Carcinoma in situ of the urinary bladder – from pathology to narrow band imaging

BOGDAN FLORIN GEAVLETE1, ALICE BRÎNZEA2, IONEL ALEXANDRU CHECHERIȚĂ3, SABINA ANDRADA ZURAC4, DRAGOS ADRIAN GEORGESCU5, ALEXANDRA EUGENIA BASTIAN6, COSMIN VICTOR ENE5, CĂTĂLIN ANDREI BULAI1, DANA-OLIVIANA GEAVLETE5, MAGDA RUXANDRA ZAHARIA1, PETRIȘOR AURELIAN GEAVLETE1

1) Department of Urology, “Sf. Ioan” Emergency Clinical Hospital, Bucharest, Romania; “Carol Davila” University of Medicine and Pharmacy, Bucharest, Romania
2) Department Pathophysiology II, No. 2 Clinical Department, “Carol Davila” University of Medicine and Pharmacy, Bucharest, Romania
3) Department of Nephrology and Dialysis, “Sf. Ioan” Emergency Clinical Hospital, Bucharest, Romania; “Carol Davila” University of Medicine and Pharmacy, Bucharest, Romania
4) Department of Pathology, Colentina University Hospital, Bucharest, Romania; “Carol Davila” University of Medicine and Pharmacy, Bucharest, Romania
5) Department of Cardiology, “Prof. Dr. C. C. Iliescu” Institute of Emergency for Cardiovascular Diseases, Bucharest, Romania; “Carol Davila” University of Medicine and Pharmacy, Bucharest, Romania
6) Department of Internal Medicine, “Sf. Ioan” Emergency Clinical Hospital, Bucharest, Romania; “Carol Davila” University of Medicine and Pharmacy, Bucharest, Romania

Abstract

Objective: A retrospective clinical analysis was performed over a time period of 10 months while aiming to establish the impact of narrow band imaging (NBI) cystoscopy and transurethral resection of bladder tumors (TURBT) in cases of carcinoma in situ (CIS). Materials and Methods: CIS tumor cells are characterized by a high cytological grade, a certain degree of cyto-nuclear pleomorphism, large, irregular, hyperchromatic nuclei, high nuclear/cytoplasmatic ratio and mitotic figures. One hundred thirty-nine patients were consecutively diagnosed with non-muscle invasive bladder cancer (NMIBC) based on standard white light cystoscopy (WLC) and NBI vision. Urinary cytology was performed in cases of flat lesions suspected by either type of cystoscopy before the TURBT staging. Conventional endoscopic resection was performed for all white light (WL) visible lesions and NBI-guided TURBT exclusively for the observed tumors. Results: At subsequent pathological analysis, 13 CIS patients were confirmed. NBI cystoscopy emphasized a superior diagnostic accuracy as compared to WLC concerning the cases’ (92.3% versus 69.2%) as well as lesions’ (93.75% versus 71.9%) detection rates. NBI-TURBT provided a higher proportion of additional tumors’ cases (53.8% versus 15.4%) when compared to classical resection but was marked by an increased frequency of false-positive results (19.9% versus 11.5%). Urinary cytology displayed an 84.6% sensitivity rate. Conclusions: NBI cystoscopy and resection substantially ameliorated the CIS-related diagnostic accuracy within a parallel to the standard endoscopic approach at the cost of a reduced specificity. NBI-TURBT was able to find more CIS patients as well as lesions, thus improving the sensitivity of standard resection and urinary cytology.

Keywords: narrow band imaging, carcinoma in situ, white light cystoscopy, transurethral resection of bladder tumors.

Introduction

Based on the present knowledge, carcinoma in situ (CIS) represents one of the most aggressive stages of non-muscle invasive bladder cancer (NMIBC) with high risk of recurrence and progression [1]. Therefore, an early and accurate diagnosis is mandatory, but unfortunately the standard white light cystoscopy (WLC) is not efficient enough to detect this type of malignancy [2, 3]. Considering these aspects and additionally increased prevalence, superior and advanced endoscopic diagnostic tools needed to be emphasized for the benefit of CIS patients regarding oncological targeted treatments and long-term survival, as well [3, 4].

As an alternative, several years ago, narrow band imaging (NBI) cystoscopy was introduced as a promising modality of ameliorating NMIBC diagnostic accuracy [4] and according to the literature, it can also remove left-a-side tumors by the classical transurethral resection of bladder tumors (TURBT) [5, 6].

