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ORIGINAL ARTICLE

# The relation between tissue galectin-3 level and platinum resistance in neoadjuvant bladder cancer treatment

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## Summary

***Purpose:*** *This study aimed to reveal the relationship between the level of galectin-3 expression and the depth of response to neoadjuvant therapy in bladder tumor tissue with muscle invasion revealed by transurethral biopsy.*

***Methods:*** *The percentage of galectin-3 staining in transurethral biopsy tissue with muscle invasion was determined by immunohistochemistry. The patients were divided into two groups: the down-staging (+) group consisting of patients with pathological complete response or non-invasive bladder cancer, and the down-staging (-) group consisting of patients with stage 2 and above.*

***Results:*** *There were 11 patients in the down-staging (+) group and 12 patients in the down-staging (-) group. There was no significant difference between the two groups in terms of median age, gender, smoking, clinical stage at the time of diagnosis, distribution of carboplatin or cisplatin used as a platinum agent. Galectin-3 was positive in 2 patients (18.2%) in the group where down-staging was achieved with neoadjuvant therapy, while it was positive in 9 patients (75%) in the other group (p = 0.01). The median follow-up period of the patients was 31.6 months (95% CI 25.1-39.3). Overall survival was 43.4 months in the down-staging (+) group (95% CI 25.1-61.6) and 31.6 months in the down-staging (-) group (95% CI 12.7-50.6). Although there was a numerical difference, it did not reach statistical significance (p=0.37).*

***Conclusion:*** *The rate of down-staging after platinum-based neoadjuvant chemotherapy is significantly higher in patients with low galectin-3 staining in transurethral bladder biopsy tissue.*

***Key words:*** *galectin-3, bladder cancer, neoadjuvant chemotherapy, platinum resistance*

## Introduction

Bladder cancer is the sixth most common cancer, constitutes 4.5% of all newly diagnosed cancers, and has a mortality rate of 3% [1]. Its frequency increases in advanced age and the mean age at diagnosis is 73 years. At the time of diagnosis, 34% of patients have localized disease (confined to the primary site) and 7% have regional disease (spread to regional lymph nodes) . Five-year survival is 69% at the localized stage and 36% at the regional stage [2]. The standard treatment approach in muscle-invasive bladder cancer (MIBC) is radical cystectomy after neoadjuvant chemotherapy [3-5]. The survival rate of neoadjuvant therapy increased by 5-10% with cisplatin-based combined chemotherapy, which has been demonstrated by randomized controlled studies and proven by meta-analyses [6-7]. Distant metastases occur within two years in half of the patients who underwent only cystectomy or radiotherapy [8]. Obtaining pathological downstaging after neoadjuvant chemotherapy is associated with prolonged overall survival [9].

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Galectins are members of the family of carbohydrate-binding proteins with a high affinity for the enzyme B-galactosidase. There is increasing evidence that galectins are associated with biological processes such as intercellular communication, inflammation, differentiation, and apoptosis [10]. Galectin-3 is the best defined one among them, and alterations in the galectin-3 expression are associated with tumorigenesis, progression and metastasis in cancer tissues [11]. Galectin-3 expression in bladder cancer is higher in muscle-invasive tumors than non-invasive tumors, and it has been reported that it can be used as a tumor biomarker [12,13]. The relationship between galectin level and chemotherapy resistance has been investigated in previous studies. It has been shown that suppression of galectin-1 level in lung cancer increases cisplatin sensitivity [14], that increased galectin expression in ovarian epithelial cells is associated with platinum resistance [15], and there is an inverse relationship between neoadjuvant chemotherapy response and galectin level in cervical cancer [16].

In this study, we aimed to reveal the relationship between galectin-3 expression level and platinum-based neoadjuvant chemotherapy response in transurethral resection (TUR) material with invasion of muscularis propria.

## Methods

*Patient characteristics*

A total of 23 patients who underwent curative surgery after neoadjuvant chemotherapy with a diagnosis of bladder cancer were included in the study.Institutional Review Board approval was obtained (TÜTF-BAEK 2019/285). All patients were diagnosed with invasive urothelial carcinoma by TUR and invasion of muscularis propria was demonstrated. Patients were clinically staged according to computed tomography (CT) and/ormagnetic resonance (MR) (the American Joint Committee on Cancer/ AJCC Cancer Staging Manual, Eight Edition). Stage 2, 3A, and 3B patients were determined as candidates for neoadjuvant chemotherapy. The patients were administered 4 cycles of neoadjuvant chemotherapy with cisplatin/carboplatin and gemcitabine regimen. After chemotherapy, patients underwent radical cystectomy. Radical cystectomy included a bilateral pelvic lymphadenectomy. Gender, age at the time of diagnosis, smoking and alcohol use, chemotherapy regimen, date of TUR and cystectomy, date of relapse, and last follow-up date were retrospectively recorded. We considered two definitions of response: tumors in the cystectomy specimen that were downstaged to non-MIBC disease (pT1pN0) and MIBC (≥T2). In previous clinical studies, pathological complete response and non-MIBC disease have been used as an endpoint that correlates with prolonged overall survival and disease-free survival [4,17,18].

