

## CLINICAL STUDY

# The prognostic value of E-cadherin and Ki-67 compared to standard histopathologic examination in non–muscle invasive bladder cancer

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**ABSTRACT**

**OBJECTIVES:** The objectives of this study were to determine the prognostic value of expression levels of selected biomarkers and their statistical analysis in relation to survival and standard histopathologic examination and other clinicopathologic variables in non-muscle invasive bladder cancer (NMIBC).

**BACKGROUND:** Worldwide, bladder cancer is a frequent malignant disease with rising incidence. Characteristic invasiveness and high recurrence rates call for more diagnostic methods to obtain more accurate information. Prognosis is affected by a significant interpersonal variability of the disease. For this reason, constant search for alternative and better diagnostic methods is essential.

**METHODS:** We analysed cancer tissue from patients with Ta and T1 bladder cancer. E-cadherin and Ki-67 expression levels were analysed using immunohistochemical staining. The expression levels quantified to a percentual amount were statistically analysed in relation to survival and their frequency distribution in the study group.

**RESULTS:** E-cadherin and Ki-67 expression levels show high association with tumor stage and grade ( $p < 0.001$ ), in contrast, the association with recurrence has proven insignificant. Patients with non-aberrant biomarker expression levels have much higher survival rates than the cases with aberrant expression.

**CONCLUSION:** Low expression levels of Ki-67 and high expression levels of E-cadherin positively affect survival of patients, whereas aberrant expressions pose poorer prognosis (*Tab. 2, Fig. 2, Ref. 33*). Text in PDF [www.elis.sk](http://www.elis.sk).

**KEY WORDS:** NMIBC, E-cadherin, Ki-67, survival, prognosis.

**Introduction**

Bladder cancer is the ninth most common malignant disease in the world. In the USA, 81 190 new cases were diagnosed and 17 240 cancer deaths were recorded in 2018 (1). In 2018, the EU reported the estimated incidence and mortality in relation to bladder cancer as 197 105 and 64 966 cases, respectively. Moreover, the incidence has a rising tendency (2). It occurs in both sexes, but men tend to be affected more often (3.2:0.9 ratio), and the disease

incidence increases with age. High prevalence with the tendency to multiple recurrences and progression despite local therapy, leads to a significant economic burden. In a study by Leal et al, this was estimated at € 4.9 billion, with health care accounting for € 2.9 billion in 2012 (3).

The majority of diagnosed cases (approximately 75 % to 85 %) are non–muscle invasive bladder cancer (NMIBC) confined to the mucosa (stage Ta) or submucosa (stage T1) (4). NMIBC with its characteristic high recurrence rate can sometimes progress to the muscle-invasive type with fast metastasizing and extremely poor prognosis (5).

Prognosis is complicated by the significant interpersonal variability of the disease. For this reason, constant search and testing of suitable biomarkers is essential in order to obtain more accurate information to allow more precise prediction of disease behavior.

Recently, E-cadherin and Ki-67 attracted attention in the mentioned context. E-cadherin is a transmembrane glycoprotein, which has an essential role in maintaining the integrity and homeostasis of the urothelial tissue. Moreover, it has been shown that E-cadherin regulates cadherin-mediated cell recognition and adhesion (6). Loss of E-cadherin expression has been associated with high grade and advanced stage of NMIBC and predicts poor prognosis (7), it also

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promotes epithelial-mesenchymal transition (EMT), which plays a pivotal role in invasiveness and metastasis progression. EMT is characterized by loss of polarity in epithelial cells and their acquisition of mesenchymal cell properties. Cadherins and their functions in a growing tumor are considered biomarkers of EMT (8).

Ki-67 (also known as MKI67) is a nuclear protein associated with ribosomal RNA transcription and cell proliferation. It can be detected in cell nuclei in the G1, S and G2 phases of the cell cycle and in mitosis. However, the exact function of Ki-67 in the cell cycle regulation is still unclear (9). Several reports showed that Ki-67 is an independent poor prognostic factor for NMIBC (10, 11). The expression level of the Ki-67 significantly correlates with recurrence rate and histopathological differentiation of the tumor (12, 13).

The main goal of the present study was to evaluate the prognostic value of immunohistochemical expression of E-cadherin and Ki-67 in NMIBC patients and to correlate their expression levels with other clinicopathological variables (stage, grade, recurrence).

