist is unable to decide between reactive, dysplastic or Cis, the term can be used. Urologists must be aware that this entity needs anti-inflammatory treatment and new biopsies to make a final diagnosis.

6. Dysplastic lesions

Low grade urothelial dysplasia (LGUD). LGUD is one of the major problems if isolated. LGUD is characterized by nuclear and architectural abnormalities with normal thickness of urothelium (14,18). LGUD is commonly seen in patients with bladder neoplasia. In patients without urothelial neoplasia the risk of developing cancer has been estimated to only 19% in an 8-year mean follow-up when LUGD are present, while observed from 32 to 83% of patients when associated to Cis (19,20). One possible explanation is the coexistence of two main molecular pathways of bladder tumor progression involving p53 (from flat dysplasia to Cis) or loss of heterozygosity on chromosome 9 (from dysplasia to low-grade papillary carcinoma but no Cis), with common interactions and overlapping (19).

Two main differential diagnoses remain: RA and Cis. Immunohistochemistry might be helpful. Aberrant CK20 expression in basal urothelial cells plus overexpression of p53 and Ki-67 and loss of CD44 expression might be helpful (21-23). Several studies have demonstrated increasing incidence if associated with Cis or papillary carcinoma, but we lack recent studies concerning prognosis of isolated lesions (1,24). One reason for the lack of data might be the problem of reproducibility in grading dysplasia (12,13,25,26).

Urothelial carcinoma in situ (Cis)/involvement of von Bruns nests/microinvasion/extensive denudation. Diagnosis of Cis is not common, features are well defined and pathologists are used to handling Cis. Frequently denuded urothelium exists underlining the discohesive nature of tumor cells. Owens and Epstein showed in a recent study that the majority of denuded lesions were of high grade. In case of important denudation urinary cytology might be helpful to determine diagnosis (27). Nevertheless, pathologists should be careful especially in case of cautery artefacts or in anatomically confined areas (28). Several different histological patterns exist, but it is probably not important to subclassify Cis in the pathology report, because different patterns do not seem to make any difference in clinical follow-up (21) (for patterns see Table III).

Differential diagnosis might be difficult with RA if cells display few atypia. Clinical history of radiotherapy has to be mentioned by urologists; otherwise reactive changing linked to the treatment might be misinterpreted.

Immunohistochemistry might be helpful to distinguish Cis from RA. In our experience a panel of p53, MIB-1 and Aurora-A, implied in early mitosis, is overexpressed in Cis, but to a lesser extent in RA (29). p53 and MIB-1 usually

Table III. Different histologic patterns in urothelial carcinoma *in situ*.

Large cell Cis
Pleomorphic
Non-pleomorphic
Small cell
Clinging
Pagetoid
Undermining/overriding

display strong nuclear staining in Cis. Aurora-A is over-expressed in the cytoplasm and perinuclear in Cis, it has also been shown that overexpression of these 3 markers predicts relapse (30). One should also think of Cis extending to von Brunn Nests, or exclusively being present in the latter. This aspect must not be mistaken for microinvasion.

7. Therapy associated atypia

Truncated papillae. This kind of lesions is not well known by many pathologists and can be observed after Mitomycin-C therapy. The top of the papillae is destroyed by chemotherapy. Together with inflammation and denudation truncated papillae might be mistaken as Cis (16,27).

Treated papillary carcinoma/granulomas. After BCG therapy granulomas containing epitheliod histocytes and multi-nucleated giant cells are common. When sending biopsies or resection material after treatment, it is important to have clinical history of the patient, otherwise differential diagnosis with urogential tuberculosis might be difficult. Ziehl-Nielsen staining is usually negative (16).

Radiation. Indication of radiation is rare nowadays, but microscopic changes might be very impressive and misleading. These lesions might be indistinguishable from Cis if no clinical history is known, they might persist for years after therapy (31).

8. Aspects of fluorodetection in bladder examination

A new ester of 5ALA (Hexvix®) has already shown its potential in FD with a short time of instillation (1 h) before cystoscopy.

