Research Report

Multi-Center Assessment of Lymph-Node Density and Nodal-Stage to Predict Disease-Specific Survival in Patients with Bladder Cancer Treated by Radical Cystectomy

Erik J. van Gennep[a,b], Francesco Claps[a−c], Peter J. Bostrom[d,e], Shahrokh F. Shariat[f−j],
Yann Neuzillet[a,k,l], Alexandre R. Zlotta[d], Carlo Trombetta[c], Markus Eckstein[m], Laura S. Mertens[a],
Rossana Bussani[n], Maximilian Burger[o], Joost L. Boormans[p], Bernd Wullich[q], Arndt Hartmann[m],
Roman Mayr[o], Nicola Pavan[c], Riccardo Bartoletti[r], M. Carmen Mir[s], Damien Pouessel[k,t],
John van der Hoeven[u], Theo H. van der Kwast[v], Yves Allory[k,w], Tahlita C.M. Zuiverloon[p],
Yair Lotan[f] and Bas W.G. van Rhijn[a,d,o,*]

[a]*Department of Surgical Oncology (Urology), Netherlands Cancer Institute – Antoni van Leeuwenhoek Hospital, Amsterdam, The Netherlands*
[b]*Department of Urology, Leiden University Medical Center, Leiden, The Netherlands*
[c]*Department of Medicine, Surgery and Health Sciences, Urological Clinic, University of Trieste, Trieste, Italy*
[d]*Department of Surgery (Urology) and Surgical Oncology, University Health Network, Princess Margaret Cancer Center, University of Toronto, Toronto, ON, Canada*
[e]*Department of Urology, Turku University Hospital and University of Turku, Turku, Finland*
[f]*Department of Urology, University of Texas Southwestern Medical Center, Dallas, TX, USA*
[g]*Department of Urology, Comprehensive Cancer Center, Medical University of Vienna, Vienna, Austria*
[h]*Department of Urology, Weill Cornell Medical College, New York, NY, USA*
[i]*Department of Urology, Second Faculty of Medicine, Charles University, Prague, Czech Republic*
[j]*Institute for Urology and Reproductive Health, I.M. Sechenov First Moscow State Medical University, Moscow, Russia*
[k]*Molecular Oncology Team, Institut Curie, CNRS, UMR144, PSL Research University, Paris, France*
[l]*Department of Urology, Hôpital Foch, UVSQ-Paris-Saclay University, Suresnes, France*
[m]*Institute of Pathology, University Hospital Erlangen, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany*
[n]*Department of Pathology, University of Trieste, Trieste, Italy*
[o]*Department of Urology, Caritas St Josef Medical Center, University of Regensburg, Regensburg, Germany*
[p]*Department of Urology, Erasmus MC Cancer Institute, University Medical Center, Rotterdam, The Netherlands*

*Correspondence to: Bas W.G. van Rhijn, MD, PhD, FEBU, Department of Surgical Oncology (Urology), Netherlands Cancer Institute – Antoni van Leeuwenhoek Hospital, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands. Tel.: +31 205122553; E-mail: b.v.rhijn@nki.nl.

^{q}*Department of Urology and Pediatric Urology, University Hospital Erlangen, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany*
^{r}*Department of Translational Research and New Technologies, Unit of Urology, University of Pisa, Pisa, Italy*
^{s}*Department of Urology, Hospital Universitario La Ribera, Valencia, Spain*
^{t}*Department of Medical Oncology, Claudius Regaud Institute, Toulouse University Cancer Center (IUCT) Oncopole, Toulouse, France*
^{u}*Department of Urology, Reinier de Graaf Hospital, Delft, The Netherlands*
^{v}*Department of Pathology, University Health Network, Princess Margaret Cancer Center, University of Toronto, Toronto, ON, Canada*
^{w}*Department of Pathology, Institut Curie, Paris, France*

Abstract.
BACKGROUND: Prognostic tools in pathological-node (pN) patients after radical cystectomy (RC) are needed.
OBJECTIVE: To evaluate the prognostic impact of lymph node (LN)-density on disease-specific survival (DSS) in patients with bladder cancer (BC) undergoing RC with pelvic lymph node dissection.
METHODS: We analyzed a multi-institutional cohort of 1169 patients treated with upfront RC for cT1-4aN0M0 urothelial BC at nine centers. LN-density was calculated as the ratio of the number of positive LNs $\times$ 100% to the number of LNs removed. The optimal LN-density cut-off value was defined by creating a time-dependent receiver operating characteristic (ROC) curve in pN patients. Univariable and multivariable Cox' regression analyses were used to assess the effect of conventional Tumor Nodes Metastasis (TNM) nodal staging system, LN-density and other LN-related variables on DSS in the pN-positive cohort.
RESULTS: Of the 1169 patients, 463 (39.6%) patients had LN-involvement. The area under the ROC curve was 0.60 and the cut-off for LN-density was set at 20%, 223 of the pN-positive patients (48.2%) had a LN-density $\geq$ 20%. In multivariable models, the number of LN-metastases (HR 1.03, $p = 0.005$) and LN-density, either as continuous (HR 1.01, $p = 0.013$) or as categorical variable (HR 1.37, $p = 0.014$), were independently associated with worse DSS, whereas pN-stage was not.
CONCLUSIONS: LN-density $\geq$ 20% was an independent predictor of worse DSS in BC patients with LN-involvement at RC. The integration of LN-density and other LN-parameters rather than only conventional pN-stage may contribute to a more refined risk-stratification in BC patients with nodal involvement.

Keywords: Bladder cancer, radical cystectomy, survival, lymph node density, urothelial neoplasm

INTRODUCTION

Radical cystectomy (RC) and pelvic lymph node dissection (PLND) with or without neoadjuvant chemotherapy (NAC) represents the standard-of-care treatment for both muscle-invasive bladder cancer (MIBC) and high-risk (Bacillus Calmette-Guerin (BCG) unresponsive) non-muscle-invasive bladder cancer (NMIBC) [1, 2]. Despite the adoption of both NAC and minimally-invasive approaches to RC, survival has remained largely unchanged [3, 4]. Moreover, approximately 25–40% of MIBC patients harbor occult lymph-node metastases (LNMs) at RC with 5-year survival rates of only 15% to 35% [5–7].

Extended or super-extended PLND represents an integral part of the surgical treatment of BC and lymph-node (LN)-related variables have been investigated as prognostic tools in RC-patients [6, 8]. Particularly, LN-density has emerged as a predictor for patients with LNMs because LN-density can potentially circumvent the inter-patient and inter-surgeon variability of PLND-yield better than conventional Tumor-Nodes-Metastasis (TNM) Nodal staging [6, 9].

