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*Original Article*

# Alternating intravesical instillation of Bacillus Calmette-Guérin and *Pseudomonas aeruginosa* effectively prevents postoperative recurrence in high-risk non-muscle-invasive bladder cancer

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**Abstract:** Objective: To evaluate the role of alternating intravesical instillation of Bacillus Calmette-Guérin (BCG) and *Pseudomonas aeruginosa* (PA) in preventing postoperative recurrence in high-risk non-muscle-invasive bladder cancer (HR-NMIBC). Methods: We retrospectively reviewed the clinical data from 115 HR-NMIBC cases who underwent transurethral resection of bladder tumors (TURBT) at Gongli Hospital of Shanghai Pudong New Area between March 2021 and January 2023. Patients were grouped based on postoperative management: a control group (n=51) treated with standard gemcitabine instillations and an intervention group (n=64) given alternating BCG and PA instillations. This study assessed 1- and 2-year recurrence, recurrence-free survival, safety (gastrointestinal reactions, fever, bladder irritation symptoms, and hematuria), serum tumor markers, and life quality. Univariate and multivariate Cox proportional hazards analyses were applied to identify the recurrence predictors. A nomogram predictive model was further developed for postoperative recurrence risk estimation, and its performance was later validated. Results: Despite an equivalent 1-year recurrence rate, the intervention group showed a lower 2-year recurrence rate, prolonged recurrence-free survival, and superior safety (fewer adverse events) than controls. The intervention group also showed decreased post-treatment serum tumor marker concentrations and greater life quality enhancement relative to the control cohort. Univariate and multivariate analyses identified tumor number ≥3 (P=0.036), high-grade tumors (P=0.040), and gemcitabine monotherapy (P=0.035) as independent predictors for 2-year recurrence. The nomogram's scoring system reliably associated elevated risk points with heightened recurrence risk, demonstrating strong discrimination and reliable calibration in medium-to-high-risk ranges. Conclusions: Alternating intravesical BCG and PA instillations markedly decreases 2-year postoperative recurrence on the premise of favorable safety in HR-NMIBC patients.

**Keywords:** High-risk non-muscle-invasive bladder cancer, postoperative recurrence, Bacillus Calmette-Guérin, Pseudomonas aeruginosa, alternating intravesical instillation

## Introduction

Bladder cancer contributes to nearly 500,000 cases annually worldwide, with the U.S. alone reporting over 80,000 diagnoses and 20,000 fatalities in 2022 [1, 2]. Approximately 75% of bladder cancer cases are non-muscle-invasive (NMIBC), where tumors remain confined to the mucosa and submucosa. These cases, however, display considerable clinical heterogeneity and a high relapse rate [3]. High-risk (HR) NMIBC (HR-NMIBC) is characterized by high-grade T1 pathology, histological variants, prior Bacillus Calmette-Guérin (BCG) failure, recurrent Ta lesions, bulky (>3 cm) high-grade Ta tumors, or carcinoma in situ (CIS). These characteristics increase the risks of disease progression and mortality [4]. HR-NMIBC is associated with unsatisfactory clinical outcomes, with 5-year recurrence-free survival (RFS) ranging from 17.0% to 89.0%, progression-free survival between 58.0% and 89.0%, and overall survival rates falling to 28.0% [5], severely compromising patients' quality of life while imposing substantial economic burdens. Despite the effective disease control achieved through transurethral resection of bladder tumor (TURBT), the primary surgical procedure used in NMIBC,

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this technique demonstrates significant limitations in HR patients, evidenced by high recurrence and progression rates, with residual tumor at the resection bed or periphery in ~30% of cases [6, 7]. To tackle this issue, the current study sought to identify and evaluate therapeutic strategies capable of mitigating postoperative recurrence in HR-NMIBC, which carries substantial clinical importance for improving patient conditions while reducing recurrence risk.

