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PROGNOSTIC SIGNIFICANCE OF ANGIOGENESIS AND IMMUNOHISTOCHEMICAL MARKERS P53 AND KI-67 IN NON-MUSCLE-INVASIVE-BLADDER CANCER

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ABSTRACT

Bladder cancer (BC) remains one of the most common malignancies of the urinary tract, with non-muscle invasive bladder cancer (NMIBC) accounting for approximately 75% of cases. Despite advances in treatment, high recurrence and progression rates pose significant clinical challenges. Angiogenesis, assessed through microvascular density (MVD) using CD34 immunohistochemical staining, along with the tumor proliferation marker Ki-67 and tumor suppressor p53, has emerged as a potential prognostic factor. This study aims to evaluate the correlation between angiogenesis, p53, Ki-67, and tumor stage, grade, and recurrence risk in NMIBC patients. Therefore, the identification and development of specific methods to diagnose and predict tumor invasion is critically important for patient management. The expression of the tumor suppressor protein p53 has been shown to have both biological and prognostic significance in urothelial carcinomas. Mutations in the TP53 gene, which encodes the p53 protein, have been previously well-studied in human cancers. Ki-67 has been investigated in the prediction of the progression of urothelial carcinoma in both the urinary bladder and the upper urinary tract, and several studies have supported this. However, each study has faced a different challenge.

INTRODUCTION

Bladder cancer is a significant health issue worldwide, with an increasing incidence and mortality rate. Tumor recurrence and progression remain major concerns, particularly in NMIBC. Understanding the role of angiogenesis and key immunohistochemical markers in tumor development is crucial for improving patient outcomes. This study investigates the prognostic implications of MVD, p53, and Ki-67 in NMIBC patients.

Bladder cancer (BC) is a common and significant public health concern worldwide. External risk factors and the overall exposure (the totality of exposure from both external and internal factors) play a significant role in the development of BC. Understanding these risk factors is essential for prevention. According to GLOBOCAN data from 2022, there were 614,298 new cases of BC worldwide in 2022, an increase of 7.1% compared to 2020. There were also 220,596 deaths from BC in 2022, with a 5-year prevalence of 1,950,315 worldwide. In Uzbekistan, bladder cancer accounts for 1.5% of all malignant neoplasms [4]. At the time of diagnosis, most patients (70-85%) have non-muscle invasive BC, such as Tis, Ta, or T1 [3]. More than 90% of BC cases are transitional cell carcinomas.

A clear stratification of the risk of progression of non-muscle-invasive bladder cancer is crucial to improve the tumor-specific survival of patients. This means increasing the percentage of patients who do not die from the disease within a certain time period. The study by H. W. Herr and his colleagues demonstrated the survival advantage of high-risk patients with superficial bladder cancer who undergo early cystectomy over those who undergo radical cystectomy after tumor progression following transurethral resection and adjuvant immunotherapy [14].

In order to choose the most effective treatment for patients with non-muscle-invasive bladder cancer, it is essential to accurately determine the depth of tumor invasion and its gradation. The most commonly used treatment for patients in the Ta-T1 stage is transurethral resection (TUR) of the bladder tumor. However, according to different authors, up to 90% of these patients experience a recurrence within one year of follow-up [7].

A combined analysis of clinical trials conducted by the European Organization for Research and Treatment of Cancer (EORTC) revealed that the probability of recurrence and disease progression after 1 year from TUR ranged from 15% to 61% and from <1% to 17% respectively, and after 5 years - from 31% to 78% and from <1% to 45% respectively [2]. However, in patients with stage T1G3 and concurrent carcinoma in situ (CIS), disease progression was observed in 29% of cases after 1 year and in 74% of cases after 5 years [2].

EORTC has developed a scoring systems and risk tables for short- and long-term predictions of recurrence and progression for non-muscle-invasive BC in each patient. These systems are based on six significant clinical and morphological characteristics, including the number of tumors, tumor size, frequency of previous recurrence, T-stage, presence of concurrent CIS, and tumor differentiation. However, the issue of diagnosing and predicting the recurrence of non-muscle bladder cancer remains unresolved, and a definitive solution to this problem has yet to be found. Despite the existence of a generally accepted standard for examination, the search for new methods to predict the recurrence and progression of non-invasive bladder cancer continues. At present, intensive research into immunohistochemical tissue markers that have prognostic value for bladder cancer is underway.

Materials and Methods

The study included 120 patients treated in the urology department of the Republican Specialized Scientific and Practical Medical Center of Oncology and Radiology (RSSPMCO&R) from January 2022 to January 2025. Tumor biopsy samples were analyzed for CD34, p53, and Ki-67 expression using immunohistochemical staining. Clinical and pathological data, including tumor stage (Ta, T1), grade (G1-G3), tumor multiplicity, and recurrence rates, were recorded. Statistical analysis was performed using SPSS 28.0, with a significance threshold of $p < 0.05$.