*Tissue sample and galectin-3 staining*

The TUR biopsy specimen obtained before registration was assessed to document eligibility (invasion of the muscularis propria). Immunohistochemical (ICH) staining, scoring, and interpretation of galectin-3 was done by pathologists. IHC was performed using the Benchmark XT/Ultra instrument (Ventana Medical Systems) according to the manufacturer's instructions, using Galectin-3 (Leica 9C4-PA0238 IgG1). Expression of galectin-3 was evaluated using light microscopy and the percentage of the positively stained cells was calculated and scored weak expression (0-20% stained cells), moderate expression (21-50% stained cells), strong expression (51-100% stained cells) [19]. Tissue samples

**Table 1.** Demographic and clinical charateristics of the study subjects

| | *Down-staging (+)* | *Down-staging (-)* | *p value* |
|---|---|---|---|
| Age, years | | | |
| Median (Interquartile range) | 64 (62-69) | 65 (58-72) | 0.92 |
| Gender, n (%) | | | |
| Female | 2 (18.2) | 4 (33.3) | 0.64 |
| Male | 9 (81.8) | 8 (66.7) | 0.64 |
| Smoking, n (%) | 9 (81.8) | 8 (66.7) | 0.59 |
| Alcohol, n (%) | 1 (9.1) | 2 (16.7) | |
| Clinical stage, n (%) | | | 0.19 |
| Stage 2 | 2 (18.2) | - | |
| Stage 3A | 7 (63.6) | 7 (58.3) | 0.46 |
| Stage 3B | 2 (18.2) | 5 (41.7) | 0.01 |
| Treatment regimen, n (%) | | | |
| Cisplatin+Gemcitabine | 8 (72.7) | 7 (58.3) | |
| Carboplatin+Gemcitabine | 3 (27.3) | 5 (41.7) | |
| Baseline Galectin-3 positivity, n (%) | 2 (18.2) | 9 (75.0) | |

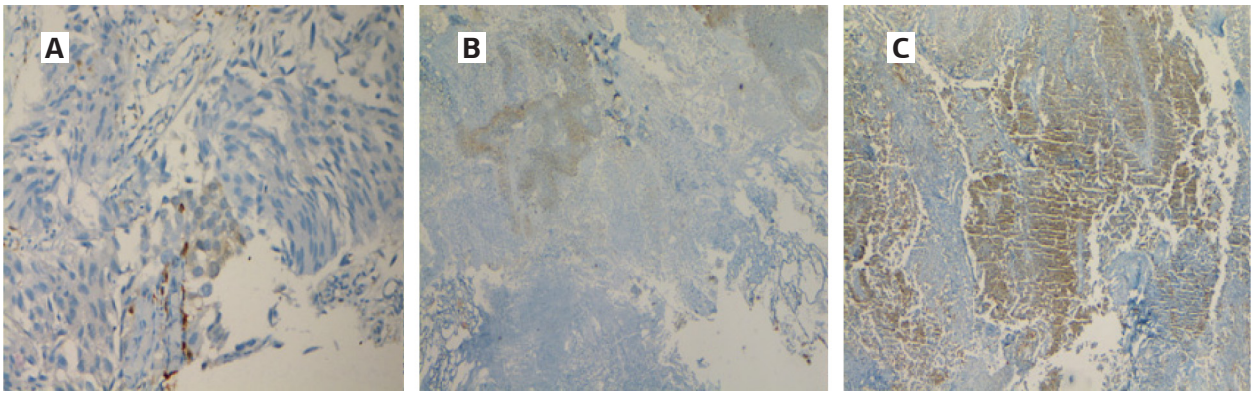


**Figure 1.** Pattern of galectin-3 staining; **A:** 5% immunoperoxidase x100. **B:** 30% immunoperoxidase x40. **C:** 90% immunoperoxidase x40.

