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INTRODUCTION

- Bladder cancer is a highly recurrent tumor, with 31% to 78% of patients experiencing recurrences [1].
- Tumor-infiltrating lymphocytes (TILs) play a crucial role in cancer immunology. However, pathologist-diagnosed TIL quantification is known to be subjective, necessitating the use of guidelines in practice [2].
- TILs have been noted as a favorable prognostic factor for bladder cancer prognosis and staging, studied in radical cystectomy specimens [3]. However, their effect have not been studied in transurethral resection of bladder (TURB) specimens alone.
- This study aimed to identify key predictors of tumor recurrence using clinicopathologic variables, including TILs assessed by an artificial intelligence (AI) model using a deep learning-based detection and segmentation algorithm.

MATERIALS AND METHODS

- Fifty-five patients diagnosed with invasive urothelial carcinoma and treated with TURB at Korea University Guro Hospital from 2015 to 2021 were included for clinicopathologic data analysis and AI model application. Hematoxylin and eosin stained WSIs were analyzed for pathologic variables and TILs were evaluated in the tumor center and invasive front, separately.

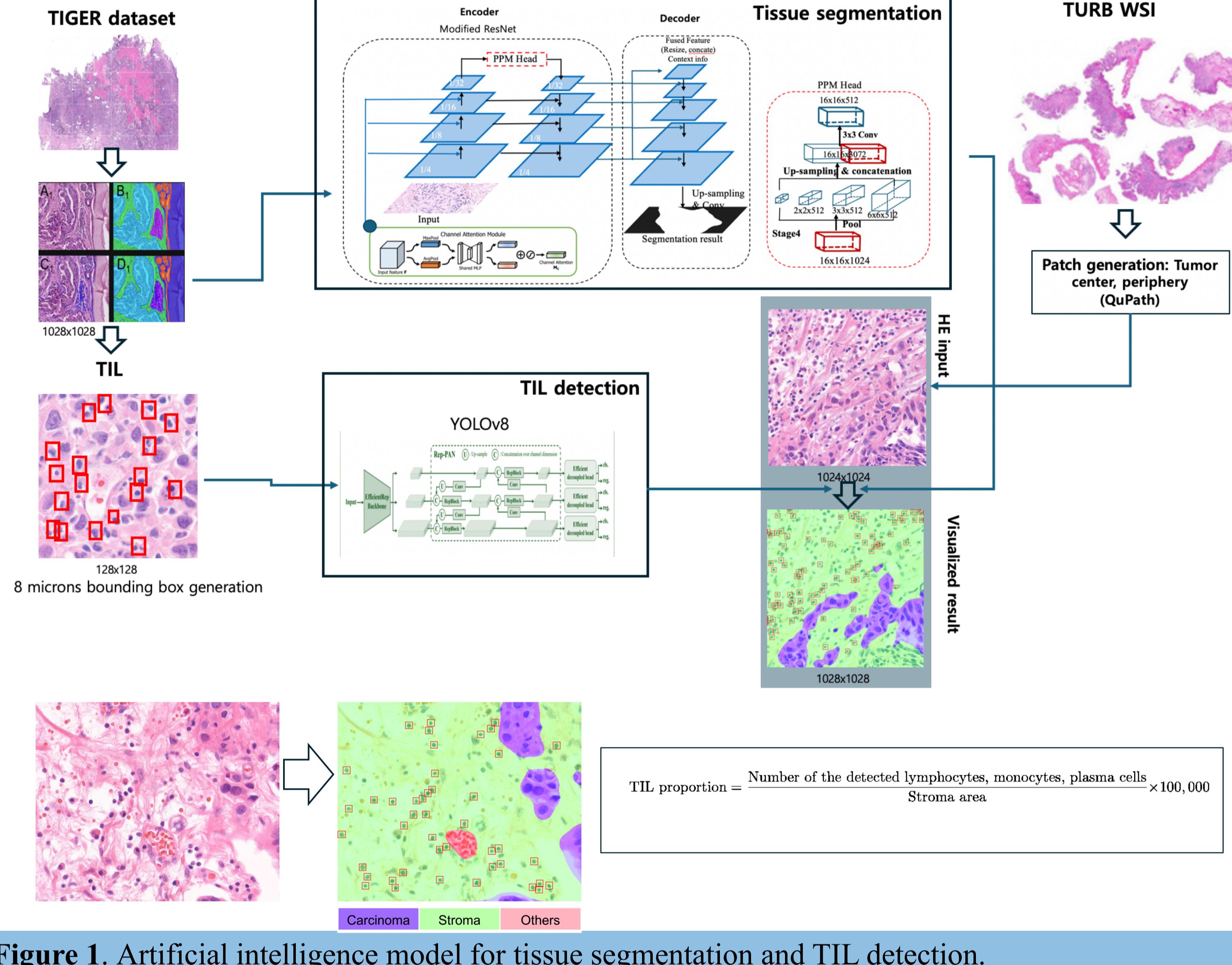


Figure 1. Artificial intelligence model for tissue segmentation and TIL detection.

Artificial intelligence model development (Figure 1)

- Our study utilized the TIGER challenge dataset, comprising 195 whole-slide images (WSIs) of breast cancer. We modified the annotations by combining tumor-associated and inflamed stroma into a single label. We extracted 1024x1024 pixel image patches from the WSIs.
- For TIL network training, with lymphocytes and plasma cells, marked within bounding boxes. The cells are initially pinpointed through point annotations; subsequently, squared bounding boxes of 8x8 microns are constructed around these points, centered on the annotations utilize an average equivalent diameter of 8 microns for lymphocytes. The training images are extracted from the WSIs at a fixed resolution of 128x128 pixels. For the training and validation phases, the image patches are divided in a 9:1 ratio, respectively.
