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The impact of different adjuvant intravesical therapy methods on tumor biology in patients with high-risk non-muscle-invasive bladder cancer

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Introduction Expression level of the cell proliferation marker Ki-67 correlates with the degree of differentiation of tumor cells and stage in primary patients with non-muscle-invasive bladder cancer (NMIBC), but the marker is currently not used in assessing the efficacy of adjuvant intravesical therapy and risk stratification in patients with recurrent bladder tumors.

Material and methods A retroprospective study included 107 patients with high-risk NMIBC; the patients were divided into 2 groups. The first group included patients who received adjuvant therapy after transurethral resection of the bladder using the Bacillus Calmette-Guérin (BCG) vaccine (BCG therapy group; n = 54), the second group consisted of patients who received hyperthermic intravesical chemotherapy (HIVEC® therapy group; n = 53) using the device for local hyperthermia Combat BRS HIVEC®.

Results Tumor recurrences were recorded in 21 (39%) patients receiving intravesical BCG therapy and in 9 (17%) patients after intravesical hyperthermic chemotherapy (p = 0.012). The expression level of Ki-67 in primary tumors did not differ; in recurrent tumors it was significantly different in both groups (32.05 ±13.80 vs 11.00 ± 6.86). The frequency of recurrence-free survival (RFS) in patients receiving chemohyperthermia was significantly higher than in patients after the BCG therapy (log-rank test result: p = 0.048).

Conclusions Assessment of Ki-67 expression in recurrent tumors can be a criterion for the effectiveness of intravesical bladder-preserving treatment. The use of hyperthermic chemotherapy can reduce the number of radical cystectomies in a separate group of patients with NMIBC.

Key Words: non-muscle invasive bladder cancer ‹› hyperthermic intravesical chemotherapy ‹› Bacillus Calmette-Guérin ‹› mitomycin-C ‹› Ki-67

INTRODUCTION

High-risk T1G3 bladder tumor is considered the non-muscle-invasive bladder cancer (NMIBC) group that is associated with a high risk of recurrence and progression after treatment with transurethral resection of bladder tumor (TURB) alone, as well as with a recurrence rate of 50–70% and a tumor progression rate of 25–50% in the first 3 years after surgery. The adjuvant intravesical therapy in patients with the NMIBC of the intermediate/high-risk reduces the probability of the recurrence and the progression to 52% and 20% after five years, respectively [1].

A separate category includes patients with the extremely high-risk NMIBC, as well as high-risk patients who had unsuccessful Bacillus Calmette-Guérin (BCG) therapy (BCG failure). In such a situation, it is recommended to perform early radical cystectomy with urine diversion, which makes the treatment of NMIBC extremely difficult [2]. The key problems of this surgical procedure are 90-day mortality at the level of 2.3–9% and the rate

of perioperative complications up to 80% [3]. In addition, bladder cancer is more common in older people with a poor comorbid status due to concomitant pathologies [4]. Therefore, there is a clear need for alternative organ-conserving therapies for patients with NMIBC who have contraindications for or who do not agree to radical cystectomy.

Chemohyperthermia is an instrumental technique that is used to increase the effectiveness of intravesical chemotherapy and has shown encouraging results in the treatment of patients with NMIBC. This method includes circulation of mitomycin-C (MMC) in the bladder and simultaneous hyperthermia (HT) of the bladder wall up to 41–44°C [5]. It is known that hyperthermia promotes both direct and indirect processes of DNA damage [6], which enhances the anticancer immune response. In addition, it increases the concentration and the depth of chemotherapeutic agents' penetration into the bladder wall tissues and potentiates their effect on cancer cells [7]. Hyperthermic intravesical chemotherapy (HIVEC®) treatment shows impressive results on disease-free survival and is well tolerated [8].

Following the current guidelines of the European Association of Urology (EAU), hyperthermic chemotherapy can be considered as device-assisted therapy for patients with 'BCG failure' who are unsuitable for or refuse radical cystectomy. However, it is not currently considered as an alternative to the BCG therapy in high-risk patients [2]. For a wider introduction of organ-preserving techniques, more predictive factors and an assessment of the effectiveness of treatment of this category of patients are required, in addition to survival rates. One of these factors can be the molecular marker of tumor cell proliferation Ki-67 [9, 10].