Hypothetically speaking, the ability to highlight the specific vascular architecture of urothelial carcinomas proves NBI superiority over WLC in observing the relatively less evident flat CIS formations [7, 8]. Still, there are very few reports specifically targeting carcinoma in situ study groups and eventual diagnostic advantages of NBI visualization when compared to WLC [8].

Based on these premises, the present retrospective clinical analysis took into account a 10 months inclusion period. As far as the actual objectives of the study were concerned, it aimed to establish the eventual evidence based impact of the NBI diagnostic and therapeutic approach within a retrospective comparison to the consecrated standard WLC-TURBT management.
The main clinical importance of the present research is intended to reside in the actual advantages of performing NBI cystoscopy as a routine adjunct investigation method for patients suspected of non-muscle invasive bladder tumors. Most importantly, the presumed novelty of the study is based on exemplifying a superior NBI related diagnostic accuracy with a specific, difficult-to-treat urothelial malignant pathology, namely carcinoma in situ.

Materials and Methods

The present report was aimed to assess a retrospective clinical analysis with regard to the use of NBI cystoscopy and resection as a complementary procedure to the standard therapeutic management in NMIBC suspected cases. The bladder cancer suspicion indicating the procedure was based on the presence of hematuria as well as a suspicion for malignancy based on ultrasound/CT imaging. These features constituted the inclusion criteria of the study and reflected on the manner in which the patients were admitted for hospitalization.

The diagnostic and therapeutic modalities were applied for patients admitted in the Department of Urology from “Sf. Ioan” Emergency Clinical Hospital, Bucharest, Romania. The multidisciplinary approach of the included cases involved a comprehensive analysis carried out in fellow clinics of nephrology, cardiology, internal medicine and pathophysiology. The pathology and immunohistochemistry analysis relied on the expertise of the Department of Pathology, Colectina University Hospital, Bucharest.

All the endoscopic procedures were based on the Visera II video system (Olympus Europa SE & Co. KG), comprising both white light as well as NBI visualization capabilities. As part of the surgical protocol applied in all the included cases, standard transurethral resection was performed for all the tumors visualized under white light. Furthermore, a switch to the NBI mode was performed, resulting in the discovery and ablation of supplementary tumors, otherwise previously left behind. A careful coagulation of the tumor bed areas was subsequently carried out, followed by a final NBI control screening for eventual residual lesions before ending the procedure.

In cases in which the presence of flat malignant lesions was suspected, urinary cytology was performed before actually beginning the resection stage of the intervention. The detection rates specific for the two types of cystoscopy were determined in accordance with the pathological analysis of the respective specimens.

After collecting, the tissue fragments were immediately immersed in 10% neutral buffered formalin for 18–66 hours; afterwards, they were washed in running tap water for 1.5 hours and submitted for routine manual histopathological processing according to specific protocols (ethanol 90° one day, ethanol 96° one day, ethanol 100° six hours, toluene 24 hours, two baths of paraffin 56°C of three hours each, and one last bath of paraffin 56°C for 18 hours) [Regulation No. 1217 from September 16, 2010 of Ministry of Health concerning approving of medical practice guidelines for Pathology, Official Gazette No. 723/October 29, 2010, Annex 1]. Immunohistochemical tests followed-up and utilized antibodies targeting cytokeratin 7 (CK7, mouse monoclonal, clone OV-TL12/30, Leica, heat-induced epitope retrieval pH 6 for 15 minutes, 1:200 dilution), collagen IV (mouse monoclonal, clone CIV 22, ThermoFischer, pretreatment Protease 1 mg/mL PBS (phosphate-buffered saline), five minutes 37°C, 1:100 dilution) and Ki67 (rabbit monoclonal, clone SP6, Leica, heat induced epitope retrieval pH 6 for 15 minutes, 1:500 dilution). All primary antibodies were detected using as detection system Novolink Polymer (Leica) and 3,3’-Diaminobenzidine (DAB) chromogen (Leica).

Histologically confirmed CIS patients were selected for this particular analysis. Differences between the two diagnostic alternatives were defined in light of the additionally discovered lesions. Comparatively outlining the sensitivity and specificity of conventional and NBI cystoscopy represented the primary endpoint of the study.

Results

Over a specific period of 10 months (between June 2014 and March 2015), a total of 139 patients were diagnosed with non-muscle invasive bladder tumors. Of these, 75.5% were male and 24.5% were female patients. Smoking was defined as the most relevant risk factor in both genders (88.6% among men and 61.8% of women).