## Materials and methods

The study group consisted of 342 patients diagnosed with NMIBC in stages Ta and T1. The median age was 68 years (range, 34–98 years), with 264 men and 78 women. The median follow-up time was 84.5 months (range 1–179 months). These patients underwent transurethral resection of the bladder tumor (TURBT) at the Department of Urology (Faculty of Medicine, Comenius University in Bratislava, Bratislava, Slovakia) in the period from 2005 to 2019. After resection, sections of the bladder tumor were histopathologically analysed by the Institute of Forensic Medicine (Faculty of Medicine, Comenius University in Bratislava, Bratislava, Slovakia). Medical history of patients and results of histopathologic examination were used as source material for this study. This study was approved by the local ethics committee. All samples were collected in accordance with The Helsinki Declaration.

### Immunohistochemical examination

The cancer tissues were graded and classified according to the World Health Organization classification from the year 1998 and the assessment of the clinical stage of cancer was based on the sixth edition of TNM (Tumor-Node-Metastasis) staging system by the International Union Against Cancer (UICC, Union Internationale Contre le Cancer) from the year 2002 (14, 15).

Tissue microarrays (TMA) were prepared from formalin-fixed paraffin blocks of tissue, whose representativeness was proved by histopathological comparison of TMA with original sections of the whole tumor. Sections of 4  $\mu$ m were prepared by microtome and then placed on poly L-lysine coated slides. Immunohistochemical (IHC) analysis was performed on prepared tissue samples using the Leica ST 5050 immunostainer using the avidin-biotin peroxidase method with diaminobenzidine as chromogen according to the instructions from the manufacturer.

Microscopic assessment of immunohistochemical staining for E-cadherin and Ki-67 was performed using primary antibodies

(E-cadherin: NCH38 clone: DAKO, Glostrup, Denmark; 1: 100 and Ki-67: murine monoclonal clone MIB-1, DAKO, Hamburg, Germany) with dilution performed using standard staining procedures (16).

Expression levels represented by the staining levels were assessed semiquantitatively by 5% steps using a modified version of previously used scoring system (17). Examination results of E-cadherin were set to three intervals: “low” when the nuclear staining of tumor cells was  $\leq 50\%$ ; “intermediate or heterogenous” when the nuclear staining of tumor cells was more than 50% and less than 75% and “high” if above 75%. Examination results of Ki-67 were set to two intervals: “low” when the nuclear staining of tumor cells was  $\leq 15\%$  and “high” if staining exceeded 15%.

### Statistical analysis

Statistical analysis was performed using SPSS v.25.0 (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.). Pearson’s chi-squared test ( $\chi^2$ ) was used to analyse the association between the frequency distribution of the staining levels and the tumor stage, grade and recurrence. Survival was calculated by the Kaplan-Meier survival analysis using the follow-up time counted in months from the initial histopathological examination or the initiation of treatment, to the death of a patient, or the last follow-up visit, which were taken as the end points of the Kaplan-Meier survival analyses. The Log-rank test was used to identify the statistical differences. The results of the statistical tests were considered significant if the p-value was lower than 0.05.

## Results

In our study, out of 342 patients, 249 (72.8%) were initially diagnosed with Ta and 93 (27.2%) with T1 bladder cancer (BC). 231 (67.5%) tumors were low-grade and 111 (32.5%) were high-grade. In 122 (35.7%) cases, patients experienced cancer recurrence and 220 (64.3%) tumors were newly diagnosed. Papillary tumors represented 316 (92.4%) cases, solid and mixed 14 (4.1%) and 12 (3.5%), respectively. Death occurred in 189 (55.3%) cases.

