

Prognostic impact of lymph node invasion levels in patients with bladder cancer undergoing radical cystectomy and pelvic lymphadenectomy

JUNICHI IKEDA¹, CHISATO OHE^{1,2}, TAKASHI YOSHIDA¹, TAKAHIRO NAKAMOTO^{1,3},
RYOICHI SAITO⁴, KOJI TSUTA³ and HIDEFUMI KINOSHITA¹

¹Department of Urology and Andrology, Kansai Medical University, Hirakata, Osaka 573-1010, Japan; ²Department of Pathology, Graduate School of Medicine, Osaka Metropolitan University, Osaka 545-8585, Japan; ³Department of Pathology, Kansai Medical University, Hirakata, Osaka 573-1010, Japan; ⁴Department of Urology, Kyoto University, Kyoto 606-8507, Japan

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Abstract. Extranodal extension in metastatic lymph nodes (LNs) is a poor prognostic factor in bladder cancer (BC). Furthermore, cancer invasion levels in sentinel LNs are associated with prognosis in melanoma. The present study aimed to evaluate the LN invasion level, defined as the extent of cancer invasion in anatomical and immunological LN substructures, and compare it with the pathological node (pN) stage of the tumor-node-metastasis staging system in BC. A total of 98 patients with BC who underwent radical cystectomy and pelvic lymphadenectomy were retrospectively assessed. The LN invasion level was classified as follows: Level 0, no cancer cell within the resected LNs; Level 1, cancer cells confined to intracapsular lymph vessels and subcapsular or transverse sinuses; Level 2, cancer cells infiltrating the cortex, paracortex or medulla; and Level 3, cancer cells infiltrating or beyond the LN capsule. The proportion of patients with Levels 0, 1, 2 and 3 was 70.4% (69/98), 8.2% (8/98), 14.3% (14/98) and 7.1% (7/98), respectively. Kaplan-Meier survival curves of recurrence-free survival (RFS), cancer-specific survival (CSS) and overall survival (OS) with LN invasion levels better stratified outcome patient when using Levels 1-3 compared with pN1-3. In addition, LN invasion levels better predicted RFS, CSS and OS, in comparison with the pN stage (c-index of 0.672 vs. 0.646, 0.688 vs. 0.665, and 0.702 vs. 0.661, respectively). Finally, multivariate analysis revealed that the predictive accuracy of the model integrating pathological tumor (pT) stage

and LN invasion levels in RFS, CSS and OS was greater than that of the conventional model that included pT and pN stage (c-index of 0.723 vs. 0.703, 0.710 vs. 0.694, and 0.725 vs. 0.692, respectively). In conclusion, the model with LN invasion levels accurately predicted the prognosis of patients with BC after radical cystectomy and pelvic lymphadenectomy.

Introduction

Radical cystectomy and pelvic lymphadenectomy are the standard treatments for muscle-invasive bladder cancer (BC) (1), and the presence of lymph node (LN) metastases is associated with poor survival outcomes (2). In the 8th American Joint Committee on Cancer/International Union Against Cancer tumor (T) node (N) metastasis (M) staging system, patients with positive LNs are stratified into three stages (N1, N2 and N3) based on the number of positive LNs and the site of metastatic LNs (3).

The number of positive LNs, LN density (defined as the number of positive LNs divided by the total number of removed LNs), maximum diameter of metastatic LNs and extranodal extension (ENE) are independent prognostic factors in BC (4-9). A previous multivariable model that included LN density, number of removed LNs, number of positive LNs and ENE, demonstrated that ENE is a strong independent prognostic factor (7).

The International Collaboration on Cancer Reporting currently recommends that the extranodal spread in regional LN status be included in the pathology reporting of the carcinoma of the bladder/cystectomy (10). Although ENE in metastatic LNs is associated with worse cancer-specific survival (CSS), an ENE assessment is not included in the current TNM staging system due to limited published data. Therefore, LN evaluation methods that include ENE are required.

