

Prognostic Value of Lymphovascular Invasion in Bladder Cancer in Patients Treated with Radical Cystectomy

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Abstract. *Background:* Bladder cancer is the fourth most frequently diagnosed cancer among males and the eleventh among females. At present, radical cystectomy is considered the standard therapy in patients with muscle invasive disease or in some cases of high-grade superficial cancer. TNM classification includes as independent predictors of disease-specific survival the grade, the pathological stage and the presence of lymph node involvement, whilst the prognostic value of lymphovascular invasion (LVI) still remains controversial. The aim of the study was to assess the prognostic role of LVI at final pathology. *Patients and Methods:* From June 1995 to January 2007, 340 consecutive patients underwent radical cystectomy. Surgical specimens were examined according to our pathological protocol. Patients with a non-transitional cell carcinoma or submitted to a salvage procedure or neoadjuvant/adjuvant chemo-radiotherapy were excluded. The follow-up consisted of abdominal ultrasound every 3 months, blood sampling every six months and both computerized tomography and urethroscopy yearly. *Results:* The median patient age was 69 years. Of the 265 patients, 218 were males and 47 females. LVI was present in 77 pathological specimens (29.1%). LVI was not significantly associated with age ($p=0.908$) or sex ($p=0.382$), but was significantly associated with high pathological grade ($p=0.028$) and stage ($p<0.001$), and the presence of node metastasis ($p<0.001$). At the multivariate analysis, pathological staging, presence of LVI and node metastasis were independent significant prognostic factors for disease-specific survival. *Conclusion:* In our series, LVI is an independent prognostic factor for disease specific survival in patients who underwent radical cystectomy for transitional cell carcinoma.

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Bladder cancer is the fourth most frequently diagnosed cancer among males and the eleventh among females, representing the 9% and the 2.7% of all cancer cases respectively. According to the Italian Network of Cancer Registries, the incidence is 70.7 new cases per 100,000 men and 16.3 per 100,000 women. It has been estimated that every year, in Italy, 15,987 new cases of urinary bladder cancer are diagnosed among males and 3,326 among females (1). At present, radical cystectomy with extended bilateral lymphadenectomy is the standard therapy in patients with muscle-invasive disease or in some cases of high-grade superficial cancer refractory to conservative treatment. The largest series published in literature, reported a 5-year recurrence-free survival and an overall survival of 68% and 60%, respectively (2). Traditionally, independent variables of disease specific survival are grading, pathological stage and presence of lymph node involvement (3-6). The prognostic value of lymphovascular invasion (LVI) has previously been investigated by others (7-14) and the aim of our study was to assess the independent prognostic role of LVI at final pathology after radical cystectomy for bladder cancer.

Patients and Methods

In our University Department, 340 consecutive patients underwent radical cystectomy with bilateral pelvic lymphadenectomy and consequently a urinary diversion for a primary muscle-invasive bladder tumor from June 1995 to January 2007. Radical cystectomy was performed with excision of the bladder, prostate and seminal vesicles in male patients, and bladder, uterus, ovaries and the proximal part of the vagina in female patients. Only in three of the group of the female patients was a monolateral ovariectomy performed. All patients received an extended bilateral pelvic lymphadenectomy from the Cloquet node to the bifurcation of the common iliac vessels. Lymphadenectomy included iliac, obturator and presacral lymph nodes. The presacral, iliac and obturator lymph node packets, divided into left and right, were sent separately for histological examination. Surgical specimens were examined according to the following protocol: the bladder was opened anteriorly through the urethra, once the lumen was opened the tumor was located and the corresponding serosa inked to assess the external surgical margin. Tumor size, configuration (papillary, flat,

ulcerated), consistency, depth of penetration in the bladder wall, location and relationship with the ureters were recorded and the tumor was submitted to histological examination performing up to four serial sections, including the possible relationships of the tumor with adjacent structures (prostate, seminal vesicles, vagina). The remainder of the mucosa was described and representative samples of the anterior, posterior, lateral walls and the dome were submitted to histological examination. In males, the prostate was processed as for radical prostatectomy, taking care to assess invasion of the prostate through the bladder neck.