Several clinical, pathological and molecular tools have prognostic value [10–12], none of these are currently used in clinical daily practice, highlighting an unmet need. Ideally, medical decisions should be tailored to the individual patient based on predicted response to local treatment and systemic agents [12]. In this context, the objective of our study was to evaluate the association between LN-density on disease-specific survival (DSS) among cN0M0 RC-patients with occult LNMs, extracted from a large multi-institutional series of individual patients' data (IPD). We hypothesized that LN-density would

serve as a more reliable prognostic tool in upfront RC-patients harboring LNMs compared to the conventional pN-staging system.

MATERIALS AND METHODS

Patient selection and data collection

We identified 1169 patients who underwent RC and bilateral PLND with curative intent for cT1-4aN0M0 urothelial BC at nine centers (Figure S1) between October 1986 and March 2020. The patients did not receive neoadjuvant treatment(s). Appropriate ethical approval was obtained at each site according to national regulations and the principles of the Declaration of Helsinki.

The collected variables included age, gender, pathological tumor (pT) stage and grade, pathological nodal (pN) status, number of positive and total number of LNs removed, lympho-vascular invasion (LVI), concomitant carcinoma-in-situ (CIS), positive surgical margins (PSMs), presence of variant histologies (VHs) next to urothelial carcinoma, adjuvant chemotherapy (AC), and adjuvant radiation.

Before RC, the patients underwent transurethral resection and computed tomography of the abdomen/pelvis and at least a chest X-ray to rule out the presence of (distant) metastases. Patients were followed in accordance with individual site surveillance protocols.

Pathological evaluation

The RC-specimens were locally reviewed by a dedicated uro-pathologist at each center and staged based on the TNM system (2017 classification, 8th edition) while tumor-grade was based on the 2004/2016 WHO system. PSMs were defined as the presence of tumor at inked areas of soft-tissue on the RC specimen. VHs were defined according to the 2016 WHO classification of bladder tumors [13]. Pure non-urothelial variants were excluded.

Endpoints

Primary endpoint of the current analysis was DSS of pN-positive patients. Disease-related death was determined by the treating physicians following chart review or corroborated by death certificates. Survival was defined as the time-interval between RC and the date of last imaging and/or clinical visit (censored) or of documented (disease-related) death.

Definition of LN-density and threshold value

LN-density was calculated as the ratio of the number of positive LNs to the total number of LNs removed $\times 100\%$. The optimal LN-density cut-off value was determined by creating a time-dependent receiver operating characteristic (ROC) curve with DSS as the endpoint to yield the highest Youden-index value among pN-positive patients. The Youden-index provides the optimal cut-off from a continuous variable by showing the score that offers the best trade-off between sensitivity and specificity.

Statistical analysis

Descriptive analyses included frequencies and proportions for categorical variables. Medians and interquartile range (IQR) were reported for continuous-coded variables. The Mann-Whitney-U test or Kruskal-Wallis was used for comparison of the continuous data and Chi-square or Fisher's exact test for categorical data. All tests were two-sided with a level of significance set at $p < 0.05$. The Kaplan-Meier method was used to estimate DSS stratified by pN-status and by LN-density groups. The log-rank method was used to determine significance. Because of the low rate of pN3 disease, pN2-3 were analyzed together.

Univariable and multivariable Cox' regression models were used to assess the Hazard Ratio (HR) with 95% confidence intervals (CI) testing the relationship between the LN-density groups and the other covariates with DSS among patients with pN-positive disease. After univariable analysis, covariates with $p < 0.25$ were entered into the multivariable model followed by backward elimination to determine the factors most associated with DSS. Beyond that, the impact of LN-density groups and pN-stages were further tested among patients who received or not AC. Cumulative and separated univariable Cox' regression models were adopted. A separate sensitivity analysis on patients with at least 14 LNs removed was performed as the median yield of PLND among pN-positive cohort. LN-density was analyzed both as a categorical and a continuous variable. Because of the colinear association between LN-density and the number of positive LNs, separate regression models were developed. Statistical analyses were performed using R-Studio (http://www.rstudio.com/).

Table 1
Characteristics and clinicopathological features of the 1169 patients with clinical localized cN0M0 urothelial bladder cancer treated with radical cystectomy and pelvic lymph-node dissection

Variable	Entire cohort
Patients, *n*. (%)	1169 (100.0)
Age (years), median (IQR)	68 (59–75)
Sex, *n*. (%)	
Male	894 (76.5)
Female	275 (23.5)
pT-stage, *n*. (%)	
NMIBC (pTa/is/1)	106 (9.1)
pT2	286 (24.5)
pT3	548 (46.9)
pT4	229 (19.6)
Grade (WHO 2004), *n*. (%)	
Low Grade	43 (3.7)
High Grade	1126 (96.3)
pN-stage, *n*. (%)	
pN0	706 (60.4)
pN1	166 (14.2)
pN2	285 (24.4)
pN3	12 (1.0)
Concomitant CIS, *n*. (%)	422 (36.1)
LVI, *n*. (%)	568 (48.6)
PSMs, *n*. (%)	60 (5.1)
LNs removed, median (IQR)	13 (8–18)
Positive LNs, median (IQR)	2 (1–4)
LN density, median (IQR)	0.0 (0.0–12.5)
VHs, *n*. (%)	298 (25.5)
Adjuvant radiotherapy, *n* (%)	60 (5.1)
Adjuvant chemotherapy, *n* (%)	343 (29.3)
Disease-specific deaths, *n* (%)	576 (49.3)
Follow-up (years), median (IQR)	2.2 (0.9–4.9)
Survivors' follow-up (years), median (IQR)	4.2 (2.1–7.4)
DSS rates, % (95% CI)	
1-year	78.8 (76.4–81.2)
2-year	63.4 (60.6–66.3)
3-year	55.5 (52.6–58.7)
5-year	46.6 (43.4–49.9)

Abbreviations are as follows: IQR: interquartile range; pT-stage: pathological tumor stage; NMIBC: non-muscle invasive bladder cancer; WHO: World Health Organization; pN-stage: pathological nodal stage; CIS: carcinoma-in-situ; LVI: Lympho-vascular Invasion; PSMs: positive surgical margins; LNs: lymph nodes; VHs: variant histologies; CI: Confidence Interval; DSS: Disease-specific Survival.

RESULTS

Patients' characteristics for the entire cohort (n = 1169)

The clinico-pathological characteristics of the 1169 patients who underwent RC and bilateral PLND are displayed in Table 1. Median age at RC was 68 (IQR, 59–75) years. Overall, 706 (60.4%), 166 (14.2%), 285 (24.4%), 12 (1.0%) patients were staged pN0, pN1, pN2, and pN3, respectively. The median number of LNs removed was 13 (IQR, 8–18).

