

Lymphangiogenesis occurs in upper tract urothelial carcinoma and correlates with lymphatic tumour dissemination and poor prognosis

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OBJECTIVE

To describe the lymphatic vessel density and to determine the functional and prognostic significance of tumoral lymphatic vessels in upper tract urothelial carcinoma (UTUC).

PATIENTS AND METHODS

The study included 65 patients who had a radical nephroureterectomy (RNU) for UTUC between 1997 and 2004. All pathological slides were re-evaluated by one reference pathologist and clinical data were reviewed. Lymphatic endothelial cells (LECs) were stained immunohistochemically using D2-40. The lymphatic vessel density (LVD) was described in representative intratumoral (ITLVD), peritumoral (PTLVD) and non-

tumoral (NTLVD) areas. Random samples were selected for double-immunostaining with D2-40 and CD-34 (to distinguish blood and lymphatic vessels) and the proliferation marker Ki-67 to detect lymphangiogenesis. The primary outcome measures were disease-specific survival (DSS) and disease recurrence (urothelial and/or distant).

RESULTS

The median (interquartile range) PTLVD was 4.0 (3.0–6.3), and significantly higher than that for ITLVD, of 0.3 (0–1.7) ($P < 0.001$), and NTLVD, of 3 (2.0–3.7) ($P < 0.001$). Both a higher ITLVD and PTLVD, the presence of lymphovascular invasion (LVI) (each $P < 0.001$) and a high tumour grade ($P = 0.004$) were associated with reduced DSS on univariate analysis. A higher PTLVD ($P = 0.028$) and the presence of LVI ($P = 0.020$) independently predicted reduced

DSS on multivariate analysis. IT and PT lymphatic vessels showed proliferating LECs in all analysed samples.

CONCLUSION

Lymphangiogenesis is present in UTUC, as shown by a significantly increased PTLVD and proliferating LECs. Our findings suggest functional relevance of PT lymphatic vessels during lymphatic tumour spread. PTLVD is a potential novel prognostic factor for DSS in UTUC, and further prospective studies will be needed to determine the effect of its routine evaluation on clinical outcomes of this malignancy.

KEYWORDS

transitional cell carcinoma, urinary tract cancer, lymphatic vessels, podoplanin, metastasis, lymphangiogenesis, prognosis

INTRODUCTION

Metastatic tumour spread to regional lymph nodes is considered as an early event in the progression of urothelial carcinoma (UC) of the bladder and upper urinary tract (UTUC). It is associated with disease progression and a poor prognosis [1–3]. Lymphatic vessels within or close to tumours serve as the primary conduit for metastatic tumour cell spread in many types of cancer [4,5]. However, the initial stages of lymphatic tumour cell progression in UTUC are poorly understood.

Factors such as the lymphatic vessel density (LVD), lymphovascular invasion (LVI) and lymph-specific growth factors (e.g. vascular endothelial growth factors, VEGF-C/-D) have been associated with lymph node metastases [6–11]. Besides the VEGF family, lymphatic endothelial cells (LECs) play a role in the complex process of LVI and cancer-cell dissemination [12]. We recently reported on the significance of lymphangiogenesis in bladder UC and found a strong association between a high peritumoral (PT) LVD (PTLVD) and the occurrence of lymphatic metastases

[13]. To assess lymphangiogenesis we used the LEC-specific marker D2-40, which binds to the transmembrane glycoprotein podoplanin on LECs and became commercially available only a few years ago [4,14,15]. The previous study also showed the existence of proliferating LECs, potentially promoting the process of lymphatic metastases [13]. Both intratumoral (IT) and PT lymphatic vessels have been associated with the occurrence of lymph-node metastases and a poor clinical outcome in several other malignancies [16–20]. However, it remains to be clarified

whether these lymphatic vessels already exist or whether de-novo synthesis, i.e. lymphangiogenesis, occurs primarily. In UTUC, the existence and clinical relevance of lymphangiogenesis is unknown to date. The prognostic impact of tumoral lymphatic vessels has rarely been investigated [6], and lymphangiogenesis has not been detected to date. The concept of lymphangiogenesis could be an area for novel therapeutic regimens comparable to those for angiogenesis previously.

The aim of the present study was to describe the LVD in UTUC and to determine the functional and prognostic significance of LECs in different representative tumour areas. Therefore, LVD was correlated with the pathological and clinical data of patients who had a radical nephroureterectomy (RNU) at our department.