Table 1 represents the frequency distribution in the study group, where the biomarker expression levels were analyzed in association with tumor stage, grade and recurrence. These results

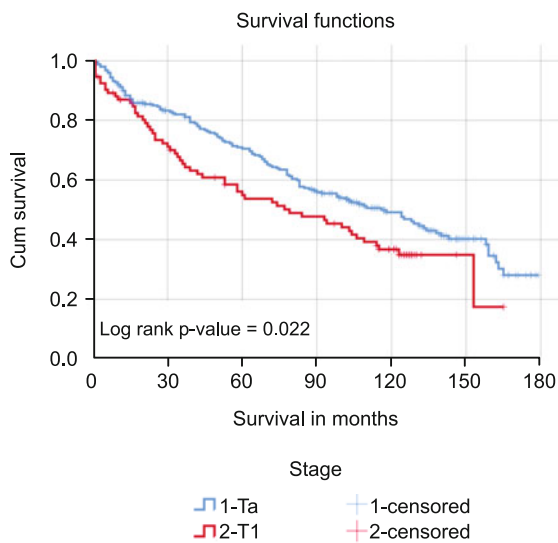
**Tab. 1. Frequency distribution in the study group. E-cadherin and Ki-67 in association with tumor stage, grade and recurrence.**

Variables	E-cadherin (%)			Ki-67 (%)		
	$\leq 50$	50-75	$> 75$	$\leq 15$	$> 15$	
Stage	pTa	26	54	169	107	
	pT1	35	27	31	62	
	$\chi^2$ p-value	$< 0.001$			$< 0.001$	
Grade	LG	19	50	162	140	91
	HG	42	31	38	33	78
	$\chi^2$ p-value	$< 0.001$			$< 0.001$	
Recurrence	Yes	25	28	69	56	66
	Newly diagnosed	36	53	131	117	103
	$\chi^2$ p-value	0.634			0.197	

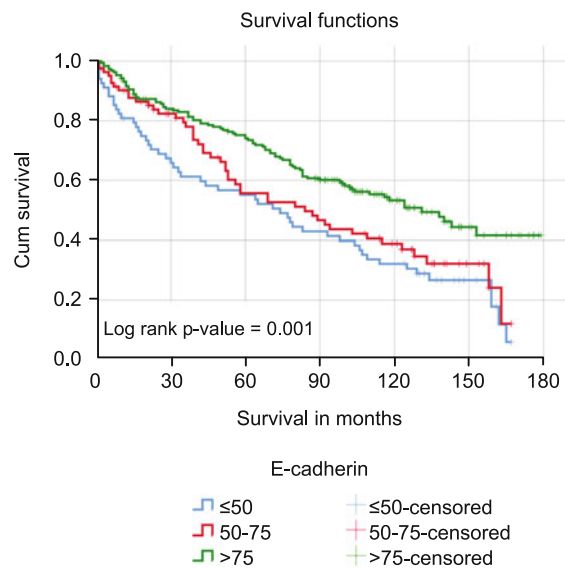
**Tab. 2. Frequency distribution within the Ta stage group. E-cadherin and Ki-67 in association with tumor grade and recurrence.**

Variables		E-cadherin (%)			Ki-67 (%)	
		≤50	50-75	>75	≤15	>15
Grade	LG	13	42	155	132	78
	HG	13	12	14	10	29
$\chi^2$ p-value		< 0.001			< 0.001	
Recurrence	Yes	11	19	64	52	42
	Newly diagnosed	15	35	105	90	65
$\chi^2$ p-value		p = 0,826			p = 0,671	

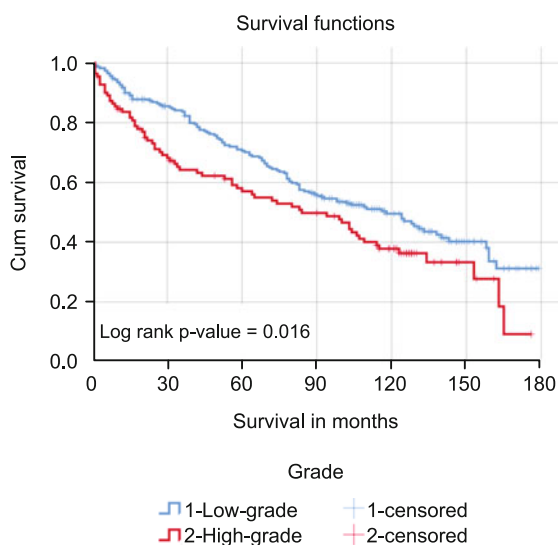
show significant association of E-cadherin and Ki-67 with tumor stage and grade ( $p < 0.001$ ), in contrast, the association with recurrence has proven insignificant. Due to the greater size of Ta stage group, we conducted one more test of frequency distribution within this group (T1 group not taken in consideration). Results presented in Table 2 show the association of biomarker expression levels in correlation with tumor grade ( $< 0.001$ ) and recurrence, but recurrence was not significantly associated with the expression levels of E-cadherin and Ki-67.



