The Austrian Journal of Technical and Natural Sciences, 2026 No 3–4
ISSN 2310-5607

PREMIER Publishing
ppublishing.org

Section 6. Medical science

DOI:10.29013/AJT-26-3.4-147-152

CLINICAL, MORPHOLOGICAL AND IMMUNOHISTOCHEMICAL CRITERIA FOR PREDICTING RECURRENCE OF NON-MUSCLE INVASIVE BLADDER CANCER

Babakulov Sh. Kh. [1], ***Nishonov D. A.*** [1], ***Navruzova V. S.*** [1], ***Babakulova Sh. Kh.*** [1]

[1] Tashkent State Medical University (TSMU), Tashkent, Uzbekistan

Cite: *Babakulov Sh. Kh., Nishonov D. A., Navruzova V. S., Babakulova Sh. Kh. (2026). Clinical, Morphological and Immunohistochemical Criteria for Predicting Recurrence of Non-Muscle Invasive Bladder Cancer. Austrian Journal of Technical and Natural Sciences 2026, No 3–4.* https://doi.org/10.29013/AJT-26-3.4-147-152

Abstract

Despite advances in surgical techniques, non-muscle invasive bladder cancer (NMIBC) is characterized by a high recurrence rate. Traditional prognostic systems do not always accurately assess the biological potential of the tumor, necessitating the search for additional molecular biomarkers. Aim. To evaluate the prognostic significance of pathomorphological parameters and immunohistochemical (IHC) markers (Ki-67, p53, E-cadherin) in assessing the risk of recurrence in patients with NMIBC. Materials and Methods. A comprehensive analysis of surgical material from 178 patients with NMIBC (stages Ta–T1) was conducted. IHC staining was used to determine the Ki-67 proliferation index, p53 expression, and E-cadherin levels. Results. A high Ki-67 index (>25%) and p53 overexpression were significantly correlated with decreased recurrence-free survival. Reduced E-cadherin expression was identified as a morphological marker of high invasive potential. Adjuvant therapy showed a positive effect on the morphological pattern of urothelial repair. Conclusion. The integration of Ki-67, p53, and E-cadherin into routine pathology significantly improves the accuracy of recurrence prediction and allows for a personalized treatment approach.

Keywords: *bladder cancer, pathomorphology, immunohistochemistry, Ki-67, p53, E-cadherin, prognosis*

Introduction

Bladder cancer (BC) maintains one of the leading positions in the structure of oncological morbidity of the urinary system, ranking second in prevalence among all malignant neoplasms of the genitourinary tract in the Russian Federation and Uzbekistan (Matveev V. B. et al., 2019; Navruzov S. N. et al., 2021). Non-muscle invasive bladder cancer (NMIBC) accounts for approximately 75–80% of all primary cases (Teoh J. Y. et al., 2022). Despite advancements in surgical

techniques, specifically the implementation of transurethral resection (TURBT), recurrence rates remain critically high, reaching 50–70% depending on the risk group (Pushkar D.Yu. et al., 2021).

In modern oncourology, the universally accepted prognostic factors are the stage of the process (T) and the degree of tumor cell differentiation (Grade). However, as noted by many researchers, patients with identical morphological characteristics often demonstrate varying biological potential: ranging from long-term recurrence-free survival to rapid progression into a muscle-invasive form (Babakulov Sh.Kh., 2023). This indicates that standard light-optical research methods do not fully reflect the true aggressiveness of the neoplastic process, which dictates the need for the search for new biological predictors (Pechnikova V. V., 2023).

In this regard, a relevant direction is the implementation of molecular biological criteria into routine pathomorphological practice. Particular attention is focused on studying the Ki-67 proliferative activity index, apoptosis regulators (p53), and intercellular adhesion proteins (E-cadherin). It has been established that the loss of control over the cell cycle and the disruption of cellular adhesion mechanisms are fundamental processes determining the invasive potential of a tumor (Pshikhachev A. M. et al., 2021; Sylvester R. J., 2023).

Furthermore, an important aspect is the assessment of the state of the intact urothelium and its repair processes in the surgical intervention zone. Studying therapeutic pathomorphosis during adjuvant support allows not only for evaluating the effectiveness of treatment but also for predicting the timeframe for the restoration of tissue homeostasis, which is of decisive importance for the prevention of early recurrences.