In a previous study, the prognostic impact of cancer invasion levels in sentinel LNs was reported in melanoma (11). In this assessment, sentinel node invasion level (SNIL) was defined as follows: SNIL 1, tumor cells confined to lymphatic vessels and subcapsular sinus or transverse sinuses; SNIL 2, tumor cells infiltrating the cortex or paracortex; and SNIL 3,

Correspondence to: Dr Chisato Ohe, Department of Pathology, Graduate School of Medicine, Osaka Metropolitan University, 1-4-3 Asahi, Abeno, Osaka 545-8585, Japan
E-mail: ohec@omu.ac.jp

Abbreviations: LN, lymph node; ENE, extranodal extension; BC, bladder cancer

Key words: LN, LN invasion level, LN metastasis, BC, ENE

tumor cells infiltrating the medulla or capsule (11). The SNIL status stratified three independent risk groups of patients who were SN-positive. This is a simple histology-based assessment of metastasis using the anatomic localization of LNs without distance measurements with a microscope (12). Nevertheless, the prognostic impact of LN invasion levels in anatomically- and immunologically-defined substructures has not been assessed in BC.

In the present study, the clinical significance of LN invasion levels in BC was assessed and the prognostic accuracy of the generated model [pathological (p)T stage and LN invasion level] and the conventional model (pT and pN stages) in patients who underwent radical cystectomy and pelvic lymphadenectomy was compared.

Materials and methods

Case selection. After receiving ethics approval from Institutional Review Board of Kansai Medical University Hospital (Osaka, Japan; approval no. 2021226), data from 131 patients with BC ($\geq$ pT1) who underwent radical cystectomy at Kansai Medical University Hospital between January 2006 and December 2017 were extracted from the institutional database. An opt-out approach was used to obtain informed consent on the hospital website. A total of 33 patients were excluded, including eight patients with nonurothelial carcinoma (small cell carcinoma, n=2; urachal carcinoma, n=2; and undifferentiated carcinoma, n=4), 14 patients with unremoved LNs, and 11 patients treated with neoadjuvant chemotherapy (NAC). Thus, 98 patients who underwent radical cystectomy and pelvic lymphadenectomy were selected for analysis. Patients with TNM clinical stage III or higher in the present study underwent an extended pelvic lymphadenectomy.

Histological evaluation. All surgical specimens were processed according to standard pathology procedures. Hematoxylin and eosin (H&E)-stained slides prepared for routine histological examination were re-evaluated by a urologic pathologist (CO) using the 2016 World Health Organization classification (13) and the 2017 Union for International Cancer Control TNM staging system (3). Clinicopathological characteristics such as pathological TNM stage, grade and histological subtypes of urothelial carcinoma, including divergent differentiation/subtypes, lymphovascular invasion and surgical margin, were reviewed. Furthermore, the association between inflammatory status and lymph node metastasis was assessed using histology-based tumor-associated immune cell status (TAICs), a methodology previously reported by our group (14).

The resected LNs of pelvic lymphadenectomy during radical cystectomy were evaluated using H&E-stained slides. Based on best practice guidelines for the routine pathology evaluation of the immune system (15), pN was assessed based on the TNM staging system (3): pN0, no cancer cells in any LNs; pN1, single regional LN metastasis in the true pelvis (perivesical, obturator, internal and external iliac, or sacral LN); pN2, multiple regional LN metastases in the true pelvis; and pN3, LN metastasis in the common iliac LNs.

The LN invasion level in each case was evaluated in the most highly invasive LNs with reference to the previous

methodology (11): Level 0, no cancer cell within the resected LNs; Level 1, cancer cells confined to intracapsular lymph vessels and subcapsular or transverse sinuses; Level 2, cancer cells infiltrating the cortex, paracortex, or medulla; and Level 3, cancer cells infiltrating or beyond the LN capsule, corresponding to the ENE (Fig. 1). Histological evaluations of LNs were performed by two independent pathologists (JI and CO) blinded to clinical outcomes, and discordant patterns were resolved by consensus. The LN invasion level was evaluated using the H&E-stained slides only.

