Clinicopathological Significance of Lymphovascular Invasion in Urothelial Carcinoma

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Lymphovascular invasion is an important prognostic marker in the assessment of bladder cancer, including both cystectomy and transurethral resection of the bladder specimens, and should routinely be reported upon in the pathological report. Strict criteria must be utilized in establishing a diagnosis of lymphovascular invasion in urothelial carcinoma to distinguish it from peritumoral stromal retraction, a common finding that often mimics a vascular space. The use of immunohistochemistry (CD31, CD34, D2-40) for the diagnosis of intra-vascular invasion in urothelial carcinoma should be used only in selected histologically equivocal cases for confirmation. Routine use of immunohistochemistry for endothelium as a screening test in all cases cannot be recommended. (Anal Quant Cytopathol Histopathol 2012; 34:173–179)

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Tumor stage represents the gold standard for prediction of tumor recurrence and progression after radical cystectomy and transurethral resection in patients with urothelial carcinoma (UC). Thus, accurate staging provides broadly applicable prognostic information and is the basis of patient management decisions. In addition to tumor stage, other pathological factors have been evaluated as possible risk factors for recurrence and survival, including lymphovascular invasion (LVI). The aim of this paper was to review the clinicopathological features of LVI in UC.

Clinical Significance of LVI
LVI in Cystectomy Specimens
LVI can be observed in about 30–50% of cystectomy specimens. Prevalence of LVI correlates significantly with increasing T classification, high tumor grade, and lymph node metastasis. However, in a considerable number of patients with histologically proven nodal disease, LVI is not detected within the bladder wall. In
the comprehensive study by Lotan et al \[7\] LVI was observed in 9.0% of T1, 23.0% of T2, 50% of T3, and 78% of T4 tumors. Likewise, the authors detected LVI in 151 of 581 (26%) node-negative and 122 of 169 (72%) node-positive cancers. In a large study based on 4,257 patients from 12 centers, Shariat et al \[12\] rendered similar data: LVI was present in 11.0% of T1, 31.3% of T2, 52.3% of T3, and 60.9% of T4 tumors, and its presence was significantly associated with high tumor grade (5.1% G1, 38.2% G2, 33.5% G3) and presence of lymph node metastasis (22.5% N0, 64.7% N1-2). We are not aware of large prospective studies investigating whether LVI in perivesical vascular/lymphatic is “more important” than LVI in the lamina propria, or in the detrusor muscle. However, the presence of isolated LVI in the perivesical fat tissue should be considered equivalent to pT3a and the patients treated accordingly, including adjuvant chemotherapy.

LVI has been associated with poor outcome in patients undergoing radical cystectomy for invasive UC. This effect is observed primarily in patients with node-negative disease.\[3,7-9,12,14,18\] In particular, LVI proved to be a predictor of local, distant, and overall tumor recurrence as well as a predictor of adverse overall and disease-specific survival, independent of tumor stage and grade.\[6,7,10-12,14,19,20\] In some studies, however, discordant results were seen. In a study by Canter et al \[3\] LVI independently predicted overall and disease-specific survival, whereas no independent influence on recurrence-free survival was seen. In other studies, some of them involving rather low numbers of patients, LVI was identified as an adverse prognostic variable only in univariate analysis, yet lost prognostic significance in multivariate analysis.\[4,5,6,21-23\] Finally, in two studies, both involving fewer than 100 patients, the presence of LVI was not found to be significantly related to outcome.\[24,25\]

Adding LVI together with other clinical (neo-adjuvant chemotherapy, adjuvant chemotherapy, adjuvant radiotherapy) and morphological (presence of carcinoma in situ) parameters to multivariate nomograms assessing risk for cancer recurrence or survival significantly improved prediction compared with the TNM (tumor, node, metastasis) staging system.\[6,11\] Adding LVI alone to a base model including tumor stage and grade, surgical margin status, number of lymph nodes removed, and adjuvant chemotherapy significantly improved predictive accuracy for disease recurrence and cancer-specific survival in node-negative cases, yet only marginally in node-positive cases.\[12\]

