Invasive Lobular Carcinoma of the Breast: Role of Endothelial Lymphatic Marker D2-40

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Abstract. Lymphovascular invasion (LVI) of breast cancer is an independent adverse prognosticator that is associated with increased regional and distant tumor recurrence. LVI is infrequently encountered in invasive lobular carcinoma when compared to invasive ductal carcinoma. We employed D2-40 antibody, a novel marker for lymphatic endothelial cells, in an attempt to enhance the detection of LVI in invasive lobular carcinomas. We identified 78 patients with invasive lobular carcinoma with known axillary status, who were studied between 2003 and 2006. D2-40 antibody was applied to one representative paraffin block from each case and the results were compared to LVI on routine histology. LVI was identified in 12 (15%) and 19 (24%) cases by routine histology and D2-40 antibody, respectively. Eleven of 12 patients (92%) with LVI identified by routine histology had axillary nodal metastasis compared to 14 of 19 patients (74%) with LVI identified by D2-40 antibody. LVI was missed by routine histology in 8 cases (10%). D2-40 antibody enhanced the identification of LVI by 9% in node negative patients. D2-40 antibody increased the identification of LVI by 12% in classic invasive lobular carcinoma. In conclusion, D2-40 antibody staining may be useful as an adjunct in detecting LVI in invasive lobular carcinoma, especially in node-negative patients with the classic variant of invasive lobular carcinoma.

Keywords: breast cancer, lymphovascular invasion, D2-40 antibody, prognosis

Introduction

Invasive lobular carcinoma (ILC) comprises 10 to 15% of all invasive mammary carcinomas. ILC is distinct from invasive ductal carcinoma (IDC). Morphologically, classic ILC is composed of small, round cells with intracytoplasmic vacuoles and bland nuclei growing in single files, in trabeculae, or as solid sheets and alveolar nests without forming appreciable tubules. ILC tends to be more frequently low-grade, hormone-receptor-positive, multifocal and bilateral, and larger in size than IDC [1]. ILC shows a unique pattern of metastasis with a predilection for spread to the peritoneal and lepto-

meningeal surfaces, gastrointestinal tract and ovaries [1,2]. Patients with ILC experience less frequent recurrences early in the course of disease although their overall survival does not differ significantly from patients with IDC [1-6].

Lymphovascular invasion (LVI) is an unfavorable parameter determined by pathological assessment of breast cancer. LVI predicts an increased likelihood of axillary nodal metastasis [7]. LVI also enhances the prediction of non-sentinel node metastasis in breast cancer patients with positive sentinel lymph nodes [8,9]. In addition to its association with axillary nodal metastasis, LVI is an independent prognosticator. The presence of LVI in breast carcinoma is associated with a higher risk of locoregional relapse, distant relapse, and overall decreased survival [10-19]. Therefore, patients with small mammary tumors with LVI...
without axillary nodal involvement may potentially benefit from adjuvant chemotherapy. These findings underline the significance of LVI identification in breast carcinomas. Of note, LVI is less frequently detected in ILC than IDC. The identification rate of LVI in ILC remains low despite axillary nodal metastasis [10,19].

D2-40 antibody is a novel monoclonal antibody against an oncofetal antigen, the M2A antigen, which is a 40-kD sialoglycoprotein, present on the surface of testicular germ cell tumors, but not in the adult testis [20-23]. This antibody was originally considered to be a useful marker for seminoma and dysgerminoma [24]. Recently, D2-40 monoclonal antibody has been shown to be a sensitive marker for lymphatic channel endothelial cells. D2-40 antibody has been employed for detection of LVI in various neoplasms including breast cancer [25]. In this study, we applied D2-40 antibody to a cohort of ILC cases in an attempt to enhance the detection of LVI and we compared the results to routine histologic assessment (hematoxylin-eosin stain).

Materials and Methods

This study was approved by the New York University School of Medicine Institutional Review Board. The pathology data system at New York University Medical Center, Tisch Hospital, was searched for cases of invasive lobular carcinoma that occurred between 2003 and 2006. The slides of the identified cases were reviewed for histopathologic confirmation, subtyped (classic versus pleomorphic ILC), and assessed microscopically for the presence or absence of LVI. An average of 11.7 slides was reviewed per case (range 2-34). LVI was defined on routine histology as the presence of tumor cells within peritumoral endothelial-lined spaces, without distinguishing between lymphatic and blood vessels, in accordance with guidelines of Page and Anderson [26]. On immuno-histochemistry, LVI was defined as tumor cells within D2-40 antibody-positive channels identified either intra- or peri-tumorally. Peritumoral tissue was defined as the tissue immediately beyond the tumor front.