Statistical analysis. Continuous data are presented as median and interquartile range (IQR). Fisher's exact test and the Mann-Whitney U test were used for comparisons between two groups. Recurrence-free survival (RFS), CSS and overall survival (OS) were assessed using the Kaplan-Meier method and the log-rank test. Harrell's concordance index (c-index) was used to compare the predictive accuracy of the Cox models. Multivariate Cox proportional hazard models were assessed to determine hazard ratio (HR). All statistical analyses were performed using EZR version 1.55 (Saitama Medical Center, Jichi Medical University) (16). $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Patient characteristics. As shown in Table I, of the 98 patients, 16 (16.3%) were female and 82 (83.7%) were male, with a median age of 71 (IQR, 67.0-77.0) years. Pathology examination revealed the proportions of pT1, pT2, pT3 and pT4 as 9.2% (9/98), 34.7% (34/98), 42.9% (42/98), and 13.3% (13/98), respectively. Divergent differentiation/subtypes on urothelial histology were observed in 33 patients (33.7%). The number of removed LNs was 22.5 (IQR, 14.3-29.5). A total of 26 (26.5%) patients experienced recurrence and 22 (22.5%) patients died due to BC. The median follow-up time was 99.0 (IQR, 72.2-129.0) months. The number of metastatic lymph nodes at each lymph node invasion level is presented in Table SI. Furthermore, a total of 12 patients (12.24%) with intravesical Bacillus Calmette-Guérin therapy were included.

Association of LN invasion level with pN stage, and TAICs with pN stage. The proportion of pN0, pN1, pN2 and pN3 was 70.4% (69/98), 12.2% (12/98), 15.3% (15/98) and 2.0% (2/98), respectively (Table I). Level 2 accounted for the majority of cases in pN1 and pN2, and Levels 2 and 3 accounted for 50% of the cases in pN3 (Fig. 2). TAICs was significantly associated with pN stages ($P = 0.03$; Table SII).

Comparison of prognostic significance between LN invasion level and pN stage. The Kaplan-Meier survival curves of RFS, CSS and OS in LN invasion Levels 1-3 and pN1-3 are presented in Fig. 3. In comparison with Level 1, LN invasion Levels 2 and 3 were associated with a higher risk of RFS (HR, 6.84; $P = 0.08$ and HR, 20.80; $P = 0.01$, respectively; Fig. 3A), CSS (HR, 5.72; $P = 0.11$ and HR, 18.90; $P = 0.01$, respectively; Fig. 3B) and OS (HR, 9.17; $P = 0.04$ and HR, 29.38; $P = 0.002$, respectively; Fig. 3C). In contrast, in comparison with pN1, pN2 and pN3 were associated with a higher risk of RFS (HR, 2.01; $P = 0.29$ and HR, 3.12; $P = 0.20$, respectively; Fig. 2D), CSS

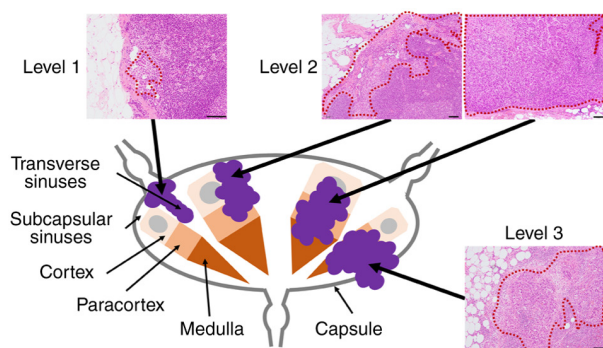


Figure 1. Schematic diagram and representative images of H&E-stained slides of invasion levels in metastatic LNs. Level 1, cancer cells confined to intracapsular lymph vessels and subcapsular or transverse sinuses; Level 2, cancer cells infiltrating the cortex, paracortex or medulla; and Level 3, cancer cells infiltrating or beyond the LN capsule. Cancer metastatic areas are indicated by red dotted lines in the H&E-stained images. Scale bar, 100 μ m. H&E, hematoxylin and eosin; LN, lymph node.