Clinically, BCG is routinely administered following TURBT in HR-NMIBC cases, leveraging its ability to trigger immune activation through lipid-based pathogen recognition mechanisms; yet, its effectiveness in preventing disease recurrence exhibits marked interpatient variability [8, 9]. Furthermore, its suboptimal effectiveness and substantial toxicities further hinder its broader clinical use [10]. *Pseudomonas aeruginosa* (PA) mannose-sensitive hemagglutinin (MSHA) strains possess therapeutic potential in treating multiple tumors, demonstrating significant anti-tumor activity. Studies demonstrate their efficacy in suppressing breast tumor growth in murine xenograft models while preventing pulmonary metastasis. In hepatocellular carcinoma, these strains impede tumor progression and lung metastasis by targeting the epidermal growth factor receptor (EGFR)/protein kinase B (Akt)/nuclear factor κB inhibitory protein β (IκBβ)/nuclear factor κB (NF-κB) axis [11, 12]. In an orthotopic mouse bladder cancer model [13], Wang et al. reported that PA-MSHA induced a protective immune microenvironment and modulated innate-adaptive immune coordination. This study hypothesized that alternating intravesical BCG and PA-MSHA instillations may provide superior recurrence prevention in HR-NMIBC compared to gemcitabine monotherapy. To our knowledge, this combinatorial approach has not been extensively explored, rendering our investigation both novel and clinically significant for shedding new insight into postoperative recurrence in HR-NMIBC patients.

## Clinical data

### *Case selection*

This study was ethically approved by the Gongli Hospital of Shanghai Pudong New Area. We retrospectively enrolled 115 HR-NMIBC patients admitted to Gongli Hospital of Shanghai Pudong New Area between March 2021 and January 2023. The control group (n=51) received intravesical gemcitabine monotherapy, while the intervention group (n=64) received alternating BCG and PA instillations.

Inclusion Criteria: bladder urothelial carcinoma confirmed by histopathologic examination [14]; TURBT as first-line therapy; Confirmed CIS or T1 disease, high-grade (G3) urothelial carcinoma, or recurrent multifocal stage Ta low-grade tumors; European Association of Urology (EAU)-defined HR-NMIBC (2021 criteria) [15]; Complete medical records.

Exclusion criteria: Concurrent malignancies; Hypersensitivity to BCG or PA; Pregnancy or lactation; Severe cardiac, pulmonary, or renal dysfunction; Psychiatric or cognitive disorders; Mortality from non-oncological causes; Concurrent bladder pathologies.

### *Intervention methods*

For the control group, gemcitabine was given as a sole perfusion agent. A solution was prepared by diluting 1 g of gemcitabine (Wuhan Bairuide Biotechnology Co., Ltd., 9003096-100) in 50 mL of normal saline (Shanghai Yaji Biotechnology Co., Ltd. HBPT034), followed by thorough mixing. Prior to instillation, patients were instructed to empty their bladders. A sterile disposable catheter was then inserted through the urethra into the bladder to ensure complete drainage of any residual urine before administering the drug solution. The solution was retained in the bladder for 1-2 hours, during which patients were assisted in rotating through four forced positions - supine, left lateral, right lateral, and prone - changing every 15 minutes to ensure optimal drug distribution. Induction Phase: Instillations were administered once weekly for eight consecutive weeks. Maintenance Phase: Following induction, treatments were given once monthly for ten consecutive months. The entire treatment course lasted 12 months across both phases.

The intervention group received alternating intravesical instillations of BCG and PA-MSHA according to the following schedule. The first bladder instillation was administered two weeks after surgery. BCG Administration: A dose of 120 mg BCG (Shanghai Yuzhuo Biological Technology Co., Ltd., hr001) was dis-

solved in 50 mL of normal saline and thoroughly mixed. The instillation method and patient positioning mirrored those of the control group, with patients changing positions every 20 minutes across four different positions. A 10 mL PA-MSHA injection (Taize (Guangzhou) Biotechnology Co., Ltd., CCTCC AB 2010174) was diluted with 40 mL of normal saline and thoroughly mixed. The instillation method, patient positioning, and retention time were identical to those used for BCG. The treatment consisted of an induction phase followed by a maintenance phase: Induction Phase (Weeks 1-8): Instillations were administered once per week for 8 consecutive weeks. BCG was given in weeks 1, 3, 5, and 7, while PA-MSHA was given in weeks 2, 4, 6, and 8. Maintenance Phase (Months 1-10): Instillations were administered once per month for 10 consecutive months. BCG instillations were scheduled for the first, third, fifth, seventh, and ninth months, while PA-MSHA was administered in the alternating months (second, fourth, sixth, eighth, and tenth months). The therapeutic course spanned twelve months in total.

For both patient cohorts, cystoscopy re-evaluations were scheduled at 3-month intervals during the first year after surgery, with 4 visits in total. In the second year after operation, re-evaluations were conducted once every six months, amounting to two visits. The follow-up period spanned two years. The loss-to-follow-up threshold was triggered at the first unattended visit, with patients exceeding two absences within 1 year classified as lost to follow-up.

