Molecular lymph node staging for bladder cancer patients undergoing radical cystectomy with pelvic lymph node dissection

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1. Introduction

Bladder cancer (BCa) is the second most common genitourinary malignancy with about 430,000 new cases worldwide every year [1]. About 25% of patients present with muscle-invasive disease at the time of diagnosis. For these patients radical cystectomy (RC) with lymph node dissection (LND) is the standard of care [2]. LND provides important prognostic information since lymph node (LN) metastases are detected in 20% to 25% of patients at the time of RC, which is the predominant risk factor for poor oncologic outcome besides pathologic tumor stage [3,4]. However, up to 37% of patients with locally advanced BCa but pathologically negative LN (>pT2, pN0) develop

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distant metastases [5]. LN metastases might remain undetected by standard histopathologic examinations at the time of RC leading to understaging. Another yet not fully addressed question is the extent of the topographic LND field necessary for thorough detection of LN metastases. Optimizing staging in BCa patients undergoing RC is therefore of crucial interest.

Besides histopathology and immunohistochemistry, molecular LN analysis using polymerase chain reaction (PCR) for the detection of cancer-enhanced transcripts shows the highest sensitivity for detection of LN metastases in solid tumors [6-10]. We recently established a new method for molecular detection of LN metastases in prostate cancer using fresh frozen tissue and quantitative real-time PCR that identified pN0-patients with high risk of postoperative tumor recurrence [6,7]. In the present study, we aimed to establish this method for BCa in patients undergoing RC with LND and sought to evaluate its clinical utility for prediction of postoperative outcome and localization of LN metastases.

For this reason, we included patients from a prospective, randomized, multicenter phase-III trial (LEA AUO AB 25/02) investigating super-extended vs. standard LND in urothelial BCa patients treated with RC [11]. The LEA-trial did not show a significant difference in recurrence-free survival (RFS) between super-extended and standard LND [11].

In these patients we prospectively evaluated 5 candidate genes with overexpression in BCa (FXYD domain-containing ion transport regulator 3 [FXYD3], cytokeratin 20 [KRT20], cytokeratin 17 [KRT17], uroplakin II [UPKII], serine peptidase inhibitor Kazal type 1 [SPINK1]) for molecular LN analysis [12-21].

In order to determine the clinical utility of molecular LN analysis in comparison with histopathology, we analyzed its diagnostic and prognostic value for the association with RFS. Moreover, we describe the topography of LN metastases in patients who received a super-extended LND.

2. Patients and methods

2.1. Patients and lymph nodes

The local ethics committee approved the present study, which was conducted according to the Declaration of Helsinki and the Good Clinical Practice guidelines of the International Conference on Harmonization. All patients provided written informed consent to participate in the study. This study was designed as translational biomarker program accompanying the prospective randomized LEA-trial (LEA AUO AB 25/02) investigating the therapeutic benefit of a super-extended vs. standard LND in 401 BCa patients treated with RC [11].

Overall, 76 patients who were enrolled within the LEA-trial at Medical Center Rechts der Isar, Technical University of Munich, Germany between 2007 and 2010 were included in the biomarker program. Main inclusion criteria were locally resectable, histologically confirmed T1G3 or muscle-invasive urothelial BCa (T2−T4a). Patients were excluded if they had radiologic evidence of a T4b tumor with infiltration of the pelvis or other organ systems, enlarged LNs (>1 cm) above the aortic bifurcation, and bone or visceral metastases. Additional exclusion criteria were neoadjuvant chemotherapy for BCa, a history of pelvic radiotherapy or pelvic LND, or coexisting malignant disease. It was mandatory to assess local tumor extension and exclude distant metastatic disease by preoperative staging with computed tomography.

Standard LND included superficial obturator, and internal and external iliac nodes as depicted in Fig. 4. The standard field was defined proximally by the bifurcation of internal and external iliac artery, distally by the pelvic floor, laterally by the genitofemoral nerve, and dorsally by the obturator nerve. Super-extended LND additionally included deep obturator, presacral, common iliac, paracaval, interaortocaval, and para-aortal nodes up to the inferior mesenteric artery. The super-extended field was defined proximally by the inferior mesenteric artery, distally by the pelvic floor, laterally by the genitofemoral nerve, and dorsally by pelvis and rectum.