ROC curve analysis and cut-off value for LN-density in pN-positive patients (n = 463)

The ROC analysis for LN-density showed that the AUC predicting DSS was 0.60 (95%CI 0.54–0.65) (Fig. 1). According to the maximum Youden-index value, the cut-off for LN-density was set at 20%. Sensitivity, specificity, accuracy and precision were 0.54 (95%CI 0.45–0.65), 0.63 (95%CI 0.57–0.74), 0.57 (95%CI 0.49–0.68), and 0.75 (95%CI 0.71–0.73), respectively. In Total, 240 (51.8%) patients had a LN-density <20%, while 223 (48.2%) had a LN-density ≥ 20%.

Patients' characteristics for the pN-positive cohort (n = 463)

The clinico-pathological characteristics of the 463 patients with LN-involvement and stratified according to LN-density cut-off (20%) are shown in Table 2. The median number of LNs removed was 14 (IQR, 9–20) and the median LN-density was 17.6 (IQR, 9.1–40.0). Median number of LNs removed was significantly higher in patients with LN-density below the cut-off of 20% (16 vs. 11, *p* < 0.001). For patients staged pN1, 146 (60.8%) had a LN-density <20%. The relationship between pN-status and LN-density is depicted in Figure S2. Patients with LN-density above the 20%-cut-off value significantly more often had features of higher tumor-aggressiveness such as non-organ confined disease, advanced pN-stage and presence of LVI. Conversely, rates of VHs were comparable among the two groups (26.7% vs. 23.8%, *p* = 0.54). Moreover, median LN-density values stratified by each VH-group were similar (Figure S3, *p* = 0.17). No significant differences were found in terms of adjuvant treatments' administration.

Comparison of survival estimates according to LN-density and pN stages in the entire cohort (n = 1169)

The median follow-up was 2.2 years (IQR, 0.9–4.9) and it was 4.2 years (IQR, 2.1–7.4) for the survivors. Overall, 576 (49.3%) disease-related deaths occurred. After 1, 3, and 5 years, the DSS rates were 78.8% (95%CI, 76.4–81.2), 55.5% (95%CI, 52.6–58.7), 46.6% (95%CI, 43.4–49.9), respectively. Kaplan-Meier curves for DSS stratified by pN-stage and LN-density (20%-cutoff) are shown in Fig. 2.
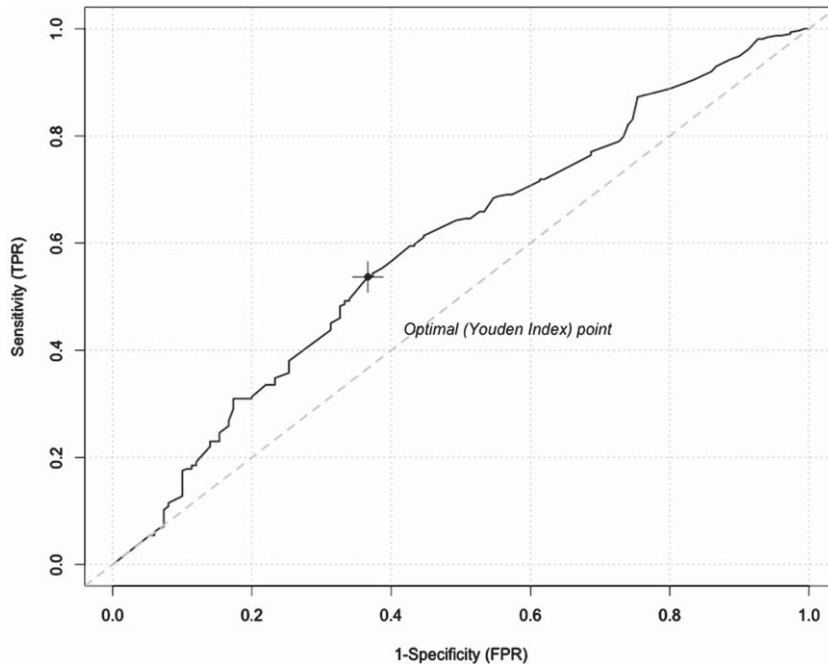
Fig. 1. ROC curve for preoperative prediction of DSS in 463 patients with node-positive BC treated with RC and PLND. Abbreviations: DSS: disease-specific survival; BC: bladder cancer; RC: radical cystectomy; PLND: pelvic lymph node dissection.

Cox' regression models in the node-positive cohort (n = 463)

Univariable and multivariable Cox' regression analyses assessing DSS in the node-positive cohort are depicted in Table 3. At univariable analysis, locally-advanced disease (pT3-4), pN2-3 stage, and presence of LVI displayed worse DSS (HR 4.50, 95%CI 1.11–18.3, $p = 0.035$), (HR 1.52, 95%CI 1.19–1.93, $p < 0.001$), (HR 1.55, 95%CI 1.19–2.03, $p = 0.001$), respectively. After adjusting for the significant prognosticators, the number of LN-metastases (HR 1.03, 1.01–1.05, $p = 0.005$) and LN-density either as continuous (HR 1.01, 95%CI 1.00–1.01, $p = 0.013$) or dichotomized variable (HR 1.37, 95%CI 1.07–1.79, $p = 0.014$) remained independently associated with worse DSS. pN-stage lost significance once the other LN-related variables were included in the models while pT3-4 disease still exhibited borderline significance. Of note, AC remained independently associated with DSS benefit in each model.

Cox' regression models among patients with at least 14 LNs removed (n = 242)

In 242 (52.3%) patients, the LN-yield of PLND was at least 14 LNs. In the multivariable Cox'

regression models, only LN-density $\geq 20\%$ (HR 1.48, 95%CI 1.03–2.14, $p = 0.035$), LN-density as continuous variable (HR 1.01, 95%CI 1.00–1.02, $p = 0.012$), and number of LN-metastases (HR 1.03, 95%CI 1.00–1.06, $p = 0.036$), revealed an independent prognostic effect on DSS. Again, AC remained independently associated with DSS benefit in each model (Table S1).

Survival estimates and Cox' regression models according to AC administration

Overall, 239 (51.6%) patients received AC. No difference was found after stratification for LN-density groups ($p = 0.9$). Kaplan-Meier curves for DSS stratified by pN-stage and LN-density groups according to AC administration are shown in Figure S4-5. Among patients in which AC was not administrated pN2-3 stage was not associated with worse DSS compared to pN1 (HR 1.31, 95%CI 0.96–1.81, $p = 0.09$). Whereas, among patients who did not received AC, LN-density $\geq 20\%$ was significantly associated with worse survival either considering pN0 and LN-density <20% as reference, (HR 3.21, 95%CI 2.63–3.90, $p < 0.001$) and (HR 1.42, 95%CI 1.05–1.92, $p = 0.02$), respectively. Among patients who received AC, LN-density $\geq 20\%$ was

Table 2
Patients' characteristics and clinicopathological features of 463 patients with pN-positive disease stratified according to lymph-node density cut-off (20%)