Normal urothelium does not emit any fluorescent signal in FD examination. The three types of metaplasia have different aspects which are characteristic in each case. SqM might be just present in white light examination as a brown plaque, but does not always display fluorescent signals. There seems to be a correlation between thickness of SqM and light signals. GM might show luminous signals which are pink and most of the time not very intense. NM is clearly seen by the urologist during FD examination, the velvet-like, crete-like aspect is preserved and typical (Fig. 1).

During FD examination FUH displays generally only slightly luminous signals. FUH represented 14.4% of the false positive biopsy findings in a large series of FD (13).
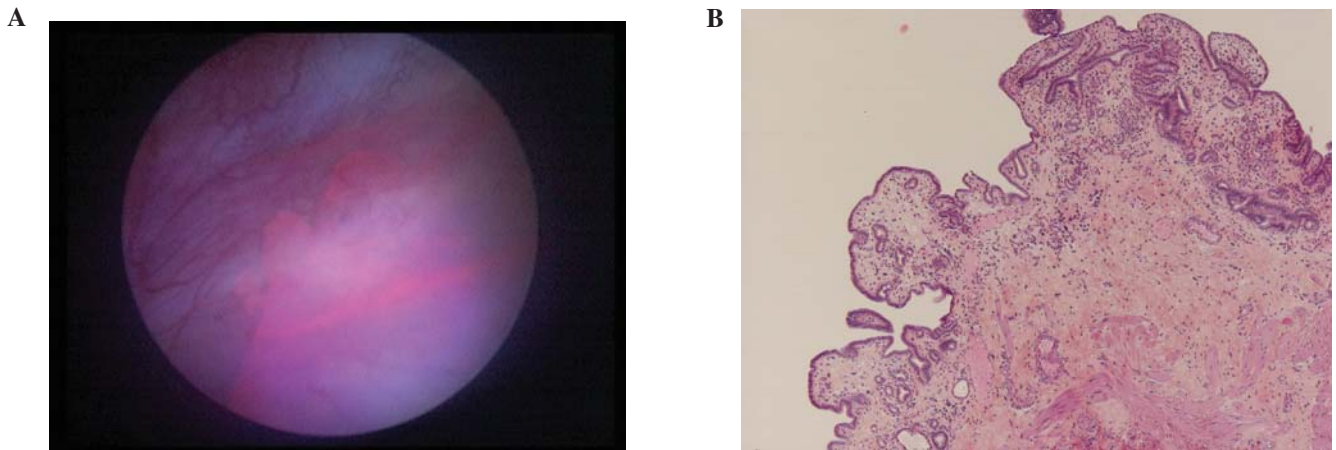
A

B

Figure 1. (A and B) FD of NM and histologically corresponding picture of NM with papillomatous, crete like slightly edematous aspects.
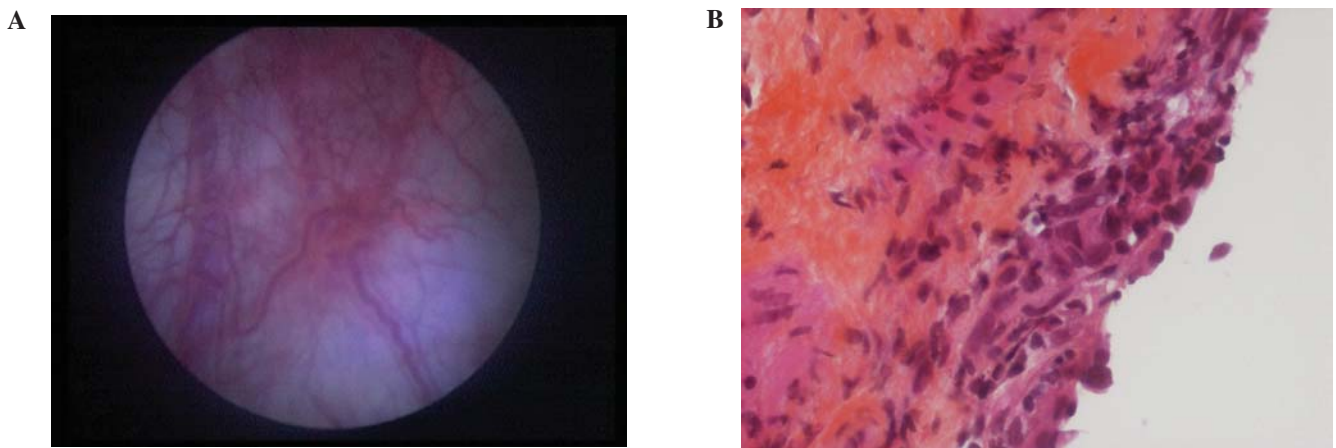
A