*Data collection*

The primary endpoints included 1-year and 2-year tumor recurrence rates, RFS, clinical safety (gastrointestinal adverse events, fever, bladder irritation symptoms, and hematuria), serum tumor markers (cytokeratin-19 [CK-19] and cytokeratin 19 fragment antigen 21-1 [CYFRA 21-1]), and quality of life in both study arms. Tumor recurrence was confirmed through cystoscopy. Any suspicious lesions identified during cystoscopy prompted immediate tissue sampling for histopathological verification. Safety assessments were conducted according to the Common Terminology Criteria for Adverse Events (CTCAE v5.0), and adverse event incidences were documented and compared across groups. Serum levels of CK-19 and CYFRA 21-1 were measured before treatment initiation and at the 12-month follow-up after treatment. Prior to testing, 3 mL of fasting venous blood was collected from each patient. The serum was then separated by centrifugation and analyzed using electrochemiluminescence immunoassay (Xi'an Tianlong Science and Technology Co., Ltd., Polaris i2400). All experimental procedures adhered strictly to the instructions provided in the kits' manuals (Shanghai Caiyou Industrial Co., Ltd., CL05541; Shanghai Aiyin Biological Technology Co., Ltd., 11820966122). Quality of life assessments were conducted at corresponding time points (pre-treatment and 12-month post-treatment) using the European Organization for Research and Treatment of Cancer Quality of Life Questionnaire Core-30 (EORTC QLQ-C30). According to the instrument's scoring system, higher scores indicate better quality of life.

Among the indicators monitored, the primary ones included 1- and 2-year recurrence rates, RFS, and clinical safety, whereas serum tumor markers and quality of life were categorized as secondary endpoints.

*Statistical analysis*

Data analysis was conducted using SPSS 23.0 (IBM Corp.) and GraphPad Prism 7.0 (GraphPad Software). Continuous variables were expressed as means ± standard errors of the means (SEM), and statistical differences were evaluated using Student's t-tests (between-groups) and paired t-tests (pre- and post-treatment within-group comparisons). Categorical data were presented as frequency (percentages), with chi-square ($\chi^2$) tests applied for group comparisons. Univariate and Cox regression analyses were employed to identify determinants of postoperative recurrence in HR-NMIBC, followed by the subsequent nomogram construction for 2-year recurrence risk. Statistical significance was defined at a two-tailed $P$-value<0.05.

## Results

*Baseline characteristics*

No significant differences were observed in baseline demographic characteristics - including gender, age, body mass index (BMI), educa-

**Table 1.** Comparison of baseline characteristics between the two groups

| Factor | n | Control group (n=51) | Intervention group (n=64) | $t/\chi^2$ | P |
|---|---|---|---|---|---|
| Gender | | | | 0.130 | 0.718 |
| Male | 72 | 31 (60.78) | 41 (64.06) | | |
| Female | 43 | 20 (39.22) | 23 (35.94) | | |
| Age (years) | 115 | 63.25±7.54 | 62.64±8.48 | 0.402 | 0.688 |
| BMI (kg/m$^2$) | 115 | 22.06±1.92 | 22.42±2.74 | 0.795 | 0.428 |
| Education level | | | | 0.232 | 0.630 |
| < High School | 66 | 28 (54.90) | 38 (59.38) | | |
| ≥ High School | 49 | 23 (45.10) | 26 (40.63) | | |
| Family history | | | | 0.132 | 0.716 |
| No | 100 | 45 (88.24) | 55 (85.94) | | |
| Yes | 15 | 6 (11.76) | 9 (14.06) | | |

Note: BMI, Body mass index.

**Table 2.** Comparison of 1- and 2-year postoperative recurrence rates between the two groups

| Factor | Control group (n=51) | Intervention group (n=64) | $\chi^2$ | P |
|---|---|---|---|---|
| 1-year postoperative recurrence | 10 (19.61) | 6 (9.38) | 2.481 | 0.115 |
| 2-year postoperative recurrence | 17 (33.33) | 10 (15.63) | 4.954 | 0.026 |

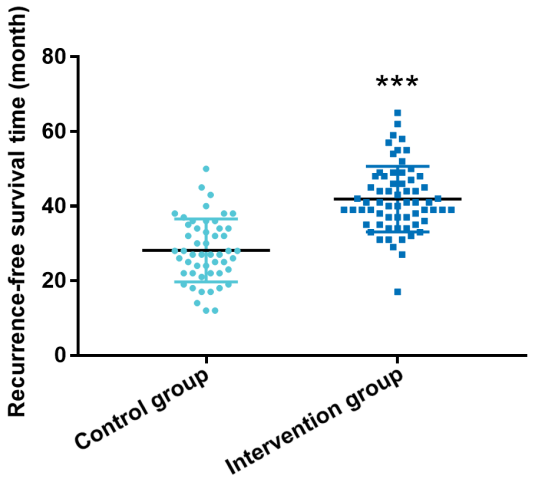


**Figure 1.** Comparison of recurrence-free survival time between the two groups. ***P<0.001.

tion level, or family history - between the control and intervention groups (P>0.05). Details are summarized in **Table 1**.