(HR, 1.55; $P=0.53$ and HR, 3.28; $P=0.18$, respectively; Fig. 3E) and OS (HR, 1.92; $P=0.22$ and HR, 2.17; $P=0.35$, respectively; Fig. 3F), but without statistical significance. Cases with low TAICs were associated with a significantly worse RFS rate (HR, 5.13; $P<0.001$; Fig. S1A), CSS rate (HR, 3.64; $P=0.008$; Fig. S1B) and OS rate (HR, 2.29; $P=0.01$; Fig. S1C) than those with high TAICs. Furthermore, 20/29 of patients with LN metastasis received adjuvant chemotherapy, which did not significantly improve CSS or OS (HR, 0.70; $P=0.60$ and HR, 0.52; $P=0.20$, respectively).

Prognostic significance of LN invasion level. The associations of clinicopathological factors with RFS, CSS and OS after radical cystectomy are presented in Tables II-IV. In the univariate analysis, pT4, pN3 and LN invasion level 3 were significantly associated with RFS ($P=0.026$, $P=0.009$ and $P<0.001$, respectively), CSS ($P=0.042$, $P=0.004$ and $P<0.001$, respectively) and OS ($P=0.023$, $P=0.017$ and $P<0.001$, respectively). In multivariate analysis, two models were evaluated: Model 1 (conventional model) with pT and pN stages; and Model 2 (proposed model) with pT stage and LN invasion level. In comparison with Model 1, Model 2 demonstrated a higher accuracy in predicting RFS (c-index, 0.703 and 0.723, respectively), CSS (c-index, 0.694 and 0.710, respectively) and OS (c-index, 0.692 and 0.725, respectively) than Model 1.

Discussion

The present study demonstrated that a histological assessment of LN invasion levels in BC better stratified patient outcome using Levels 0, 1, 2 and 3 in comparison with the pN stage of the TNM staging system. In addition, the LN invasion level better predicted RFS, CSS and OS compared with the pN stage, and multivariate analysis revealed that the predictive accuracy of the proposed model was greater than that of the conventional model. Thus, LN invasion levels may provide a better prognostic prediction for patients with BC after radical cystectomy and lymphadenectomy. Furthermore, LN invasion level may be a useful criterion for implementing adjuvant therapy.

Table I. Patient characteristics (n=98).

Variable	Value
Age, years	71 (67.0-77.0)
Sex	
Female	16 (16.3)
Male	82 (83.7)
Tumor grade	
Low	0 (0.0)
High	98 (100.0)
Divergent differentiation/subtype ^a	
Absent	65 (66.3)
Present	33 (33.7)
pT stage	
1	9 (9.2)
2	34 (34.7)
3	42 (42.9)
4	13 (13.3)
pN stage	
0	69 (70.4)
1	12 (12.2)
2	15 (15.3)
3	2 (2.0)
Surgical margin	
Negative/X	91 (92.8)
Positive	7 (7.1)
Lymphovascular invasion	
Absent	22 (22.5)
Present	76 (77.6)
Number of removed LNs	22.5 (14.3-29.5)
Adjuvant chemotherapy	
No	64 (65.3)
Yes	34 (34.7)
Recurrence	26 (26.5)
Cancer-specific mortality	22 (22.5)
Overall mortality	40 (40.8)
Follow-up time, months	99 (72.2-129.0)

Data are presented as n (%) or median (interquartile range). pT, pathological tumor; pN, pathological node; LNs, lymph nodes.