PATIENTS AND METHODS

We reviewed the data from 76 consecutive patients with UTUC who had RNU at our department between 1997 and 2004. Patients without sufficient follow-up records (five) and those in which no adequate tissue samples could be obtained for sectioning of paraffin blocks (six) were excluded from the analysis; the study population therefore comprised 65 patients.

All pathological haematoxylin and eosin-stained sections were re-evaluated by one genitourinary reference pathologist (P.S.) unaware of the clinical data. Pathological stage was assigned according to the 2002 TNM classification of the American Joint Committee on Cancer [21]. Tumour grade was assessed according to the 1998 WHO/International Society of Urologic Pathology consensus classification [22]. LVI was defined as the presence of UC cells within vessels with an unequivocal single-cell endothelial lining. The presence of tumour necrosis was considered significant when >10% of the tumour tissue was affected. Urothelial recurrence was defined as any recurrence of UC within the remaining urinary tract.

UC tumour specimens were fixed using standardized procedures. One to three representative UTUC blocks with normal tissue surrounding the tumour area were selected from each patient. For all immunohistochemical analysis, serial 3- μ m sections were cut, then placed on

SuperFrost®Plus slides (R. Langenbrinck, Tenningen, Germany) and dried at 37 °C overnight. All slides were evaluated by two investigators (C.B. and K.H.) who were unaware of the pathological and clinical data before any statistical analysis.

We previously described all immunohistochemical procedures in detail and briefly summarize them here. Appropriate control sections were identified through the pathology reports, selecting paraffin blocks in which only non-malignant tissue was present microscopically. Normal human UT urothelial specimens and bladder specimens were used as positive controls for D2-40, CD-34 and Ki-67 (clone MIB-1) antibody expression. Negative controls were produced by replacing the primary antibody with Tris-buffered saline.

For D2-40 staining, after deparaffinizing and rehydrating the sections, the epitopes were retrieved. Endogenous peroxidase activity was blocked using 3% hydrogen peroxide in methanol. The slides were incubated with D2-40 (#730-26, ready-to-use, Signet Laboratories, Inc., Dedham, USA) as primary antibody overnight at 4 °C. Sections were incubated with a goat antimouse secondary antibody (EnVision+ System, Dako, Glostrup, Denmark) for 30 min, with aminoethylcarbazole as the chromogen.

For D2-40 double-staining we used the endothelial marker CD-34 (to distinguish blood and lymphatic vessels) and the proliferation marker Ki-67 (cell proliferation, clone MIB-1) in 10 randomly selected samples, respectively. Therefore, the monoclonal mouse antihuman CD-34 antibody (Clone QBEnd, Dako Code 7165) was applied in conjunction with D2-40 (#730-26) for simultaneous visualization of blood and lymphatic vessels (EnVision Doublestain System, Dako). CD-34 positivity was visualized using diaminobenzidine. The slides were then incubated with D2-40 overnight at 4 °C and visualized with alkaline phosphatase and Fast Red chromogen (EnVision). A mouse monoclonal Ki-67 antibody (Clone MIB-1; Dako M7240) and the EnVision goat antimouse antibody was used for Ki-67 staining, with diaminobenzidine as the chromogen before applying the D2-40 monoclonal secondary antibody.

LVD was analysed in hot spots, as previously described [13,23]. Briefly, lymphatic vessels

were quantified using a counting grid (0.34 mm²) at $\times 200$ in three areas, i.e. IT, PT and non-tumoral (NT). The PT area was defined as NT tissue at the tumour periphery (maximum distance 580 μ m and not surrounded by tumour tissue). Normal tissue was defined as the area with at >580 μ m from the tumour. Ki-67 positivity in the nucleus of LECs was considered as a sign of lymphangiogenesis.

Data on LVDs are given as the median (interquartile range, IQR, and range); the Wilcoxon test for paired samples was used to compare the LVD values in different areas. We used the Kruskal–Wallis test, Mann–Whitney *U*-test and the chi-squared test to assess the correlation between LVD in the different areas and pathological or clinical variables. Survival was analysed using the Kaplan–Meier method. Pathological (including LVD values) and clinical data were correlated with the occurrence of urothelial disease recurrence (reflected by recurrence-free survival), the first documented presence of distant metastases (metastasis-free survival) and death from UC (defined as disease-specific survival, DSS) to identify prognostic factors. The log-rank test was used to compare survival curves of several groups and to test the influence of continuous variables on failure times. For the multivariable analysis of prognostic factors we used Cox regression models (with forward selection). Hazard ratios (HRs) and 95% CI were determined to assess the associations. All reported *P* values are two-sided and statistical significance was assumed at *P* < 0.05.