Variable	Overall	LN-density <20%	LN-density ≥ 20%	p
Patients, *n*. (%)	463 (100.0)	240 (51.8)	223 (48.2)	
Age (years), median (IQR)	67 (58–73)	67 (58–72)	66 (58–74)	0.62
Sex, *n*. (%)				0.56
Male	359 (77.5)	183 (76.2)	176 (78.9)	
Female	104 (22.5)	57 (23.8)	47 (21.1)	
pT-stage, *n*. (%)				0.003
NMIBC (pTa/is/1)	9 (1.9)	3 (1.2)	6 (2.7)	
pT2	79 (17.1)	48 (20.0)	31 (13.9)	
pT3	243 (52.5)	137 (57.1)	106 (47.5)	
pT4	132 (28.5)	52 (21.7)	80 (35.9)	
Grade (WHO 2004), *n*. (%)				0.74
Low Grade	6 (1.3)	4 (1.7)	2 (0.9)	
High Grade	457 (98.7)	236 (98.3)	221 (99.1)	
pN-stage, *n*. (%)				<0.001
pN1	166 (35.6)	146 (60.8)	20 (9.0)	
pN2	285 (61.6)	91 (37.9)	194 (87.0)	
pN3	12 (2.6)	3 (1.3)	9 (4.0)	
Concomitant CIS, *n*. (%)	174 (37.6)	101 (42.1)	73 (32.7)	0.05
LVI, *n*. (%)	342 (73.9)	167 (69.6)	175 (78.5)	0.04
PSMs, *n*. (%)	36 (7.8)	13 (5.4)	23 (10.3)	0.07
LNs removed, median (IQR)	14 (9–20)	16 (11–21)	11 (6–17)	<0.001
Positive LNs, median (IQR)	2 (1–4)	1 (1–2)	4 (2–7)	<0.001
LN-density, median (IQR)	17.6 (9.1–40.0)	9.5 (6.7–13.3)	40 (25–64.5)	<0.001
VHs, *n*. (%)	117 (25.3)	64 (26.7)	53 (23.8)	0.54
Adjuvant radiotherapy, *n* (%)	31 (6.7)	15 (6.3)	16 (7.2)	0.84
Adjuvant chemotherapy, *n* (%)	239 (51.6)	124 (51.7)	115 (51.6)	0.9
Disease-specific events, *n* (%)	313 (67.6)	145 (60.4)	168 (75.3)	<0.001
Follow-up (years), median (IQR)	1.5 (0.7–3.1)	1.7 (0.8–3.7)	1.3 (0.7–2.6)	0.01
Survivors' follow-up (years), median (IQR)	3.2 (1.5–6.9)	3.8 (1.5–7.3)	3.0 (1.4–5.1)	0.28
DSS rates, % (95% CI)				<0.001*
1-year	69.6 (65.4–74.0)	73.0 (67.5–78.9)	65.9 (60.0–72.5)	
2-year	47.7 (43.2–52.7)	53.8 (47.6–60.9)	41.3 (35.1–48.6)	
3-year	34.9 (30.5–39.9)	42.8 (36.5–50.2)	26.8 (21.3–33.8)	
5-year	24.9 (20.8–29.9)	32.7 (26.6–40.3)	16.7 (11.8–23.6)	

Abbreviations are as follows: IQR: interquartile range; pT-stage: pathological tumor stage; NMIBC: non-muscle invasive bladder cancer; WHO: World Health Organization; pN-stage: pathological nodal stage; CIS: carcinoma-in-situ; LVI: Lympho-vascular Invasion; PSMs: positive surgical margins; LNs: lymph nodes; VHs: variant histologies; CI: Confidence Interval; DSS: Disease-specific Survival. *Log-rank statistic p-value.

significantly associated with worse survival either considering pN0 and LN-density <20% as reference, (HR 2.18, 95%CI 1.51–3.14, $p < 0.001$) and (HR 1.69, 95%CI 1.21–2.36, $p = 0.002$) whereas no difference was found between pN0 and LN-density <20% (HR 1.29, 95%CI 0.88–1.88, $p = 0.2$).

DISCUSSION

In this multi-institutional analysis, we evaluated the prognostic impact of LN-density in a large cohort of patients with node-positive BC. We found that LN-density with a cut-off value greater than 20% was an independent prognostic factor for worse DSS.

Although risk-stratification after RC is currently based on TNM staging, several reports have ques-