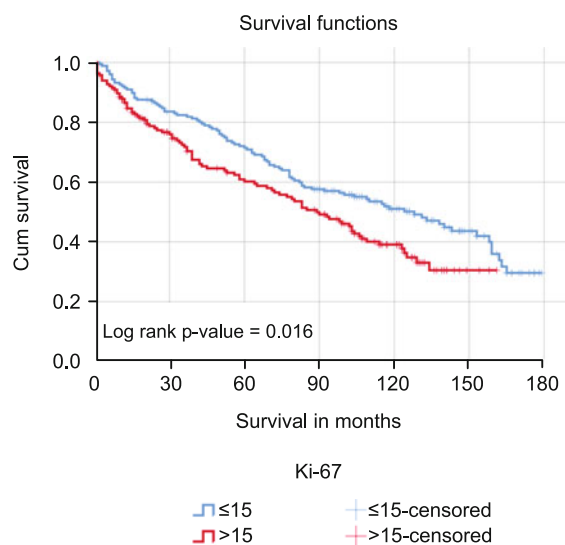
**Fig. 1. Kaplan–Meier survival curve showing a statistically significant difference in survival between Ta and T1 stage groups.**



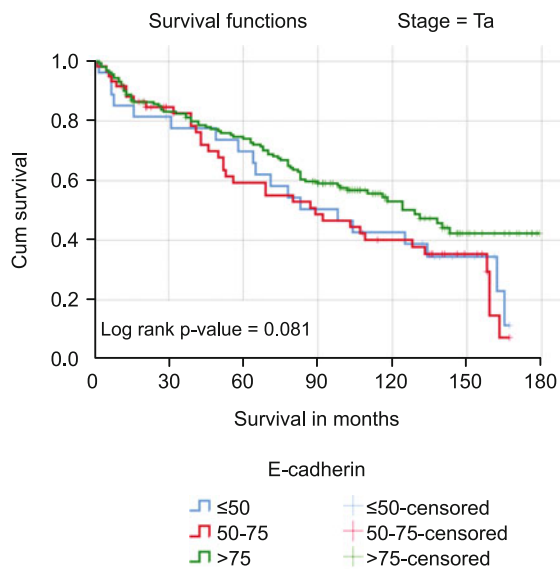
**Fig. 3. Kaplan–Meier survival curve showing a statistically significant difference in survival of patients in association to E-cadherin expression levels.**



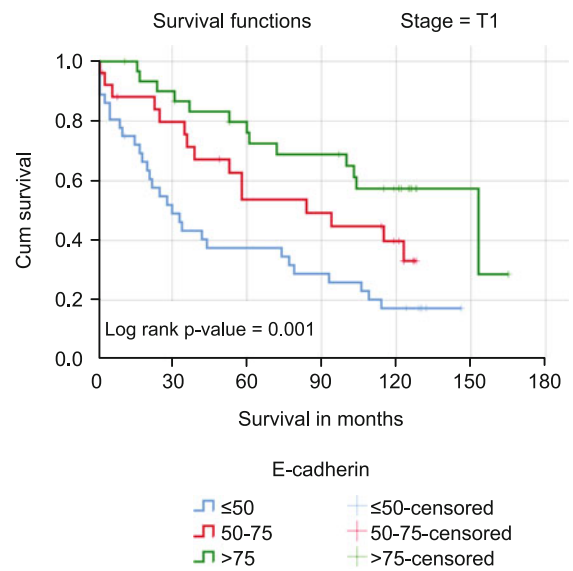
**Fig. 2. Kaplan–Meier survival curve showing a statistically significant difference in survival of patients initially diagnosed with Low-grade and High-grade tumors.**