RESULTS

The median (range) patient age was 67 (34–89) and the male : female ratio was $\approx 2:1$ (45 men). The clinical and pathological data of all patients are listed in Table 1. No neoadjuvant therapy was used and one patient had adjuvant chemotherapy.

D2-40-positivity specifically identified lymphatic vessels, providing visualization of the typical single thin, lymphatic endothelial-cell layers. Vessels containing red blood cells remained unreactive for D2-40. Tumour cells were consistently negative for D2-40, as was the urothelium along the ureter and in the renal pelvis, apart from tumoral areas (Fig. 1). Stromal cells such as fibroblasts or myofibroblasts in the tumour periphery occasionally showed D2-40-positivity,

TABLE 1 Demographic, clinical and pathological variables

Variable	n (%)
Previous bladder UC	
Yes	12 (20)
No	48 (80)
Tumour location	
Renal pelvis	46 (71)
Ureter	19 (29)
Tumour architecture	
Papillary	47 (72)
Sessile	18 (28)
Multifocality	
Yes	18 (28)
No	47 (72)
Concomitant carcinoma <i>in situ</i>	
Yes	12 (19)
No	53 (81)
Pathological T stage	
pTa	4 (6)
pTis	2 (3)
pT1	13 (20)
pT2	13 (20)
pT3	27 (42)
pT4	6 (9)
Pathological grade	
Low	31 (48)
High	34 (52)
Tumour necrosis (threshold 10%)	
Yes	9 (14)
No	56 (86)
LVI	
Yes	21 (32)
No	44 (68)
Pathological N stage	
pN0	9 (14)
pN+	8 (12)
pNx	48 (74)

whereas differentiation to lymphatic vessels was possible in all sections. Lymphatic vessels were often localized beyond the urothelial lamina propria and typically paralleled blood vessels (Figs 1,2). Also, they were present within smooth muscle or fatty tissue. In all sections, lymphatic vessels were present in the NT and in the PT area (Fig. 3). In 36 sections (55.4%), IT lymphatic vessels were detected (Fig. 4). Most lymphatic vessels identified both in the IT and in the PT areas were collapsed (Figs 3,4). Occasionally there was LVI of malignant cells in both the IT and PT areas. Double-staining with D2-40 antibody and CD-34 antibody distinguished D2-40-positive lymphatic vessels from adjacent D2-40-negative/CD-34 positive

FIG. 1. Paraffin sections showing the expression of D2-40 in suburothelial LECs (red). Lymphatic vessels (black arrows) are located within the lamina propria of the normal renal pelvis; blood vessels containing erythrocytes were unreactive (blue arrow) ($\times 100$).

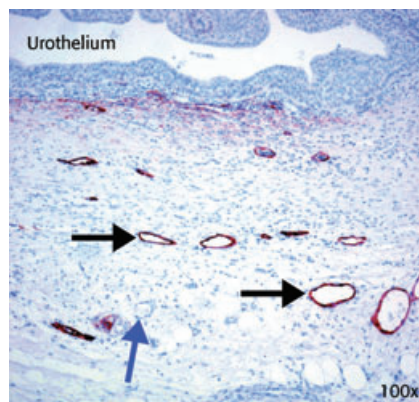
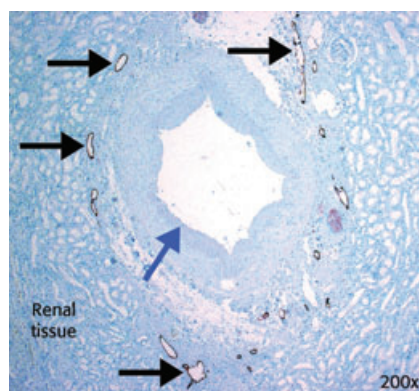


FIG. 2. D2-40 positive lymphatic vessels (black arrows) in parallel with a large blood vessel (blue arrow) within normal renal tissue ($\times 200$).



blood vessels, which contained red blood cells in nearly all cases (Fig. 5).