^aDivergent differentiation (squamous/glandular) and subtype (micro-papillary/sarcomatoid).

Recently, inflammation and nodal state have attracted attention in muscle invasive BC, and new biomarkers have been identified (17). Although the number of positive LNs and the site of metastatic LNs are the standard methods of evaluation in the TNM staging system (3), several LN parameters, such as the number of positive LNs, LN density, the maximum diameter of metastatic LNs and ENE, are also prognostic indicators (4-9). Consistent with previous studies (7), Level 3 corresponding to the ENE was significantly associated with a worse prognosis compared with other levels in RFS, CSS and

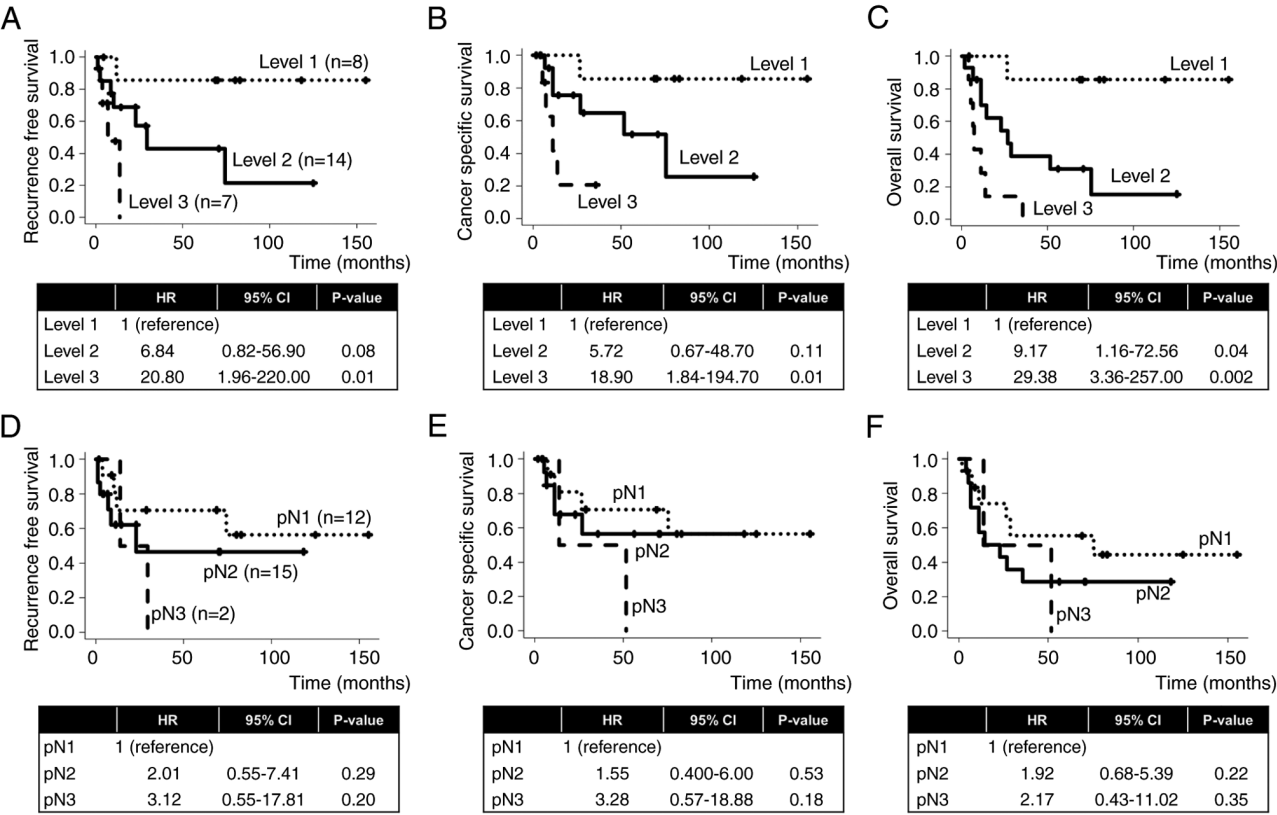
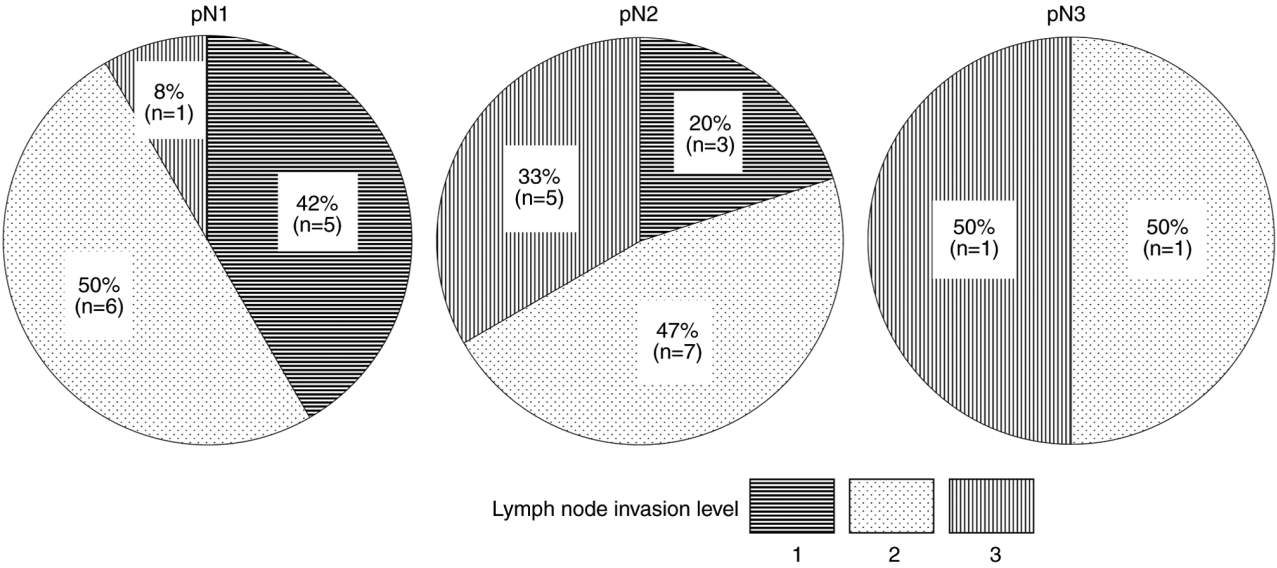