tioned the reliability of such a system among node-positive patients. Thus, the accuracy of LN-density and other LN-based parameters have been explored to better characterize node-positive BC [6, 9, 14–19]. Kassouf et al. demonstrated that LN-density was superior to the 6th edition of TNM classification in predicting DSS for node-positive patients after RC [6]. Expanding their cohort to 1038 BC patients, Kassouf et al. externally validated the prognostic relevance of LN-density [14]. Considering 181 patients with low volume pN-positive disease (defined as 1 or 2 positive-LNs), in which neoadjuvant treatments were allowed, Bruins et al. found that LN-density was an independent prognosticator of both recurrence-free and overall survival [15]. Within a multicentre analysis, May et al.
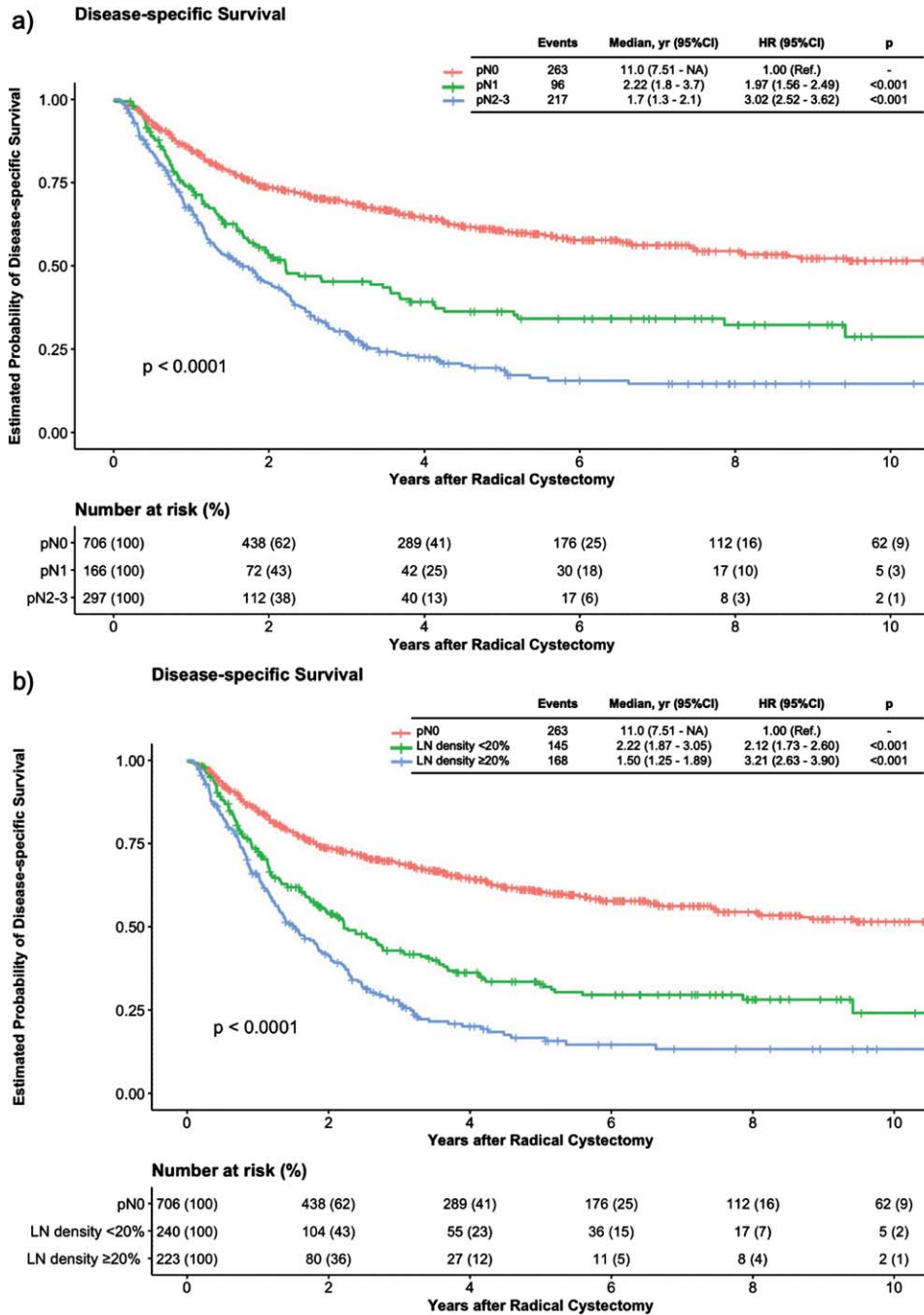
Fig. 2. (a) Kaplan-Meier survival curves of disease-specific survival (log-rank, $p < 0.0001$) stratified by pN status among 1169 patients with non-metastatic bladder cancer undergoing radical cystectomy and pelvic lymph node dissection are shown. Univariable Cox's regression analysis assessed the HRs with their 95% CI: pN0 vs. pN1 (HR 1.97, 95%CI 1.56–2.49, $p < 0.001$), pN0 vs. pN2-3 (HR 3.02, 95%CI 2.52–3.62, $p < 0.001$), respectively. (b) Kaplan-Meier survival curves of disease-specific survival (log-rank, $p < 0.0001$) stratified by LN-density group among 1169 patients with non-metastatic bladder cancer undergoing radical cystectomy and pelvic lymph node dissection are shown. Univariable Cox's regression analysis assessed the HRs with their 95% CI: pN0 vs. LN-density <20% (HR 2.12, 95%CI 1.73–2.60, $p < 0.001$), pN0 vs. LN-density $\geq$ 20% (HR 3.21, 95%CI 2.63–3.90, $p < 0.001$), respectively. Abbreviations are as follows: pN: pathological nodal stage; HR hazard ratio, CI confidence interval.

Table 3
Univariable and multivariable Cox' regression analysis for disease specific survival for the 463 pN-positive patients

| Variable | Univariable | | Multivariable | | | | | |
| | | | Model 1 | | Model 2 | | Model 3 | |
	HR 95% CI	p	HR 95% CI	p	HR 95% CI	p	HR 95% CI	p
Age (years), as cont.	1.01 (1.00–1.03)	0.011	1.00 (0.99–1.01)	0.9	1.00 (0.99–1.01)	0.9	1.00 (0.99–1.01)	0.9
Sex								
Male	1.00 (Ref.)	–	–	–	–	–	–	–
Female	1.07 (0.82–1.40)	0.6						
pT stage								
NMIBC -pT2	1.00 (Ref.)	–	1.00 (Ref.)	–	1.00 (Ref.)	–	1.00 (Ref.)	–
pT3-4	4.50 (1.11–18.3)	0.035	3.51 (0.85–14.5)	0.08	3.40 (0.82–14.0)	0.09	3.42 (0.83–14.1)	0.089
pN stage								
pN1	1.00 (Ref.)	–	1.00 (Ref)	–	1.00 (Ref)	–	1.00 (Ref)	–
pN2-3	1.52 (1.19–1.93)	<0.001	1.15 (0.86–1.54)	0.3	1.19 (0.90–1.54)	0.2	1.24 (0.94–1.62)	0.12
LVI								
Absence	1.00 (Ref.)	–	1.00 (Ref.)	–	1.00 (Ref.)	–	1.00 (Ref.)	–
Presence	1.55 (1.19–2.03)	0.001	1.26 (0.95–1.67)	0.11	1.24 (0.93–1.64)	0.14	1.22 (0.92–1.62)	0.2
Concomitant CIS								
Absence	1.00 (Ref.)	–	1.00 (Ref.)	–	1.00 (Ref.)	–	1.00 (Ref.)	–
Presence	0.78 (0.62–0.99)	0.038	0.81 (0.64–1.03)	0.087	0.81 (0.64–1.02)	0.073	0.80 (0.63–1.01)	0.65
PSMs								
Absence	1.00 (Ref.)	–	1.00 (Ref.)	–	1.00 (Ref.)	–	1.00 (Ref.)	–
Presence	0.99 (0.98–1.01)	0.25	1.22 (0.81–1.84)	0.25	1.23 (0.82–1.85)	0.25	1.25 (0.83–1.88)	0.25
VH								
Absence	1.00 (Ref.)	–	–	–	–	–	–	–
Presence	1.09 (0.84–1.40)	0.5	–	–	–	–	–	–
N. of LNs removed, as cont.	1.00 (0.99–1.01)	0.6	–	–	–	–	–	–
N. of positive LNs, as cont.	1.03 (1.01–1.05)	0.005	–	–	–	–	1.03 (1.00–1.06)	0.027

	HR (CI)	p	HR (CI)	p	HR (CI)	p	HR (CI)	p
LN-density (%), as cont.	1.02 (1.00–1.01)	<0.001	–	–	1.01 (1.00–1.01)	0.013	–	–
LN-density								
<20%	1.00 (Ref.)	–	1.00 (Ref.)	–	–	–	–	–
≥20%	1.51 (1.21–1.88)	<0.001	1.37 (1.07–1.79)	0.014				
Adjuvant radiotherapy								
Absence	1.00 (Ref.)	–	–	–	–	–	–	–
Presence	0.88 (0.57–1.37)	0.6						
Adjuvant chemotherapy								
Absence	1.00 (Ref.)	–	1.00 (Ref.)	–	1.00 (Ref.)	–	1.00 (Ref.)	–
Presence	0.44 (0.35–0.55)	<0.001	0.45 (0.35–0.57)	<0.001	0.45 (0.35–0.57)	<0.001	0.44 (0.35–0.56)	<0.001
Center								
Center 1	1.00 (Ref.)	–	–	–	–	–	–	–
Center 2	1.51 (0.66–3.52)	0.3						
Center 3	1.23 (0.52–2.95)	0.6						
Center 4	0.71 (0.27–1.86)	0.5						
Center 5	0.66 (0.32–1.31)	0.2						
Center 6	1.43 (0.64–3.24)	0.4						
Center 7	0.71 (0.30–1.65)	0.4						
Center 8	0.65 (0.37–1.28)	0.2						
Center 9	2.59 (0.61–5.95)	0.2						