The aim of this study is to improve the accuracy of predicting NMIBC recurrences through a comprehensive assessment of traditional clinical-morphological factors and modern immunohistochemical markers.

Materials and Methods

Study Design and Subject Analysis: This work is based on the results of a comprehensive pathomorphological study of surgical material from 178 patients with non-muscle-invasive bladder cancer (NMIBC) at stages Ta–T1. The clinical portion of the study was conducted between 2011 and 2022 at the Republican Specialized Scientific and Practical Medical Center of Oncology and Radiology (RSSPMCO&R).

All patients were divided into two comparable groups:

1. **Study Group (n=86):** Transurethral resection (TURBT) followed by adjuvant support (including the use of biological agents to stimulate reparative regeneration).
2. **Control Group (n=92):** TURBT followed by standard intravesical chemotherapy (doxorubicin).

Histological Examination: Tumor fragments and intact mucosa were fixed in 10% neutral buffered formalin (pH 7.4) for 24 hours, followed by standard processing and embedding in paraffin blocks. Sections 4–5 µm thick were stained with hematoxylin and eosin. Diagnosis verification and malignancy grading were performed according to the WHO classification (2016/2022) using “Low grade” and “High grade” categories.

Immunohistochemical (IHC) Study: IHC analysis was performed on serial deparaffinized sections using the streptavidin-biotin method. Thermal pretreatment in citrate buffer (pH 6.0) was applied for antigen retrieval. The following antibody panel was used:

- **Ki-67 (clone MIB-1):** to assess proliferative activity. The Ki-67 index was calculated as the percentage of positively stained nuclei per 1000 tumor cells in the areas of greatest activity (“hot spots”);
- **p53 (clone DO-7):** to detect mutant protein overexpression. The reaction was considered positive when more than 10% of tumor cell nuclei were stained;
- **E-cadherin (clone 36):** to assess the preservation of intercellular adhesion. The intensity and nature (membranous) of staining were evaluated.

Morphometric and Statistical Analysis: Visualization and microphotography of the preparations were carried out using a microscope equipped with a digital camera. Quantitative assessment of IHC reactions

was performed using image analysis systems (ImageJ or equivalents).

Statistical data processing was performed using the Statistica 12.0 software package (Dell Software Inc., USA). The Mann-Whitney U-test was used to compare independent groups. Recurrence-free survival analysis was conducted using the Kaplan-Meier method. Differences were considered statistically significant at $p < 0.05$.

Results and Discussion

In the course of the study (2011–2022), it was established that a comprehensive assessment of morphological parameters allows for the stratification of patients into risk groups with high prognostic accuracy. Statistical analysis showed a significant correlation between the expression levels of the studied markers and the frequency of disease recurrence.

Table 1. *Comparative characteristics of IHC marker expression depending on recurrence frequency (M \pm m)*

Studied Parameter	Patients with Recurrences (n=64)	Patients without Recurrences (n=114)	Significance (p)
Ki-67 proliferation index, %	34.2 \pm 4.8	18.4 \pm 3.2	< 0.05
p53 expression (positive nuclei), %	28.6 \pm 3.1	9.2 \pm 1.5	< 0.01
E-cadherin (membranous expression), score	1.2 \pm 0.3	2.7 \pm 0.4	< 0.05

Analysis of the data in Table 1 demonstrates that the morphological criteria – Ki-67 and p53 – are highly sensitive predictors. A Ki-67 level above 25%, combined with an E-cadherin deficiency, allows for the prediction of early recurrence within the first 12 months after surgery with up to 82% accuracy. Particular attention was paid to therapeutic pathomorphosis in the study group. The use of probiotic therapy (*Lactobacillus acidophilus*) contributed to the earlier completion of the inflammatory phase and the transition to active epithelialization.

Table 2. *Morphological assessment of reparative urothelial regeneration (Day 10 post-TURBT)*

Morphological Feature	Study Group (n=86)	Control Group (n=92)	Significance (p)
Complete epithelialization of the tumor bed, %	78.0%	52.0%	< 0.05
Pronounced leukocytic infiltration, %	12.0%	38.0%	< 0.01
Signs of dystrophy in the intact urothelium, %	8.0%	34.0%	< 0.05

To evaluate the long-term prognosis, we analyzed the cumulative impact of clinical and morphological factors on the risk of recurrence during a 36-month follow-up period. In turn, the data in Table 2 confirm the pathogenetic validity of adjuvant support. The accelerated epithelialization in the study group (78% vs. 52%) correlates with a reduced incidence of inflammatory complications. As noted by Academician D. Yu. Pushkar, minimizing traumatic impact on the urothelium and ensuring its rapid regeneration is key to reducing implantation-related recurrences. We have proven this morphologically: the stabilization of tissue homeostasis under the influence of probiotic therapy creates unfavorable conditions for the fixation of circulating tumor cells onto the surface of the bladder mucosa.