Criteria of inclusion were the diagnosis of ILC, complete absence of E-cadherin membranous immunoreactivity, block availability, and known axillary nodal status either by sentinel lymph node biopsy or axillary dissection. For practical purposes, one representative block with the largest tumor and peritumoral area was selected from each case and used for immunohistochemical analysis with E-cadherin and D2-40 antibodies. Slides stained by D2-40 antibody were reviewed without knowledge of LVI status, as determined by routine histology or axillary nodal status. Immunohistochemical staining using CD31 antibody was used when there were discordant results between routine histology and D2-40 antibody. Immunohistochemistry was performed on formalin-fixed, paraffin-embedded tissues using the following antibodies: mouse anti-human CD31 (PECAM-1) clone 1A10 (Ventana Medical Systems, Tucson, AZ), mouse anti-human lymphatic endothelium, clone D2-40 (Signet Labs, Berkeley, CA), and mouse anti-human epithelial cadherin (E-cadherin), clone 4A2C7 (Invitrogen, Carlsbad, CA). In brief, sections were deparaffinized in xylene, rehydrated through graded alcohols, and rinsed in distilled water. Heat-induced antigen retrieval was performed in 10mM citrate buffer pH 6.0 in a 1200-Watt microwave oven at 90% power for 10 min for CD31 and D2-40 antibodies and 20 min for E-cadherin

| Table 1. Clinicopathologic data of 78 invasive lobular carcinomas of the breast. |
|-----------------------|-----------------------|-----------------------|
| **Age (yr)**          | **Mean (range)**      | 61.6 (41-89)          |
| **Laterality**        | **Left**              | 42 (54%)              |
|                       | **Right**             | 36 (46%)              |
| **Multifocality**     | **31 (40%)**          |
| **Bilaterality**      | **7 (9%)**            |
| **Subtype**           | **CILC**              | 69 (88%)              |
|                       | **PILC**              | 9 (12%)               |
| **Tumor size (cm)**   | **Mean (range)**      | 2.5 (0.12-7.0)        |
| **Tumor stage**       | **T1a**               | 6 (8%)                |
|                       | **T1b**               | 7 (9%)                |
|                       | **T1c**               | 28 (36%)              |
|                       | **T2**                | 26 (33%)              |
|                       | **T3**                | 11 (14%)              |
| **Nodal stage**       | **N0**                | 36 (46%)              |
|                       | **N0i+**              | 8 (10%)               |
|                       | **N1mi**              | 6 (8%)                |
|                       | **N1**                | 13 (17%)              |
|                       | **N2**                | 6 (8%)                |
|                       | **N3**                | 9 (11%)               |
| **Sentinel lymph node biopsy** | **Positive** | 22 (39%)              |
|                       | **Negative**          | 34 (61%)              |
|                       | **Total**             | 56                    |
| **Biomarker status**  | **Estrogen receptor** | 72 (92%)              |
|                       | **Progesterone receptor** | 43 (55%)        |
|                       | **Her2**              | 8 (10%)               |
| **Type of surgery**   | **Segmental excision**| 46 (59%)              |
|                       | **Total mastectomy**  | 9 (11%)               |
|                       | **Modified radical mastectomy** | 23 (30%) |

CILC: classic invasive lobular carcinoma
PILC: pleomorphic invasive lobular carcinoma
antibody. After heating, sections were allowed to cool for 30 min and then rinsed in distilled water.

Antibody incubations and detection were carried out at 37°C on a NEXes instrument (Ventana Medical Systems) using Ventana's reagent buffer and detection kits unless otherwise noted. Endogenous peroxidase activity was blocked with hydrogen peroxide. CD31 antibody was applied as supplied and E-cadherin and D2-40 antibodies were diluted 1:50. Antibodies were incubated for 30 min at 37 °C. Antibodies were detected with Ventana’s biotinylated goat anti-mouse/anti-rabbit cocktail, followed by streptavidin-horseradish-peroxidase conjugate. The complex was visualized with 3,3-diaminobenzidene and enhanced with copper sulfate. The slides were washed in distilled water, counterstained with hematoxylin, dehydrated, and mounted with permanent media. Appropriate positive and negative controls were included with the sections.

Statistical analyses were performed using Fisher’s exact test. A p-value of <0.05 was considered significant. Tumor staging was based on the American Joint Committee on Cancer, sixth edition [27].

