Laser en-bloc resection of non-muscle-invasive bladder cancer: clinical and morphological specificities

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Objectives *to enhance the morphological diagnostic complex in order to predict postoperative outcomes in a more accurate way and to optimize patients with non-muscular invasive bladder cancer treatment.*

Materials and methods. *The study included 34 patients from 25 to 71 years old underwent laser en-block resection, the most of them were males – 28. In 9 cases multiple carcinomas (2 or more) were found. Huge tumors (2 cm or more in one dimension) were resected in 6 patients.*

Results. *The major part of tumors removed (n = 22) histologically appeared to be papillary urothelial carcinomas with low grade of malignancy and PUNLMP; in 6 cases G_2 was verified, one tumor with high malignancy potention – G_3. In 3 patients intramuscular invasion was found (invasive carcinoma T2) excluding them from the study.*

Discussion. *Laser en-block resection of non-muscle-invasive bladder cancer appears to be the most optimal approach in operative treatment that provides representative histological material. For correct morphological estimate we recommend either to expand the resection zone to 1 cm which allows to remove circular resection margin or to take extra pinch biopsy from tumor crater (vertical margin). In 3 patients from our study positive circular margin was revealed histologically whereas foci of perineural and perivascular invasion were found in one case. A new subgrading of stage T1 depending on intramuscular invasion depth was suggested whereas the main criteria is the muscularis mucosae involvement.*

Conclusion. *Morphological estimate of circular resection margin provides an ability to predict postoperative outcomes and correct the treatment in one or another way. Subgrading for T1-stage tumors is recommended for following correct postoperative prognosis and possibility of tumor recurrence.*

Key words: *non-muscular invasive bladder cancer, papillary urothelial carcinoma, laser en-bloc resection, substaging of bladder cancer*

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Background

Bladder cancer is the second most common genitourinary malignancy. Approximately 75 % of all newly diagnosed bladder carcinomas show no muscle wall invasion [1]; therefore, their removal with subsequent intravesical instillation of a chemotherapeutic agent is probably the most effective treatment strategy. In this case, surgery pursues two essential goals: removal of all visible bladder tumors and accurate tumor staging after morphological examination of surgical specimens.

Currently accepted guidelines on the surgical management of non-invasive bladder cancer allow both layer-by-layer removal of the tumor using standard transurethral resection (sTUR) and laser en-bloc resection of bladder tumor (L-ERBT), which is so far the most promising and innovative technique. On the one hand, this type of surgery is the least traumatic, because it allows avoiding tumor damaging during sTUR by its fragmentation and subsequent flotation of tumor cells in the bladder cavity. Researchers are still discussing the possibility of their subsequent implantation into the mucous membrane and recurrence [2]. On the other hand, unlike sTUR, L-ERBT gives large surgical specimens that include the whole tumor and a relatively large fragment of the bladder wall with mucosa and submucosa (tumor + tumor base). Moreover, L-ERBT has clinical advantages over sTUR, for example, complete absence of the obturator reflex and associated accidental perforations and bleeding [3].

Histological assessment of the detrusor muscle is a mandatory requirement for accurate T-staging, since it allows to exclude the diagnosis of muscle-invasive cancer (stage T2 or greater). Thus, the presence of muscle cells in the tumor basement is an indicator of a high-quality resection and is always reflected in the morphological report. The absence of detrusor elements is often observed in tumor fragments removed using sTUR (in up to 30%–50% of cases), providing no information on the depth of invasion and indicating non-radical tumor resection, thus, directly affecting treatment outcomes, since such patients require repeated surgery. On the contrary, multiple studies have

reported that L-ERBT provides high-quality specimens with detrusor elements found in 97%–100% of tumors [4].

Maximum preservation of the tumor integrity and its papillary structures is an important requirement for assessing the tumor grade (G). The native tumor structure with unchanged vertical architectonics in specimens obtained during L-ERBT ensures more accurate morphological examination, in contrast to sTUR, when a pathologist have to analyze scattered fragments of tumor tissue. Thus, only histological examination of the entire complex including tumor + tumor base provides maximally reliable results.

Objective: to analyze specific characteristics of morphological evaluation of bladder tumors removed using the L-ERBT technique.