Perioperatively, LNs with a size >5 to 20 mm were bisected. One half and the lateral edge of the second half were formalin fixed and stained with hematoxylin and eosin for histopathologic examination (pN0 or pN+). The remainder of the same LN was snap frozen within 30 minutes after removal and stored at −80°C for later RNA extraction. LNs with a size >20 mm were bisected, and the resulting 2 pieces were examined like singular LNs <20 mm. LNs with a size <5 mm were only assessed by histopathology as tissue was insufficient for examination by both techniques. Standard histopathology comprised 1 section per 5 mm of LN tissue.

2.2. qPCR assay

For molecular LN analysis, we used an analytically validated quantitative PCR (qPCR) assay established by our group [6,7]. Briefly, LNs were homogenized and lysed for RNA extraction and complementary DNA was generated. We used commercially available Taqman probe and primer sets for detection of the molecular marker genes FXYD3 (HS00254211_m1), KRT17 (Hs01588578_m1), KRT20 (Hs00300643_m1), SPINK1 (HS00162154_m1), UPKII (HS00171854_m1), and normalized qPCR results to endogenous reference gene expression of HPRT1 (Hs01003267_m1) and UBC (Hs00824723_m1) in relation to a calibrator sample (647V-cells in 10^7 peripheral blood mononuclear cells [PBMC] with a given relative gene expression] of 1.0) using the ΔΔCT method. Sensitivity was determined by the detection of serial dilutions with defined copy numbers ($0-10^6$) of cloned marker genes as well as 0 to 10^4 647V-cells in 10^7 PBMCs using triplicates.

As control, 136 LNs from 45 male prostate cancer patients without BCa treated with radical prostatectomy

and LND were analyzed to determine a threshold for physiologic expression of FXYD3, KRT17, KRT20, SPINK1, and UPKII. These LNs were assessed as negative by histopathology and qPCR for KLK3 expression in order to exclude prostate cancer metastasis.

2.3. Statistical analysis

Data were analyzed using IBM SPSS Statistics version 25.0 and the statistical software package R [22]. A threshold for molecular results (molN0 or molN1) was calculated identifying 99% of histopathologic true-negative LNs with a 99% level of confidence by using the R-package "tolerance" for estimating tolerance intervals based on a gamma distribution of FXYD3, KRT17, KRT20, SPINK1, or UPKII expression [23,24].

Cox regression analyses and Kaplan-Meier curves were used to assess survival rates. All statistical tests were performed 2-sided, and a P value of <0.05 was considered statistically significant.

The Kruskal-Wallis test was applied to assess the association of LN status with pathologic tumor stage. The Spearman correlation coefficient rho was used to assess presence and magnitude of monotonous trend between the level of evidence of LN metastases (pN0/molN0 <pN0/molN+ <pN+/molN+) and pathologic tumor stage.

3. Results

3.1. Patients and histopathology

Overall, 76 patients were enrolled in the accompanying translational biomarker program of the LEA-trial between May 2007 and August 2010. In this cohort 37 patients underwent a standard LND and 39 patients a super-extended LND. Overall, 40 of 76 (53%) patients presented with pT3/4 and/or pN+ tumors of which 17 (22%) patients received adjuvant chemotherapy. Patient characteristics are shown in Table 1.

In total, 2,612 LNs in 76 patients (median 31 LNs/patient; interquartile range 30−45) were dissected, of which 1,319 LNs were >5 mm and were analyzed by both methods with histopathologic and molecular examination (Fig. 1). In LNs >5 mm histopathology detected 39 LN metastases in 17 (22%) patients. In LNs <5 mm histopathology detected 23 histopathologic positive LNs in 4 patients. These LNs were found in patients that were already staged as node-positive in LNs >5 mm.