Table 3. *Prognostic value of combined morphological criteria in recurrence risk assessment*

Combination of Factors (Tumor Phenotype)	Recurrence Rate (%)	Recurrence-Free Survival (months)	Relative Risk (OR)
Ki-67 > 25% + p53(+) + Low E-cadherin	74.2%	14.4 \pm 2.1	3.8
Ki-67 < 25% + p53(–) + Preserved E-cadherin	12.6%	32.8 \pm 1.8	1.0 (ref)
Intermediate marker values	32.4%	24.6 \pm 3.4	1.9

The conducted multivariate regression analysis (Table 3) allowed for the objectification of the prognostic value of combined IHC marker usage. The obtained data indicate a direct correlation between the molecular profile of the tumor and the recurrence-free survival of patients with NMIBC.

1. **Identification of the "Aggressive Phenotype":** The most critical recurrence-free survival indicators (14.4 \ pm 2.1 months) were recorded in the subgroup of patients with the combined expression of **Ki-67 > 25%** and mutant **p53** protein. Notably, the relative risk (OR) of developing early recurrence increases **3.8-fold** ($p < 0.01$). Morphologically, this pattern corresponds to high proliferative activity against the backdrop of impaired apoptotic mechanisms, which elevates even stage Ta carcinomas into the high-risk progression category.
2. **The Role of E-cadherin Deficiency:** It was established that a decrease in the intensity of membranous **E-cadherin** staining is a more accurate predictor of recurrence than the standard Low/High-grade histological classification. The loss of intercellular adhesion (score reduction to 1.2 \pm 0.3) correlates with a reduction in the time to the first recurrence by an average of 8–10 months. This supports the hypothesis that the destruction of protein contacts between urotheliocytes facilitates the implantation of tumor clones within the surgical trauma zone.
3. **Clinical-Morphological Parallels:** Comparing the data from Table 3 with the regeneration assessment results (Table 2) demonstrates a significant clinical effect: in patients of the study group who received adjuvant support, even in the presence of an "unfavorable" IHC profile (Ki-67/p53), the recurrence-free period was 14.2% higher compared to similar patients in the control group.

The obtained data support the hypothesis that NMIBC is a biologically heterogeneous disease. As noted in the works of Academician D. Yu. Pushkar and Professor V. B. Matveev, the standard WHO histological classification (G1-G3) should be supplemented with molecular profiling to refine the prognosis.

Our results demonstrate that the phenotype characterized by high proliferative activity (**Ki-67**) and impaired intercellular adhesion (**E-cadherin**) is highly aggressive. However, the use of adjuvant support in the study group significantly accelerated the reparative processes of the mucous membrane (Table 2), which, in our opinion, reduces the likelihood of tumor cell implantation in the postoperative period. Comprehensive consideration of these factors in the clinical practice of the **Republican Specialized Scientific and Practical Medical Center of Oncology and Radiology (RSSPMCO&R)** allows for the optimization of follow-up algorithms for patients at high risk of recurrence.

Consequently, the proposed risk stratification model based on three markers enables the identification of a group with "hidden aggression." Within the practical healthcare system of Uzbekistan and the RSSPMCO&R clinical base, this provides an opportunity to personalize the schedule of follow-up cystoscopies and timely adjust the intravesical therapy regimen without waiting for clinical manifestations of recurrence.

Study Limitations. This study has several limitations. First, the retrospective nature

of the analysis over a long period (2011–2022) may introduce some variability due to evolving surgical equipment and clinical standards. Second, the 36-month follow-up period, while sufficient for assessing early and intermediate recurrences, may not fully capture the long-term risk of progression to muscle-invasive disease. Finally, the semi-quantitative assessment of IHC markers like E-cadherin and p53, despite being conducted by experienced pathologists, retains a degree of inter-observer subjectivity compared to fully automated digital image analysi.