The follow-up consisted of abdominal ultrasound every 3 months, blood sampling every six months in order to assess renal function, hemoglobin and serum electrolytes, and both computerized tomography and urethroscopy yearly. Urine cytology was also assessed in some cases. The follow-up data, obtained by medical examination and from information from the referring physicians, were included in a dedicated database. Seventy-five patients with a non-transitional cell carcinoma or submitted to a salvage procedure or neoadjuvant/adjuvant chemoradiotherapy were excluded. Patients without histological assessment of lymphovascular status were also excluded.

We evaluated 265 patients with muscle-invasive bladder cancer and high grade superficial transitional cell carcinoma (pT1, G3 and/or carcinoma *in situ*) refractory to conservative management. Pathological staging was performed according to the 2002 TNM while grading was assessed according to Mostofi's classification. The clinical outcome was cancer-specific survival.

The study was performed in line with the Helsinki declaration and national regulations. According to usual clinical care and under the approval of our Ethics Committee, all patients signed an informed consent for the storage of clinical data.

Pathological evaluation. Two uro-pathologists evaluated all surgical specimens. LVI is defined as the presence of neoplastic cells within an endothelium-lined space. According to the general trend, no attempt was made to differentiate between lymphatic and vascular invasion. In fact vessels are identifiable more accurately by immunohistochemical markers (such as CD34, CD31) which are rarely used in current clinical practice. Stained sections in hematoxylin and eosin were used to evaluate the presence of LVI that could be classified as intratumoral, peritumoral or extratumoral.

Statistical analysis. Cancer-specific survival was considered from the day of cystectomy to the day of bladder cancer-specific death. Survival probabilities (\pm SE) at 5 and 10 years were estimated using the Kaplan-Meier actuarial method. Mann-Whitney, Fisher exact tests and linear-by-linear association chi-squared tests were used to assess the correlation between different variables and LVI. The prognostic significance of the pathological variables for overall and cancer specific survival was assessed by using univariate and multivariate Cox proportional hazards regression analysis. A two-tailed *p*-value of less than 0.05 was considered statistically significant. The SPSS (Statistical Package for Social Science, Chicago, IL, USA) software was used for the statistical analysis.

Results

The median patient age was 69 years (ranging from 46 to 93 years). Of the 265 patients, 218 were males and 47 females with a 4.6:1 male-to-female ratio. The majority of patients had tumor of grade 3 (81.1%). LVI was present in 77

pathological specimens (29.1%). Demographic and histopathological features stratified according to LVI are listed in Table 1. LVI was not significantly associated with age ($p=0.908$), nor sex ($p=0.382$) but was significantly associated with higher pathological grade ($p=0.028$), stage ($p<0.001$) and presence of node metastasis ($p<0.001$). LVI was present in 20 out of the 140 patients (14.3%) with organ-confined disease and in 57 out of the 125 patients (45.6%) with extravesical disease ($p<0.001$). Moreover LVI was present in 38/61 patients with node metastasis (62.3%) and 39/204 patients without node metastasis (19.1%). The presence of node metastasis was significantly ($p<0.001$) higher in patients with extravesical disease than in patients with organ-confined disease (41.6% and 6.4% respectively).