3.2. Molecular lymph node analysis

A selection of 5 genes (FXYD3, KRT17, KRT20, SPINK1, and UPKII) were identified as potential molecular markers by literature research [12-21] and preclinical

Table 1
Baseline patient characteristics and final histopathology

Characteristic	Standard LND ($n = 37$)	Super-extended LND ($n = 39$)	Overall ($n = 76$)
Gender, number of patients (%)			
Male	32 (86)	33 (85)	65 (86)
Age, years			
Median	67	69	67
Interquartile range	59−73	63−77	59−75
Removed lymph nodes, number			
Total	914	1698	2612
Used for PCR	500	819	1319
Median, per patient	25	42	31
Interquartile range, per patient	15−31	28−53	30−45
pN-status, number of patients (%)			
pNx	0	0	
pN0	31 (84)	28 (72)	59 (78)
pN+	6 (16)	11 (28)	17 (22)
pN1	1 (3)	6 (15)	7 (9)
pN2	5 (14)	5 (13)	10 (13)
pN3	0	0	0
Max. pT-status, number of patients (%)			
pT1	8 (22)	8 (21)	16 (21)
pT2	12 (32)	14 (36)	26 (34)
pT3	11 (30)	15 (38)	26 (34)
pT4	6 (16)	2 (5)	8 (11)
R-status, number of patients (%)			
R0	34 (92)	35 (90)	69 (91)
R1	3 (8)	2 (6)	5 (7)
Rx	0	2 (6)	2 (3)
Adjuvant chemotherapy, number of patients (%)	6 (16)	11 (28)	17 (22)

LND = lymph node dissection; PCR = polymerase chain reaction.

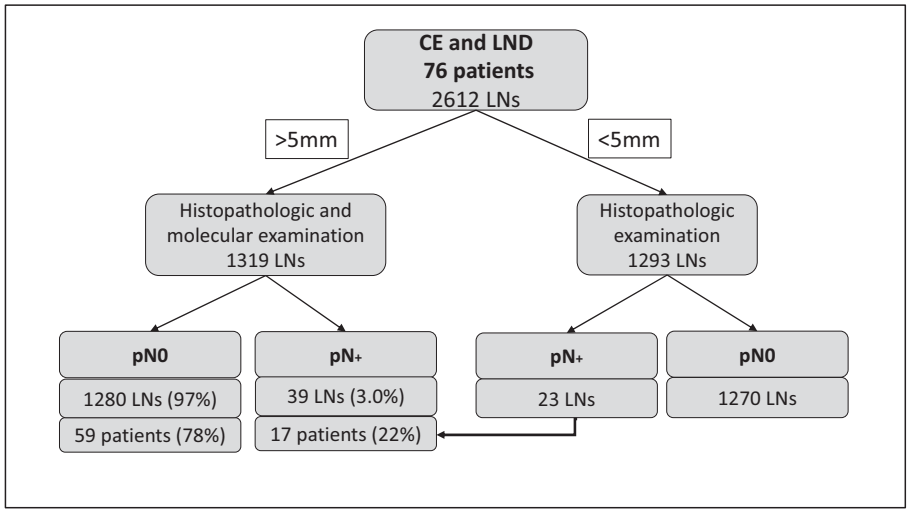


Fig. 1. Flow chart depicting the classification of LNs and patients according to histopathologic (pN0 vs. pN+) LN examination. About 1,293 LNs were smaller than 5 mm and therefore only analyzed by histopathology. These included 23 LN metastases in 4 patients, but all 4 patients also had metastases in LNs >5 mm, which were also analyzed with qPCR. LND = lymph node dissection; RC = radical cystectomy.

examination. Sensitivity tests resulted in the detection of 10 BCa cells (647V) and of 10 transcript copies in 10^7 PBMCs for all marker genes (Suppl. Fig. S1).

Based on a control group with 136 LNs from 45 patients, thresholds for each gene (FXYD3: 5.9; KRT17: 0.2; KRT20: 1.7; SPINK1: 49.7; UPK2: 3.4) were calculated as cut-off for discrimination between the absence (molN0) and presence (molN+) of metastatic BCa cells in LNs. Thus, combination of histopathologic and molecular results classified the LN status as negative (pN0/molN0), positive only by molecular examination (pN0/molN+), positive by histopathologic and molecular analysis (pN+/molN+), or positive only by histopathologic examination (pN+/molN0). As an example, the gene expression for FXYD3 in LNs of the control group and patients is given in Fig. 2, results for the other markers are shown in the supplementary data (Suppl. Fig. S2).