Material and methods

This prospective study included 34 patients with newly diagnosed non-invasive bladder cancer (tumors up to 3 cm; stage Ta and T1) and treated at the M.R. Bronstein Urology Clinic. The diagnosis was based on the results of cystoscopy and radiologic examination. Each participant had 1 to 6 tumors. The exclusion criteria were muscle-invasive and other (non-urothelial) bladder cancers. During L-ERBT, we used a two-wavelength laser device FiberLase U1 (Urolaz) produce by «IRE-Polus» and implemented into clinical practice in 2017. The device has thulium and erbium fiber laser operating at 1.94 μm and 1.56 μm respectively and output power of 120 W (1.94 μm) and 15 W (1.56 μm). We used the mode with a wavelength of 1.94 μm, pulse energy 1 J, and frequency of 10 Hz. The biopsy specimens (tumor + tumor basement) obtained during surgery were sent to the Central Department of Pathology for histological examination.

According to currently accepted guidelines on the morphological evaluation of biopsy specimens (European Association of Urology (EAU) guidelines and World Health Organization (WHO) guidelines 1973 and 2004), we assessed tumor structure, differentiation, presence of detrusor elements, invasion through the basement membrane into bladder submucosa and muscle layer at the tumor base, presence of perivascular, perineural, venous, and lymphovascular invasion.

In addition to morphological characteristics, we evaluated resection margins (horizontal and vertical). After the resection of small tumors with adjacent and underlying tissues, it is advised to check whether the tumor was completely removed from healthy tissue. Thus, we recommend at least 10 mm indention from the tumor base during L-ERBT to ensure accurate morphological evaluation of separately collected specimens of the circumferential (horizontal) resection margin. We dissected the resection margin away from the tumor immediately after surgery prior to its fixation in formalin in order to avoid excessive dryness, rigidity and loss of elasticity, while the tumor was stretched on medical gauze. However, if the tumor forms short and smooth papillary structures or demonstrates creeping growth, the risk of false positive results in the circumferential resection margin is quite high. In these cases, we used a special technique: the removed fragment of the bladder wall with the tumor was immediately stretched by needles on a special styrofoam basis and then fixed in formalin ('styrofoam' fixation method) (Fig. 1); such dissection of the circumferential resection margin appears optimal.

This technique allows stretching the tumor base with adjacent fragments of the bladder wall to make the specimen look like it looked intraoperatively, when the wall was stretched by filling of the bladder. The advantages of this fixation method include the possibility of assessing horizontal resection margin using both o'clock positions and correct excision of the entire perimeter of the tumor base. Moreover, such a rigid fixation allows applying a special dye on the external side of the tumor base, which ensures correct identification of the external side of the circumferential margin during morphological examination and allows avoiding false positive results.

At the end of surgery, we performed random excisional biopsy of the muscle layer in the resection crater (vertical resection margin). To ensure correct positioning of samples and longitudinal section through the middle of the removed fragment, we marked the tumor base, which is particularly important for small tumors (< 0.5 cm). All specimens were fixed in 10% buffered formalin and embedded into paraffin; slides were stained with hematoxylin and eosin.

All patients underwent single immediate instillation of a chemotherapeutic agent (mitomycin C at a dose of 40 mg

Рис. 1. *Макропрепарат: удаленная опухоль со стелящимся характером роста, обработанная по методике «пенопласт» – фиксация к предварительно подготовленной основе с помощью игл для облегчения отсечения циркулярного края резекции*

Fig. 1. *Gross: removed tumor with superficial spreading type of growth; "on foam plastic fixation" method with needles for circular margin of resection cutting*

or doxorubicin at a dose of 50 mg). Patients diagnosed with G3 or T1 tumors in the postoperative period underwent a course of intravesical chemotherapy as follows: 1 instillation once a week for 2 months with the same dosages.

All patients were actively followed up during the postoperative period. Postoperative management included follow-up cystoscopy with biopsy of suspicious areas 3, 6, 9 and 12 months postoperatively, and then annually during the next 4 years.