**LVI in Transurethral Resection of the Bladder Specimens**

The significance of LVI has additionally been addressed in patients undergoing transurethral tumor resection or partial cystectomy. Zhang et al \[26\] presented 100 patients with muscle-invasive UC who underwent partial cystectomy. LVI was present in 16% of cases and was independently associated with cancer-specific survival but not with recurrence-free survival. In all, six studies have so far evaluated the significance of LVI in transurethral resection of the bladder (TURB) material.\[16,18,27-30\] The reported prevalence of LVI is 6–28%,\[16,27-30\] i.e., lower than in radical cystectomy specimens. According to a recent investigation involving matched TURB and radical cystectomy material from 75 patients, concordance between LVI diagnoses of TURB and cystectomy material is good in muscle-invasive cancers, while it is only low in superficial tumors.\[16\] Overall sensitivity and specificity of LVI detection in TURB samples were 37% and 87%, and positive predictive values were 65% and 67%, respectively. Similar to the situation in cystectomy specimens, presence of LVI in TURB material proved to be an independent predictor of tumor recurrence\[28\] and progression\[27,28\] as well as patients’ overall\[29\] and disease-specific survival,\[27\] particularly in clinical stage I or II.\[18\]

**Stage pT0 Carcinoma**

Stage pT0 tumor is a condition in which there is no evidence of residual carcinoma in the cystectomy specimen after an initial cancer diagnosis in the biopsy or transurethral resection specimens. The incidence of stage pT0 bladder carcinoma is approximately 10%. Unlike stage pT0 cancer of the prostate (so-called “vanishing cancer phenomenon”), the clinical outcome among patients with stage pT0 bladder cancer is variable. In the largest series of pT0 carcinomas (120 patients), the 5-year recurrence-free, cancer-specific, and overall survivals were 84%, 88%, and 84%, respectively. Multivariate analysis showed that LVI and carcinoma in situ in the TUR specimens were independent predictors of adverse clinical outcome. The 5-year overall survival for patients with LVI was 70%, compared to 89% for those patients without LVI. The incidence of lymph node metastasis among pT0 patients was 3–7%.
**Venous Invasion Versus Lymphatic Invasion**

Whether or not lymphatic invasion and venous invasion should be considered separately has not been fully addressed. In fact, most studies have not attempted to differentiate between venous and lymphatic invasion and distinguish between different endothelial cell types. Lotan et al. refer to difficulty and lack of reproducibility when using routine light microscopic examination of hematoxylin and eosin–stained slides.

Some studies have, however, differentiated between lymphatic and venous invasion and defined venous invasion as tumor present in vessels with a thick vascular wall and blood cells within the lumen. Using this morphological definition, the prevalence of venous invasion was generally lower than that of lymphatic invasion. With respect to prediction of outcome, venous invasion proved to be superior to lymphatic invasion in two of these studies, whereas a stronger prognostic effect for lymphatic invasion was noted in one study.

**Morphological Identification of LVI**

Despite unanimity regarding the relationship between presence of LVI and UC aggressiveness, there are major issues of concern that have hindered its integration in treatment guidelines for UC and clinical decision making. In fact, it appears that the major factor is poor diagnostic reproducibility: reported prevalence and levels of prognostic significance vary considerably, differing from one study to another. The lack of reproducibility when using routine light microscopic examination of hematoxylin and eosin–stained slides is well-known. These differences have been attributed mainly to lack of standardized assessment of LVI, as demonstrated by divergent, often poorly described histological criteria used by pathologists to identify LVI. If histological criteria are mentioned, most studies refer to presence of tumor cells within endothelium-lined spaces without underlying muscular wall (Figure 1). In fact, identification of a clear-cut endothelial lining is considered crucial in differentiating LVI from stromal retraction, a frequent finding around tumor cell nests (Figure 2), particularly at the leading edge of invasion. Stromal retraction is commonly overdiagnosed as vascular invasion; however, at least in the breast cancer literature, there is strong evidence that stromal retraction is not a processing artifact, has independent prognostic significance in node-negative disease on its own merit, and may possibly be early phase of vascular invasion. Finally, varying levels of experience of pathologists evaluating LVI, and knowledge of the potential problems and mimics, may influence accuracy of diagnosis, illustrating the need for strict morphological criteria to diminish the problem of interobserver variability. Another related problem that is not commonly discussed is that the general approach to assessing LVI in diagnostic surgical pathology does not recognize the varied biologic mechanisms involved in tumor vascularization, some of which are not readily visible at the light microscopic level.

**Algaba Criteria for LVI**

In 2006 Algaba suggested the following morphological criteria for the diagnosis of LVI on hematoxylin and eosin–stained slides: morphological features in favor of LVI are tumor cell thrombi with...
in isolated spaces with an unequivocal endothelial lining, preferably located next to arterioles and with a normal surrounding stromal tissue. The tumor thrombus is usually floating, completely free, within the vessel lumen and may show some fibrin precipitate and/or blood cells around it. The tumor cells are usually tightly cohesive and display a smooth border, the cells in the periphery of the thrombus having a shell-like morphology. In contrast, morphological features arguing against LVI are tumor cell clusters, particularly with invasion of multiple spaces, surrounded by an ectatic capillary network inherent to an abnormal stroma. Moreover, in pseudoinvasion due to tissue retraction, the pseudothrombus usually displays a surface with a blurred outline and shreds of cytoplasm may be present between the pseudothrombus and the supposed vessel wall.