*Postoperative recurrence rates*

The intervention group exhibited 1-year and 2-year postoperative recurrence rates of 9.38% and 15.63%, respectively. While the 1-year recurrence rate did not differ significantly from the control group (P>0.05), the 2-year rate was significantly lower (compared with 33.33% in control group; P<0.05) (**Table 2**).

*RFS analysis*

RFS duration was significantly prolonged in the intervention group compared to the control group (P<0.001, **Figure 1**).

*Safety outcomes*

The intervention group demonstrated a markedly lower overall incidence of adverse events (9.38% vs. 23.53%; P<0.05), including gastrointestinal reactions, fever, bladder irritation symptoms, and hematuria (**Table 3**).

*Serum tumor marker levels*

No significant differences in baseline CK-19 and CYFRA21-1 levels were observed between the two groups prior to treatment (P>0.05). Following treatment, both groups exhibited significant reductions in these tumor markers (P<0.01). Notably, the intervention group demonstrated significantly lower post-treatment levels of CK-19 and CYFRA21-1 compared to the control group (P<0.05) (**Figure 2**).

*Quality of life assessment*

Quality of life was evaluated using the EORTC QLQ-C30 questionnaire. Baseline scores did not differ significantly between groups (P>0.05). Posttreatment assessment revealed significant improvement in QLQ-C30 scores for both gro-

**Table 3.** Comparison of clinical safety between the two groups

| Adverse event | Control group (n=51) | Intervention group (n=64) | $\chi^2$ | P |
|---|---|---|---|---|
| Gastrointestinal reactions | 1 (1.96) | 1 (1.56) | | |
| Fever | 3 (5.88) | 1 (1.56) | | |
| Bladder irritation symptoms | 7 (13.73) | 3 (4.69) | | |
| Hematuria | 1 (1.96) | 1 (1.56) | | |
| Total | 12 (23.53) | 6 (9.38) | 4.307 | 0.038 |

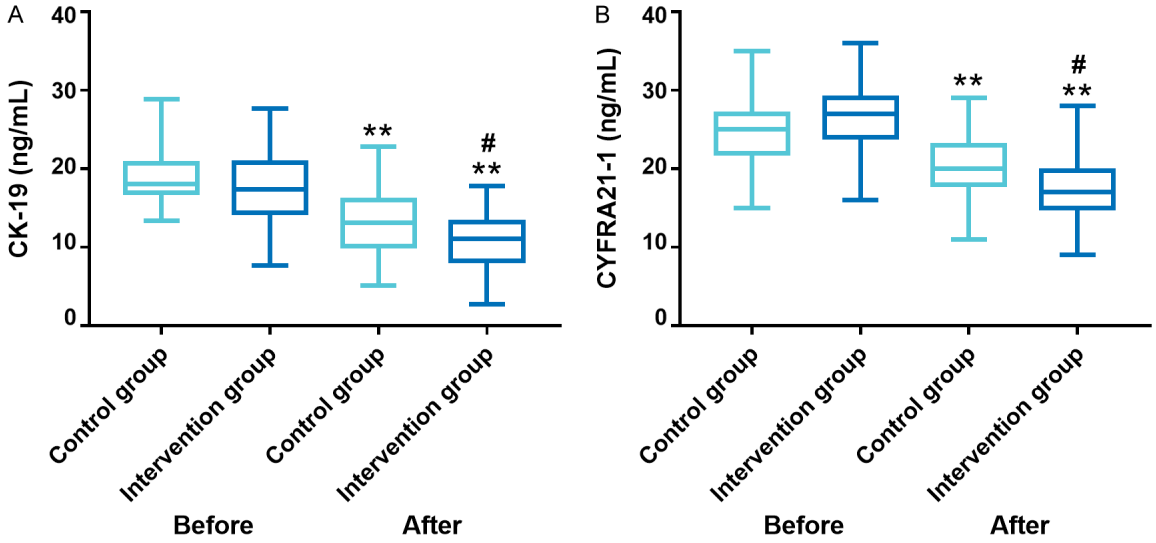


**Figure 2.** Comparison of serum tumor marker profiles between the two groups before and after treatment. A. CK-19 levels pre- and post-treatment. B. CYFRA21-1 levels pre- and post-treatment. Notes: **P<0.01 vs. pretreatment level; #P<0.05 vs. control group. CK-19, cytokeratin-19; CYFRA21-1, cytokeratin 19 fragment antigen 21-1.