Subsequent to the pathological analysis, 13 (9.4%) of these cases were confirmed with the diagnostic of carcinoma in situ, both high grade CIS (Figure 1) and low-grade CIS (Figure 2). Immunohistochemical stainings for collagen IV and CK7 were used in order to confirm the non-invasive nature of the proliferation (lack of invasion in lamina propria); collagen IV stain revealed uninterrupted basement membranes beneath the tumor epithelium and no CK7-positive small nests or isolated cells were present within lamina propria in all lesions, irrespective of their method of identification (WLC or NBI vision). Ki67 stain was used in order to evaluate the proliferation index of tumor population and therefore offered a better overview of the degree of dysplasia. No specific pattern of CK7 expression or differences in Ki67 index were noticed between WLC or NBI vision resected tumors.

The following data analysis of this trial was exclusively targeted on this specific category of patients.

To begin with, NBI vision was characterized by an improved patients’ detection rate when compared with the classical WLC (92.3% versus 69.2%). The advantages of NBI cystoscopy, in terms of diagnostic accuracy and capability of revealing the abnormal vascular architecture of a flat carcinoma in situ urothelial lesion (Figure 3), were maintained on tumors related basis as well (93.75% versus 71.9% detection rates, respectively) (Figure 4). The superior outcomes in terms of more numerous cases as well as lesions being found in NBI vision mode outlined the increased sensitivity of this optical enhancement method while drawing a parallel to the classical endoscopy.

From another perspective, after one of the cystoscopic modalities had been completed, the proportion of cases in which additional CIS lesions were discovered and improved
visual characterization of the intensified capillary architecture specific for the CIS tumors (Figure 5), was substantially larger for narrow band imaging (53.8% versus 15.4%) (Figure 4).

On the other hand, the NBI visualization displayed somewhat of a drawback while considering the evidence-based specificity of the additional investigation method, as it was marked by a higher frequency of false-positive results when comparing to WLC (18.9% versus 11.5%) (Figure 4).

Additionally, from the point of view of urinary cytology, this investigation modality showed a rather satisfactory sensitivity (84.6%). Most importantly, for the two cases of false negative findings, NBI cystoscopy was able to accurately establish the CIS diagnostic (only one of these patients was correctly diagnosed in white light cystoscopy).

As far as for the rest of the studied series, 85 (61.2%) of the patients were diagnosed with pTa stage tumors while pT1 lesions were found in 41 (29.5%) cases. The NBI specific diagnostic accuracy was further examined while referring to papillary malignant formations within a parallel to standard cystoscopy (Figure 6).

Figure 1 – High-grade papillary urothelial carcinoma: (A) Papillary proliferation with distorted architecture and loss of linear orientation perpendicular to the basement membrane – prominent cellular dyscohesion (HE staining, ×200); (B) Neoplastic cells present prominent cytoplasmic and nuclear hyperchromasia – significant nuclear pleomorphism with occasional monstrous nuclei (HE staining, ×400); (C) Relatively numerous nuclei positive for Ki67 within the tumor (Ki67 immunostaining, ×200); (D) Several mitoses (arrows) are highlighted by Ki67 immunomarking (Ki67 immunostaining, ×400); (E) No invasion in lamina propria can be accounted for (Collagen IV immunostaining, ×200); (F) Tumor cells are heavily positive for CK7 (CK7 immunostaining, ×200).
Figure 2 – Low grade papillary urothelial carcinoma: (A) Papillary proliferation with central fibrovascular cores and randomly distribution of cells in this proliferating urothelium – readily identifiable variation of cytological features (HE staining, ×200); (B) Obvious loss of cellular polarity – neoplastic cells present more crowding and layering; cytoplasmic and nuclear hyperchromasia; however, tumor cells are relatively uniform in size with no significant nuclear pleomorphism (HE staining, ×200); (C) Low Ki67 index within the tumor (Ki67 immunostaining, ×200); (D) Few mitoses (arrow) are highlighted by Ki67 immunomarking (Ki67 immunostaining, ×200); (E) No invasion in lamina propria can be accounted for (Collagen IV immunostaining, ×100); (F) Tumor cells are heavily positive for CK7 – no tumor cells are present in lamina propria (CK7 immunostaining, ×400).

Figure 3 – A single CIS lesion case solely diagnosed in NBI vision. The image on the left shows a “classical” aspect of the bladder mucosa visualized in “normal” white light; the picture on the right of the figure exemplifies a narrow band imaging aspect capable of revealing the abnormal vascular architecture of a flat, carcinoma in situ type of urothelial lesion.
Consequently, a significantly increased patients’ related detection rate was underlined for the NBI vision mode with regard to both pTa (95.3% versus 87.1%) and pT1 (97.6% versus 92.7%) (Figure 7).

