

Epithelial Displacement Into the Lymphovascular Space Can Be Seen in Breast Core Needle Biopsy Specimens

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Upon completion of this activity you will be able to:

- discuss the diagnostic difficulties caused by mechanical epithelial displacement in breast core needle biopsies.
- describe the histopathologic features and immunohistochemical methods that help discriminate between true lymphovascular invasion and mechanical epithelial displacement.
- discuss the reasons why epithelial displacement may be present in initial breast core needle biopsy specimens.

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Abstract

Breast core needle biopsy (BCNB) has been reported to cause mechanical epithelial displacement into adjacent tissues, leading to diagnostic difficulties. However, epithelial displacement into lymphovascular spaces (EDLS), mimicking true lymphovascular invasion, may also be seen in the initial BCNB specimen itself. We retrospectively reviewed 218 BCNB specimens diagnosed as ductal carcinoma in situ (DCIS) and searched for the presence of EDLS. The subsequent surgically resected specimens for all cases were also reviewed. EDLS was demonstrated in 7 (3.2%) of 218 initial BCNB specimens. It could be differentiated from detached clusters of DCIS cells within preexisting ducts by morphologic features and immunohistochemical stains for D2-40 and p63. EDLS can occur at initial BCNB, and, therefore, the presence of tumor cell clusters within lymphovascular spaces in a BCNB specimen with DCIS may not represent true lymphovascular invasion.

The breast core needle biopsy (BCNB) is frequently performed on nonpalpable, mammographically “suspicious” abnormalities to differentiate cases requiring surgical management from those that can be managed with clinical and mammographic follow-up.¹⁻⁵ However, BCNB has its limitations. For example, it can result in the underestimation of a malignant lesion owing to the small sample obtained by this procedure, the target lesion can be missed during sampling,¹ and it can also cause mechanical epithelial displacement into the BCNB tract, leading to difficulties in the interpretation of the subsequent surgically excised specimen.⁶⁻¹⁰ Epithelial displacement is mostly seen in stromal tissues in association with post-BCNB change, including hemorrhage, fibrosis, and hemosiderin-laden macrophages, but may also be located within lymphovascular spaces, leading to misinterpretation as true lymphovascular invasion. The discrimination of epithelial displacement into lymphovascular spaces (EDLS) from true lymphovascular invasion can be even more problematic when the displaced epithelia happen to be malignant cells.¹⁰ EDLS has been well described in surgically excised specimens after BCNB,^{8,9} but the presence of EDLS in initial BCNB tissues has not been reported.

The aims of this study were to explore the presence of EDLS in BCNB specimens and to discuss the clinicopathologic implications of this phenomenon.

Materials and Methods

Samples from 218 patients diagnosed with ductal carcinoma in situ (DCIS) on initial BCNB at Severance Hospital (Yonsei University Health System, Seoul, South Korea) from

January 1996 to December 2008 were included in this study. All patients underwent subsequent surgical excision at the same institution, and, therefore, surgically excised specimens were available for histopathologic evaluation in all cases. This study was approved by the Yonsei University Severance Hospital Institutional Review Board.

Cases demonstrating invasive components in the biopsied tissue and those with papillary lesions for which definitive diagnoses could not be made on biopsy were excluded from the study. Samples from patients who had undergone needling procedures such as fine-needle aspiration before BCNB were also excluded from the study. BCNBs were performed by breast imaging specialists under ultrasonographic guidance with a 14-gauge Tru-Cut needle (SACN Biopsy Needle, Medical Device Technologies, Gainesville, FL) under local anesthesia. According to our standard protocol, 4 or 5 core samples were obtained from each lesion, and the tissue cores were submitted for histopathologic examination and immunohistochemical studies for hormone receptor status (estrogen receptor [ER] and progesterone receptor [PR]) and HER-2 overexpression status.