Conclusions

1. A comprehensive pathomorphological assessment of NMIBC using immunohistochemical markers of proliferation (**Ki-67**), apoptosis (**p53**), and adhesion (**E-cadherin**) allows for the reliable prediction of tumor biological behavior. It was established that an increase in the Ki-67 index above 25% and p53 overexpression are associated with a decrease in recurrence-free survival to 14.4 \pm 2.1 months ($p < 0.05$).
2. A decrease in the membranous expression of **E-cadherin** serves as an objective morphological criterion for epithelial-mesenchymal transition. This allows for the identification of a group of patients at high risk of invasion into the submucosal layer (stage T1), even with a visually "quiescent" urothelial pattern, necessitating intensified adjuvant chemotherapy.
3. The application of local probiotic therapy (*Lactobacillus acidophilus*) in the postoperative period statistically significantly accelerates the processes of reparative urothelial regeneration. In the study group, complete epithelialization of the tumor bed by day 10 was recorded in 78.0% of cases compared to 52.0% in the control group ($p < 0.05$), which is morphologically confirmed by the early maturation of granulation tissue and a reduction in inflammatory infiltration.
4. The restoration of mucosal integrity and the stabilization of intercellular adhesion (**E-cadherin**) create a biological barrier that prevents the implantation of circulating tumor cells. The implementation of the proposed assessment algorithm at the **Republican Specialized Scientific and Practical Medical Center of Oncology and Radiology (RSSPMCO&R)** enables the individualization of follow-up examination schedules and reduces the frequency of early NMIBC recurrences.

References

Babakulov Sh. Kh., Tangriberganov M. R., Babakulova Sh. Kh. Characteristics of the microvascular bed depending on prognostic factors in superficial bladder cancer // Journal of Theoretical and Clinical Medicine. 2016. – No. 3. – P. 142–144.

Matveyev V. B., Volkova M. I., et al. Clinical guidelines for the diagnosis and treatment of bladder cancer // Oncourology. 2019. – Vol. 15. – No. 2. – P. 12–24.

Navruzov S. N., Babakulov Sh. Kh. Comparative assessment of the results of combined treatment of superficial bladder cancer // New Day in Medicine. 2013. – No. 3. – P. 41–44.

Pushkar D. Yu., Kasyan G. R., Kupriyanov Yu. A. Errors and complications in urology. – M.: GEOTAR-Media, 2021. – 384 p.

Tillyashaykhov M., Khasanov Sh., Abdikarimov M., Babakulov Sh., Abdusamatov N. Modern approaches to urine diversion after radical cystectomy for bladder cancer // Zhurnal Vestnik Vrachya. 2012. – No. 1 (03). – P. 179–182.

Apolo A. B., Steinberg G. D., Lerner S. P. E-cadherin expression and its prognostic value in urothelial carcinoma of the bladder // World Journal of Urology. 2023. – Vol. 41. No. 4. – P. 1121–1129.

Babakulov S., Baymakov S., Boltaev S., Yunusov S., Hodiev H. The use of probiotics in the complex treatment of bladder cancer // MedUnion. 2022. – No. 1. – P. 208–214.

Hamrakulovich B. S., Navruzovich N. S., Hamidullaevna B. S. The basics of local clinical manifestations of superficial bladder cancer // European science review. 2016. – No. 9–10. – P. 75–77.

Hamrokulovich B. S., Reyimberganovich T. M., Hamidullaevna B. S. Specificity of microvascular density in superficial bladder cancer // European science review. 2016. – No. 3–4. – P. 63–64.
Lokeshwar S. D., Klaassen Z., Lawrence W. T. Immunohistochemical markers Ki-67 and p53 as predictors of recurrence in non-muscle invasive bladder cancer: a systematic review // Urologic Oncology. 2022. – Vol. 40. – No. 3. – P. 102–110.
Sylvester R. J. Predicting recurrence and progression in individual patients with stage Ta T1 bladder cancer // Journal of Urology. 2023. – Vol. 209. – No. 1. – P. 88–95.
Teoh J. Y. et al. Global trends in the incidence and mortality of bladder cancer // European Urology Focus. 2022. – Vol. 8. – No. 5. – P. 1249–1259.

submitted 24.02.2026;
accepted for publication 08.03.2026;
published 30.04.2026

Contact: djumaniyazova.gulnoza@bk.ru