Results

The majority of study participants (28 out of 34) were males. Patient age varied between 25 and 71 years. Nine patients had multiple (2 and more) bladder tumors. Large tumors (2 cm and greater) were removed in 6 patients. In 5 cases, the diagnosis of bladder cancer was rejected after morphological examination: 2 individuals were found to have urothelial papillomas, another 2 patients had chronic cystitis (typical cystitis glandularis with intestinal metaplasia and intestinal-type cystitis glandularis with cystic lesions), and 1 patient had a true diverticulum removed. Thus, the total number of patients was reduced to 29.

Histological examination showed that the majority of tumors removed (n = 22) were low-grade papillary urothelial carcinomas (G1) and papillary urothelial neoplasms of low malignant potential (PUNLMP). Six biopsy samples demonstrated G2 stage and 1 sample demonstrated G3 stage.

We paid particular attention to estimating the depth of tumor invasion: layers of the bladder wall (including the detrusor muscle) could be clearly identified in all biopsy samples in the tumor basement. Some difficulties arose only in 2 cases, when the tumor was separated from the underlying base due to pronounced artificial changes. Three patients had tumors that had invaded the detrusor muscle and, therefore, they were diagnosed with invasive bladder cancer (T2) and excluded from the study. One of these invasive tumors presented histological picture of G2 papillary adenocarcinoma, which is a rare type of bladder cancer. Its development is likely to be caused by glandular metaplasia in the urothelium in response to chronic inflammation in the area of tumor base. One patient with multiple (3) bladder tumors and relatively favorable characteristics (G1 stage, no invasion to the basement membrane) was found to have tumor emboli in small veins of the submucosa in one biopsy specimen and an area of perivascular invasion in the submucosa, which is assumed to be the factors of poor prognosis by the EAU.

In 7 patients, we found an evidence of tumor invasion not reaching the muscle layer: affecting the submucosa (n = 4) and small foci penetrating the basement membrane and invading the lamina propria without affecting the muscularis mucosae (MM) (n = 3).

Three patients had positive circumferential (horizontal) resection margin (these patients underwent surgery without photodynamic diagnosis (PDD)). Foci of perineural and perivascular invasion were observed in one participant. The vertical resection margin was represented by an intact fragment of the muscle layer in all participants (always negative).

С учетом того, что пациенты находились на лечении с сентября по апрель 2018 г., в данное время их повторное обследование не было проведено. Since all study participants were on treatment between September 2017 to April 2018, none of them has undergone re-examination. However, in the nearest future we will be able to detect recurrence and disease progression in patients with positive resection margin, foci of perineural and perivascular invasion, and intravenous tumor emboli. Moreover, we will change the strategy of postoperative management for these patients.

Discussion

The main goals of bladder cancer surgery are radical tumor removal and providing high-quality biopsy specimens to ensure accurate morphological examination. For urothelial carcinomas, there are two most important morphological criteria that directly affect the disease prognosis and further patient management: tumor grade and the presence of tumor invasion into the underlying layers.

EAU guidelines recommend being more cautious about the term ‘non-invasive bladder cancer’, whereas the term ‘superficial bladder cancer’ should be avoided, because even Ta G1 bladder tumors might have malignant potential. Moreover, even patients with relatively favorable PUNLMP can have disease progression (in up to 8% of cases) and develop relapses (in up to 60% of cases) [5]. Some authors reported that stage Ta bladder tumors may demonstrate various degree of malignancy. Wolters et al. examined 6 non-invasive stage Ta tumors removed using LERBT with thulium YAG laser and found that only one tumor was well differentiated (G1), whereas the remaining tumors were either moderately differentiated (G2, n = 2) or poorly differentiated (G3, n = 3). Thus, in this relatively small sample of non-invasive urothelial carcinomas, the majority of tumors were histologically classified as intermediate and high grade types [6].

Invasive bladder cancer includes all urothelial carcinomas that penetrate the basement membrane with any depth of invasion. The type of invasion is important: tumor invasion along a wide front is associated with better prognosis, whereas tentacle-like growth of the tumor is far more dangerous. This type of tumor growth was observed in some of our patients [7].