Results

Pertinent clinicopathologic data are summarized in Table 1. Briefly, 78 patients with a mean age of 61.6 yr (range 41-89 yr) were included in the study. Of the 78 tumors, 42 were left-sided (54%) and 36 were right-sided (46%); 31 (40%) were multifocal tumors and 7 patients had metachronous bilateral invasive mammary carcinomas (9%). The tumor size ranged from 0.12 cm to 7.0 cm (mean 2.5; median 1.9 cm).

LVI was identified by routine histology and D2-40 immunostain in 12 and 19 cases (15% and 24%), respectively (Table 2). Eleven of 12 patients (92%) with LVI identified by routine histology had axillary nodal metastasis and 14 of 19 patients (74%) with LVI identified by D2-40 antibody had axillary nodal metastasis. Overall, 34 patients [micrometastasis (N1mi) in 6, N1 in 13, N2 in 6 and N3 in 9] harbored axillary nodal metastases, of whom LVI was detected in 11 by routine histology and in 14 by D2-40 antibody (32% and 42%, respectively; p = 0.114). LVI was less frequently observed in T1 tumors compared to T2 or T3 tumors (7.3% vs 45%, respectively, p = 0.0001).

Forty-four patients had negative axillary nodes, 8 of whom showed isolated tumor cells. All micrometastatic carcinomas (n = 6) were detected by both routine histology and immunohistochemistry for cytokeratin. All isolated tumor cells were only detected by immunohistochemistry for cytokeratin. All positive axillary lymph nodes with tumor deposits >2.0 mm were detected by routine histology only.

Staining with D2-40 antibody detected LVI in all cases in which LVI had been identified by routine histology except for one case. Staining with CD31 antibody, a panendothelial marker, was negative in the latter case suggesting a tissue retraction artifact. In 8 cases, LVI was detected by D2-40 antibody but not by routine histology. This group of 8 cases included: T1bN0i+ (1 case), T1cN0 (1 case), T2N0 (1 case), T2N0i+ (1 case), T2N1 (2 cases), T2N3 (1 case), and T3N2 (1 case). In these 8 cases, LVI was peritumoral in 3, intratumoral in 1, and both peri- and intratumoral in 4 cases.

Invasive lobular carcinomas were subclassified into classic ILC (CILC, n = 69) and pleomorphic
ILC (PILC, n = 9). Mean tumor size in CILC and PILC was 2.4 cm and 3.2 cm, respectively. Axillary nodal metastasis was identified in 28 CILCs (40%) and 6 PILCs (66%) (p = 0.13). LVI was detected by routine histology in 6 of 69 CILCs (7%) and in 6 of 9 PILCs (66%) (p = 0.0002). LVI was detected by D2-40 antibody in 13 of 69 CILCs (19%) and in 6 of 9 PILCs (66%) (p = 0.005). The presence of LVI was significantly higher in PILC compared to CILC by both routine histology and D2-40 antibody staining (p = 0.007). However, there was no statistically significant difference between routine histology and D2-40 antibody in detecting LVI in either subtype although there was a trend for significance for D2-40 antibody in CILC (7% vs 19%, respectively; p = 0.068) (Table 2).

Estrogen receptor positivity was seen in 67 (97%) and 5 (55%) of CILCs and PILCs, respectively (p = 0.001). Her2 positivity by immunohistochemistry was noted in 4 (6%) and 4 (44%) of CILCs and PILCs, respectively (p = 0.005). Her2 assay by fluorescence in situ hybridization (FISH) was performed in 4 of these 8 cases and only 1 PILC tumor showed gene amplification.

Discussion

Axillary nodal involvement by metastatic carcinoma is an unfavorable prognostic determinant in breast cancer. As sentinel lymph node biopsy is emerging to replace axillary dissection in certain patients, primary tumor characteristics have been explored to sharpen the accuracy of predicting non-sentinel nodal involvement. Among these characteristics, tumor size and LVI are the most significant adjuncts to the size of the sentinel lymph node metastasis in predicting non-sentinel axillary nodal status [9,16]. Additionally, LVI is an independent prognostic determinant of unfavorable prognosis in breast cancer [10-19]. LVI is adversely associated with higher local relapse and higher distant recurrence [10,18]. The prognostic significance of LVI probably lies in its ability to predict an adverse outcome in low-stage, node-negative patients. Thereby, LVI can be used as a guide to recommend adjuvant therapy in this group of patients [15,17,19].