We currently have data on the prognostic value of the cell proliferation marker Ki-67, which has been well studied in breast cancer. Ki-67 expression correlates with the degree of differentiation of tumor cells and tumor stage [11]. During the interphase, protein is determined exclusively in the nucleotide matrix associated with chromosomes. In the active phases of the cell cycle, the matrix and nucleic acids are dissociated and the expression of the protein and its content in the cytoplasm grow, especially when the cell progresses through the synthetic stage. The Ki-67 antigen is rapidly degraded in cells passed into a non-proliferative state; antigen expression is also absent during DNA repair [12].

There are a number of studies assessing the prognostic value of the Ki-67 antigen in stratification of the recurrence and progression risks in primary patients with the NMIBC, but the marker is currently not used in assessing the efficacy of adjuvant intravesical therapy and risk stratification in patients with recurrent bladder tumors [13, 14]. In the present study, we aimed to assess the prognostic significance of the Ki-67 tumor marker expression for evaluating the adjuvant therapy effectiveness in patients with high-risk non-muscle invasive bladder cancer.

MATERIAL AND METHODS

Study design

A retroprospective study included 107 patients with high-risk NMIBC with an average age of 65.01 ±12.23 (95% CI: 62.72–67.35) years, who received adjuvant intravesical therapy in the period from 2013 to 2021. All studies were carried out in accordance with the Council of Europe Convention "Convention for the protection of Human Rights and Dignity of the Human Being with regard to the Application of Biology and Medicine: Convention on Human Rights and Biomedicine" (ETS No. 164) dated 04.04.1997, and the Declaration of Helsinki by the World Medical Association (2008). Before including patients to the study protocol, their personal written voluntary consent to participate in the study were obtained and all precautions were taken to ensure their anonymity.

Depending on the type of treatment, patients were divided into 2 groups. Group 1 included patients who received adjuvant therapy after TURB with BCG vaccine (BCG therapy group; n = 54). Group 2 included patients, who received adjuvant intravesical chemotherapy using a Combat BRS HIVEC® device for local hyperthermia (HIVEC® therapy group; n = 53).

The treatment protocol for the BCG group patients included the Uro-BCG vaccine solution instillation (a lyophilisate containing from 2×10^8 to 8×10^8 viable BCG bacteria), at a temperature of 20–21ºC, once a week into an empty bladder via a disposable urethral catheter. Patients had to withstand an exposure time of 1 hour before urinating. The main course of treatment consisted of 6 weekly treatments. The maintenance course involved 1 instillation of BCG once a month for 6 months.

The treatment protocol for patients in Group 2 consisted of a six-week course of mitomycin-C instillations at a dosage of 40 mg using an apparatus for hyperthermic chemotherapy which extravesically heated the chemotherapy solution to a temperature of 41–43°C and recirculated it for 60 minutes with stable pressure and rate of 200 ml/minute. All instillations were performed using the Combat BRS system V2.0, which was used according to the manufacturer's instructions (Combat Medical, Withamstead, UK).

Patient characteristics

Patients and tumors characteristics are presented in Table 1. There were no significant differences between the groups.

Every three months, all patients underwent cystoscopy as well as cytological analysis of the urine samples for atypical cells was performed. In case of endoscopic suspicion for the recurrent process or positive cytological examination result, a bladder biopsy was performed. Spiral computed tomography of the urinary tract was performed once a year. The primary outcome was recurrence-free survival (RFS), defined as the time from the start of the first intravesical chemohyperthermia procedure to the recurrence of the bladder wall tumor. Recurrence was defined as a histologically confirmed diagnosis of urothelial carcinoma. Recurrent tumor was assessed by the depth of invasion, the degree of differentiation; the therapeutic pathomorphosis was also investigated. Investigation of Ki-67 antigen expression was carried out in recurrent tumors in comparison with primary ones.

Immunohistochemistry

Immunohistochemical investigation were performed using monoclonal antibodies against the Ki-67 antigen (clone MIB-1, 1: 100, Thermo Fisher Scientific, USA). Antibodies were diluted in a solvent with a background reducing component. All reactions were followed by appropriate control reactions in which specific antibodies were replaced by a universal negative control union for the complex of primary N9106-S series antibodies (Thermo Fisher Scientific, USA). A fraction of 1000 tumor cells was selected as the minimal number to obtain representative results for evaluation. The value of IHC markers was expressed as a percentage of tumor cells with a positive stain. The Ki-67 index was adopted according to the recommendations of the St. Gallen Consensus 2017: low proliferative tumor – expression level of Ki-67 <14%, moderately proliferative – from 14 to 19%, high Ki-67 status – ≥20% [11].