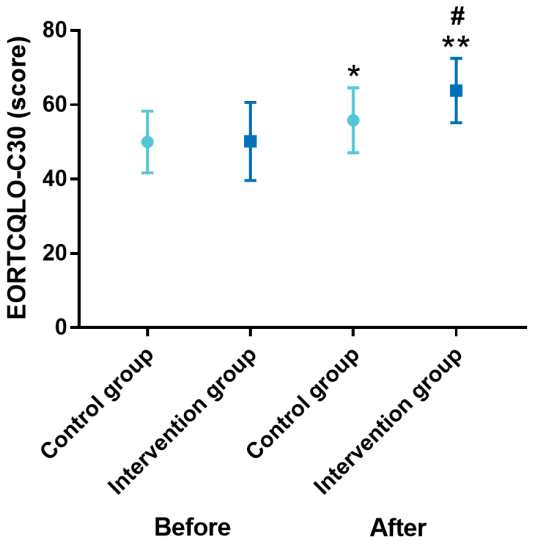


**Figure 3.** Comparison of quality of life scores between the two groups before and after the treatment. Notes: *P<0.05, **P<0.01 vs. pretreatment score; #P<0.05 vs. control group. EORTC QLQ-C30, European Organization for Research and Treatment of Cancer Quality of Life Questionnaire Core 30.

ups (P<0.05), with the intervention group achieving significantly higher scores than the control group (P<0.05) (**Figure 3**).

*Univariate analysis of factors associated with postoperative recurrence in HR-NMIBC patients*

Preliminary univariate analysis demonstrated no significant association (P>0.05) between postoperative recurren-ce and demographic factors, including gender, age, BMI, education level, or family history. In contrast, significant differences (P<0.05) were identified in clinical and pathological characteristics, such as clinical stage, tumor diameter, tumor multiplicity, tumor grade, diabetes status, and treatment regimen. Detailed results are presented in **Table 4**.

*Multivariate analysis of postoperative recurrence determinants in HR-NMIBC*

Risk factors identified as significant in univariate analysis were included as indepen-

**Table 4.** Univariate analysis of factors influencing postoperative recurrence in HR-NMIBC patients

| Factor | n | Recurrence group (n=27) | Non-recurrence group (n=88) | $\chi^2$ | P |
|---|---|---|---|---|---|
| Gender | | | | 0.750 | 0.387 |
| Male | 72 | 15 (55.56) | 57 (64.77) | | |
| Female | 43 | 12 (44.44) | 31 (35.23) | | |
| Age (years) | | | | 0.313 | 0.576 |
| <65 | 65 | 14 (51.85) | 51 (57.95) | | |
| ≥65 | 50 | 13 (48.15) | 37 (42.05) | | |
| Body mass index (kg/m$^2$) | | | | 1.744 | 0.187 |
| <22 | 43 | 13 (48.15) | 30 (34.09) | | |
| ≥22 | 72 | 14 (51.85) | 58 (65.91) | | |
| Education level | | | | 0.049 | 0.826 |
| < High School | 66 | 15 (55.56) | 51 (57.95) | | |
| High School | 49 | 12 (44.44) | 37 (42.05) | | |
| Family history | | | | 0.933 | 0.334 |
| No | 100 | 22 (81.48) | 78 (88.64) | | |
| Yes | 15 | 5 (18.52) | 10 (11.36) | | |
| Clinical stage | | | | 4.486 | 0.034 |
| Ta | 63 | 10 (37.04) | 53 (60.23) | | |
| T1 | 52 | 17 (62.96) | 35 (39.77) | | |
| Tumor diameter (cm) | | | | 6.712 | 0.010 |
| <3 | 75 | 12 (44.44) | 63 (71.59) | | |
| ≥3 | 40 | 15 (55.56) | 25 (28.41) | | |
| Tumor multiplicity (n) | | | | 7.187 | 0.007 |
| <3 | 60 | 8 (29.63) | 52 (59.09) | | |
| ≥3 | 55 | 19 (70.37) | 36 (40.91) | | |
| Tumor grade | | | | 10.517 | 0.001 |
| Low-grade urothelial carcinoma | 80 | 12 (44.44) | 68 (77.27) | | |
| High-grade urothelial carcinoma | 35 | 15 (55.56) | 20 (22.73) | | |
| Comorbid diabetes | | | | 6.202 | 0.013 |
| No | 40 | 4 (14.81) | 36 (40.91) | | |
| Yes | 75 | 23 (85.19) | 52 (59.09) | | |
| Treatment regimen | | | | 4.954 | 0.026 |
| Gemcitabine instillation | 51 | 17 (62.96) | 34 (38.64) | | |
| Alternating intravesical instillation of BCG and PA | 64 | 10 (37.04) | 54 (61.36) | | |