The latest WHO guidelines [8] recommend assessing not only the depth of invasion, but also other characteristics, including type of invasion and its approximate volume. All the recommendations mentioned above suggest that the issue of more precise evaluation of tumor invasion (T1 substaging) remains highly relevant in terms of its impact on

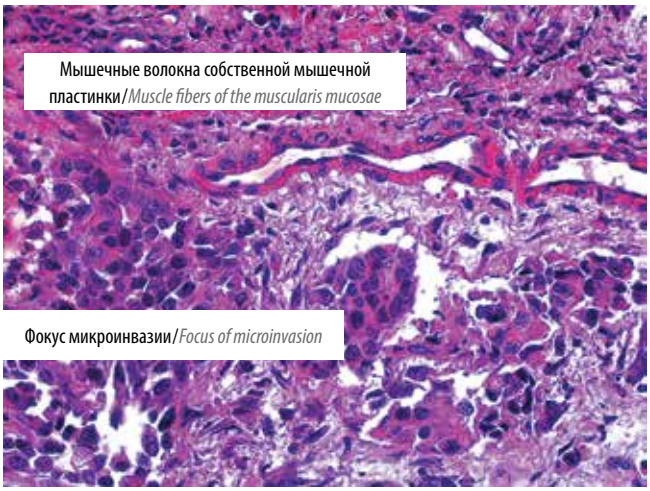

Рис. 2. *Фокус микроинвазии опухоли за базальную мембрану до собственной мышечной пластинки (окраска гематоксилином и эозином, × 200)*
Fig. 2. *Microinvasion focus through basement membrane till muscularis mucosae (staining with hematoxylin and eosin, × 200)*

the disease prognosis, probability of relapses, optimal strategy of postoperative examination, criteria for choosing an appropriate chemotherapy regimen, and Bacillus Calmette–guérin (BCG) therapy.

Many currently used variants of subclassification are based on the subclassification system developed by M. Younes in 1990 (includes three T1-substages: T1-a, b, c) [9]. The MM is considered the main criteria for substaging. Stage T1a tumors are characterized by invasion into connective tissue over the MM; stage T1b tumors directly invade the MM; stage T1c tumors invade connective tissue between the MM and the muscle layer (submucosa). This subclassification system has one significant drawback

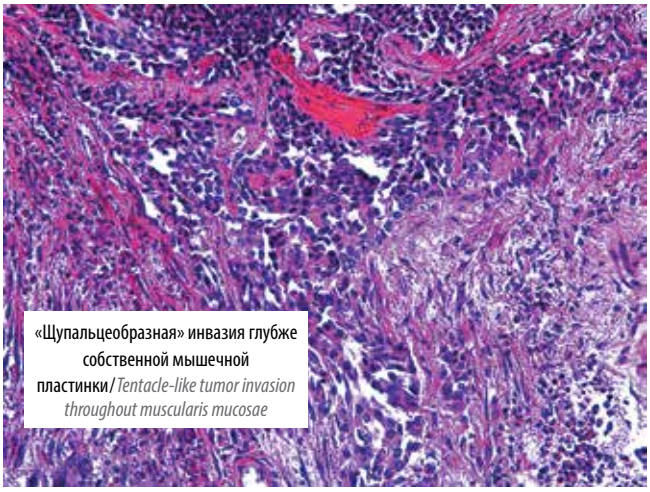

Рис. 3. *Фокус инвазии опухоли через толщу собственной мышечной пластинки в поверхностные отделы подслизистого слоя (окраска гематоксилином и эозином, × 200)*
Fig. 3. *Focus of tumor invasion throughout muscularis mucosae into submucosal layer superficial part (staining with hematoxylin and eosin, × 200)*