**Role of Immunohistochemistry**

The role of immunohistochemistry in the differentiation of LVI from retraction artifacts remains to be defined. Almost all published reports on UC rely solely on hematoxylin and eosin–stained slides, and immunohistochemistry seems to be reserved for equivocal cases. However, data obtained from other types of cancer clearly demonstrate that additional immunohistochemical staining may help to identify LVI and increase the accuracy of identification with LVI by proving true vascular invasion and/or avoiding false-positive reporting due to overinterpretation of stromal retraction artifacts. Thus, immunostaining using monoclonal antibodies directed against endothelial markers (Figure 3), such as CD31, CD34 and podoplanin (D2-40), the latter specific for lymphatic endothelial cells, has been shown to increase the detection rate of LVI compared to conventional hematoxylin and eosin staining and to render clinically significant information in breast, colorectal, endometrial, gastric and oral squamous cell carcinoma.

Two studies indicated the potential value of these ancillary techniques: angioinvasion diagnosed on hematoxylin and eosin–stained slides was confirmed by immunohistochemistry in only 5 of 36 or 2 of 5 cases, respectively. Both studies are fairly old and did use antibodies directed against Ulex europaeus agglutinin I, von Willebrand factor, and QBEND/10, which have generally been replaced with more specific markers in current practice. Therefore, it cannot be excluded that some technical aspects inherent to the immunohistochemical staining evaluation may have caused the high rate of putative false-positive reporting of LVI on hematoxylin and eosin–stained slides. In 2009 Afonso et al published the first systematic study investigating the potential value of CD31 and D2-40 immunostaining in UC including 83 patients with radical cystectomy. Using hematoxylin and eosin–stained slides, blood and lymphatic vessel invasion were diagnosed in 19 (23%) and 18 (22%) cases, respectively. Immunohistochemistry has significantly improved the recognition of vascular invasion for both lymphatic and blood vessels, regarding particularly for identification of intravascular single tumor cells (40% for CD31, 37% for D2-40). Overall agreement between the different methods used to identify LVI was 42.2%. Univariate analysis rendered vascular invasion diagnosed on hematoxylin and eosin–stained slides as well as blood and lymphatic vessel invasion diagnosed by immunohistochemistry as significant prognostic variables regarding for overall survival. On multivariate analysis, blood vessel invasion detected by CD31 immunostaining proved to be the only independent prognostic factor. Finally, another recent study demonstrated a significant association between lymphatic invasion, assessed by D2-40 immunohistochemistry, and presence of lymph node metastasis.

**Conclusion**

The incidence of vascular invasion is variable and has been reported to be as high as 50%. The presence of lymphovascular invasion predicts poor out-
come, and this finding should be included in the pathology report. The 5-year cancer-specific survival was 87% and 65%, respectively, for those without and with lymphovascular invasion. Identification of lymphovascular invasion can be difficult due to frequent artifactual clefting around nests of invasive carcinoma. Retraction artifact is prominent and almost uniformly present in the micropapillary variant of urothelial carcinoma. In suspicious cases, blood vessels can be highlighted by immunohistochemical staining for CD31 or CD34. The presence of vascular or lymphatic invasion, and whether immunohistochemical stains assisted in identifying that finding, should be included in the report. Immunohistochemical studies directed against endothelial cells have found that fewer than 40% of cases with purported vascular invasion on routine hematoxylin and eosin examination are confirmed immunohistochemically.

References

27. Andius P, Johansson SL, Holmang S: Prognostic factors in stage T1 bladder cancer: Tumor pattern (solid or papillary) and vascular invasion more important than depth of invasion. Urology 2007;70:758–762
37. Marinho VO, Metze K, Sanches FS, Rocha GF, Gobbi H: Lymph vascular invasion in invasive mammary carcinomas identified by the endothelial lymphatic marker D2-40 is associated with other indicators of poor prognosis. BMC Cancer 2008;8:64
44. Larsen MP, Steinberg GD, Brendler CB, Epstein JI: Use of Ulex europaeus agglutinin I (UEA1) to distinguish vascular and “pseudovascular” invasion in transitional cell carcinoma of bladder with lamina propria invasion. Mod Pathol 1990;3:83–88