Abbreviations are as follows: HR: hazard ratio; CI: confidence interval; pT stage: pathological tumor stage; pN stage: pathological nodal stage; LVI: lympho-vascular invasion; CIS: carcinoma in situ; PSMs: positive surgical margins; VHs: variant histologies; LN: lymph node.

supported the prognostic relevance of LN-density in 477 chemotherapy-naïve patients with node-positive BC over conventional pN-stages [16]. Similar to our results, LN-density either as a continuous or dichotomized variable remained significant at multivariable models [16]. Among 130 RC candidates receiving an extended PLND (≥15 LNs removed), Jeong et al. confirmed the independent ability of LN-density to predict DSS over traditional pN-stages [17]. Similarly, Lee et al. showed that LN-density was a better prognostic tool compared to pN-stages in patients receiving at least an extended PLND [9]. Furthermore, considering overall survival among 1381 NAC-naïve patients with pathological node-positive BC, Afferi et al. identified three prognostic groups as follows: a favorable (≤pT2, positive-LNs ≤ 2), an intermediate (≥pT3, positive-LNs ≤ 2) and a poor prognosis group (pTany, positive-LNs ≥ 3), respectively [18]. By doing this, they demonstrated that pN-positive patients harbored variable survival rates depending on pT-stage and other LN-related variables beyond conventional pN-stages. Such stratification was proposed to further refine patients who may benefit from cisplatin-based AC [18]. In line with the previous reports, we found that LN-density was an independent prognostic factor for worse DSS in multivariable analyses with 463 pN-positive, cN0M0 RC-patients whereas conventional pN-stage was not significant anymore in these multivariable models. Hence, ours and results of several other reports suggest that the integration of LN-density and other LN-parameters beyond conventional pN-stages may contribute to a more refined risk-stratification among patients with LN-involvement.

Furthermore, these reports further highlighted the low incidence of pN3-disease in the context of NAC-naïve patients undergoing RC [6, 16, 17]. Likewise, our cohort comprised only 12 (2.6%) pN3 patients that were analyzed together with pN2 group because of this low rate. Here, Bruins et al. found that neither the 6th nor the 7th TNM staging system performed well as a prognostic tool [5]. Furthermore, Tarin et al. considering 114 patients, in which NAC administration was allowed, found that patients who harbored pN3 disease had similar DSS compared to those with node-disease limited to the true pelvis [19]. Although the prognostic role of LN-density in the context of NAC remains to be elucidated, it appears plausible that the administration of such a strategy could even minimize the incidence of pN3-stage by making the adoption of other LN-related variables more useful in the clinical setting.

Adjuvant treatments represent a crucial step in this clinical scenario and a trend towards a greater benefit from AC in the pN-positive population has been noticed [20]. Controversy exists about who benefit the most from adjuvant treatments administration. Afferi et al. showed that cisplatin-based AC might be most effective in patients with ≥ 3 LNMs irrespectively of pT-stages. Conversely, no survival improvement was described among for patients with less than two LNMs [18]. Here, we tested the reliability of both LN-density groups and pN-stages for DSS estimation whether or not AC was administrated (Figure S4-5). Beyond that, AC administration remained independently associated with survival benefit in pN-positive patients across all the multivariable models. Thus, as reported by the Advanced Bladder Cancer Meta-analysis Collaborators Group, cisplatin-based AC is a valid option for improving outcomes of patients who harbored LN-involvement after RC [21].

Several cut-off points for LN-density have been proposed. Kassouf et al. arbitrarily categorized LN-density into quintiles and further defined LN-density <6% as the best survival scenario in node-positive patients [14]. However, the authors highlighted the independent prognostic impact of LN-density only as continuum risk-factor [14]. A German experience analyzed the best threshold value for LN-density using the maximal selected chi^2-statistics. A threshold (>20% comparable to ours) was associated with worse DSS regardless of the number of LNs removed (cut-off 12 LNs) [16]. In contrast, Fajkovic et al. did not demonstrate an independent prognostic value for LN-density as continuous variable [8]. Moreover, Fleischmann et al. found that LN-density ≥ 20% failed as an independent prognostic discriminator at multivariable analysis [22]. To the best of our knowledge, only one study based the LN-density cut-off selection on a ROC analysis as we did [17]. Although limited by the retrospective single-center design and the small sample size, Jeong et al. highlighted the independent prognostic impact of LN-density (>18% cut-off) [17]. However, no information on the performance of the ROC analysis was provided. Assessing the prognostic ability among LN parameters, Oszwald et al. demonstrated that LN-density had the best predictive performance (AUC 0.83) [23]. We obtained a 20% cut-off as Youden-index showing a strong link between LN-density and worse DSS regardless of the extent of PLND or AC administration.

Presence of VHs next to urothelial carcinoma showed no influence on DSS among node-positive

BC patients. Furthermore, median values of LN-density were not significantly different among pure UC and urothelial-variants. To the best of our knowledge, this is the largest series evaluating the relationship between VHs and LN-density in such a setting. In general, presence of VHs has been associated with features of aggressive disease at time of RC [24]. However, only few reports evaluated such patterns in node-positive BC. Considering 65 VH patients with LN involvement, Rice et al. demonstrated that different VHs showed distinct lymphatic spread patterns [25]. Particularly, higher median values of LN-density for micropapillary and clear-cell variants were highlighted [25]. A recent French single-center experience among 34 node-positive individuals described the increased metastatic potential among micropapillary and squamous variants [23]. Conversely, Marks et al. found that extra-nodal extension (ENE) and LN-density were predictors of worse outcome regardless of VHs presence in a retrospective cohort of 138 patients [26]. In line with Marks et al., we also found no difference; neither in terms of rates of VHs among the entire and node-positive cohort (25.5% vs. 25.3%) nor any contribution in the Cox' models. Hence, the prognosis of these VH-patients might be driven by other LN-related characteristics rather than by the presence of VHs itself.