Figure 3. Comparison of prognostic significance between lymph node invasion levels and pN stages. Kaplan-Meier curve of (A) recurrence-free survival, (B) cancer-specific survival and (C) overall survival for lymph node invasion levels, and of (D) recurrence-free survival, (E) cancer-specific survival and (F) overall survival for pN stages. pN, pathological node; HR, hazard ratio; CI, confidence interval.

OS in the present study. In addition to the prognostic impact of ENE the present study demonstrated that LN invasion levels could be used to classify patients into three independent risk groups according to the degree of cancer infiltration in metastatic LNs.

To the best of our knowledge, LN invasion levels have not been previously assessed in BC. Although a similar evaluation

method was first proposed for metastatic sentinel nodes in melanoma (11), the definition of Levels 2 and 3 in the present study also integrated the ENE. Notably, the present study demonstrated that LN invasion levels were more significantly associated with prognosis compared with pN stages. By using LN invasion levels, the present study evaluated the metastatic infiltration in their anatomically and immunologically defined

Table II. Cox regression analysis of prognostic factors for predicting recurrence-free survival.

Variable	Univariate		Model 1		Model 2	
	P-value	c-index	HR (95% CI)	P-value	HR (95% CI)	P-value
Age, years	0.418	0.542				
Sex (female vs. male)	0.411	0.533				
pT stage		0.687				
1	-		1 (reference)		1 (reference)	
2	0.800		1.16 (0.13-10.07)	0.892	1.29 (0.15-11.16)	0.815
3	0.270		2.11 (0.26-17.04)	0.486	2.25 (0.28-18.11)	0.446
4	0.026		7.53 (0.88-64.42)	0.065	5.73 (0.66-50.00)	0.115
pN stage		0.646				
0	-		1 (reference)			
1	0.159		2.00 (0.64-6.26)	0.235		
2	0.004		2.34 (0.81-6.78)	0.116		
3	0.009		6.90 (1.39-34.18)	0.018		
Lymph node invasion level		0.672				
0	-				1 (reference)	
1	0.713				0.71 (0.09-5.47)	0.740
2	0.001				2.90 (1.06-7.96)	0.038
3	<0.001				7.25 (1.94-27.15)	0.003

HR, hazard ratio; CI, confidence interval; c-index, Harrell's concordance index.

substructures. Thus, the results of the present study are consistent with the assessment that the natural route of metastatic spread in LNs reflects a more accurate prognosis compared with the current pN stage (11).

Anatomically, LNs are surrounded by capsules, and multiple lymph lobules are demarcated by lymph-filled sinuses. The superficial cortex is composed of follicles and the inter-follicular cortex of all adjacent lobules, and the paracortex is formed by their deep cortical units. The medulla is composed of the paracortex and its medullary cords and sinuses (18). In the first step of LN metastasis, cancer cells enter the LNs through the subcapsular sinus; from there, they invade the cortex to the medulla and break the LN capsule (19). The leakage of tumor cells through holes in the LN capsule into nearby soft tissues results in the ENE (20-22). Due to the metastatic process in LNs, it was reasonable to expect that a higher LN invasion level was significantly associated with poor prognosis.

Univariate analysis revealed that there were no significant differences between invasion levels 0 and 1 (Tables II-IV); cancer cells confined to intracapsular lymph vessels and subcapsular or transverse sinuses corresponded to no parenchymal metastasis in LNs. Contrary to the findings in the present study, the cause-specific survival rate, which is defined as the mortality rate owing to bladder cancer in patients with molecularly detected micrometastases, has been reported to be markedly lower than in those without micrometastases, independent of pathologically-positive LNs (23). In contrast, it has been reported that there is no notable difference in survival between the pN micro+ and pN0 groups of patients with immunohistochemically-detected micrometastases in LNs, nor between the pN micro+ and pN+ groups (24,25). The

issue is controversial, and larger studies are needed to evaluate the clinical significance of micrometastases and parenchymal metastasis in LNs as the number of patients with Level 1 LN metastases remains too small for a definitive conclusion to be reached.

The present study has several limitations; i) The present study was a retrospective, single-center study with a small sample size. Therefore, validation analysis across multiple institutions is needed; ii) LN invasion levels were assessed in the most highly invasive LNs, not the sentinel LNs, using H&E-stained slides prepared for routine pathology examinations; iii) lymphadenectomy was performed by different surgeons, although a pelvic lymphadenectomy was performed and the mean number of resected LNs was 22.5, which was comparable to the number of dissected lymph nodes reported previously (26,27). If the dissection range is determined in advance and the procedure is performed reliably, there should be little difference between surgeons; iv) no patients treated with NAC were included in the present study. Lymph node status may change when NAC is administered, and the pN+ status after NAC may have a negative impact compared with a pN+ status without NAC (28). Therefore, the present study excluded patients who received NAC to adjust LN status. Del Bene *et al* (28) reported that NAC reduces pN+ status and improves prognosis; therefore, further investigation in to the association between LN invasion levels and prognosis after NAC treatment is needed; and v) sentinel LNs were not detected in Kansai Medical University Hospital. For a more detailed assessment of sentinel LNs, indocyanine green fluorescence should be considered (29). Despite these limitations, the present study demonstrated the clinical impact of a

Table III. Cox regression analysis of prognostic factors for predicting cancer-specific survival.