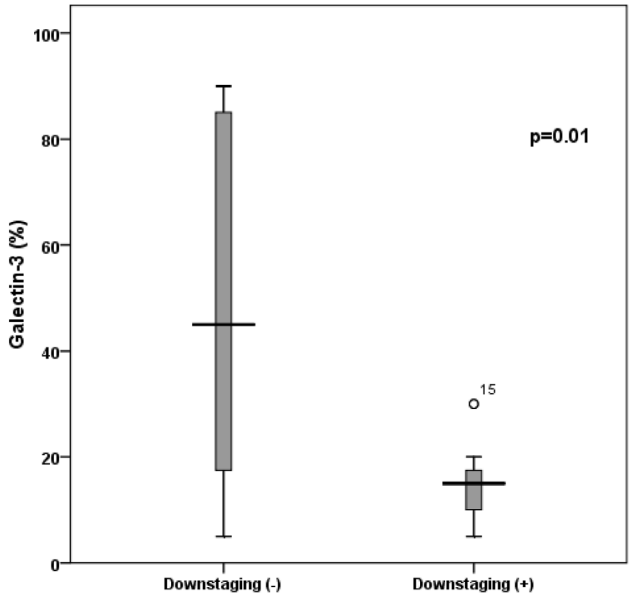


**Figure 2.** Boxplot graph of the association between galectin-3 and neoadjuvant response.

with galectin-3 staining level ≤20% were accepted as galectin-3 (-) and samples stained with >20% galectin-3 as galectin-3 (+) (Figure 1). Cystectomy specimens were reviewed to assess the pathological stage and to determine the rate of down-staging.

*Statistics*

23 patients included in the study were divided into two groups as down-staging (+) and down-staging (-). The disease-free survival (months) showed the time between radical cystectomy and the date of relapse, while the time between the date of TUR and date on which the patient died constitutes the overall survival (months). Quantitative data were calculated as median (interquartile range) and compared with the Student t-test and Mann-Whitney U-test. Categorical data were compared with the chi-square test. Overall survival was calculated as the time between diagnosis date and death. Disease-free survival was calculated as the time between cystectomy date and progression date. Kaplan-Meier and log-rank tests were used in survival analysis. P <0.05 was accepted as statistically significant.

## Results

There were 23 patients in the study who met the inclusion criteria. According to the radical cystectomy material, there were 11 patients in the down-staging (+) group and 12 patients in the down-staging (-) group. The median age was 64 years in the positive group and 65 in the negative group (p=0.92). The number of female patients was 2 (18%) in the positive group and 4 (33%) in the negative group (p=0.64). There was no difference between the two groups in terms of smoking and alcohol use. There was no statistical difference in the distribution of stage 2, 3A, and 3B patients included in the study with clinical staging. The number of cisplatin-ineligible patients was 3 in the positive group and 5 in the negative group (p=0.46). Galectin (-) patients were 9 (82%) in the down-staging (+) group and 3 (25%) in the down-staging (-) group (p = 0.01) (Table 1).

As a result of staining with ISH on the TUR material of the patients, only two patients (30%, 30%) were found to be galectin-3 positive in the group in which down-staging was achieved with neoadjuvant therapy. In the group where down-staging could not be achieved, galectin-3 negative (5%, 5%, 5%) was stained in 3 patients (Figure 2). In the group with down-staging, a T1 tumor was detected in 5 patients in the cystectomy material, while a pathological complete response was observed in 6 patients.

The median follow-up period of the patients was 31.6 months (95% CI 25.1-39.3). While the number of patients with recurrence was 6 in the down-staging (+) group, it was 8 in the other group. Disease-free survival was 8.5 months (95%