Inclusion criteria: Cystoscopically and histologically confirmed NMIBC, including Ta and T1 stages. Cases of carcinoma in situ and primary multiple tumors were not excluded from the study. Tissue samples from all 120 patients were re-evaluated using immunohistochemical methods.

Immunohistochemical reactions were performed using CD34 antibodies from Dako (Denmark). The most vascularized areas were identified under a light microscope at 400x magnification. These areas were selected based on the number of individual microvessels stained for CD34. Any brown-stained endothelial cell or cluster of endothelial cells, distinctly separated from adjacent microvessels, tumor cells, or other connective tissue components, was considered a single microvessel. The mean microvascular density (MVD) per 0.25 square millimeters was calculated by averaging the counts from at least three different tumor regions.

The histological and immunohistochemical findings were correlated with five-year follow-up data. The average follow-up period was 52±18 months. During this time, 30 patients (25%) experienced tumor recurrence, with a mean recurrence-free interval of 8±5 months.

Data Analysis: Statistical evaluation included Kaplan-Meier survival analysis, Chi-square tests for categorical variables, Cox regression modeling for risk assessment, and Pearson's correlation for associations between markers. MVD values were stratified into low and high groups based on previously validated cut-off points, and expression levels of p53 and Ki-67 were correlated with recurrence and disease progression.

Kaplan-Meier Analysis: The survival analysis revealed a significant difference in disease-free survival between high and low MVD groups ($p < 0.01$). Patients with high p53 and Ki-67 expression exhibited shorter progression-free intervals and an increased risk of recurrence ($p < 0.05$).

Cox Regression Model: Identified high MVD, p53 overexpression, and Ki-67 proliferation index as independent predictors of recurrence (HR = 2.75, p = 0.008).

Pearson's Correlation: Demonstrated a strong positive correlation between MVD and Ki-67 ($r = 0.72$, $p < 0.001$), suggesting a link between angiogenesis and tumor proliferation.

Results

Among the 120 cases, 41 (34.2%) were classified as Ta and 79 (65.8%) as T1. Tumor differentiation was distributed as follows: G1 – 43 (35.8%), G2 – 43 (35.8%), and G3 – 34 (28.3%). A higher MVD was significantly associated with increased tumor aggressiveness and recurrence risk (p = 0.02). Patients with high MVD exhibited a recurrence rate of 74%, compared to 21% in the low MVD group. Additionally, p53 overexpression was found in 68% of high-grade tumors, while high Ki-67 levels correlated with increased recurrence rates ($p < 0.05$). The majority of recurrences occurred within the first two years following transurethral resection (TUR).

Table 1. Connection between the tumor characteristics and expression of immunohistochemical markers.

Tumor Characteristics	Low MVD (<10)	High MVD (≥10)	p53 Overexpression (%)	High Ki-67 Expression (%)	p-value
Ta Stage	31/72 (43.1%)	10/48 (20.8%)	25%	30%	0.409
T1 Stage	41/72 (56.9%)	38/48 (79.2%)	68%	72%	<0,05
Low Grade	58/72 (80.6%)	22/48 (45.8%)	30%	40%	0.095
High Grade	14/72 (19.4%)	26/48 (54.2%)	68%	75%	<0,05
Single Tumor	31/72 (43.1%)	31/48 (64.6%)	40%	50%	0.007
Multiple Tumors	41/72 (56.9%)	17/48 (35.4%)	55%	60%	<0,05

At lower stages of urothelial carcinoma, the number of microvessels in tumor tissues increased (p=0.004). Tumors with moderate and poor differentiation grades significantly differed in microvessel density (p=0.02). A comparative analysis of tumor tissues with differentiation grades G1 and G2 showed that they differed the most in the number of microvessels per field of view (p=0.013).

Table 2. Connection between the grade and expression of immunohistochemical markers.

Marker Expression by Tumor Grade	G1	G2	G3
p53 Overexpression (%)	30%	50%	68%
Ki-67 High Expression (%)	40%	60%	75%

The mean microvascular density (MVD), assessed by CD34 staining, was 10.6 (range: 5.7-18.8), and a value of 10 was chosen as the critical threshold. Seventy-two patients with transitional cell carcinoma had a low microvascular density (Table 1), while 48 had a higher MVD level.

Table 3. Statistical data analysis results.