Note: HR-NMIBC, high-risk non-muscle-invasive bladder cancer; BCG, Bacillus Calmette-Guérin; PA, *Pseudomonas aeruginosa*; T, Tumor.

dent variables in the Cox proportional hazards model. Multivariate analyses identified ≥3 tumors (P=0.036), high-grade urothelial carcinoma (P=0.040), and gemcitabine monotherapy (P=0.035) as independent predictors for disease recurrence in HR-NMIBC patients. Based on these findings, we developed a nomogram incorporating all significant predictors for individualized 2-year recurrence probability. For internal validation purposes, we applied 1000 Bootstrap resampling procedures. The resulting C-index was 0.791, with a 95% CI of (0.686, 0.879), indicating the model's better-than-moderate discriminatory performance. The calibration curve analysis showed good calibration in medium-high risk areas, although slight overestimation was observed in low-risk regions and minor underestimation in medium-to-low risk regions. The assignment of variables, Cox regression results, as well as the predictive nomogram and relevant performance validation results are presented in **Tables 5**, **6** and **Figure 4**, respectively.

**Table 5.** Variable assignments

| Factor | Variable | Assignment |
|---|---|---|
| Clinical stage | X1 | Ta=0, T1=1 |
| Tumor diameter (cm) | X2 | <3=0, ≥3=1 |
| Tumor multiplicity (n) | X3 | <3=0, ≥3=1 |
| Tumor grade | X4 | Low-grade urothelial carcinoma =0, high-grade urothelial carcinoma =1 |
| Comorbid diabetes | X5 | No =0, yes =1 |
| Treatment regimen | X6 | Alternating intravesical instillation of BCG and PA=0, Gemcitabine instillation =1 |

Note: BCG, Bacillus Calmette-Guérin; PA, *Pseudomonas aeruginosa*.

**Table 6.** Multivariate Cox regression analysis of independent risk factors for recurrence in HR-NMIBC patients

| Factor | B | SE | Wald | P | HR | 95.0% CI |
|---|---|---|---|---|---|---|
| Clinical stage | 0.522 | 0.414 | 1.594 | 0.207 | 1.686 | 0.749-3.794 |
| Tumor diameter (cm) | 0.688 | 0.400 | 2.951 | 0.086 | 1.990 | 0.908-4.361 |
| Tumor multiplicity (n) | 0.896 | 0.427 | 4.410 | 0.036 | 2.450 | 1.062-5.654 |
| Tumor grade | 0.843 | 0.410 | 4.234 | 0.040 | 2.324 | 1.041-5.190 |
| Comorbid diabetes | 0.759 | 0.556 | 1.865 | 0.172 | 2.136 | 0.719-6.350 |
| Treatment regimen | 0.845 | 0.401 | 4.430 | 0.035 | 2.328 | 1.060-5.113 |

Note: HR-NMIBC, high-risk non-muscle-invasive bladder cancer; B, Regression Coefficient; SE, Standard Error; HR, Hazard Ratio; CI, Confidence Interval.