A comparative analysis of the studied parameters in the compared groups was carried out according to the $\chi 2$ method and using the t-test for independent samples. The Kaplan-Meier method was used to obtain estimates of the RFS. The indicators were compared with each other using a log-rank test. The risk assessment was carried out using the Cox proportional hazards model. Patients were censored by the date of recurrence, or by the date of latest cystoscopy. IBM SPSS Statistics for Windows (version 26.0) was used as a program for calculating statistical indicators. A p-value <0.05 was considered an indicator of statistical significance.

RESULTS

The median follow-up period in Group 1 was 27 months (range 4–36), in Group 2 it was 18.0 months (range 2–36), respectively (p = 0.147). Tumor recurrence was reported in 21 patients received intravesical BCG therapy and in 9 patients after intravesical hyperthermic chemotherapy. Recurrence rates were significantly different between these two groups (39% versus 17%, p = 0.012). The mean recurrence-free survival rate in patients received intravesical hyperthermic chemotherapy [31.2 ±1.40 (95% CI: 28.5–33.9)] was significantly higher than in patients after intravesical BCG therapy [26.2 ±1.72 (95% CI: 22.8–29.6)] (result of the log-rank test is p = 0.048) (Figure 1).

In all patients, primary tumors were highly proliferative in terms of Ki-67 expression, the difference in the obtained indicators of the nuclear antigen Ki-67 expression level in recurrent tumors after the adjuvant treatment was statistically significant (p <0.001) (Table 2).

After adjuvant treatment in the BCG group, 14 (66.7%) patients had the Ki-67 marker expression ≥20% in recurrent tumors, in 3 (14.3%) cases the expression was in the range from 14% to 19%,

Table 1. *Comparison characteristics of EORTC stratification*

Parameter	Group of the BCG n (%)	Group of the HIVEC® n (%)	P value
Sex:			
Male	44 (81.5)	40 (75.5)	0.45
Female	10 (18.5)	13 (24.5)	
Age	64.76 ±12.1 (95%CI: 61.43–68.79)	65.26 ±12.476 (95%CI: 61.96–68.79)	0.83*
Primary	45 (83.3)	40 (75.5)	0.147
Recurrent	9 (16.7)	13 (24.5)	
Ta	5 (9.3)	3 (5.7)	0.48
T1	49 (90.7)	50 (94.3)	
N of tumors			
Single	30 (55.6)	31 (58.5)	0.76
Multiple	24 (44.4)	22 (41.5)	
Diameter of tumors			0.29
< 3	29 (53.7)	23 (43.4)	
≥ 3	25 (46.3)	30 (56.6)	
Concomitant CIS	11 (20.4)	10 (18.9)	0.85
G1			
G2	28 (51.9)	24 (45.3)	0.50
G3	26 (48.1)	29 (54.7)	
Low-grade	30 (55.6)	24 (45.3)	0.159
High-grade	24 (44.4)	29 (54.7)	

EORTC – European Organisation for Research and Treatment of Cancer; HIVEC – hyperthermic intravesical chemotherapy; BCG – Bacillus Calmette-Guérin; N – number; CIS – carcinoma in situ

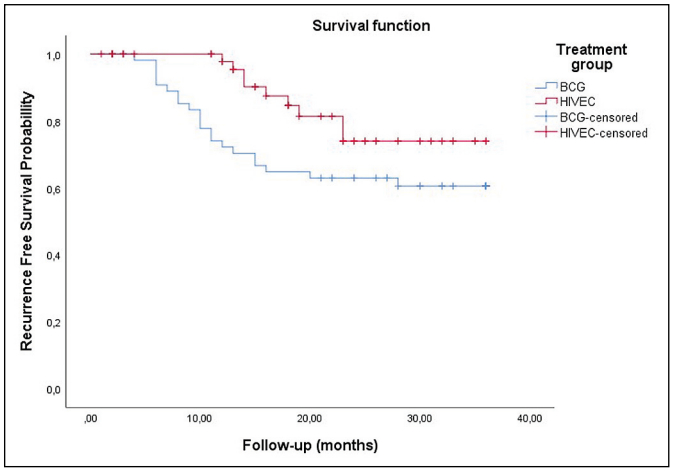

Figure 1. *Kaplan-Meier curves of indicators of the RFS in the studied groups.*

RFS – recurrence-free survival; HIVEC – hyperthermic intravesical chemotherapy; BCG – Bacillus Calmette-Guérin