- To segment invasive tumor and stroma regions, we developed a novel encode-decode network architecture including an encoding phase integrated a channel attention (Woo et al.[4]) module into bottlenecks of ResNet architecture, and Pyramid Pooling Module from UperNet [5] while the decoding phase adopted a UNet-like expansive path [6]. We trained the model using the mmSegmentation framework with an AdamW optimizer, achieving Dice scores of 78.52% for invasive tumor and 82.44% for stroma in the test dataset.
- For the detection of lymphocytes and plasma cells, we employed the YOLOv8 algorithm [7], which achieved a free-response operating characteristic of 48.2%.

RESULTS

Descriptive Statistics by Recurrence Status			
Variable	No Recurrence (N=17)	Recurrence (N=38)	p-value
Age	73.3 ± 8.4	69.8 ± 11.5	0.273
Sex			0.205
Female	0 (0.0%)	6 (15.8%)	
Male	17 (100.0%)	32 (84.2%)	
Carcinoma in situ			0.655
Absent	15 (88.2%)	30 (78.9%)	
Present	2 (11.8%)	8 (21.1%)	
pT category			0.012
1a	1 (5.9%)	12 (31.6%)	
1b	6 (35.3%)	5 (13.2%)	
1c	5 (29.4%)	2 (5.3%)	
2	5 (29.4%)	18 (47.4%)	
3a	0 (0.0%)	1 (2.6%)	
Histologic subtype			0.925
Conventional	14 (82.4%)	31 (81.6%)	
Micropapillary	1 (5.9%)	3 (7.9%)	
Plasmacytoid	1 (5.9%)	1 (2.6%)	
Sarcomatoid	1 (5.9%)	3 (7.9%)	
Lymphovascular invasion			0.963
Absent	16 (94.1%)	34 (89.5%)	
Present	1 (5.9%)	4 (10.5%)	
Invasive Front_TIL	24.6 ± 7.6	20.7 ± 8.9	0.124
Central_TIL	25.2 ± 7.6	19.7 ± 8.6	0.026
Months to recurrence	50.1 ± 40.3	16.4 ± 23.8	0.004

Table 1: Comparison of clinical and pathological characteristics between patients with and without tumor recurrence.
 Continuous variables are presented as mean ± standard deviation. Categorical variables are presented as count (percentage). P-values are derived from t-tests for continuous variables and chi-square tests for categorical variables. Statistically significant p-values ($p < 0.05$) are in bold and red.

- Our study of 55 bladder cancer patients revealed significant associations between tumor characteristics and recurrence risk. Notably, tumor central TILs were significantly higher in patients without recurrence compared to those with recurrence (25.2 ± 7.6 vs. 19.7 ± 8.6 , $p=0.026$). Additionally, tumor stage (pT category) showed a significant association with recurrence status ($p=0.012$), with pT1a tumors more prevalent in the recurrence group (31.6% vs. 5.9% in non-recurrence).