Several retrospective series have shown a survival benefit if a higher LN-count was retrieved [27, 28]. Particularly, Dhar et al. highlighted that the adoption of extended PLND was associated with 5-year survival benefit over the limited PLND in patients with node-positive BC [29]. These results were further confirmed in a meta-analysis comprising 2824 patients [30]. So far, only one prospective randomized trial compared a super-extended template versus standard PLND [31]. Although limited by the exclusion of NAC and the low rate of adjuvant treatment (28%), this trial failed to show any therapeutic advantage of a more extended PLND [31]. In our Cox' regression models, the number of LNs removed at PLND was also not associated with DSS. Conversely, an higher median number of LNs removed was correlated with LN-density <20%. To overcome the dilution effect of a more extensive PLND, we performed a Cox' regression sub-analysis in patients with at least 14 LNs removed. Again, LN-density, either continuous or categorical variable, remained independently associated with DSS (Table S1). As future perspective, more unified criteria in histological processing for counting LNs together with a prospectively evaluated standardized PLND-template might enhance the performance of LN-density ensuring a more refined risk-stratification for LN-positive patients.

Our study is not devoid of limitations that must be acknowledged. This study was limited by the retrospective nature and a time-span of over 30 years, in which different temporal practice patterns may have existed. Template of pelvic lymph node dissection varied between centers and was not included in this analysis. The low rate of AC (51.6%) is a concern for our pN-positive population. The extension of PLND-templates across different institutions was variable as well as the number of surgeons involved. Competing risks were not captured for Overall Survival estimation. Moreover, the rate of disease-specific events was high, potentially representing an aggressive cohort, which may not be representative of more contemporary RC-series. Furthermore, we did not include patients treated with neoadjuvant chemotherapy as currently recommended by international Guidelines. Nevertheless, a recent meta-analysis of the Advanced Bladder Cancer (ABC) Group with Individual Participant Data from Randomized Controlled Trials found a similar absolute survival benefit at 5 years (6%) of AC compared to NAC (5–8%). Thus, our data still might be informative among candidates for upfront RC [32]. It should be noted that administration of NAC is supported by level I evidence (positive RCTs and meta-analyses) and AC only by level II evidence (meta-analysis) [1, 32]. Strengths are the local pathology review of the RC-specimens of a relatively large set of homogeneously treated patients with IPD extracted from nine tertiary referral centers.

CONCLUSIONS

LN-density with a cut-off value greater than 20% was an independent prognostic factor for worse DSS in BC patients treated with RC and PLND. Furthermore, the integration of LN-density and other LN-parameters rather than conventional pN-stages may contribute to a more refined risk-stratification for patients with LN-involvement at time of RC.

ACKNOWLEDGMENTS

FUNDING

AUTHOR CONTRIBUTIONS

Conceptualization and design: Erik J. van Gennep, Francesco Claps, Theo H. van der Kwast, M. Carmen Mir, Yves Allory, Yair Lotan, Bas W.G. van Rhijn.

Methodology: Erik J. van Gennep, Francesco Claps, Roman Mayr, Laura S. Mertens, Theo H. van der Kwast, Arndt Hartmann, Yair Lotan, Bas W.G. van Rhijn.

Data curation: all authors.

Writing the original draft: Erik J. van Gennep, Francesco Claps.

Writing, review and editing: all authors.

Administrative, technical or material support: Peter J. Bostrom, Shahrokh F. Shariat, Yann Neuzillet, Alexandre R. Zlotta, Carlo Trombetta, Markus Eckstein, Laura S. Mertens, Rossana Bussani, Maximilian Burger, Joost L. Boormans, Bernd Wullich, Arndt Hartmann, Roman Mayr, Nicola Pavan, Riccardo Bartoletti, M. Carmen Mir, Damien Pouessel, John van der Hoeven, Theo H. van der Kwast, Yves Allory, Tahlita C.M. Zuiverloon, Yair Lotan, Bas W.G. van Rhijn.

Project Administration: Bas W.G. van Rhijn.

ETHICAL CONSIDERATIONS

Appropriate Ethical approval was obtained at each site according to national regulations and the principles of the Declaration of Helsinki.

Site specific approval and protocol numbers:

Amsterdam: The Institutional Review Board of the Netherlands Cancer Institute – Antoni van Leeuwenhoek hospital (CFMPB-160 & IRBd18126).

Trieste: The University of Trieste Ethical Committee; Trieste (ID 113/2021)

Regensburg: Medical ethical board of the University of Regensburg (16-101-0218).

Paris: The regional ethics board of Ile-de-France IX – Comité de protection des personnes – Ile-de-France IX - Créteil (11-052).

Toronto: The regional ethics board of the University Health Network, Toronto (02-0515-C, 08-0263-T, 09-0826-CE and 09-0556-TE).

Turku: Ethical committee of the hospital district of South-West Finland, No: ETMK 6/2006.

Erlangen: The ethical and translational boards, Erlangen (217_18 Bc and 329_16-B).

Dallas: The Institutional Review Board for the Protection of Human Subjects at University of Texas Southwestern Medical School: STU 102014-008.

Rotterdam: The medical-ethical board of Erasmus MC, Rotterdam (MEC 168.922/1998/55).

CONFLICT OF INTEREST

DATA AVAILABILITY

The data supporting the findings of this study are available within the article and/or its supplementary material.

SUPPLEMENTARY MATERIAL

The supplementary material is available in the electronic version of this article: https://dx.doi.org/10.3233/BLC-230086.

REFERENCES

[1] Witjes JA, Bruins HM, Cathomas R, et al. European Association of Urology Guidelines on Muscle-invasive and Metastatic Bladder Cancer: Summary of the 2020 Guidelines. Eur Urol. 2021;79:82-104.

[2] Babjuk M, Burger M, Compérat EM, et al. European Association of Urology Guidelines on Non-muscle-invasive Bladder Cancer (TaT1 and Carcinoma In Situ) – 2019 Update. Eur Urol. 2019;1-19.

[3] Zehnder P, Studer UE, Skinner EC, et al. Unaltered oncological outcomes of radical cystectomy with extended lymphadenectomy over three decades. BJU Int. 2013. https://doi.org/10.1111/bju.12215

[4] Psutka SP, Barocas DA, Catto JWF, Gore JL, Lee CT, Morgan TM, Master VA, Necchi A, Rouprêt M, Boorjian SA. Staging the Host: Personalizing Risk Assessment for Radical Cystectomy Patients. Eur Urol Oncol. 2018;1:292-304.

[5] Bruins HM, Dorin RP, Rubino B, Miranda G, Cai J, Daneshmand S, Skinner EC. Critical evaluation of the American Joint Committee on cancer TNM nodal staging system in patients with lymph node-positive disease after radical cystectomy. Eur Urol. 2012;62:671-6.

[6] Kassouf W, Agarwal PK, Herr HW, Munsell MF, Spiess PE, Brown GA, Pisters L, Grossman HB, Dinney CP, Kamat AM. Lymph node density is superior to TNM nodal status in predicting disease-specific survival after radical cystectomy for bladder cancer: analysis of pooled data from MDACC and MSKCC. J Clin Oncol. 2008;26:121-6.