From the perspective of the actual number of discovered tumors, the respective proportions remained substantially higher subsequent to the use of narrow band imaging (96.9% versus 90.8% detection rate for pTa and 97.4% versus 94.9% for pT1 stage lesions) (Figure 7).

While gathering the above-mentioned data, it became clear that a distinctively larger category of cases were identified as presenting supplementary lesions during the NBI examination (Figure 8) when compared to conventional cystoscopy (17.6% versus 5.9% in the pTa group and 9.7% versus 4.8% among pT1 patients) (Figure 7).

From a different point of view, as previously shown for CIS lesions, the frequency of negative biopsies remained elevated for the NBI-guided TURBT by comparison to the classical resection (14.3% versus 9.6% rate of false positive results for the pTa series and 8.4% versus 5.1% for pT1 cases) (Figure 7).
In accordance with a part of the available literature, expressions as useful early predictors of progression [12]. VEGF and HIF-1α (hypoxia inducible factor) tumor tissue levels in urine or plasma [20] or promising findings [12–19].

Regarding the CIS histopathological aspect, it has been observed that it represents a transitional cell carcinoma (TCC), known also as urothelial cell carcinoma, with normal urothelium; the growth pattern can be papillary, sessile or as carcinoma in situ [9–11]. Furthermore, one significant contributing factor of bladder cancer development is tobacco smoking with a high prevalence of more than 50% in men and one-third in women [9–11]. Consequently, a clear association was noticed between active smoking and elevated TCC risk, while quitting smoking improved the overall outcome [9].

CIS pathology

Urothelial CIS presents several cytological anomalies such as flat and disordered proliferation of cells, and additionally, during standard cystoscopy it is noticed as a reddish patch of mucosa located proximal to an exophytic tumor; usually this focal lesion is discovered in a patient without other macroscopic malignant entities (maybe endoscopically invisible) [16]. In almost 50% of cases, multifocal injuries are detected with higher affinity for the trigon, lateral wall and bladder dome [16]. Because the involved mucosa has a common aspect, standard cystoscopy highlights the diagnostic importance of urinary cytology [16].

Because CIS represents a highly invasive form of malignancy, numerous studies showed the significant contribution of angiogenesis in bladder tumors natural evolution and, therefore, the density of newly developed microvessels could be considered as a useful and powerful progression biomarker [12–19]. Still, because most of the published trials focused simultaneously on both types of tumors (CIS and pT1a), the results were mainly controversial, creating the opportunity for future experimental more applied studies towards a clear validation of these promising findings [12–19].

Other researchers considered vascular endothelial growth factor (VEGF) levels in urine or plasma [20] or VEGF and HIF-1α as useful early predictors of progression [12]. In accordance with a part of the available literature, these elements represent quite relevant parameters while assessing the risk of progression to muscle invasive bladder cancer [12, 20, 21].

Mechanisms of malignant cell transformation

During the course of the histopathological examination, low-grade papillary neoplasms of the bladder were frequently observed in association with a decreased risk of invasion by comparison to carcinoma in situ lesions. Currently, the understanding of the underlining pathological mechanisms are still a matter of debate with constant effort of revealing new significant data regarding molecular basis of urothelial bladder cancer [12, 22].

It has been already established that there are two different possibilities (corresponding to each type of bladder neoplasia) of malignant tumor development of the urothelium [12]; according to several experimental studies [12] CIS formation is correlated to p53 protein impairment (explaining also the invasive features [23]) that occur concomitant with H-ras expression [24] or deletion of specific markers such as pRb [25] or Pten [26].

Macroscopic and microscopic assessment

The macroscopic analysis of CIS emphasizes large areas of mucosa injuries (e.g., flat, erythematous, granular or cobblestone) – no specific tumoral mass – with cells located in the middle to the upper epithelium, which is often demodulated [27].