An overview for all marker results for histopathologic and molecular LN status is given in Table 2. FXYD3 showed the highest concordance with histopathologic positive results and

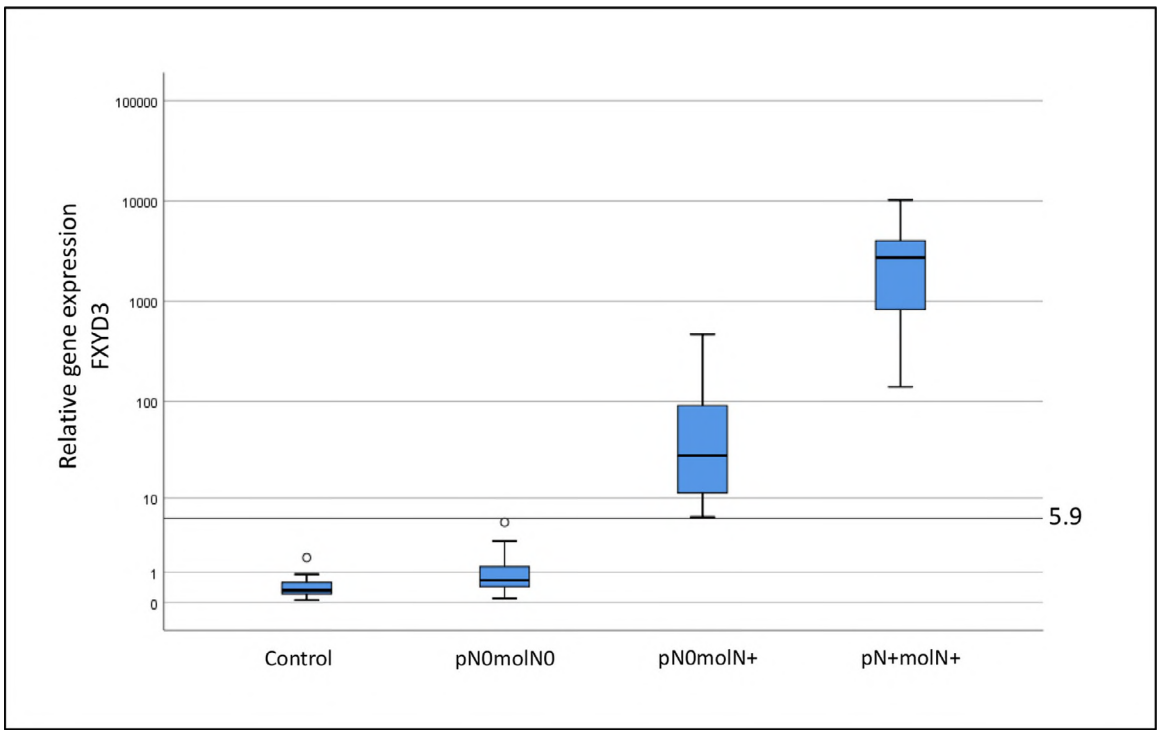


Fig. 2. Logarithmic box plot of the maximum relative gene expression (RGE) values for FXYD3 in lymph nodes (LNs) of the control group ($n = 45$) as well as patients in the study group classified as histopathologic and molecular negative (pN0/molN0; $n = 51$), histopathologic negative but molecular positive (pN0/molN+; $n = 7$), and both histopathologic and molecular positive (pN+/molN+; $n = 17$).

Table 2
Stratification by individual markers for patients ($n = 76$) and LNs ($n = 1,319$)