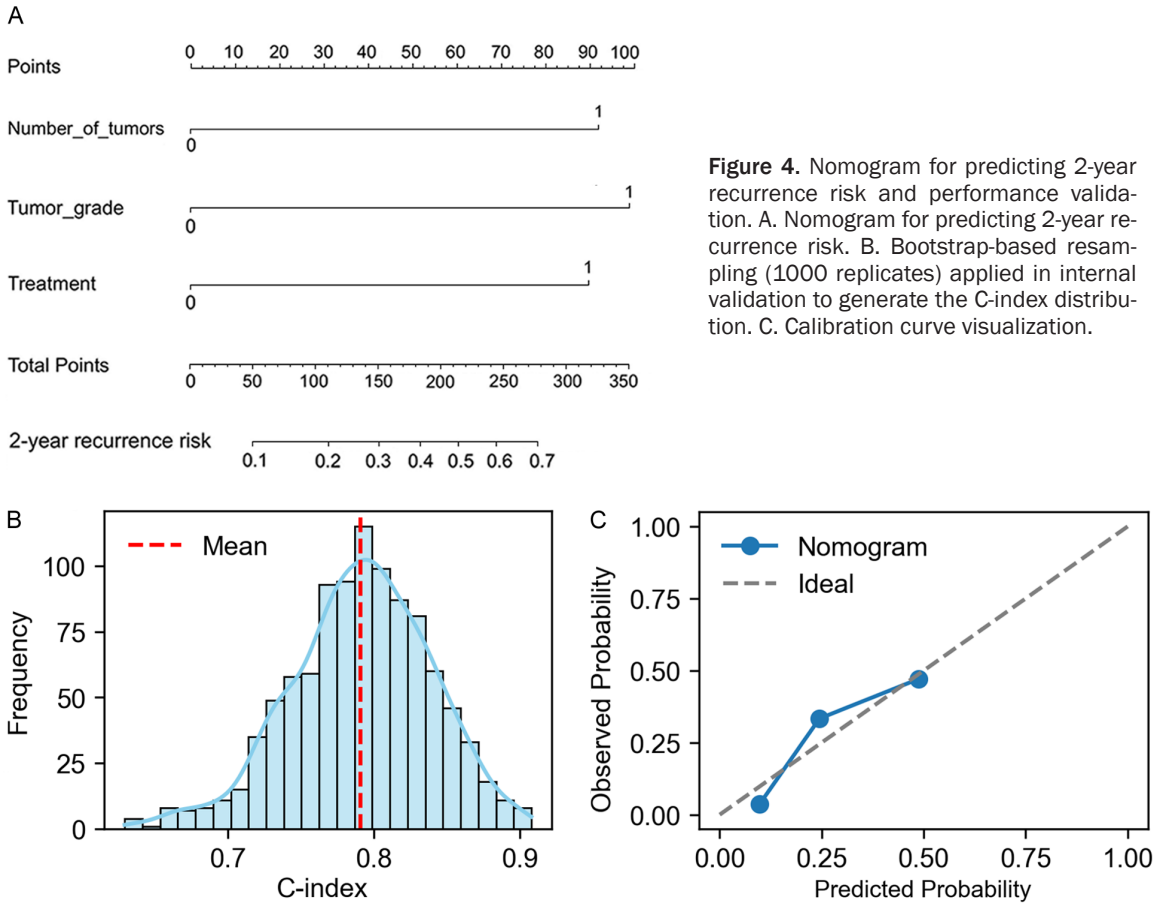


**Figure 4.** Nomogram for predicting 2-year recurrence risk and performance validation. A. Nomogram for predicting 2-year recurrence risk. B. Bootstrap-based resampling (1000 replicates) applied in internal validation to generate the C-index distribution. C. Calibration curve visualization.

## Discussion

Gemcitabine is a cell cycle phase-specific chemotherapeutic agent and a pyrimidine antimetabolite. Its antitumor mechanism primarily involves selective killing of cells in the deoxyribonucleic acid (DNA) synthesis phase (S phase) and inhibition of cell cycle progression at the G1/S checkpoint [16]. Clinical applications of gemcitabine extend to a variety of oncologic diseases, including localized unresectable intrahepatic cholangiocarcinoma and inoperable head and neck squamous cell carcinoma, where improved outcomes and controlled toxicity have been documented [17, 18]. For NMIBC patients, gemcitabine functions as an intravesical perfusion drug with some therapeutic effects, though it comes with toxic side effects such as bladder irritation [19]. HR-NMIBC patients often undergo BCG instillation post-TURBT as a first-line therapy. While this intervention contributes to reduced mortality and mitigates the risk of muscle invasion [20], its drawbacks include a substantial non-response rate (41%) and the potential for high-grade (grade 3 or 4) toxicities [21]. Given these challenges, this study attempts to explore improved therapies that could enhance prognosis in HR-NMIBC patients.

Our study results revealed no notable intergroup difference in 1-year postoperative recurrence. However, alternating BCG and PA instillations yielded superior prophylaxis against disease relapse in both groups. BCG and PA-MSHA are known to exert anti-tumor effects through distinct pathways, and their combined application may therefore enhance anti-tumor activity synergistically by acting through different mechanisms [22]. This synergy may help explain, to some degree, the pronounced recurrence-preventing effect of the alternating instillation approach in HR-NMIBC patients two years post-surgery. Hence, while alternating intravesical BCG and PA-MSHA initially suppressed immune activity - leading to no 1-year recurrence benefit - it ultimately lowered recurrence risk at the 2-year follow-up in HR-NMIBC patients.