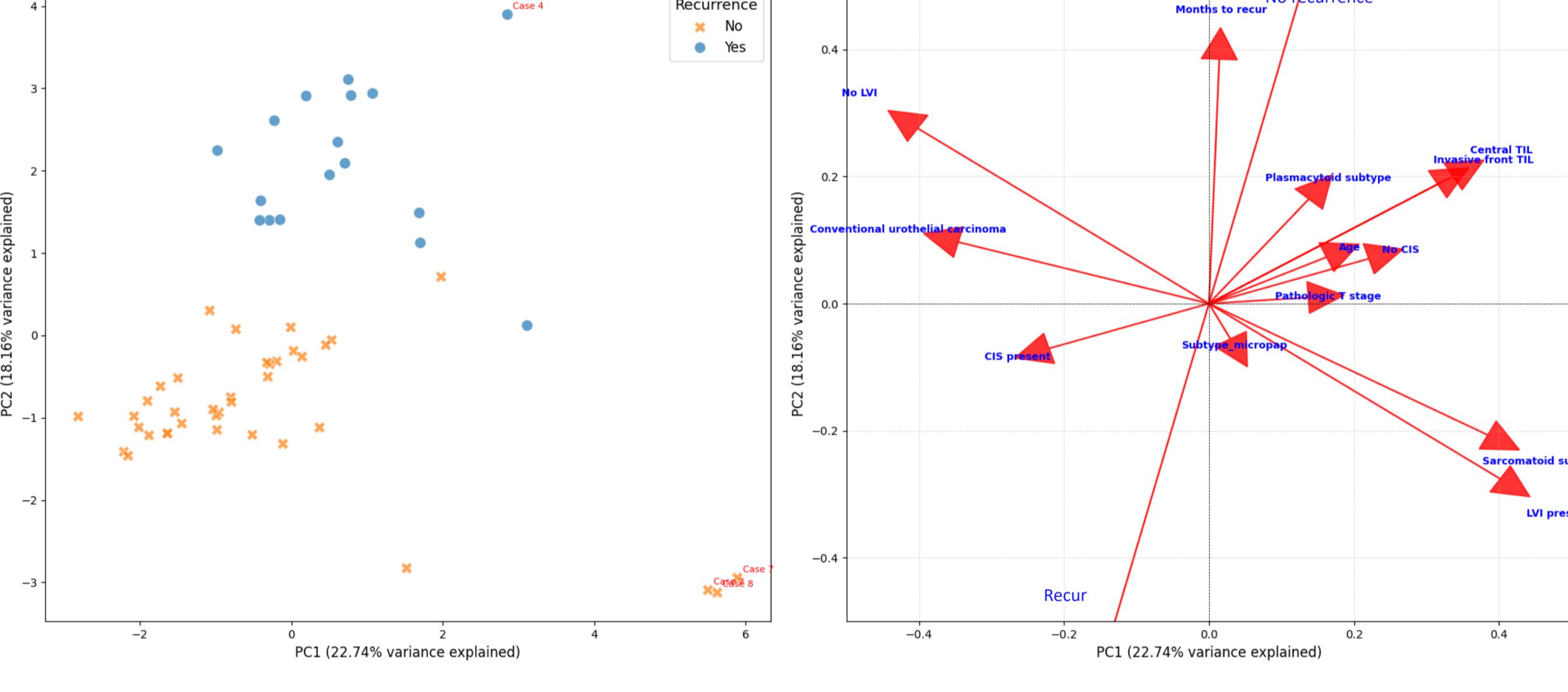


Figure 2. Principal component analysis of the clinicopathologic variables and recurrence.: TIL levels, particularly tumor central TILs, histological subtype (sarcomatoid), and factors such as LVI plays significant roles in the landscape of bladder cancer recurrence.