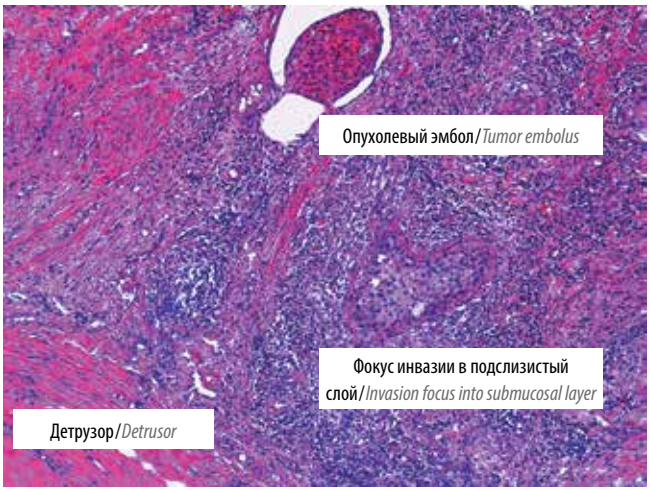

Рис. 4. *Инвазия опухоли в глубокие отделы подслизистого слоя: расположенные ниже пучки детрузора интактны; в просвете зияющего лимфатического коллектора – опухолевый эмбол; в подслизистом слое – признаки резко выраженного хронического воспаления (окраска гематоксилином и эозином, × 100)*
Fig. 4. *Tumor invasion into submucosal layer deep area: detrusor muscle bundles located below are intact; tumor embolus is seen in gaping lymphatic vessel lumen; prominent chronic inflammation is present in submucosal layer (staining with hematoxylin and eosin, × 100)*

associated with need for very thorough examination of tumor invasion into the MM (stage T1b), which is often absent in biopsy specimens or represented by intermittent thin clusters of muscle fibers, and, therefore, can be reliably identified in 72% of cases only. Moreover, this system does not exclude the subjective factor, because the MM is a thin and quite delicate structure, so pathologists may have different opinions on the location of tumor cells in the MM itself. Therefore, in this study, we used a simplified system for assessing the depth of tumor invasion: stage T1a tumors penetrate the basement membrane and invade the lamina propria without affecting the MM (microinvasion) (Fig. 2), whereas stage T1b tumors invade the submucosa (Fig. 3). Thus, subclassification of T1 tumors is possible even without an excessive detalization, when the MM is too intermittent and poorly represented in the specimen; large vessels of the submucosa located under the MM and over the muscle layer of the bladder wall can be used as a reliable checkpoint (Fig. 4).

According to the criteria described above, four patients from our study had tumors invading beyond the MM into the submucosa (stage T1b), whereas three patients had microinvasion beyond the basement membrane without affecting the MM (stage T1a). In the future, we hope to identify statistically significant correlations between the depth of invasion and characteristics of the disease course (particularly probability of relapse) using the results of follow-up examination in patients with various T1 substages. Van Rhijn et al. developed an additional system for stage T1 tumors substaging and analyzed long-term postoperative outcomes in 129 patients (follow up for 3–9 years) [10, 11]. This substaging

system discerns stage T1m (microinvasion) and stage T1e (extensive invasion) tumors based on the maximum size, location (T1m tumors has invasions ≤ 0.5 cm in diameter located in the lamina propria), and number of invasion foci. According to the results of long-term postoperative follow up, patients with stage T1e cancer demonstrated disease progression (relapse or transformation into T2 stage) significantly more often than those with stage T1m cancer. Immunohistochemical examination for Ki-67 and p53 confirmed these findings. Stage T1e tumors demonstrated Ki-67 expression in > 25% of cells 3 times more often than stage T1m tumors. Patients with stage T1e had expression of p53 in > 10% of tumor cells twice as often as patients with stage T1m cancer. Of note, both markers indicate proliferative activity of cancer cells, which is directly related to tumor progression. However, we think that substaging of T1 tumors with estimating all invasion foci creates additional difficulties for a morphologist. We believe that evaluation of tumor invasion based on assessing the MM involvement is most sensible. Anyway, subclassification of T1 tumors is a promising strategy for morphological evaluation of biopsy specimens.

Our findings strongly suggest that close cooperation between clinicians and pathologists is needed to increase the quality of surgical specimens and the accuracy of histological examination as well as to develop an optimal treatment strategy. It implies not only an individual approach to each biopsy, but also the improvement of the specimen processing algorithm.

In 2017, the Research Institute of Uronephrology and Human Reproductive Health of Sechenov University together with the Central Pathology Department initiated a comprehensive clinical and morphological study analyzing intraoperative biopsy specimens of bladder tumors taken during L-ERBT. In addition to unifying resection schemes, marking, and fixing gross specimens, we have developed more detailed criteria for morphological assessment: all patients after L-ERBT should undergo evaluation of circumferential and vertical resection margins along with additional substaging of stage T1 tumors.

Conclusion

We believe that the implementation of T1 substaging will allow more accurate distribution of patients according to risk factors, which will affect the treatment strategy and improve postoperative management of patients with non-invasive bladder cancer.

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