All cases included in the study were retrospectively reviewed independently by experienced breast pathologists (J.S.K. and W.-H.J.), and the following data were recorded: architectural subtype, nuclear grade, presence of comedo-type necrosis, presence of EDLS, hormonal receptor expression (ER and PR), and HER-2 overexpression status. A cutoff value of 1% or more positively stained nuclei was used to define ER and PR positivity. HER-2 staining was scored according to the American Society of Clinical Oncology/College of American Pathologists guideline using the following categories: 0, no immunostaining; 1+, weak, incomplete membranous staining in any proportion of tumor cells; 2+, complete membranous staining, nonuniform or weak in at least 10% of tumor cells; and 3+, uniform, intense membranous staining in more than 30% of tumor cells.¹¹

We also retrospectively reviewed the subsequent surgically excised specimens and recorded the histologic diagnosis, the presence of EDLS, and any post-BCNB changes, including hemorrhage, hemosiderin-laden macrophages, stromal fibrosis, and granulation tissue formation.

Immunohistochemical Staining

Immunohistochemical stains were performed for D2-40 (dilution 1:20; DAKO, Glostrup, Denmark) and p63 (4A4, dilution 1:50; DAKO) in the BCNB cases showing detached epithelial cells within otherwise empty spaces. Briefly, 4- μ m-thick sections were deparaffinized in xylene, rehydrated in graded alcohol, and quenched in 3% hydrogen peroxide. Antigen retrieval was performed in 0.1 mol/L citrate buffer (pH 6.0), and incubation with primary antibodies was performed overnight at 4°C. After rinsing, a secondary

antibody (EnVision Rabbit/Mouse kit, DAKO) was applied and then developed with 3,3'-diaminobenzidine. Slides were then counterstained with hematoxylin. Dark brown cytoplasmic and dark brown nuclear staining were counted as positive for D2-40 and p63, respectively.

Statistical Analysis

Data were statistically processed using SPSS for Windows, version 12.0 (SPSS, Chicago, IL). The Student *t* test and Fisher exact test were used for continuous and categorical variables, respectively. Statistical significance was assumed when the *P* value was less than .05.

Results

Clinicopathologic Features

Table 1 shows the clinicopathologic characteristics of our 218 cases. Of 218 cases, 11 (5.0%) showed tumor cell clusters within lymphovascular spaces in the BCNB specimen. Four of these cases were subsequently diagnosed as invasive ductal carcinoma in surgical excision, and because the possibility of true lymphovascular invasion could not be completely excluded, only the remaining 7 pure DCIS cases (3.2%) were regarded as EDLS in our study. Of the 7 cases with EDLS in the BCNB specimen, the predominant histologic subtype of DCIS was the solid type (5/7 [71%]). The cribriform type was most frequently observed in the 211 cases without EDLS (81/211 [38.4%]; *P* = .104). There was a tendency for a lower nuclear grade in EDLS+ cases compared with EDLS- cases (*P* = .062). ER and PR expression were more frequently seen in EDLS+ cases (*P* = .211 and *P* = .134, respectively). HER-2 overexpression (3+) was demonstrated in 1 (14%) of the 7 EDLS+ cases, and in 80 (37.9%) of the 211 EDLS- cases (*P* = .438).

Review of the subsequent surgical excision specimens showed that 53 cases (24.3%) were diagnosed as invasive ductal carcinomas, and the remaining 165 as DCIS (75.7%). In addition, lymph node metastasis was found in 10 cases that belonged to the EDLS- group. All 10 cases with lymph node metastases were cases finally diagnosed as invasive ductal carcinoma.

Pathologic Features of Epithelial Displacement in BCNB Specimens

There were 18 cases with groups of neoplastic epithelial cells inside clear spaces on histologic examination; however, immunohistochemical staining for D2-40 and p63 revealed that these spaces were actually lined by myoepithelial cells and not lymphatic endothelia in 7 cases, and, therefore, 11 of the 18 cases were regarded as tumor cell clusters within true

Table 1
Comparison of Clinicopathologic Characteristics of BCNB Cases With and Without EDLS*