The median follow-up was 108 months (range: 1-216 months). A total of 88 of 265 patients (33.2%) died from progression of their disease. In addition, 11 patients (4.2%) died from other causes (4 patients died of pulmonary embolism, 4 of acute coronary syndrome, 2 of acute renal failure and the last of oesophageal carcinoma). The probability of disease-specific survival at 5 and 10 years of the entire cohort was $60.6\pm 3.8\%$ and $46.7\pm 5.6\%$, respectively. The disease-specific survival was inversely related to the tumour grade (hazard ratio, HR: 1.72; 95% confidence interval, CI: 1.41-2.11, $p<0.001$), in fact the 5- and 10-year disease-specific survival rates were $82.5\pm 4.8\%$ and $57.7\pm 11.2\%$ in pT1, $64.8\pm 8.1\%$ and $43.8\pm 11.4\%$ in pT2, $44.9\pm 8.3\%$ and $44.9\pm 8.3\%$ in pT3, and $27.9\pm 0.10\%$ and $27.9\pm 0.10\%$ in pT4 patients. The disease-specific survival was inversely related to the histopathological grading (HR: 1.74; 95% CI: 1.12-2.70, $P=0.014$), the probability of disease-specific survival at 5 and 10 years was $85.6\pm 9.5\%$ and $76.0\pm 12.3\%$ in G1 patients, $72.5\pm 8.5\%$ and $47.5\pm 13.8\%$ in G2 patients, and $55.8\pm 4.6\%$ and $45.2\pm 6.1\%$ in G3 patients, respectively. Moreover, the disease-specific survival was also inversely related to the lymph node status (HR: 3.60; 95% CI: 2.35-5.49, $p<0.001$). When data were stratified according to the presence of node metastases, the 5- and 10-year disease-specific survival was $70.1\pm 4.2\%$ and $52.2\pm 7.3\%$ in patients without positive nodes, while it was $30.1\pm 6.9\%$ and $25.8\pm 7.2\%$ in patients with node metastasis, respectively. When the entire cohort was stratified according to the presence of LVI the 5- and 10-year disease-specific survival was $36.1\pm 7.8\%$ and $28.9\pm 9.0\%$ in patients with and $69.5\pm 4.2\%$ and $53.2\pm 6.9\%$ in those without LVI (HR: 2.85; 95% CI: 1.85-4.40, $p<0.001$). The prognostic significance of LVI disease-specific survival was also evaluated by stratification of the patients into three groups: group 1 (N1 patients), group 2 (N0 LVI 0) and group 3 (N0 LVI 1) (Figure 1). Group 1 patients with node metastasis had a significantly increased risk of cancer-specific death (HR: 3.96; 95% CI: 2.53-6.21, $p<0.001$) compared to patients without LVI and node metastasis (N0, LVI0). Group 3 (N0 LVI 1) patients had a significant

Table 1. Demographic and histopathological features of the entire cohort, as well as after stratification according to LVI (a Mann-Whitney test, b Fisher exact test, c linear-by-linear association chi-squared).

Characteristic (n=265)	Total (n=77)	LVI+ (n=188)	LVI- (n=188)	p-Value
Median age (years)	69	70	69	0.908
Range	46-93	47-93	46-89	
Gender				0.382 ^b
Male	218	66 (85.7%)	152(80.9%)	
Female	47	11 (14.3%)	36 (19.1%)	
Grading				0.028 ^c
G1	15	1(1.3%)	14 (7.4%)	
G2	35	8 (10.4%)	27 (14.4%)	
G3	215	68 (88.3%)	147 (78.2%)	
Pathological stage				<0.001 ^c
T stage				
pT1/pTis	85	5 (6.5%)	80 (42.6%)	
pT2	55	15 (19.5%)	40 (21.3%)	
pT3	82	30 (36.6%)	52 (27.7%)	
pT4	43	27 (35.1%)	16 (8.5%)	
pN+	61	38 (49.4%)	23 (12.2%)	<0.001 ^b
M+	0	0	0	-

increased risk of cancer-specific death compared with group 2 (HR: 1.69; 95% CI: 0.86-3.32, $p=0.04$). At the multivariate backward stepwise Cox regression analysis, the following variables: age, patho-logical staging, presence of LVI and node metastasis, were significant independent prognostic factors for disease-specific survival; pathological grading did not reach statistical significance as an independent prognostic factor ($p=0.098$).