LVD differed significantly in the areas of interest; the lowest LVD was detected in IT areas (ITLVD), being significantly lower than in areas of NT (NTLVD) and in the PT area (both $P < 0.001$). The median overall ITLVD was 0.3 (IQR 0–1.7, 0–17.0). A higher ITLVD was significantly associated with a poor histological differentiation, and with the presence of LVI and a positive pN status (Table 2).

The PTLVD was consistently the highest; the overall median PTLVD was 4.0 (3.0–6.3, 0.3–18.3), correlating positively with a higher pT stage, poor histological differentiation, the presence of tumour necrosis, LVI and a

FIG. 3. Peritumoral D2-40 positive lymph vessels (black arrows) within stromal tissue with a high PTLVD. A few collapsed IT vessels are present (blue arrow). This pattern of distribution was the most common ($\times 100$).

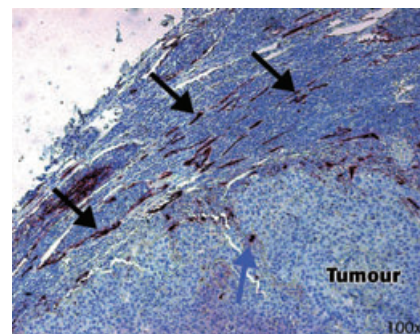


FIG. 4. D2-40-positive IT, nearly collapsed lymphatic vessels (black arrows). There were more vessels in patients with lymph node metastases and very few in patients with no nodal tumour involvement ($\times 100$).

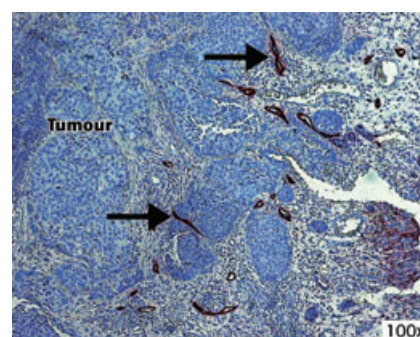
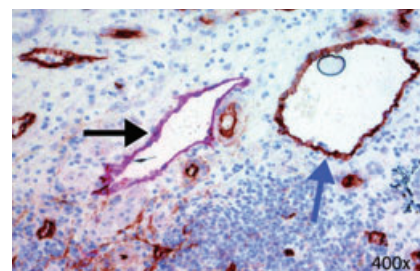


FIG. 5. D2-40-positive lymphatic vessels (pink endothelium; black arrow) and CD-34 positive blood vessels (bay-coloured endothelium; blue arrow) within stromal tissue in normal renal pelvis. Clear distinction was possible in all double-stained slides ($\times 400$).



positive pN status (Table 2). The presence of LVI correlated significantly with the presence of lymphatic metastases (positive pN status; $P = 0.049$; Fisher's exact test).

TABLE 2 The association between LVDs in different areas (IT, PT, NT) and pathological variables

Variable	No. of patients	Median (IQR) LVD	P*
ITLVD			
Pathological T stage			
≤pT2	32	0.3 (0–1.7)	0.405
≥pT3	33	0.3 (0–2.7)	
Pathological grade			
Low	31	0 (0–1.0)	0.024
High	34	0.8 (0–3.3)	
Tumour necrosis			
Absent	56	0.3 (0–1.7)	0.572
Present	9	1.0 (0–2.7)	
LVI			
Absent	44	0 (0–1.0)	<0.001
Present	21	2 (0.3–4.0)	
Pathological N stage*			
pN0	9	0.3 (0–2.0)	0.007
pN+	8	7.2 (3.5–10.7)	
PTLVD			
Pathological T stage			
≤pT2	32	3.5 (2.2–4.7)	0.003
≥pT3	33	5.7 (3.3–8.3)	
Pathological grade			
Low	31	3.3 (2.3–5.0)	0.033
High	34	5.2 (3.0–8.3)	
Tumour necrosis			
Absent	56	3.7 (2.5–5.7)	0.026
Present	9	8.0 (6.0–8.3)	
LVI			
Absent	44	3.3 (2.3–5.0)	<0.001
Present	21	8.0 (4.3–8.3)	
Pathological N stage*			
pN0	9	5.6 (3.0–6.3)	0.038
pN+	8	10.5 (8.8–13.8)	
NTLVD			
Pathological T stage			
≤pT2	32	3.0 (2.0–3.8)	0.571
≥pT3	33	2.7 (2.0–3.7)	
Pathological grade			
Low	31	2.7 (2.0–3.7)	0.889
High	34	3.0 (1.7–3.7)	
Tumour necrosis			
Absent	56	2.8 (2.0–3.7)	0.756
Present	9	3.0 (2.3–3.7)	
LVI			
Absent	44	2.8 (2.0–3.8)	0.849
Present	21	3.0 (2.0–3.3)	
Pathological N stage*			
pN0	9	4.3 (2.0–5.0)	0.809
pN+	8	3.2 (2.3–4.3)	