Target	Stratification by	Combination of histopathologic and molecular analysis		
		pN0/monN+ *N* (%)	pN+/molN+ *N* (%)	pN+/molN0 *N* (%)
FXYD3	Patients	7 (9)	17 (22)	0 (0)
	Lymph nodes	41	32 (2)	7 (1)
KRT17	Patients	10 (13)	16 (21)	1 (1)
	Lymph nodes	61 (5)	32 (2)	7 (1)
KRT20	Patients	8 (11)	16 (21)	1 (1)
	Lymph nodes	57 (4)	21 (2)	18 (1)
SPINK1	Patients	11 (15)	15 (20)	2 (3)
	Lymph nodes	79 (6)	32 (2)	7 (1)
UPK2	Patients	6 (8)	16 (21)	1 (1)
	Lymph nodes	84 (6)	31 (2)	7 (1)

classified all 17 pN+ patients correctly as node-positive by molecular examination (KRT17, KRT20, and UPK2 correctly classified 16 and SPINK1 correctly classified 15 of 17 pN+-patients). Thus, no patient was staged positive by histopathologic but negative by molecular LN examination (pN+/molN0) using FXYD3. ROC curve analyses confirmed highest AUC of 0.94 for FXYD3 (KRT17: 0.89; KRT20: 0.90; Spink1: 0.85; UPK2: 0.91) on patient level (Suppl. Fig. S3). Therefore, we chose the marker FXYD3 for further analyses of molecular LN examination.

Most importantly, in addition to correctly identifying histopathologic positive LNs (pN+/molN+), molecular analysis using FXYD3 detected pathologic marker expression in 41 histopathologic negative LNs (pN0/molN+) and thus additionally classified 7 (9%) patients as node-positive.

3.3. Association with pathologic tumor extension

A statistically significant association of LN status (pN0/molN0 vs. pN0/molN+ vs. pN+/molN+) was observed with pathologic tumor extension at RC ($P = 0.03$, Kruskal-Wallis test; Table 3). Pathologic tumor extension was most favorable in patients without LN metastases (pN0/molN0) and most unfavorable in patients with histopathologic and molecular LN metastases (pN+/molN+), while patients with exclusively molecular LN metastases (pN0/molN+) exhibited intermediate features (test for the correlation pN0/molN0 < pN0/molN+ < pN+/molN+: Spearman rho +0.31; $P = 0.006$).

3.4. Clinical outcome

At a median follow-up of 46.5 months (interquartile range 18.8−67.5 months) tumor recurrence was observed in 21 (28%) patients, 18 (24%) patients died from BCa and 9 (12%) patients died from other causes.

According to LN status using FXYD3 as molecular marker, 38 of 52 (73%) node-negative patients (pN0/molN0), 3 of 7 (43%) only molecular LN-positive patients (pN0/molN+), and 5 of 17 (29%) histopathologic and molecular LN-positive patients (pN+/molN+) were alive without tumor recurrence. LN status (pN0/molN0 vs. pN0/molN+ vs. pN+/molN+) was significantly associated with RFS ($P < 0.001$; Fig. 3). Median RFS was not reached in LN-negative patients (pN0/molN0), 45 months (95%CI 8−83 months) in exclusively molecular LN-positive

Table 3
Statistical analysis with Kruskal-Wallis test and Spearman rho of LN status for histopathology and the molecular marker FXYD3 with pathologic tumor extension

No. of patients	Groups				pN0/molN0 < pN0/molN+ < pN+/molN+	
	pN0/molN0 52	pN0/molN+ 7	pN+/molN+ 17	*P* value	Spearman rho	*P* value
Pathologic tumor stage, No. of patients (%)				0.02	+0.31	0.006
pT1	16 (31)	0 (0)	3 (18)			
pT2	18 (35)	3 (43)	2 (12)			
pT3	15 (29)	3 (43)	8 (47)			
pT4	3 (6)	1 (14)	4 (24)			