Discussion

In this study, we present a cohort of patients treated with radical cystectomy and extended pelvic lymphadenectomy for transitional cell carcinoma of the urinary bladder without neoadjuvant/ adjuvant therapy. We found that 53% of patients had organ-confined disease, in which 9 patients (6%) had node metastasis and 20 patients (14%) LVI compared to those with extravesical disease, 42% of whom had positive lymph nodes and 46% LVI. In this series, we report a 5- and 10-year disease-specific survival of 61% and 47% respectively. These data are similar to those of the largest reported series in literature. In a cohort of 1,054 patients Stein *et al.* reported a disease-specific survival rate at five and ten years of 68% and 60% (15). They also reported a 23% rate of node metastasis. Moreover as we reported in this paper, Stein *et al.* demonstrated that LVI was a significant and independent prognostic factor for cancer-specific survival, as well as pathological staging and presence of node metastasis. Since immunohistochemical markers available for differentiation between lymphatic and vascular invasion in pathological specimens are rarely used in current clinical practice,

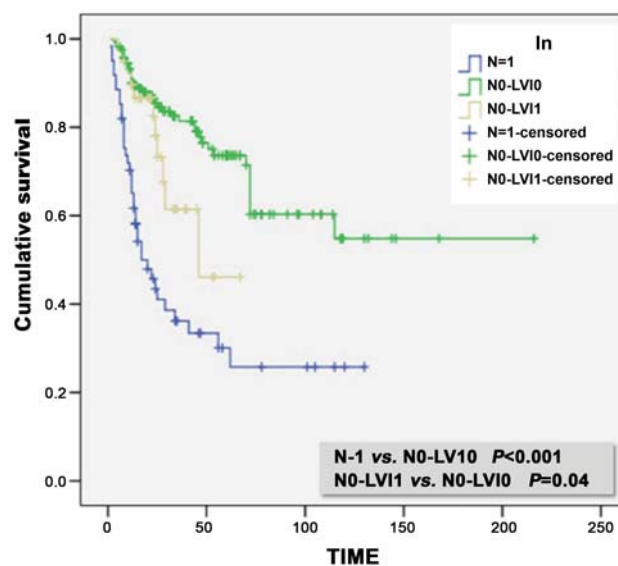


Figure 1. Evaluation of the prognostic significance of lymphovascular invasion disease-specific survival by stratification of the patients into three groups: group 1 (N1 patients), group 2 (N0 LVI 0) and group 3 (N0 LVI 1).

pathologists did not make any attempt to characterize the two different type of invasion. Moreover, as reported elsewhere, a pathologist trained in urological malignancies can easily describe LVI with appropriate sections stained in hematoxylin and eosin.

At present the prognostic value of LVI remains controversial. Herrmann *et al.* reported that pathological tumour stage ($p<0.0001$), lymph node status ($p=0.004$) and

LVI ($p=0.001$) were independent prognostic factors associated with unfavourable overall survival in a cohort of 833 patients (4). Canter *et al.* analysed data from 356 patients treated with radical cystectomy at the University of Pennsylvania and at univariate analysis found that the presence of LVI conferred a risk for decreased overall, cancer-specific and recurrence-free survival ($p<0.0001$) (12). They found that only pT3 patients, when stratified by presence of LVI, showed statistical differences in clinical outcomes ($p=0.005$, $p<0.001$ and $p<0.001$ for overall, disease-specific and recurrence-free survival, respectively). At multivariate analysis, these authors also reported that LVI was an independent predictor of poor overall and disease-specific survival ($p<0.01$ and $p=0.007$, respectively) but not for recurrence-free survival ($p=0.1$). Lotan *et al.* reported data from a multi-institutional retrospective study of 958 patients who had undergone radical cystectomy with pelvic lymphadenectomy between 1984 and 2003. Overall they found LVI in 36% of patients, with 26% node-negative and 72% node-positive patients. At multivariate Cox regression analysis (considering age, stage, grade and number of nodes removed), LVI was an independent predictor of local ($p=0.0003$), distant ($p=0.0011$) and overall ($p=0.0003$) recurrence in node-negative patients. In their series, LVI was not a predictor of survival and recurrence in node-positive patients (11). Bassi *et al.* found that only tumor stage and nodal involvement were independent prognostic variables on multivariate analysis (3).

Conclusion

In our retrospective study, LVI was found to be an independent prognostic factor for disease-specific survival in patients submitted to radical cystectomy with extended lymphadenectomy.

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