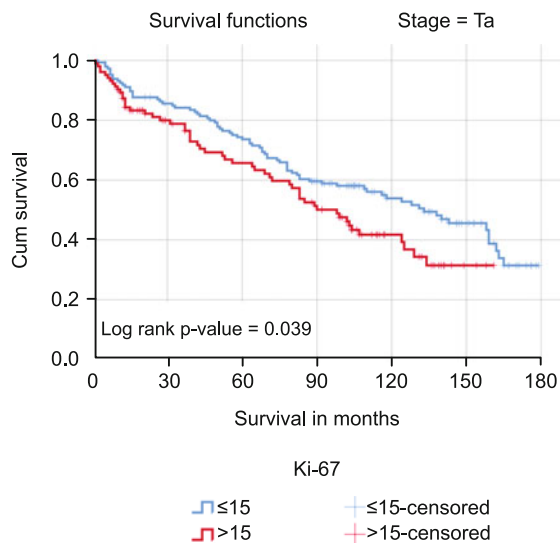
**Fig. 4. Kaplan–Meier survival curve showing a statistically significant difference in survival of patients in association to Ki-67 expression levels.**



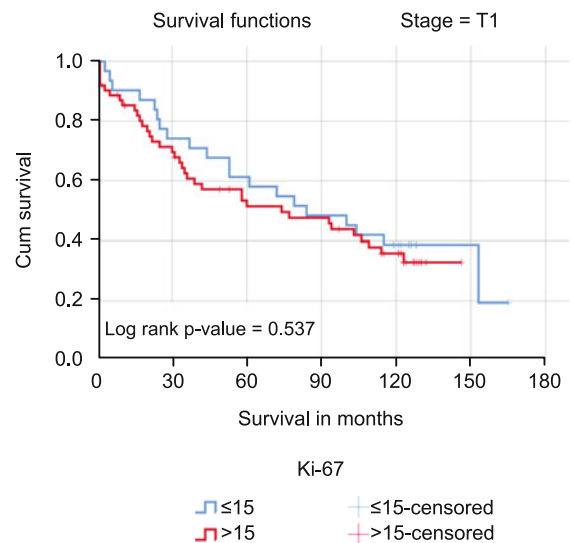
**Fig. 5.** Kaplan–Meier survival curve showing a marginally significant difference in survival of patients within the Ta stage group in association to E-cadherin expression levels.



**Fig. 7.** Kaplan–Meier survival curve showing a statistically significant difference in survival of patients within the T1 stage group in association to E-cadherin expression levels.



**Fig. 6.** Kaplan–Meier survival curve showing a statistically significant difference in survival of patients within the Ta stage group in association to Ki-67 expression levels.



**Fig. 8.** Kaplan–Meier survival curve showing an insignificant difference in survival of patients within the T1 stage group in association to Ki-67 expression levels.

In both cases the “high” expression group of E-cadherin and “low” expression group of Ki-67 contained the higher (statistically significant) amount of lower risk tumors. This fact shows that E-cadherin and Ki-67 are not simply replicating the results of standard histopathologic examination but work effectively as their extension and may help with risk stratification of these patients.

Figures 1–4 present the association of chosen variables (stage, grade, E-cadherin, Ki-67) with overall survival of patients in the study group. Results show, that all the chosen variables are significantly associated with survival. Ta stage and low-grade along

with non-aberrant biomarker expression levels have much higher survival rates than the cases with aberrant expression or worse histopathologic result. Low expression levels of Ki-67 positively affect overall survival of patients, whereas high expression poses poorer prognosis. Patients with high expression levels of E-cadherin have survived longer than patients with intermediate or low expression.

Figures 5–8. show the comparison of impact of biomarker expression levels within individual stage groups of Ta and T1. Significance for E-cadherin is observed in both stage groups, although,

the statistical significance for Ta group is marginal ( $p=0.081$ ). Observed results suggest, that higher levels of expression positively affect the length of survival. In the case of Ki-67 stage group Ta shows significantly longer survival in patients with low expression levels. Patients with low expression of Ki-67 within the T1 group have experienced slightly higher survival rates than patients with high expression, which shows the clinical significance, but fails to prove statistically significant.

## Discussion

Non-muscle invasive bladder cancer with its many recurrences and mostly unforeseeable progression, is a very heterogeneous and variable disease. Clinicians and researchers experience cases where even after previously successful treatment the recurrence in the future remains uncertain. The existence of sex-related differences in mortality of cancer patients is a fact, that also affects bladder cancer patients, but shows little effect on carcinomas in the upper urinary tract (18). First thought passive and without any importance, nowadays stromal cells are under research for their role in tumorigenesis and progression of bladder cancer. Experiments have found supportive and restraining functions of stromal cells, while a recent study analyzing five stromal markers in association with survival shows high association with survival rates, but for their positive relation to tumor stage failed to stand as independent markers (19).