| | Total (n = 218) | EDLS in BCNB Specimen | | P |
|---|------------------|-----------------------|------------------|------|
| | | Yes (n = 7) | No (n = 211) | |
| Mean \pm SD age (y) | 48.48 \pm 9.79 | 55.00 \pm 9.53 | 48.26 \pm 9.75 | .073 |
| Dominant architecture | | | | .104 |
| Cribriform | 82 (37.6) | 1 (14) | 81 (38.4) | |
| Solid | 65 (29.8) | 5 (71) | 60 (28.4) | |
| Comedo | 52 (23.9) | 1 (14) | 51 (24.2) | |
| Micropapillary | 19 (8.7) | 0 (0) | 19 (9.0) | |
| Nuclear grade | | | | .062 |
| Low | 47 (21.6) | 4 (57) | 43 (20.4) | |
| Intermediate | 90 (41.3) | 2 (29) | 88 (41.7) | |
| High | 81 (37.2) | 1 (14) | 80 (37.9) | |
| Comedo necrosis | 130 (59.6) | 3 (43) | 127 (60.2) | .358 |
| Estrogen receptor | | | | .211 |
| Positive | 138 (63.3) | 6 (86) | 132 (62.6) | |
| Negative | 80 (36.7) | 1 (14) | 79 (37.4) | |
| Progesterone receptor | | | | .134 |
| Positive | 127 (58.3) | 6 (86) | 121 (57.3) | |
| Negative | 91 (41.7) | 1 (14) | 90 (42.7) | |
| HER-2 | | | | .438 |
| Positive (3+) | 81 (37.2) | 1 (14) | 80 (37.9) | |
| Equivocal (2+) | 26 (11.9) | 1 (14) | 25 (11.8) | |
| Negative (1+, 0) | 111 (50.9) | 5 (71) | 106 (50.2) | |
| Final diagnosis after surgical excision | | | | |
| IDC | 53 (24.3) | 0 (0) | 53 (25.1) | |
| DCIS | 165 (75.7) | 7 (100) | 158 (74.9) | |
| Lymph node metastasis | 10 (4.6) | 0 (0) | 10 (4.7) | |

BCNB, breast core needle biopsy; DCIS, ductal carcinoma in situ; EDLS, epithelial displacement into lymphovascular spaces; IDC, invasive ductal carcinoma.

* Data are given as number (percentage) unless otherwise indicated.

lymphovascular spaces **Image 1** and **Image 2**. In addition, as described, 7 of these 11 cases were subsequently diagnosed as pure DCIS after excision and, therefore, were regarded as EDLS in this study.

The clinicopathologic features of the 7 cases showing EDLS in BCNB specimens are shown in **Table 2**. The number of EDLS foci ranged from 1 to 3. The clear spaces containing EDLS were located in the interlobular or periductal stroma and were associated with other vascular structures (Images 1B and 1C). The morphologic appearance of EDLS varied from dyscohesive and scattered epithelial cells to epithelial cell clusters forming secondary lumina. The majority of epithelial cell clusters did not conform to the contour of the lymphovascular spaces, and, in some cases, tumor cell necrosis was seen within the EDLS. Lymphovascular spaces were lined by flattened D2-40+ and p63– endothelial cells (Images 1D, 1E, and 1F). However, some cells floating within the lymphovascular spaces demonstrated both p63 and D2-40 positivity and, therefore, were likely to be detached myoepithelial cells (Image 1F). In 4 cases, tumor cells in the EDLS demonstrated expression of ER and PR, as in the corresponding DCIS.

The 7 cases demonstrating lymphovascular-like spaces containing tumor cells were most likely detached clusters of DCIS cells within preexisting ducts (Images 2A, 2B, and

2C). The tumor cell clusters within the spaces were larger than the clusters seen in EDLS+ cases, and the cells lining the spaces were more tightly packed together and demonstrated a hobnail-like pattern, with the nuclei protruding into the lumen. The lining cells were positive for both D2-40 and p63, suggesting that they were, in fact, myoepithelial cells and not lymphatic endothelia.

Pathologic Features of Subsequent Surgically Excised Specimens

Post-BCNB changes were recognized in all surgically resected specimens along the previous BCNB tracts. The histologic features included stromal fibrosis, granulation tissue formation, hemosiderin-laden macrophage accumulation, fat necrosis, and acute and chronic inflammation. Of 7 EDLS+ cases on BCNB, 2 (29%) also demonstrated EDLS in the BCNB tract of subsequent surgically excised specimens.