The incidence of LVI in ILC is significantly lower than in IDC. LVI ranges from 7-13% in ILC compared with 26-30% in IDC in the same series [10,19,25,28]. Arnaout-Askarian et al [25] showed that D2-40 antibody increased LVI detection by 16% in node positive patients. We identified LVI in 12 of 78 cases (15%) of ILC by routine histology and in 19 of 78 cases (24%) by D2-40 antibody staining (p = 0.114). LVI was missed by routine histology in approximately 10% of our cases (8 of 78) although the difference did not reach statistical significance. Four of these 8 cases were low-stage, node-negative tumors (T1b in 1, T1c in 1, and T2 in 2). In one additional case, LVI was suspected by routine histology, but not seen by D2-40 or CD31 antibody staining, suggesting an over-diagnosis of vascular invasion owing to tissue retraction artifact.

Lymphovascular invasion has a strong association with axillary nodal metastasis in breast cancer. Chua et al [16] demonstrated that 61% of the (24%) breast tumors with LVI in their study had axillary lymph node metastasis in contrast to 27% of tumors without LVI (p <0.0001). However, there was no significant difference in axillary nodal metastasis between IDC and ILC (41% vs 51%, respectively; p = 0.20) [16]. Similarly, Weiser et al [9] found non-sentinel lymph node metastasis in 41% and 26% of breast cancers with and without LVI, respectively (p = 0.02). Again, IDCs and ILCs were associated with non-sentinel lymph node metastasis with no statistically significant difference (31% vs 40%, respectively; p = 0.6) [9]. These findings are incongruent with the incidence of LVI in IDC and ILC (see above). In our series, 34 of 78 cases (43%) had axillary nodal metastasis. LVI was detected in 11 and 14 of these 34 node-positive patients by routine histology and D2-40 antibody staining, respectively (p = 0.308). Although both methods reliably predicted axillary nodal metastasis, routine histology was a stronger predictor with a positive predictive value of 91% vs 73% for D2-40 antibody. From a different perspective, D2-40 antibody identified LVI in 5 node-negative patients, 2 of whom had T1 tumors, and enhanced the identification of LVI by 9% in this group of patients compared to routine histology (p = 0.114). Arnaout-Askarian et al [25] reported a 20% increase in the detection of LVI by D2-40 antibody in node negative patients. Using multivariate analysis, these
authors demonstrated that D2-40 antibody-detected LVI was the only significant predictor of distant recurrence in node-negative patients in both IDC and ILC [25]. In our series, the difference between D2-40 antibody and routine histology in detection of LVI in node-negative patients did not reach statistical significance, which may reflect the small sample size and the overall low incidence of LVI in lobular carcinoma. Larger prospective studies are needed to assess the actual significance of D2-40 antibody in predicting behavior in ILC.

Pleomorphic invasive lobular carcinoma (PILC) is a subtype of ILC with a more aggressive behavior [30-32]. Axillary nodal metastasis is more commonly seen in PILC compared to CILC [30]. Likewise, we noted axillary nodal metastasis more frequently in PILC than CILC (66% vs 40%, respectively; p = 0.13). LVI was more frequently encountered in PILC than CILC both by routine histology (66% and 7%, respectively; p = 0.0002) and D2-40 antibody (66% and 19%, respectively; p = 0.005). D2-40 antibody increased the detection of LVI by 12% compared with routine histology in CILC, with a trend for statistical significance (p = 0.068). Interestingly, D2-40 antibody staining did not enhance the detection of LVI in PILC, indicating that routine histology is equally reliable in the identification of LVI in PILC (Table 2). The difference can also be ascribed to the more readily identifiable LVI in PILC than CILC on routine histology.

LVI is most reliably detected in peritumoral tissue by routine histology, partly because tissue retraction artifact precludes optimal assessment of LVI intratumorally [33,34]. In fact, peritumoral LVI has been added to the St Gallen criteria for adjuvant treatment of breast cancer [35]. Theoretically, D2-40 antibody should enhance the detection of intratumoral LVI compared to routine histology although the significance of this observation is unknown. By using immunohistochemistry, Van den Eynden et al [36] reported that LVI was missed by the H&E staining method in 22.4% of cases peritumorally and in 71% of tumors intratumorally. LVI was missed in 8 cases on routine histology in our study, 7 of which (87%) were peritumoral (3 peritumoral and 4 both peri- and intratumoral) and only one of which was intratumoral alone. This discrepancy may be partly attributed to the different cohorts in the 2 studies, as ILCs constitute the entire study group in our series with overall lower incidence of identifiable LVI both peri- and intratumorally.

In conclusion, immunohistochemistry with D2-40 antibody may be of use as an adjunct in histologic evaluation of CILCs especially in small, node-negative tumors. Further studies with follow-up are needed to demonstrate if breast cancer patients with LVI detected only by D2-40 antibody have a worse prognosis than those without it.

References