In the search of a better and more viable diagnostic tool for BC, various proteins including proliferation markers (Ki-67), tumor suppressors (p53), inhibitors of apoptosis (survivin), cell adhesion molecules (cadherins - E-cadherin, N-cadherin), supplementary adhesion molecules ( $\alpha$ -catenin,  $\beta$ -catenin,  $\gamma$ -catenin), cytokeratins (CK20), transcription factors (twist-related protein 1), proliferating cell nuclear antigen (PCNA) and structural proteins (vimentin) have already been studied in association with BC.

Based on previous studies, we conducted our study on the expression levels of E-cadherin and Ki-67, as they show very promising, yet not thoroughly studied diagnostic potential, for patients with NMIBC. Our results confirm the prognostic value of these biomarkers and show considerable association with prognosis, where aberrant levels of expression negatively influenced the survival of patients. However, our study did not explain the recurrence of NMIBC, which is a problematic topic for researchers with mainly inconsistent results. Recurrence has been connected to focality, multiplicity, past recurrences and general compliance of patients (20). A study on HER-2 expression in association to bladder cancer recurrence has shown significantly increased expression level between the timepoint of initial diagnosis and recurrence, where the overexpression of HER-2 was associated with higher recurrence rates and could serve as predictive biomarker for NMIBC (21).

Studies on E-cadherin show conflicting results. Low expression levels of E-cadherin were reported with a poor prognosis in UBC patients, but some studies suggest that there is no association between E-cadherin expression and prognosis in bladder cancer patients (22 - 24). Moderately and less differentiated cancer cells

exhibit decreased E-cadherin expression, whereas a well differentiated cell line has higher expression (25). Survival analysis showed a significant difference between normal and aberrant expression of E-cadherin, which may be a good prognostic marker (26).

Significant correlation has been found between Ki-67 expression levels and progression, survival and recurrence in NMIBC (27, 28). Aberrations in Ki-67 expression are associated with worse results of histopathology and clinical outcome in patients (29). Expression of Ki-67 may provide additional information that will allow more accurate stratification of the risk of NMIBC recurrence after TURBT (13).

Apart from previously mentioned biomarkers, considerable effort is put into researching all the elements that have some impact on tumorigenesis, progression and recurrence. Nervous system and alterations to signalization between the nervous system and peripheral tissue have also been reported in association to initiation and cancer progression (30).

Molecular diagnostic method has brought progress not only in research, but also in the diagnostic process of many diseases, including various types of cancer. Lately, real-time polymerase chain reaction (PCR) has proven very effective in examining genes and DNA. Identification of cell-free DNA (cfDNA) in urine samples has showed diagnostic potential in a study where five-gene panel for urine supernatant and a seven-gene panel for urine sediments were identified with promising options for identifying bladder cancer in hematuria patients (31). Long non-coding RNA (lncRNA) has been studied in association with tumorigenesis and metastasizing of bladder carcinomas. Tumor growth is highly affected by lncRNA H19, also upregulated H19 promotes cell migration and enhances bladder cancer metastasis by inhibiting E-cadherin expression and associating with EZH2 enzyme (Enhancer of zeste homolog 2) that participates in histone methylation (32). A recent study on transcriptional regulatory elements reported, that the expression of Histone deacetylase 1 (HDAC1) in cancerous tissue was more than five times higher, which shows that HDAC1 overexpression has an effect on bladder cancer tumorigenesis and the over-expressed HDAC1 mRNA might be a potential diagnostic marker (33).

In conclusion, based on obtained results it can be emphasized that low expression levels of Ki-67 and high expression levels of E-cadherin positively affect survival of patients, whereas aberrant expressions pose poorer prognosis.

## Learning points

- The study results indicate that there is a benefit in the detection of studied biomarkers, because there is a high association of E-cadherin and Ki-67 expression levels with tumor stage and grade ( $p<0.001$ ).
- The association with recurrence has proven insignificant, meaning that studied biomarkers are unsuitable for the prediction of recurrence.
- Low expression levels of Ki-67 and high expression levels of E-cadherin positively affect survival of patients, whereas aberrant expressions pose poorer prognosis.



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