*Mann–Whitney–U–test; †As lymphadenectomy was only done in 17 of 65 patients (26%), 48 (74%) had to classified as pNx.

For NTLVD the median was 3.0 (2.0–3.7, 0.3–9.0), but there was no significant correlation with any pathological variable (Table 2). Cycling LECs were detected in all sections of the subgroup double-stained with D2-40/Ki-67 in a variable proportion (Fig. 6a,b). Whereas Ki-67-positive lymphatic vessels were detected in IT and PT LECs in each selected slide, there was LEC proliferation in the NT areas in only six of 10 samples.

Valid follow-up data were available for 63 patients (96.9%), over a median of 35 (1–115) months. There was urothelial recurrence in 26 of 60 patients (43%), with 22 having a recurrence within 24 months after RNU. Only tumour grade (high vs low) showed a slightly significant correlation with urothelial recurrence ($P = 0.075$; Table 3).

Factors associated with the occurrence of distant metastases were higher values for PTLVD, ITLVD and the presence of LVI (each $P < 0.001$), and a high tumour grade ($P = 0.005$; Table 3). A higher PTLVD ($P = 0.016$; HR 1.16, 1.03–1.31) and the presence of LVI ($P = 0.044$; 3.88, 1.03–14.62) were independently associated with distant metastases on multivariable analysis.

At the follow-up, 38 of 63 patients (60%) were alive; 16 patients died from UTUC (25%). The mean (SD) 2- and 5-year actuarial DSS probabilities were 81.2 (5)% and 66.1 (8)%, respectively. Both a higher ITLVD and PTLVD (both $P < 0.001$; Fig. 7a) and a higher tumour grade ($P = 0.004$) and the presence of LVI ($P < 0.001$; Fig. 7b) translated into a reduced DSS on univariable analysis (Table 3). A higher PTLVD ($P = 0.028$; HR 1.15, 1.02–1.29) and the presence of LVI ($P = 0.020$; 4.84, 1.29–18.16) were the only significant predictors for reduced DSS on multivariable analysis.

DISCUSSION

The presence of LVI and lymph node metastases have previously been associated with a poor clinical outcome in patients with UTUC [7,24]. Understanding the lymphatic dissemination process in UTUC is crucial for the development of novel therapeutic strategies, both in surgical procedures and targeted drug therapy. The present findings offer insights into the mechanisms of lymphatic tumour cell spread by studies of LVD and lymphangiogenesis occurring in UTUC. To our knowledge, the present study is

FIG. 6. Paraffin sections showing double-staining for D2-40 and Ki-67, showing PT (A) and IT (B) lymphatic vessels (black arrows) with Ki-67-positive endothelial cell nucleus and proliferating tumour cells (B) expressing Ki-67 (green arrows in both pictures) (x200).

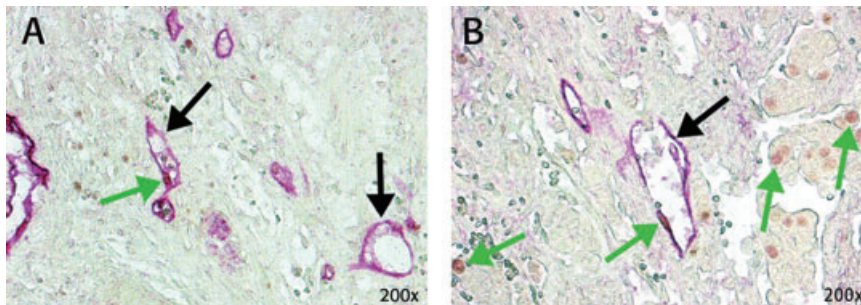


TABLE 3 Associations between pathological variables and endpoints on univariable analysis (P values derived by log-rank test) and remaining significant variables on multivariable analysis (Cox regression model with forward selection) as independent predictors for the defined endpoints