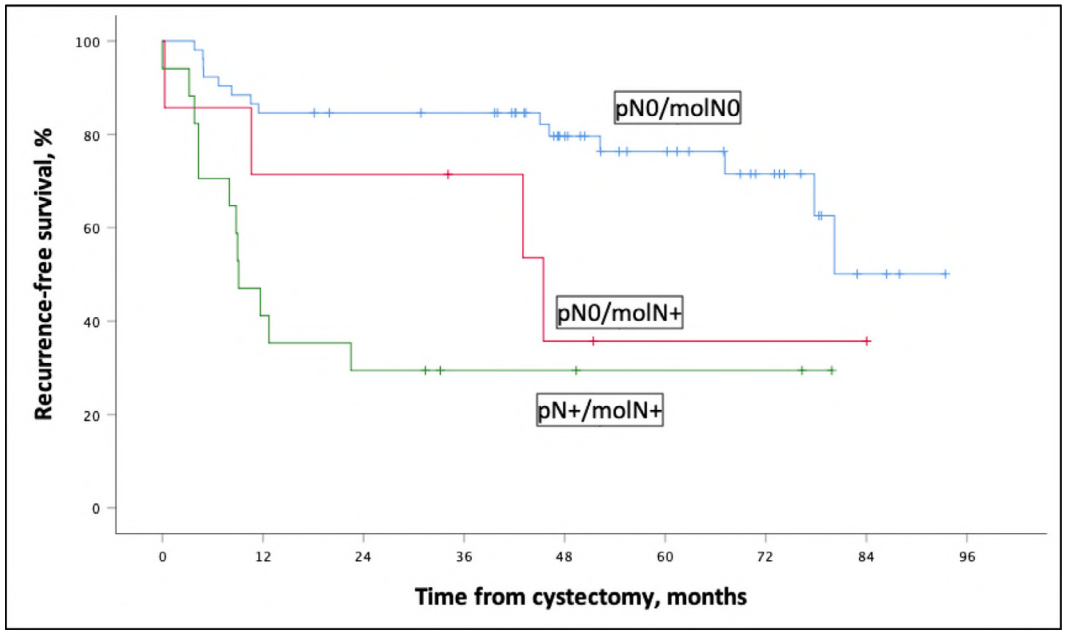


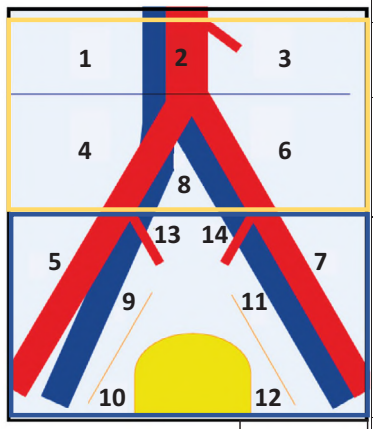
Fig. 3. Kaplan-Meier curve for recurrence-free survival with combination of histopathology and molecular analysis for FXYD3 (pN0/molN0 vs. pN0/molN+ vs. pN+/molN+), $P < 0.001$.

patients (pN0/molN+) and 9 months (95%CI 5−13 months) in patients with histopathologic and molecular positive LNs (pN+/molN+). Compared to LN-negative patients (pN0/molN0) the risk of tumor recurrence was significantly elevated in patients with histopathologic and molecular positive LNs (pN+/molN+) (hazard ratio= 4.8 95%CI 2.2−10.5; $P < 0.001$). In patients with only molecular positive LNs (pN0/molN+) a trend for an elevated risk of tumor recurrence was observed, which did not reach conventional levels of significance (hazard ratio = 2.5 95%CI 0.8−7.7; $P = 0.11$). Kaplan-Meier curves for RFS for the additional 4 markers are shown in Supplementary Fig. S4.

3.5. Topography of lymph node metastases

In 39 patients with extended LND, the topography of LN metastases was analyzed using histopathologic and molecular examination (Fig. 4). Overall, 15 of 39 (38%) patients were node-positive (pN0/molN+ or pN+/molN+). In total, 5 patients (13%) had LN metastases above the common iliac bifurcation, of which 2 patients (5%) had LN metastases exclusively above the common iliac bifurcation.

Overall, 1,698 LNs were removed in 39 patients with extended LND. On LN level, 66 LNs (3.9%) were positive by either histopathology or molecular analysis (pN0/molN+ or pN+/molN+). In total, 35 of 66 positive LNs (53%) were located below the common iliac bifurcation and 31 LNs (47%) were located above the common iliac bifurcation of which 20 LNs (30%) were located above the aortic bifurcation.



	pN0/molN+ or pN+/molN+ (%)	
	Pat	LN
1, 2, 3	4 (10)	20 (1.2)
4, 6, 8	4 (10)	11 (0.6)
5, 7, 9, 10, 11, 12, 13, 14	13 (33)	35 (2.1)
Total	15 (38)	66 (3.9)