[7] Mertens LS, Meijer RP, Meinhardt W, van der Poel HG, Bex A, Kerst JM, van der Heijden MS, Bergman AM, Horenblas S, van Rhijn BWG. Occult lymph node metastases in patients with carcinoma invading bladder muscle: incidence after neoadjuvant chemotherapy and cystectomy vs after cystectomy alone. BJU Int. 2014;114:67-74.

[8] Fajkovic H, Cha EK, Jeldres C, et al. Extranodal extension is a powerful prognostic factor in bladder cancer patients with lymph node metastasis. Eur Urol. 2013;64:837-45.

[9] Lee D, Yoo S, You D, Hong B, Cho YM, Hong JH, Kim CS, Ahn H, Ro JY, Jeong IG. Lymph node density vs. the American Joint Committee on Cancer TNM nodal staging system in node-positive bladder cancer in patients undergoing extended or super-extended pelvic lymphadenectomy. Urologic Oncology: Seminars and Original Investigations. 2017;35:151.e1-e7

[10] Claps F, van de Kamp MW, Mayr R, et al. Risk factors associated with positive surgical margins' location at radical cystectomy and their impact on bladder cancer survival. World J Urol. 2021;39:4363-71

[11] Mertens LS, Claps F, Mayr R, et al. Prognostic markers in invasive bladder cancer: FGFR3 mutation status versus P53 and KI-67 expression: a multi-center, multi-laboratory analysis in 1058 radical cystectomy patients. Urol Oncol. 2022;40:110.e1-e9.

[12] Claps F, Mir MC, Zargar H. Molecular markers of systemic therapy response in urothelial carcinoma. Asian J Urol. 2021;8:376-90.

[13] Humphrey PA, Moch H, Cubilla AL, Ulbright TM, Reuter VE. The 2016 WHO classification of tumours of the urinary system and male genital organs—Part B: Prostate and bladder tumours. Eur Urol. 2016;70:106-19.

[14] Kassouf W, Svatek RS, Shariat SF, et al. Critical analysis and validation of lymph node density as prognostic variable in urothelial carcinoma of bladder. Urol Oncol. 2013;31:480-6.

[15] Bruins HM, Huang GJ, Cai J, Skinner DG, Stein JP, Penson DF. Clinical outcomes and recurrence predictors of lymph node positive urothelial cancer after cystectomy. J Urol. 2009;182:2182-7.

[16] May M, Herrmann E, Bolenz C, et al. Lymph node density affects cancer-specific survival in patients with lymph node-positive urothelial bladder cancer following radical cystectomy. Eur Urol. 2011;59:712-8.

[17] Jeong IG, Park J, Song K, Ro JY, Song C, Hong JH, Ahn H, Kim CS. Comparison of 2002 TNM nodal status with lymph node density in node-positive patients after radical cystectomy for bladder cancer: Analysis by the number of lymph nodes removed. Urologic Oncology: Seminars and Original Investigations. 2011;29:199-204.

[18] Afferi L, Lonati C, Montorsi F, et al. Selecting the best candidates for Cisplatin-based Adjuvant chemotherapy after radical cystectomy among patients with pN+Bladder cancer. Eur Urol Oncol. 2022. https://doi.org/10.1016/J.EUO.2022.04.001

[19] Tarin TV, Power NE, Ehdaie B, Sfakianos JP, Silberstein JL, Savage CJ, Sjoberg D, Dalbagni G, Bochner BH. Lymph node-positive bladder cancer treated with radical cystectomy and lymphadenectomy: Effect of the level of node positivity. Eur Urol. 2012;61:1025-30.

[20] Galsky MD, Stensland KD, Moshier E, et al. Effectiveness of adjuvant chemotherapy for locally advanced bladder cancer. Journal of Clinical Oncology. 2016;34:825-32.

[21] Burdett S, Fisher DJ, Vale CL, et al. Adjuvant chemotherapy for muscle-invasive bladder cancer: A systematic review and meta-analysis of individual participant data from randomised controlled trials. Eur Urol. 2022;81:50-61.

[22] Fleischmann A, Thalmann GN, Markwalder R, Studer UE. Extracapsular extension of pelvic lymph node metastases from urothelial carcinoma of the bladder is an independent prognostic factor. Journal of Clinical Oncology. 2005;23:2358-65.

[23] Oszwald A, Wasinger G, Larnaudie L, Varinot J, Sebe P, Cussenot O, Compérat E. Pathological reporting of cystectomy lymph nodes: a retrospective analysis of experience in Paris. World J Urol. 2021. https://doi.org/10.1007/s00345-021-03630-8

[24] Stroman L, Nair R, Russell B, Malik N, Desai A, Chandra A, Thurairaja R, Dasgupta P, Khan MS, Malde S. The impact of non-urothelial variant histology on oncological outcomes following radical cystectomy. BJU Int. 2019;124:418-23.

[25] Rice KR, Koch MO, Kao CS, Pedrosa JA, Kaimakliotis HZ, Masterson TA, Bihrle R, Cheng L. Lymph node metastases in patients with urothelial carcinoma variants: Influence of the specific variant on nodal histology. Urologic Oncology: Seminars and Original Investigations. 2015;33:20.e23-e29.

[26] Marks P, Gild P, Soave A, et al. The impact of variant histological differentiation on extranodal extension and survival in node positive bladder cancer treated with radical cystectomy. Surg Oncol. 2019;28:208-13.

[27] May M, Herrmann E, Bolenz C, et al. Association between the number of dissected lymph nodes during pelvic lymphadenectomy and cancer-specific survival in patients

with lymph node-negative urothelial carcinoma of the bladder undergoing radical cystectomy. Ann Surg Oncol. 2011;18:2018-25.

[28] Leissner J, Hohenfellner R, Thüroff JW, Wolf HK. Lymphadenectomy in patients with transitional cell carcinoma of the urinary bladder; Significance for staging and prognosis. BJU Int. 2000;85:817-23.

[29] Dhar NB, Klein EA, Reuther AM, Thalmann GN, Madersbacher S, Studer UE. Outcome after radical cystectomy with limited or extended pelvic lymph node dissection. Journal of Urology. 2008;179:873-8.

[30] Bi L, Huang H, Fan X, et al. Extended vs non-extended pelvic lymph node dissection and their influence on recurrence-free survival in patients undergoing radical cystectomy for bladder cancer: A systematic review and meta-analysis of comparative studies. BJU Int. 2014. https://doi.org/10.1111/bju.12371

[31] Gschwend JE, Heck MM, Lehmann J, et al. Extended versus limited lymph node dissection in bladder cancer patients undergoing radical cystectomy: Survival results from a prospective, randomized trial (Figure presented.). Eur Urol. 2019;75:604-11.

[32] Adjuvant chemotherapy for muscle-invasive bladder cancer: A systematic review and meta-analysis of individual participant data from randomised controlled trials. Eur Urol. 2022. https://doi.org/10.1016/J.EURURO.2021.09.028