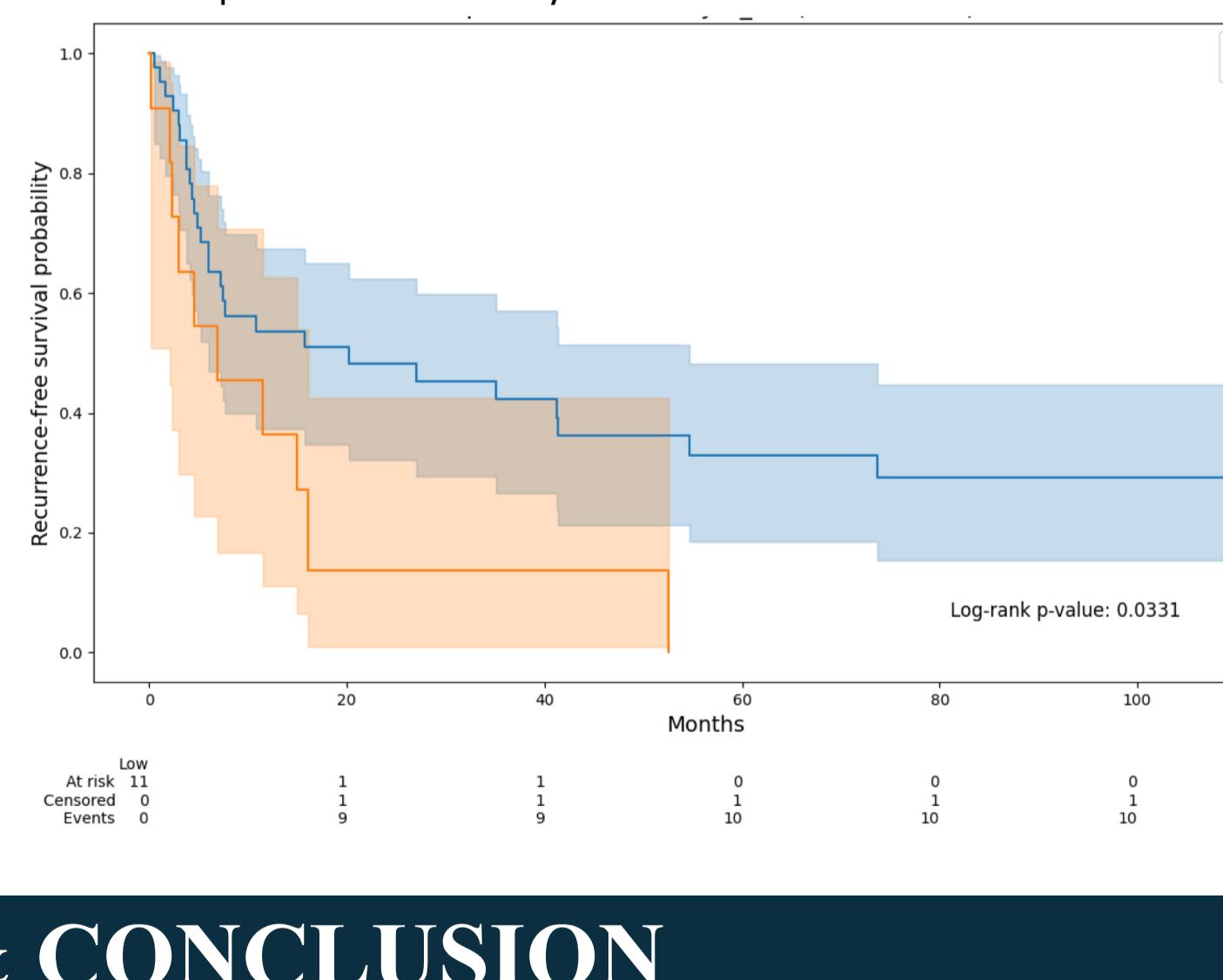


Figure 3. Kaplan-Meier analysis of the recurrence free survival (RFS) of the TURB patients (TIL proportion cutoff : 12.24): Patients were stratified into high and low central TIL groups using a cutoff value of 12.24, revealing a statistically significant difference in recurrence-free survival between the high and low central TIL groups (Log-rank p-value: 0.0331).

DISCUSSION & CONCLUSION

- Our study demonstrated a significant association between high tumor central TIL proportions with low recurrence in bladder cancer patients treated with TURB.
- The protective effect of tumor central TIL proportions observed in our study corroborates recent research suggesting that robust immune infiltration may suppress tumor recurrence in various cancer types, including bladder cancer.

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