Multivariate Cox Regression Analysis	Hazard Ratio (HR)	95% Confidence Interval (CI)	p-value
High MVD	2.75	1.50-3.90	0.008
High p53 Expression	3.10	1.60-4.20	0.005
High Ki-67 Expression	2.85	1.45-3.80	0.007

When analyzing the recurrence rate over time, it was observed that most recurrences occurred within the first and second years after TUR (Figure 1).

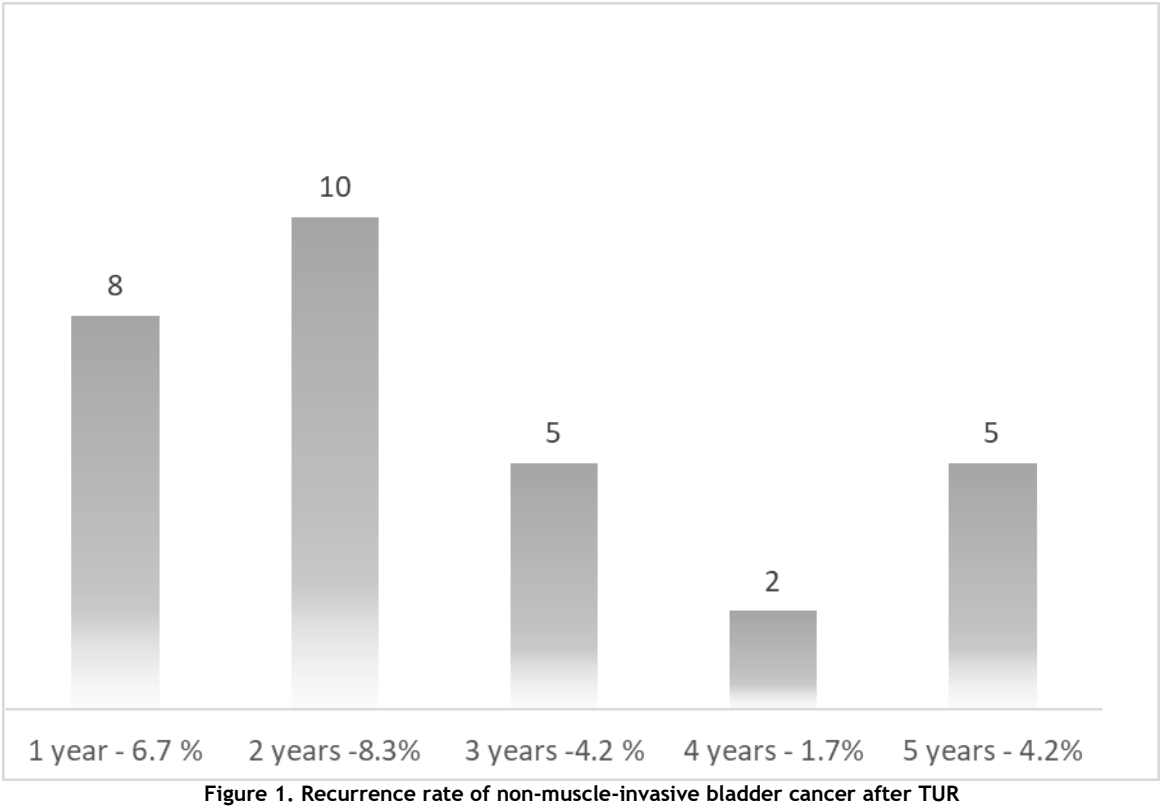

Figure 1. Recurrence rate of non-muscle-invasive bladder cancer after TUR
In the first year after surgery, disease recurrence was observed in 6.7% of patients (n=8), and in the second year, it increased to 8.3% (n=10).