B

Figure 2. (A and B) Weak fluorescence signals and the histologic aspect with inflammatory urothelium displaying some proeminent nuclei. No more umbrella cells are present, inflammatory cells colonize the urothelium.
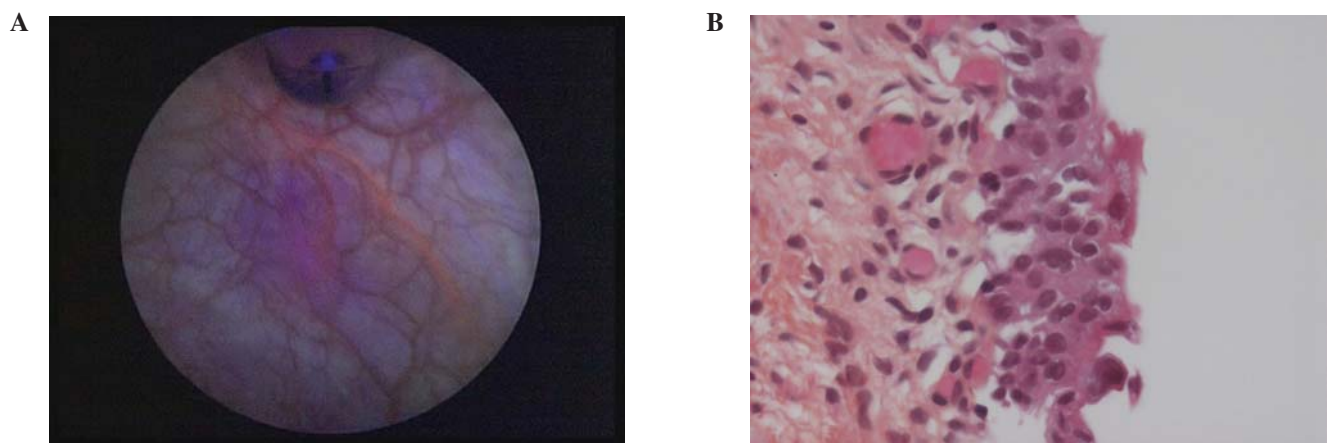
A

B

Figure 3. (A and B) LGUH displays sheet-like stronger signals than the previous picture. On the histological slide loss of polarity and enlarged nuclei can be seen. Slight nuclear pleomorphism exists, no inflammation is present.

These data support the hypothesis FUH displays some of the genetic alterations commonly found in bladder cancer. Should positive fluorescent findings of FUH be considered as false-positive findings or as detection of an early premalignant urothelial lesion with low-risk profile? Several authors have demonstrated in FUH early genetic changes, and FD seems to indicate early but already visible intraurothelial changes (32).
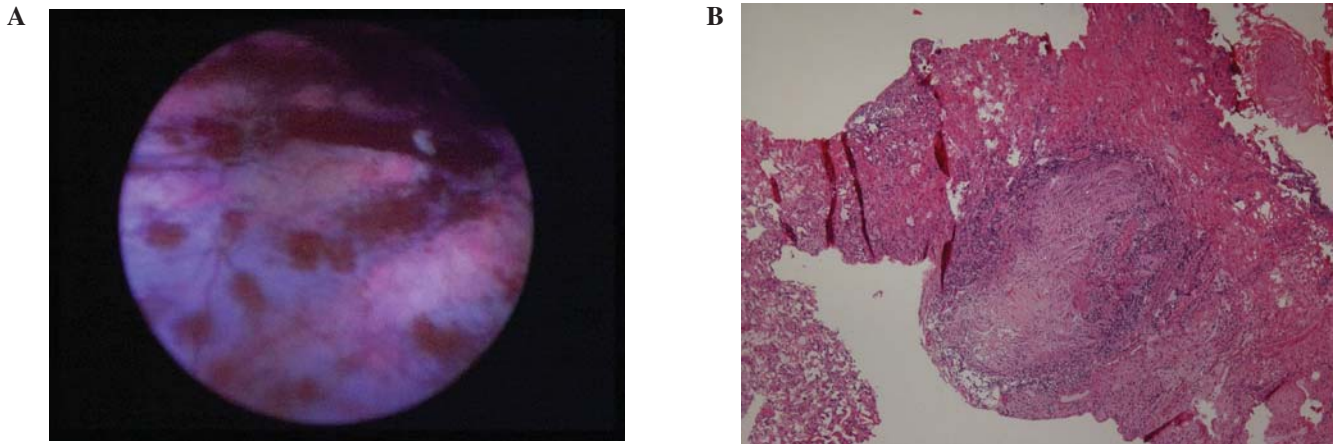
A