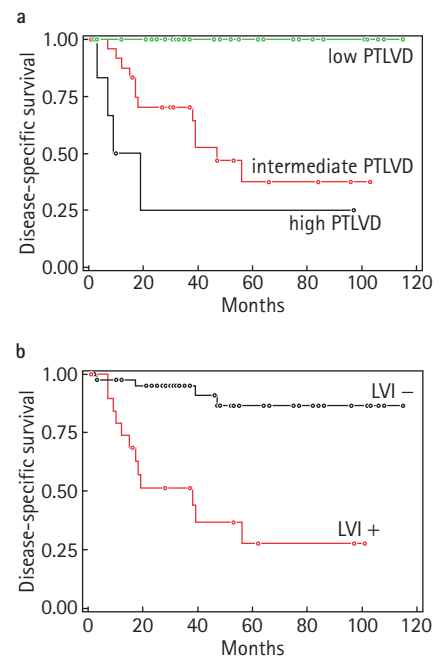
Variable	P for		
	Urothelial recurrence	Metastases	DSS
Univariable analysis			
ITLVD	0.891	<0.001	<0.001
PTLVD	0.715	<0.001	<0.001
NTLVD	0.863	0.762	0.418
LVI	0.127	<0.001	<0.001
Tumour:			
Necrosis	0.917	0.365	0.112
Multifocality	0.185	0.094	0.332
Grade (low vs high)	0.075	0.005	0.004
Stage ($\leq$ pT2 vs $\geq$ pT3)	0.676	0.244	0.162
N stage (pN0 vs pN+)	0.191	0.244	0.203
Multivariate analysis			
PTLVD	–	0.016	0.028
(95% CI)	–	1.16 (1.03–1.31)	1.15 (1.02–1.29)
LVI	–	0.044	0.020
(95% CI)	–	3.88 (1.03–14.62)	4.84 (1.29–18.16)

the first to strongly suggest the existence of lymphangiogenesis in UTUC by showing the proliferation of LECs with a specific proliferation marker. D2-40/Ki-67 double-staining is a valuable tool to detect lymphangiogenesis and has been used by others to detect proliferation of LECs in lung cancer, head and neck squamous cell carcinoma (HNSCC), and breast cancer specimens [17,18,25].

In the present samples, there was induced lymphangiogenesis particularly in the PT areas, but also in IT areas from patients with lymph node metastases. The notably high PTLVD and the presence of proliferating LECs in these areas can be interpreted as de-novo synthesis of lymphatic vessels. Furthermore,

with increasing ITLVD and PTLVD, the incidence of LVI increased, being likely to facilitate penetration and dissemination of UTUC cells via the lymphatics. This hypothesis is supported by earlier reports on other malignancies, showing that activation of the lymphatic endothelium by factors secreted by tumour cells promoted an increase in lymphatic vessel size and facilitated LVI of tumour cells and even aggregates [5,8,26]. On the other hand, the absence of a high LVD and active lymphangiogenesis was reported in some tumours that metastasize via lymphatics, including breast and prostate cancer, raising the question of the functional role of pre-existing lymphatics in promoting lymphatic metastases [23,27–29]. However, for breast

FIG. 7. Kaplan–Meier estimates for DSS in 65 patients with UTUC after RNU stratified by: **a**, the PTLVD (low, 0–4, green curve; intermediate, 5–10, red curve; high, ≥ 10 , black curve; the threshold for low was 4, representing the median PTLVD). Patients with an intermediate or high PTLVD (red and black curve) were at significantly greater risk of disease-specific death than those with a low PTLVD, in which the DSS was 100% (green curve; $P = 0.028$); **b**, the presence (red curve, LVI+) or absence (black curve, LVI–) of LVI. Patients with LVI were at significantly greater risk of disease-specific death than those without LVI ($P = 0.020$).



cancer, contradictory results were reported and evidence of proliferating IT lymphatics was reported also for inflammatory tissue [25].