Regarding the microscopic analysis, while aiming to establish the existence of one of the six patterns of urothelial CIS, certain features should be assessed, such as the type of cell proliferation with pagetoid or nest-like appearance [28], as well as the eventual presence of desmosplasia [28, 29]. The CIS tumor cells are characterized by cytological atypia without invasion within the lamina propria [28]. Usually, the CIS lesions are composed of cells with variable polymorphism (according to tumor grade – low-grade CIS or high grade CIS) [28, 30]. The tumor cells have [28] large, irregular, hyperchromatic nuclei, usually with prominent nuclear pleomorphism, and high nuclear/cytoplasmatic ratio [31]. However, nuclear pleomorphism is not a prerequisite feature, as it is absent in the large cell type of CIS [32]; mitotic figures are often present. Of course, less important, are underlined by specific urothelium alterations: loss of polarity, nuclear size a few time larger compared to lymphocytes (normally double the size) and irregular thickness [31].

The fact that the cells in question are not cohesive leads to the process of shedding into the urinary flow, thus creating the premises for a positive cytology outcome [33]. The CIS specific sensitivity of the urinary cytology is reaching towards 95%, while nuclear changes are usually...
described, involving elements such as minimal pleomorphism, numerous high-grade neoplastic cells and a relatively clean background [33]. The image analysis of bladder wash cytology could be comparable to the “expert” standard cytological review providing superior results [33, 34].

**Comparative clinical data assessment**

When reviewing the literature data, urothelial carcinoma in situ represents a constant poorly diagnosed malignancy [35], and additionally, standard white light cystoscopy and related classical TURBT are usually followed by high short-term residual tumors of this particular type of bladder cancer [36]. Therefore, the need of correctly detecting CIS formation imposed the search for alternative investigation techniques aimed to complete the already established protocol in NMIBC management [37]. When narrow band imaging was firstly introduced, the perspective of an improved diagnostic option was highlighted because of its remarkable technique for presenting detailed images of capillary architecture of non-invasive bladder tumors and CIS flat lesions [38].

Although the amount of clinical data supporting the NBI superior efficacy is still limited, several studies confirmed the clear step forward made in accurate diagnosis of NMIBC [39]. The specific bladder CIS field of interest was not actually targeted by clinical trials, but there are various findings outlining the superiority of NBI as diagnostic and treatment approach when compared to conventional cystoscopy [40].

Overall, there are important studies concluding the efficiency of NBI versus standard WLC regarding CIS detection and tumor resection (92.7–94.7% versus 57.3–79.2%) [41, 42]. A substantially improved CIS-related diagnostic accuracy was confirmed by the NBI-TURBT therapeutic approach by comparison to the exclusively white light guided resection. During the course of the present study, the subsequent pathological analysis revealed a significantly higher precision for the narrow band imaging endoscopic tumor ablation (93.75% versus 71.9%) [43].

Furthermore, the diagnostic advantages of NBI cystoscopy over WLC were underlined by the significant amount of subjects in which additional CIS lesions were discovered, both according to the available literature (36.4% versus 9.1%) [42], as well as to the presently discussed clinical analysis (53.8% versus 15.4%).

While considering a different point of view, the specificity of the NBI assessment of the bladder mucosa proved to be affected by an increased rate of false positive results when drawing a parallel to the classical cystoscopy [7]. This idea was supported by the published literature findings (25.5 – 28% versus 16.4 – 21%) [41, 43], as well as by the outcomes of this study (18.9% versus 11.5%, respectively).

Summarizing, although presenting an inferior specificity, it may be stated that NBI endoscopy represents a superior diagnostic tool compared to white light for CIS detection [7]. In any case, the already available clinical data supporting the perspective of significantly higher NBI related sensitivity remain rather scarce. Ultimately, the eventual acknowledgement of NBI guided resection as integrant part of the standard NMIBC therapeutic approach will largely depend on the future, more extensive trials to come, targeting bladder cancer in general and CIS in particular.

**Conclusions**

For the difficulty to diagnose CIS bladder pathology, the NBI endoscopic visualization created the conditions for superior tumor ablation compared to the conventional approach. In support of this principle, clearly improved patients’ as well as lesions’ detection rates were obtained as a result of NBI resection while drawing a parallel to the classical technique. Furthermore, a substantial proportion of cases were defined as presenting additional tumors in NBI mode, otherwise left behind by the white light assessment of the bladder mucosa. From a different point of view, narrow band imaging seemed more susceptible to false positive findings, thus creating the premises for more numerous unnecessary biopsies to be taken. Last but not least, this alternative type of cystoscopy appears to constitute a good completion to the already acknowledged urinary cytology, as cases missed by this investigation were successfully discovered by NBI guided TURBT.

**Conflict of interests**

Potential conflict of interests declared by Bogdan Florin Geavlete, who received consultancy fees as a speaker for Olympus Company.

**Author contribution**

All the authors had equal contributions to the article.

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