Subsequent analysis demonstrated a favorable safety profile in HR-NMIBC patients administered with alternating BCG and PA-MSHA, as evidenced by statistically fewer adverse events in total (e.g., gastrointestinal reactions, fever, bladder irritation, hematuria). This enhanced tolerability may be attributed to the use of PA-MSHA, an inactivated bacterium that eliminates the risk of live bacterial infections. While BCG may cause side effects like bladder irritation and fever, alternating it with PA-MSHA helps reduce cumulative toxicities. These findings confirm that alternating BCG and PA-MSHA in bladder instillation is safe for HR-NMIBC patients.

Cytokeratin (CK), a key component of epithelial intermediate filaments, plays a significant role in bladder cancer progression. CK-19 has been strongly associated with NMIBC, demonstrating marked overexpression in affected patients. Besides, CYFRA21-1 up-regulation correlates with advanced tumor staging and higher histologic grades, further underscoring their utility in disease severity assessment [23-25]. In our study, HR-NMIBC patients treated with alternating intravesical BCG and PA-MSHA exhibited a significant reduction in abnormal CK-19 and CYFRA21-1 elevations, suggesting superior efficacy compared to gemcitabine monotherapy in mitigating disease progression. The enhanced therapeutic effect may stem from the synergistic action of BCG and PA-MSHA, which likely optimizes treatment efficacy, thus suppressing malignant progression while significantly reducing serum CK-19 and CYFRA21-1 concentrations. Furthermore, this alternating regimen led to greater patient-reported quality-of-life improvements, which can be attributed, at least in part, to the favorable safety profile of the combined therapy, minimizingpostoperative toxicity and speeding up recovery. All these findings support the marked inhibition of serum tumor markers (CK-19 and CYFRA21-1) as well as life quality enhancement in HR-NMIBC patients undergoing alternating BCG and PA-MSHA therapy.

In the univariate analysis, significant differences were observed between relapsed and non-relapsed patients regarding clinical stage, tumor diameter, tumor multiplicity, tumor grade, diabetes comorbidity, and treatment regimen. Subsequent multivariate Cox proportional hazards analysis validated several independent predictors for recurrence: tumor multiplicity, high tumor grade (G3), and treatment regimen. Tumors that develop in multiple foci typically indicate greater heterogeneity and a higher probability of residual disease, factors that may

increase the risk of postoperative recurrence. Additionally, high-grade (G3) tumors are characterized by a more invasive nature and a stronger inclination toward early recurrence. Yet, alternating use of BCG and PA-MSHA in intravesical immunotherapy instillations has shown a significant drop in recurrence risk, likely through synergistic multi-mechanistic therapeutic effects [26]. Building upon these findings, we developed a nomogram to predict the individual 2-year recurrence probability for HR-NMIBC patients. This predictive model demonstrated a positive correlation between each predictor's cumulative score and the estimated recurrence risk, providing a valuable tool for risk stratification and clinical decision-making. Internal validation using the Bootstrap resampling technique with 1000 iterations, combined with calibration curve analysis, confirmed moderate-to-high predictive capacity of this predictive model. The calibration analysis indicated good calibration performance in the medium-high risk range. These analytical efforts not only pinpointed the independent factors affecting 2-year postoperative recurrence in HR-NMIBC patients but also led to the development of a nomogram, serving as a quantitative risk assessment aid.

Several limitations in this study should be addressed in subsequent research. The first drawback is the lack of extended follow-up data (e.g., spanning 5-10 years). Future research should include prolonged tracking periods to better evaluate the long-term prognostic outcomes of this regimen. Next, no fundamental research was conducted to explore the underlying mechanisms of the treatments. Supplementing such analyses would undoubtedly enhance our comprehension of the therapeutic mechanisms at play. Furthermore, no comparative study was done on different doses of BCG and PA when used alternately for intravesical instillation. Further assessment in this aspect would assist in identifying the optimal doses and refining treatment protocols.

To conclude, alternating BCG and PA-MSHA by intravesical instillation demonstrates efficacy in preventing post-TURBT recurrence in HR-NMIBC patients, while maintaining patient safety. Patients presenting with clinical risk factors (e.g., multifocal tumors, high-grade disease, previous gemcitabine monotherapy instillation) are at an increased risk of recurrence within the first two years postoperatively. Additionally, incorporating factors such as tumor multiplicity, tumor grade, and treatment regimen enhances the predictive accuracy for 2-year postoperative recurrence risk in -NMIBC.

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## Disclosure of conflict of interest

None.

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