The present results confirm our previous findings on LVD in bladder UC and are in concordance with a recently published study on tumoral lymphatic vessels in UTUC by Miyata *et al.* [6]. They studied lymphatic vessels in UTUC and reported the presence of IT lymphatic vessels as the only independent prognostic factor for a reduced DSS. They also found a correlation between an increased PTLVD and the presence of lymph node metastases. Furthermore, a higher PTLVD correlated with the occurrence of distant metastases, but not with a reduced DSS. Our results complement these findings in terms of assessing the functional role of tumoral lymphatic vessels. Moreover, in the present

patients, a higher PTLVD independently predicted a reduced DSS, thus becoming a potential prognostic marker for UTUC. We were unable to show a significant association between the presence of lymph node metastases and poor outcome. This might be explained by the fact that regional lymphadenectomy in conjunction with RNU is not a standardized procedure at our institution. It was only done in patients with enlarged lymph nodes on preoperative axial imaging or with adenopathy detected during surgery, leading to many (48) patients with pNx status.

In several cancers, PT lymphatic vessels have been associated with regional nodal metastasis and reduced DSS, including lung cancer, HNSCC, prostate cancer, UTUC and colorectal cancer [17–20]. It was suggested that these lymphatic vessels might pre-exist and could be simply compressed into the tumour periphery by increased interstitial pressure induced by proliferating tumour cells, thus leading to artificially high LVDs [30]. However, besides proliferating PT lymphatics, we also identified several proliferating IT lymphatic vessels, particularly in patients with nodal metastases. This again suggests an induced synthesis of novel lymphatic vessels. Nonetheless, it does not exclude potential concomitant activation of pre-existing lymphatics by specific growth factors (e.g. VEGF) produced by tumour cells, as suggested by others [9–11,26].

The role of IT lymphatic vessels has been controversial. In several studies, only a few IT lymphatic vessels have consistently been detected, whereas others suggest an important functional role of these vessels in tumour progression [6,16,23,25,29]. However, findings on IT lymphatics seem to vary in different malignancies and depend on different stages within the same malignancy. For example, Miyata *et al.* [10] found a low incidence of IT lymphatic vessels (16%) in a recent study on bladder UC. This could be explained by the inclusion of a high proportion of noninvasive bladder UCs having a lower malignant potential (only 18.4% of patients presented with muscle-invasive tumours). There might be fewer lymphatic vessels involved in tumour progression. Our findings support the hypothesis of a significant correlation between a high ITLVD and increased tumour aggressiveness, as there were higher tumour grades, and more LVI and nodal metastases with increasing

ITLVD. As to the increased likelihood of lymph node metastases and reduced DSS in patients with a higher ITLVD, our results are consistent with previous reports on cutaneous melanoma and HNSCC [16,17]. Thus, there is evidence that IT lymphatic vessels also play a role in tumour cell dissemination via lymphatics.

There are several limitations to the present study. The 'ideal' marker for the specific detection of lymphatic vessels remains to be established, as there are other markers, such as LYVE-1, for morphological studies on lymphatics, that might yield different results. Another limitation might be the interobserver variability of all immunohistochemical studies. However, we used a well-established method for lymphatic vessel counts [13,23] and furthermore, as recommended by the authors of an international consensus [30], we assessed LEC proliferation as an objective criterion, suggesting the de-novo synthesis of lymphatic vessels. As there are very few studies on LVD in UTUC to date, we consider the present investigation as a basic study contributing to a better understanding of the onset of lymphatic metastases in UTUC.

In conclusion, we showed that there was a high PTLVD and proliferating IT and PT lymphatic vessels, strongly suggesting induced lymphangiogenesis in UTUC. Furthermore, a higher PTLVD was associated with the presence of lymph node metastases and translated into a reduced DSS on multivariable analysis. LVD must be considered as a potential novel prognostic marker complementing the use of the TNM system in patients with UTUC undergoing radical surgery. A multi-institutional validation and prospective evaluation of our findings is necessary to estimate the clinical usefulness of LVD measures. Furthermore, studies on lymph-specific growth factors and their receptors are required to assess their role in triggering UTUC lymphangiogenesis.

CONFLICT OF INTEREST

Mario I. Fernández is an Investigator for Pfizer.

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Abbreviations: DSS, disease-specific survival; HR, hazard ratio; HNSCC, head and neck squamous cell carcinoma; IQR, interquartile range; LVD, lymphatic vessel density; IT, intratumoral; PT, peritumoral; NT, non-tumoral; LEC, lymphatic endothelial cell; LVI, lymphovascular invasion; RNU, radical nephroureterectomy; UC, urothelial carcinoma; UT, upper urinary tract; VEGF, vascular endothelial growth factor.