Table 2. *Expression levels of the Ki-67 nuclear antigen in primary and recurrent tumors in patients of the studied groups*

Groups	Group of the BCG Mean (%)	Group of the HIVEC® Mean (%)	P value*
Ki-67 primary	65.00 ±10.47 (95%CI: 60.74–69.06)	67.33 ±7.98 (95%CI: 61.92–72.40)	0.556
Ki-67 recurrent	32.05 ±13.80 (95%CI: 26.10–37.48)	11.00 ±6.86 (95%CI: 6.90–15.64)	<0.001

RFS – recurrence-free survival; HIVEC – hyperthermic intravesical chemotherapy; BCG – Bacillus Calmette-Guérin

in 4 cases (19%) the expression level decreased less than 14%. In the HIVEC® therapy group, 6 (66.6%) patients had a decrease the Ki-67 marker below 14%, in 2 cases (22.2%) the expression level was in the range from 14% to 19% in recurrent tumors (Table 3). Recurrent tumor, concomitant carcinoma in situ (CIS), high-grade of differentiation and BCG treatment in compare with HIVEC® treatment were independent prognostic factor for tumor recurrence after adjuvant treatment by the Cox hazards regression model.

Before the introduction of the HIVEC® therapy in patients with failed BCG-based treatment who could not undergo radical cystectomy due to their age and comorbidity status, treatment options were systemic chemotherapy, external beam radiation therapy, and palliative endoscopic disease control (Table 4).

Of the 7 cystectomies performed for recurrent tumors in the BCG treatment group, 4 (19%) were performed in cases of muscle-invasive recurrences, and 3 (14%) for BCG refractory/unresponsive recurrence. Palliative chemotherapy was given to 2 (10%) elderly patients with bladder recurrence and progression to regional lymph nodes, 1 (5%) elderly patient with BCG-refractory recurrence underwent external beam radiation therapy. Palliative endoscopy was performed in both groups of patients with late non-muscular-invasive relapses who refused other treatment. In 4 (19%) cases of late recurrence with an extremely low level of Ki-67 expression, a repeated course of BCG therapy was carried out. In the HIVEC® therapy group, radical cystectomy was performed in 1 (11) case of muscle-invasive recurrence, in 2 cases of muscle-invasive recurrent tumors patients preferred palliative chemotherapy and external beam therapy. Four patients in the BCG group and 5 patients in the HIVEC® group (21% of all recurrent tumors) with low Ki-67 expression received HIVEC® therapy as a second line of intravesical therapy. For these patients, HIVEC® was an alternative to radical cystectomy, no repeated recurrences have been identified in this subgroup yet.

DISCUSSION

Our data are consistent with most researchers' conclusions, who also noted a correlation between the Ki-67 expression and the stage and grade of malignancy. The presence of a high Ki-67 expression might be a prognostic factor of recurrence in the near future [15, 16].

In study involving 332 patients with NMIBC, Ding et al. revealed the Ki-67 overexpression and a close relationship of the tumor marker with more aggressive manifestations of the oncological process: multifocal growth, concomitant carcinoma in situ, higher stage of the disease and low differentiation of the tumor, as well as a greater progression risk according to the European Organisation for Research and Treatment of Cancer (EORTC) tables [17]. In our study, the Ki-67 marker high level also was revealed in all primary tumors, because the study was performed in patients of high and extremely high-risk groups according to EORTC stratification.

He et al made a systematic meta-analysis of 11 studies with 1321 cases of the NMIBC. They tried to evaluate the predictive value of the Ki-67 marker in patients with the NMIBC after the TURB and intravesical BCG therapy. The results of this meta-analysis showed that the Ki-67 expression did not have a statistically significant association with the recurrence free survival, but was significantly associated with progression-free survival (PFS) [14].