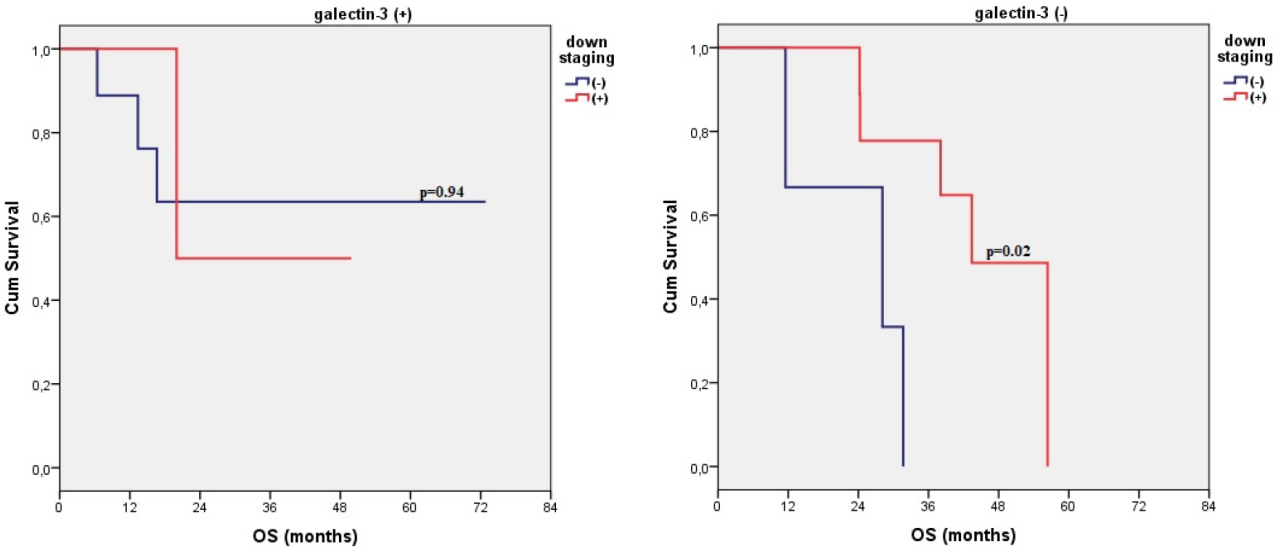


**Figure 3.** The relation between down-staging and OS according Galectin-3 staining.

CI 3.9-13.1) in the down-staging (+) group and 5.5 months (95% CI4.5-6.5) in the down-staging (-) group (p=0.67). Six patients in the down-staging (-) group died and 6 patients from the down-staging (+) group died due to cancer-related causes. Overall survival was 43.4 months (95% CI 25.1-61.6) in the down-staging (+) group, 31.6 months (95% CI 12.7-50.6) in the down-staging (-) group. Although there was a numerical difference, it did not reach statistical significance (p=0.37). In particular, patients with negative galectin-3 and down-staging achieved the best overall survival with 43.4 months (95% CI 33.0-53.7) (Figure 3).

## Discussion

This study is the first to evaluate the relationship between galectin-3 expression levels in muscle-invasive bladder cancer and platinum-based neoadjuvant chemotherapy response. Patients with low galectin-3 expression in TUR material were found to have a high rate of down-staging after neoadjuvant chemotherapy. This difference did not show a disease-free survival and overall survival benefit for the patients in the study.

The standard treatment approach in muscle-invasive bladder cancer is radical cystectomy and pelvic lymph node dissection after combined cisplatin-based neoadjuvant chemotherapy. The complete pathological response is the most important prognostic indicator after cystectomy [20]. However, this response can be achieved in only 30% of patients [4]. For this reason, the specific clinical, pathological, and molecular characteristics of the patient group that will respond to treatment should be revealed. In the study of Culp et al the presence of hydroureteronephrosis, pathological vascular invasion, neuroendocrine subtype and T3b-T4a disease were defined as poor clinicopathological prognostic factors in patients receiving neoadjuvant therapy [21]. Cisplatin, the main drug of neoadjuvant therapy, is an alkylating agent that disrupts DNA synthesis by DNA fragmentation. DNA damage repair defects are the most important mechanism in cytotoxic chemotherapy efficacy. Studies have shown that DNA repair genes (BRCA1, ATM, RB1, ERCC2) can be biomarkers in neoadjuvant therapy in bladder cancer [22,23]. The galectin-3 expression has been found to be higher in invasive bladder cancer compared to non-invasive lesions [12]. This data suggests that the galectin-3 molecule can be investigated as a biomarker in bladder cancer. Zhang et al reported that galectin overexpression was found to be associated with cisplatin resistance in lung cancer and epithelial ovarian cancer [14,15]. In addition, in the study of Zhu et al, investigating the role of galectin in neoadjuvant therapy in cervical cancer, found a negative correlation between chemotherapy response and galectin expression level, and they attributed this to apoptosis suppression in cervical cancer cells. Similarly, in our study, a negative correlation was found between galectin-3 expression level and chemotherapy response. In studies investigating the relationship between galectin and neoadjuvant treatment response, no clinical data on overall survival and disease-free survival were found [16,24]. In our study, although there was a significant numerical difference between galectin-3 level and overall survival and disease-free survival in analyses performed with a low number of patients, it did not reach statistical significance.

The most important limitation of our study is the small number of patients. Therefore, there is a possibility of error in survival analysis. Besides, galectin-3 staining was also applied to the cystectomy material, but these data were not presented in the study, because the tumor group was heterogeneous and no tumor tissue would be detected in those with a complete response.

In conclusion, a galectin-3 level <20% in TUR tissue in patients with muscle-invasive bladder cancer for whom neoadjuvant therapy is planned indicates that a better response will be obtained with platinum-based combined therapies. The addition of galectin-3 staining to the tissue sample in clinical practice may be a good response predictor for the clinician who decides on neoadjuvant therapy. Besides, galectin-3 could be a treatment target for future clinical trials.

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## Ethical approval

Institutional Review Board approval was obtained. All procedures performed in studies involving participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

## Author contribution

AG: data analysis, manuscript writing/editing; AK: Manuscript writing/editing; OK: Project development; SS: Manuscript writing/editing; NC: Data collection/management; BH: Manuscript writing/editing; BE: Manuscript writing/editing; SU: Manuscript writing/editing; IC: Supervisor, Project development

## Conflict of interests

The authors declare no conflict of interests.

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