Fig. 4. On the left side anatomic template for lymph node dissection is shown. In this study, standard lymph node dissection-field (LND) includes obturator (9, 11), internal (13, 14) and external iliac (5, 7), and deep obturator (10, 12) nodes (blue). The standard field was defined proximally by the bifurcation of internal and external iliac artery, distally by the pelvic floor, laterally by the genitofemoral nerve, and dorsally by the obturator nerve. The super-extended LND-field additionally include presacral (8), common iliac (4, 6), paracaval (1), interaortocaval (2), and para-aortal (3) lymph nodes up to inferior mesenteric artery (yellow). The right table shows the topography of positive lymph nodes on patient level (Pat) and LN level (LN). (Color version of figure is available online.)

4. Discussion

BCa patients with LN metastases at the time of RC and LND have a high risk for tumor recurrence [3,4]. Improving LN staging in BCa is of importance since it triggers adjuvant chemotherapy, which improves overall survival [2,25,26]. Also, many patients with locally advanced BCa but pathologically negative LN develop distant metastases indicating the need to improve sensitivity in detection of LN metastases [5].

The randomized phase-III LEA-trial (LEA AUO AB 25/02) investigated the therapeutic role of an extended vs. a limited LND in urothelial BCa patients undergoing RC and showed no statistically significant difference in RFS [11]. As part of this trial, molecular LN status was assessed in a subgroup of 76 patients.

There are no established markers for the detection of distant urothelial BCa cells in LNs, thus we prospectively evaluated 5 transcripts (FXYD3, KRT17, KRT20, SPINK1, and UPKII) for molecular LN analysis. FXDYD3, KRT20, and UPKII have previously been described for molecular LN analysis and KRT17 as well as SPINK1 were additionally identified from literature search [12-21]. All transcripts were preclinically tested showing high sensitivity in dilution series of urothelial BCa cell lines and cloned marker copies in PBMCs.

Common study limitations of previous findings on molecular LN analysis in BCa are a lack of direct comparison between uniquely described biomarkers, retrospective trial design, small numbers of included patients, and analyzed LNs as well as missing data on associations with survival [12-21]. Moreover, the lack of independent validation of biomarkers is a requirement often discussed.

Based on our results, FXYD3 was identified as an optimal transcript for molecular LN analysis in BCa providing 100% concordance with histopathologic positive LNs and additional identification of metastases in histopathologic LN-negative patients. FXYD3, also named Mat-8 (mammary tumor marker 8), codes for a transmembrane protein overexpressed in BCa [27] and has been described as BCa-specific molecular marker in blood and LNs [13,16,21,28].

Two previous studies evaluated FXYD3-mRNA levels by quantitative real-time PCR to detect LN metastases on a molecular level [13,16]. In these studies, a combination of FXYD3 and KRT20 was used for molecular LN analysis and led to upstaging in 20.5% of patients primarily classified as pN0 by histopathology. However, no significant association between molecular LN status and RFS or CSS was observed [13,16]. When comparing these studies to the current one, a main limitation is the extent of LND and amount of analyzed LNs. Our study analyzed in average 17 LNs per patient with PCR, whereas the former studies analyzed a very small amount of LNs per patient (1.8 and 5.6 LNs in average per patient, respectively). But all 3 reports raise a rationale for pursuing FXYD3 as a biomarker for molecular LN analysis in BCa.

In our study, molecular and histopathologic LN status was significantly associated with locally advanced tumor stage and poor RFS. Molecular and histopathologic LN examination stratified patients with negative LNs (pN0/molN0) in a low-risk group being associated with a higher rate of locally confined tumors and longer RFS. Patients with histopathologic and molecular positive LNs (pN+/molN+) were stratified in a high-risk group being associated with the highest rate of locally advanced tumors and shortest RFS, while patients with only molecular positive LNs (pN0/molN+) exhibited intermediate-risk features with a median RFS located between the low- and high-risk group. Thus, molecular LN analysis using FXYD3 might be useful to identify additional patients with LN metastasis who have an elevated risk of tumor recurrence and might benefit from adjuvant chemotherapy [26]. The statistical significance for RFS in the pN0/molN+ group was probably not reached due to the small group size of only 7 patients. Molecular changes might be detected earlier with PCR than morphologic changes can be detected by histopathology. Therefore, another reason for the missing significant difference of the survival endpoint might be the therapeutic impact of LND when removing small-volume metastases [16].