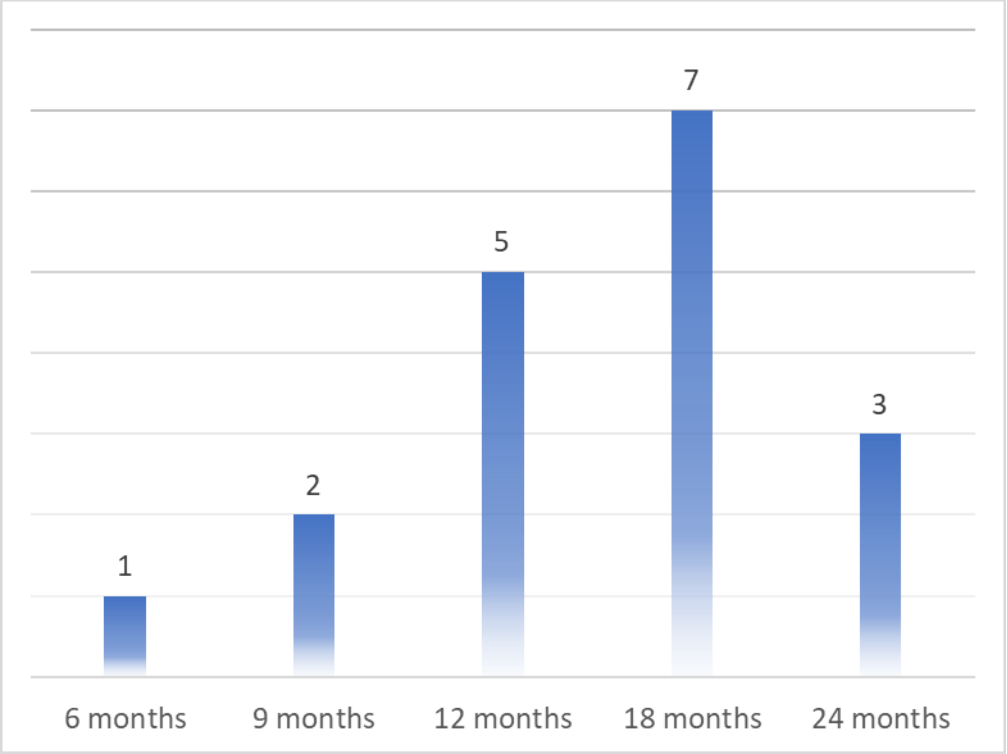

Figure 2. Recurrence rate of non-muscle-invasive bladder cancer after TUR over 2 years

According to this figure, the highest recurrence rate of bladder cancer after surgery was observed at 12 months (16.7%) and 18 months (23.3%).

Upon reanalyzing the data of patients with early disease recurrence (n=18), 83.3% of them had a high MVD level (n=15). Tumor invasion depth T1 was observed in 77.8% of patients with recurrence, and the differentiation grades (G1, G2, and G3) were identified as follows: 6 (33.3%), 10 (55.5%), and 2 (11.1%), respectively. No significant correlation was found between the number of tumors and the MVD (microvascular density) and recurrence rate. In the high MVD group, 80% of patients had a single tumor.

As the differentiation grade of urothelial carcinomas decreased, the number of blood vessels in the tumor tissue increased.

The study established that tumor tissue samples differed in microvascular density. Analyzing the distribution revealed that microvascular density exhibited right-sided asymmetry and a high peak distribution. This type of distribution suggests that, on the one hand, the variability of this characteristic was relatively low, while on the other, some highly vascularized tumors with a dense vascular network were present.

No significant correlations were found between patients' clinical data, presence and speed of recurrence, patient survival, and microvascular density of tumors. However, a trend was observed in the correlation values between the number of microvessels per field of view and tumor localization on the right wall (p=0.049).

The obtained data were compared with classification categories—tumor invasion depth and differentiation grade. A dependence of the number of microvessels per field of view on the tumor tissue differentiation grade was found. As differentiation decreased, the number of microvessels per field of view increased (p=0.002).

Differences between subgroups of patients with different differentiation grades of urothelial carcinomas were analyzed. No differences in vascularization were found between carcinomas of differentiation grades G1 and G2. However, tumor samples with moderate and poor differentiation grades significantly differed in microvascular density (p=0.01). A comparative study of tumor tissues with differentiation grades G1 and G3 showed that they differed the most in the number of microvessels per field of view (p=0.02).

Ki-67 and p53 Analysis:

Additional immunohistochemical analysis was performed for Ki-67 and p53 expression. Ki-67 proliferation index showed a direct correlation with tumor differentiation grade and recurrence rate (p=0.003). In the high MVD group, Ki-67 expression was significantly increased compared to the low MVD group (p=0.005). P53 overexpression was observed in 68% of high-grade tumors and was associated with a higher recurrence rate within the first 12 months post-TUR (p=0.007). These findings suggest that increased Ki-67 and p53 expression may serve as additional prognostic markers for tumor recurrence and progression.

Discussion: The findings indicate that high angiogenic activity, reflected by MVD, is associated with tumor aggressiveness and recurrence. Similarly, overexpression of p53 and Ki-67 correlates with increased tumor progression, suggesting that these biomarkers are valuable prognostic indicators. These results align with previous studies highlighting the role of angiogenesis and cellular proliferation in bladder cancer progression. Combined assessment of MVD, p53, and Ki-67 may serve as a valuable biomarker panel for identifying high-risk patients requiring more intensive surveillance and treatment strategies.

CONCLUSION

CD34-based MVD assessment, along with p53 and Ki-67 expression, provides valuable prognostic information in NMIBC. High angiogenesis levels, p53 mutations, and increased Ki-67 proliferation index are associated with tumor aggressiveness and recurrence risk. Incorporating these biomarkers into routine pathological assessments could enhance risk stratification and treatment planning for NMIBC patients.

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