In our study, we also did not reveal any statistically significant relationship between the Ki-67 expression in primary tumors and recurrence-free survival, but the Ki-67 expression level in recurrent tumors

Table 3. *Main characteristics of primary and recurrent tumors*

Case	TURBT1						TURBT2					
	T	Number	Diameter	Grade	CIS	Ki-67	T	Number	Diameter	Grade	CIS	Ki-67
						BCG						
1	T1	Multiple	≥3	High		57	T1	Multiple	<3	Low		18
2	T1	Multiple	<3	High	+	76	T2	Single	≥3	High	+	34
3	T1	Multiple	<3	Low		65	T1	Single	<3	Low		14
4	T1	Single	<3	Low		63	T1	Single	<3	Low		19
5	T1	Multiple	<3	High	+	74	T2	Single	≥3	High	+	44
6	T1	Single	<3	High		67	T2	Single	<3	High		45
7	Ta	Multiple	≥3	High	+	73	T1	Single	<3	Low	+	38
8	T1	Single	≥3	High	+	54	T1	Single	≥3	Low	+	33
9	T1	Single	≥3	High		78	T2	Single	<3	High		48
10	T1	Single	<3	High		80	T2	Single	<3	High		40
11	T1	Multiple	<3	Low		64	T1	Single	<3	Low		44
12	T1	Single	≥3	Low		55	T1	Single	<3	Low		12
13	T1	Multiple	<3	High	+	78	T2	Multiple	<3	High	+	44
14	T1	Single	<3	Low	+	60	T1	Single	<3	Low	+	43
15	T1	Multiple	≥3	High		62	T1	Single	<3	High		32
16	T1	Multiple	≥3	Low	+	74	T1	Multiple	<3	Low		25
17	T1	Multiple	<3	High	+	54	T2	Single	<3	High	+	41
18	T1	Multiple	<3	Low		43	T1	Single	<3	Low		11
19	T1	Single	<3	High	+	76	T1	Single	<3	High	+	53
20	T1	Single	≥3	High		64	T1	Single	<3	High		18
21	T1	Single	<3	Low		48	T1	Single	<3	Low		11
						HIVEC®						
1	T1	Single	≥3	High	+	68	T1	Single	<3	Low		10
2	T1	Multiple	≥3	High		62	T2	Multiple	<3	High		17
3	T1	Single	≥3	High	+	74	T2	Single	≥3	High	+	19
4	T1	Multiple	≥3	High		59	T1	Single	<3	Low		6
5	T1	Multiple	<3	Low		54	T1	Single	<3	Low		5
6	T1	Multiple	<3	High	+	76	T2	Multiple	<3	High	+	20
7	T1	Multiple	≥3	High	+	67	T1	Single	<3	Low		7
8	T1	Multiple	≥3	Low	+	78	T1	Single	<3	Low		10
9	T1	Single	≥3	High		68	T1	Single	<3	Low		5

TURBT – transurethral resection of bladder tumor; CIS – carcinoma in situ; HIVEC – hyperthermic intravesical chemotherapy; BCG – Bacillus Calmette-Guérin

after adjuvant treatment was a significant risk factor in BCG group patients [HR 1.06 (95% CI: 1.01–1.11); p = 0.021] (Table 5).

The difference between the expression of Ki-67 in primary and recurrent tumors was statistically significant, both in the BCG therapy group and in the HIVEC® therapy group (p <0.001). When comparing the groups with each other, there was no difference between the expressions of Ki-67 in primary tumors, but there was a significant difference in recurrent tumors (p <0.001) (Figure 2). This indicates that recurrent tumors after chemohyperthermia have a low proliferative potential, which may have a positive effect on progression-free survival after HIVEC®.

Its might be due to the mechanism of medicinal agents' action. BCG vaccine has an antitumor effect on the bladder wall, causing a local immune response. It increases the CD4+ and CD8+ lymphocytes, natural killer cells, macrophages and dendritic cells activity, and by this way induced local antitumor immunity [18].

Table 4. *Treatment modalities in recurrent patients*

	Group of the BCG n (%)	Group of the HIVEC® n (%)
Cystectomy	7 (33)	1 (11)
Repeated BCG course	4 (19)	
Systemic chemotherapy	2 (10)	1 (11)
Repeated HIVEC® course	4 (19)	5 (56)
External beam therapy	1 (5)	1 (11)
Palliative endoscopy	3 (14)	1 (11)

HIVEC – hyperthermic intravesical chemotherapy; BCG – Bacillus Calmette-Guérin; n – number

Table 5. *Relationship between recurrence-free survival and indicators of Ki-67 nuclear antigen expression*

Parameter		HR	95%CI	P-value
Group of the BCG	Ki-67 primary	1.02	0.97–1.06	0.520
	Ki-67 recurrent	1.06	1.01–1.11	0.021
Group of the HIVEC®	Ki-67 primary	1.06	0.95–1.18	0.288
	Ki-67 recurrent	1.05	0.95–1.18	0.336