B

Figure 4. (A and B) Very typical scattered aspect in fluorescence examination of post BCG granulomas. Histologically important granulomas under the lamina propria can be seen, urothelium is not present on this slide.

RA might display a slight reactivity in FD examination, probably linked to inflammation, they represented 45.8% of the false positive biopsy findings in recent series. We lack recent data to determine, whether RA is a precancerous lesion and its influence on bladder carcinogenesis (Fig. 2) (13). Previous transurethral resections in patients under surveillance is one of the main causes of false positive findings and can be observed up to 3 months (17).

With the development of FD examination, LGUD that is usually cytoscopically and clinically silent, becomes a diagnostic problem. Therefore, prognosis of isolated LGUD lesions is still a matter of debate as detection rate of LGUD by FD is 30% greater than with conventional white light endoscopy (13,20). We consider important to mention LGUD, as LGUD can progress towards Cis and neoplasia (Fig. 3) (24,33). Longer follow-up data are required to show whether it is useful to indicate LGUD in pathologic reports. In FD examination Cis is easy to detect and shows a particular, strong and bright light signal. One question remains, whether different subtypes of Cis have different aspects in FD examination. With the increased Hexvix use in urology this problem might be resolved soon.

Granulomas post-BCG therapy can be observed in FD examination, one suggests that the product affects the superficial layer of the submucosa (Fig. 4). The lesions after radiation display atypical fluorescence signals in FD probably linked to inflammation. Many FD aspects still have unknown diagnosis, histology remains the gold standard.

9. Discussion

We show that flat lesions correspond to strictly defined criteria and explain some pitfalls in diagnosis of urothelial lesions. Sometimes the frontiers might be fluent and difficult even for experienced pathologist. FD will certainly change the patient management. Several studies have shown an improvement in the diagnosis of bladder carcinoma and Cis (34). Soloway and Kriegmair tried to resolve this problem already in 1996, considering that dysplasia was not cancer and did not require therapy. Therefore, they suggested not to over-treat patients, who in case of papillary tumor, would

have been treated few months later (35). The question of clinical relevance is not resolved in our opinion. Filbeck *et al* clearly showed in a recent study that FD leads to an improvement in the diagnosis of bladder carcinoma. It allows the early selection of the best treatment option and thus has a potentially positive effect on the prognosis of the affected patients (34). Jocham *et al* made two sets of records and compared white light cystoscopy and FD. They demonstrated that FD improved treatment in 21.7% (36). There is evidence that early detection changes patient treatment. Several studies have shown that dysplasia leads to Cis and invasive tumors in 15% (37). In a recent study Witjes and Douglass (38) suggest that more appropriate treatments might be less aggressive. In the study of Jocham *et al* (36), two patients had Cis simultaneous to other lesions, which was only detected in FD. Without this information Cis could have been interpreted as treatment failure leading to cystectomy. Several studies have shown that FD trans-urethral resection reduces residual tumor and recurrence rates and avoid unnecessarily aggressive therapy (34,39-41).

10. Conclusion

Several questions remain; we consider that pathologists should try to be as precise as possible in the biopsy or resection report. We think that it is very important to have accurate definitions of flat lesions and with FD experience we will probably change our reports going more into detail. Precise description and schemata are required from urologists to superpose the two experiences, those of urologists, but also those of pathologists, to be aware of problems of the two sides. Short formation courses for pathologists and urologists could be one step forward.

Studies of large patient cohorts are required to validate different ways of bladder cancer treatment and to improve our knowledge of the natural history of bladder cancer and its precursor lesions.

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