Variable	Univariate		Model 1		Model 2	
	P-value	c-index	HR (95% CI)	P-value	HR (95% CI)	P-value
Age, years	0.100	0.600				
Sex (female vs. male)	0.145	0.565				
pT stage		0.675				
1	-		1 (reference)		1 (reference)	
2	0.815		1.06 (0.12-9.24)	0.958	1.23 (0.14-10.65)	0.850
3	0.423		1.29 (0.15-11.08)	0.816	1.38 (0.16-11.73)	0.769
4	0.042		5.90 (0.66-52.68)	0.112	4.08 (0.44-37.53)	0.215
pN stage		0.665				
0	-		1 (reference)			
1	0.070		3.01 (0.92-9.88)	0.070		
2	0.007		2.35 (0.72-7.66)	0.157		
3	0.004		10.81 (2.05-57.08)	0.005		
Lymph node invasion level		0.688				
0	-				1 (ref.)	
1	0.878				0.93 (0.12-7.35)	0.946
2	0.002				3.37 (1.09-10.43)	0.035
3	<0.001				13.04 (3.31-51.37)	<0.001

HR, hazard ratio; CI, confidence interval; c-index, Harrell's concordance index.

Table IV. Cox regression analysis of prognostic factors for predicting overall survival.

Variable	Univariate		Model 1		Model 2	
	P-value	c-index	HR (95% CI)	P-value	HR (95% CI)	P-value
Age, years	0.019	0.610				
Sex (female vs. male)	0.110	0.542				
pT stage		0.660				
1	-		1 (reference)		1 (reference)	
2	0.784		1.04 (0.23-4.79)	0.962	1.21 (0.26-5.55)	0.804
3	0.275		1.45 (0.32-6.55)	0.632	1.34 (0.29-6.14)	0.708
4	0.023		3.36 (0.66-17.00)	0.143	2.50 (0.48-12.94)	0.273
pN stage		0.661				
0	-		1 (reference)			
1	0.071		2.20 (0.87-5.59)	0.100		
2	<0.001		3.41 (1.45-8.05)	0.005		
3	0.017		5.60 (1.21-25.82)	0.027		
Lymph node invasion level		0.702				
0	-				1 (reference)	
1	0.450				0.47 (0.06-3.54)	0.465
2	<0.001				3.81 (1.60-9.06)	0.003
3	<0.001				14.81 (5.13-42.73)	<0.001

HR, hazard ratio; CI, confidence interval; c-index, Harrell's concordance index.

novel LN assessment in BC considering the natural route of metastatic spread in LNs.

In conclusion, the present study demonstrated the usefulness of the histological assessment of LN invasion levels,

considering their anatomical and immunological substructures. The proposed model integrating pT and LN invasion levels accurately predicted the prognosis of patients with BC undergoing radical cystectomy and pelvic lymphadenectomy.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

JI, CO and TY designed the present study. JI, CO, TY and TN performed data collection and pathological assessments. JI and CO confirm the authenticity of all the raw data. JI performed the statistical analyses. JI, CO, TY, RS, KT and HK interpreted the data. JI and CO drafted the manuscript. JI, CO, TY, TN, RS, KT and HK critically revised the manuscript for important intellectual content. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Institutional Review Board of Kansai Medical University Hospital (Osaka, Japan; approval no. 2021226). Kansai Medical University Hospital is one of the affiliated medical facilities of Kansai Medical University. Informed consent was obtained as an opt-out on the Kansai Medical University Hospital website (https://hp.kmu.ac.jp/about/research/diagnostic_pathology/). No patients expressed a refusal.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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