When performing RC in patients with BCa an adequate LND is crucial for diagnostic and therapeutic reasons. However, the extent of LND for therapeutic reasons is still controversial. The LEA-trial performed by Gschwend et al. evaluated for the first time in a randomized-controlled trial whether super-extended vs. standard LND prolongs RFS [11]. It showed a trend with a small survival benefit in the super-extended LND group but did not reach conventional levels of significance. As we here present data on the accompanying biomarker program of the LEA-trial, molecular LN staging in BCa was for the first time evaluated prospectively and with patients receiving an adequate LN dissection (median of 25 and 42 LNs per patient in the standard and super-extended arm, respectively). Another prospective, randomized phase-III trial evaluating limited vs. extended LND in BCa patients treated with RC by the Southwest Oncology Group (Southwest Oncology Group S1011; Clinical-Trials.gov number, NCT01224665) might further elucidate the therapeutic role of super-extended LND.

So far, all published mapping studies of LN metastases in BCa with LND were based on histopathologic findings. To our knowledge, the present study is the first to determine the topography of LN metastases via molecular and histopathologic examination in BCa patients treated with RC and LND. Analyzing the topography in patients treated with super-extended LND, 13% of patients had LN metastases above the common iliac bifurcation. Remarkably, on LN level, 47% of detected metastases were located above the common iliac bifurcation. Thus, our analyses of topography of LN metastases are consistent with previous findings and therefore support the importance of a LND above

the common iliac bifurcation in order to identify and remove LN metastases in patients with BCa [29,30]. In the provided study many LN metastases were only identified using a super-extended LND above the common iliac bifurcation and molecular LN analysis (31 LN metastases in 5 patients [13%] in the super-extended field). Since super-extended LND does not lead to relevant additional morbidity, except for lymphoceles, a greater extent of LND seems reasonable in patients with muscle-invasive BCa [11].

As a limitation of standard histopathology, small-volume metastases can be missed due to a sampling error on nodal slicing, or they may be missed on microscopic examination. Importantly, FXYD3 as a molecular marker detected all patients that were histopathologic node-positive, although on LN level 7 histopathological positive LNs were negative in the molecular analysis. This might be due to bisection of LNs since each half was only examined by one technique. For that reason, small-volume metastases only present in one half of the LN might have been missed by the other method—an argument that also applies to LNs staged as histopathologic negative but molecular positive. Furthermore, heterogeneous gene expression or loss of mRNA expression might have led to FXYD3-negative metastases. Therefore, application of a marker panel might be necessary to further improve sensitivity and specificity of molecular LN detection. A limitation of our study is that no pathological re-evaluation of pN0/molN+ LNs was performed to confirm the findings. A limitation of the molecular LN analysis is that morphologic information such as size of LN metastasis and extracapsular extension cannot be determined. However, a molecular analysis by qPCR is independent of a pathologist's experience and, once established, might be more time and cost effective in the long term when analyzing large numbers of samples achieving higher sensitivity than histopathology.

Future perspectives should include the validation of FXYD3 and a panel of molecular markers in a prospective larger cohort. Further studies should also include patients after neoadjuvant chemotherapy, since it is now the recommended treatment for patients with muscle-invasive BCa [2].

5. Conclusion

This study validates the marker FXYD3 for molecular LN analysis in BCa leading to additional identification of patients with LN metastases despite negative histopathology. Thus, molecular LN analysis using FXYD3 could supplement histopathologic LN staging to identify patients with higher risk for tumor progression as a diagnostic tool to guide adjuvant treatment. Moreover, our study supports the role of a proper LND above the common iliac bifurcation to identify and remove LN metastases at the time of RC in BCa.

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Conflict of interest

No potential conflicts of interest were disclosed.

Supplementary materials

Supplementary material associated with this article can be found in the online version at https://doi.org/10.1016/j.urolonc.2020.01.018.

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