HIVEC – hyperthermic intravesical chemotherapy; BCG – Bacillus Calmette-Guérin

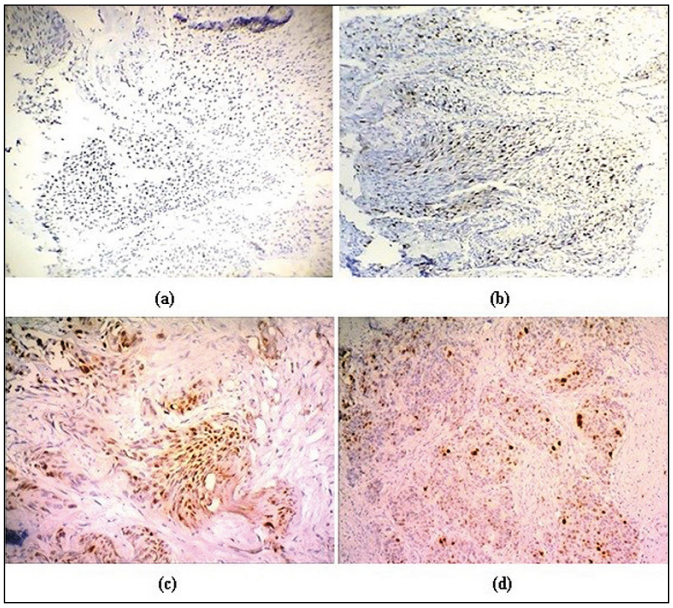

Figure 2. *Ki-67 example BCG HIVEC.*
BCG – Bacillus Calmette-Guérin; HIVEC – hyperthermic intravesical chemotherapy

The mechanism of the heated mitomycin C action is mainly explained by the direct cytotoxic effect on tumor cells: 1) linear growth arrest in the M and S phases of the cell cycle; 2) short-term decrease in RNA synthesis; 3) long-term decrease in DNA synthesis; 4) damage to DNA repair systems [19]. Accordingly, chemohyperthermia directly affects the Ki-67 nuclear antigen expression, markedly reducing it while maximal concentration in the cytoplasm.

In the future, as data accumulate, the determination of the Ki-67 expression level may be an additional prognostic factor for the primary stratification of patients. In moderate and high-risk groups NMIBC with a Ki-67 level of 20% and above, an intravesical adjuvant chemohyperthermia might be is a preferable therapeutic option.

The expression level of the Ki-67 marker might be a factor of changing the tactics of bladder-preserving treatment in the recurrent NMIBC. In case of the BCG-unresponsive tumor and the Ki-67 level ≥20% no doubt radical cystectomy is the option of choice. But in case of Ki-67 expression level less than 20%, chemohyperthermia might be used after BCG failure. In case of late recurrence after a full course of BCG therapy, hyperthermic chemotherapy is possible in patients with a Ki-67 expression level of 14% and higher. In case of recurrence after hyperthermic chemotherapy with a Ki-67 marker level ≥20%, radical cystectomy is the option of choice. When the expression level is below 20%, a full repeated course of hyperthermic chemotherapy or BCG therapy might be recommended.

Limitations of this study include the small cohort of patients and its retroprospective non-randomized nature. Given the retrospective nature of the study, the BCG therapy group began to form before the introduction of the method of hyperthermic chemotherapy into practice; this is associated with the differences in the medians of observation of patients in both groups. In the future, an increase in the number of relapses can be expected in the group of hyperthermic intravesical chemotherapy.

CONCLUSIONS

Assessment of Ki-67 expression in recurrent tumors can be a criterion for the effectiveness of intravesical bladder-preserving treatment and influence the choice of subsequent therapy tactics. The use of hyperthermic chemotherapy can reduce the number of radical cystectomies in a selected group of patients with high risk NMIBC. The high expression level of Ki-67 after adjuvant BCG therapy is a risk factor for recurrence-free survival (HR 1.06; p = 0.021).

a) High Ki-67 antigen expression in primary tumor; b) High Ki-67 antigen expression in recurrent tumor in the same patient after intravesical adjuvant BCG therapy; c) High Ki-67 antigen expression in primary tumor; d) Decreased Ki-67 antigen expression in recurrent tumor in the same patient after intravesical adjuvant hyperthermic chemotherapy.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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