Discussion

Although the biologic significance of epithelial displacement is unknown, it not infrequently poses significant diagnostic problems in surgical pathology practice and also significant management implications. For example, its presence

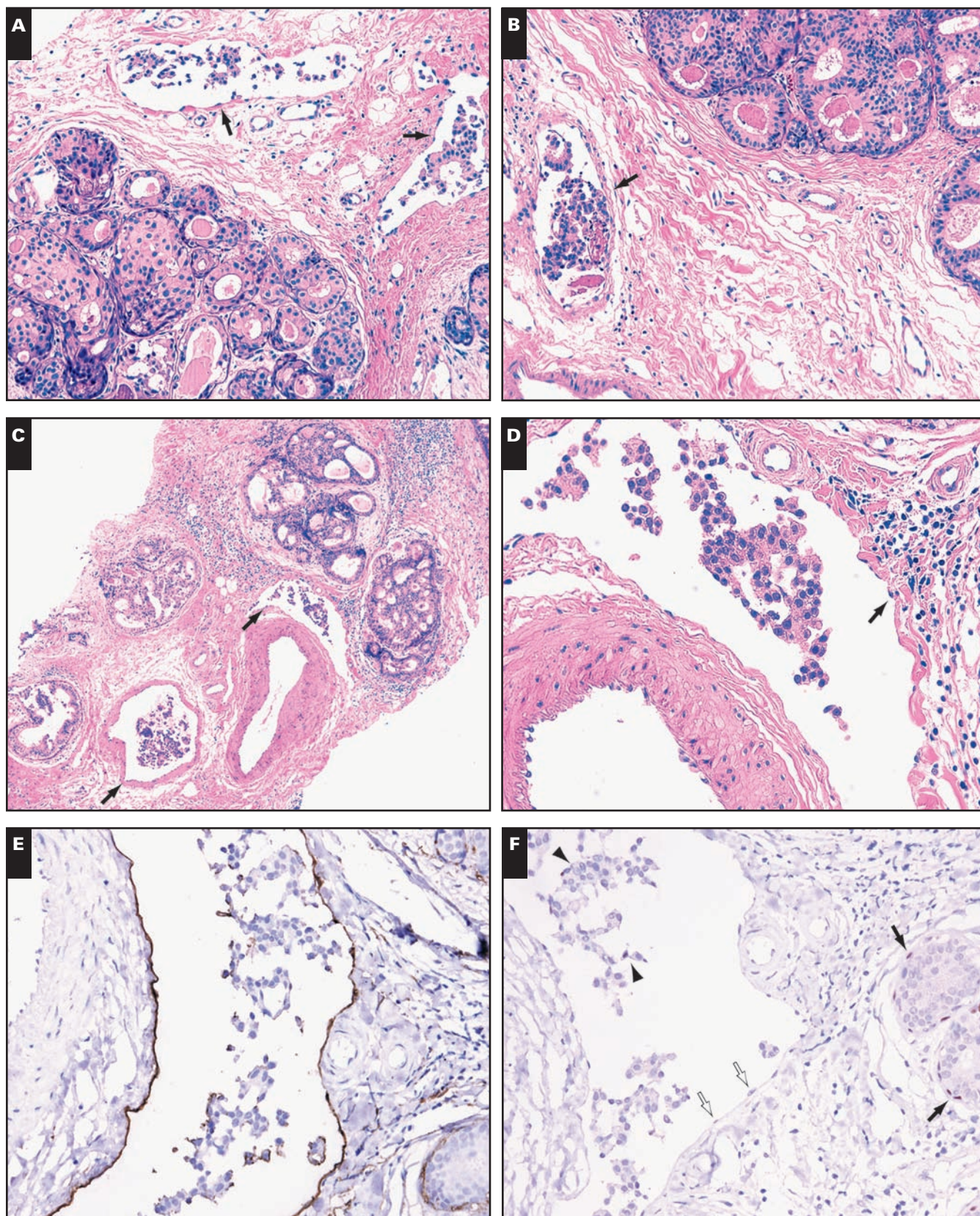


Image 1 Histologic features of epithelial displacement in lymphovascular spaces. Foci of epithelial displacement (arrows) are noted next to the ductal carcinoma in situ (**A** and **B**, H&E, $\times 100$; **C**, H&E, $\times 40$). Higher power magnification of **C** demonstrates that the space containing the tumor cell clusters is lined by flattened endothelial cells (arrow) (**D**, H&E, $\times 200$). The lymphovascular space is stained by D2-40 (**E**, $\times 200$), but not by p63 (**F**, $\times 200$, white arrows). p63 positivity is seen in a few cells within the lymphovascular space (arrowheads) and in the myoepithelial cells in surrounding nonneoplastic ducts (black arrows).

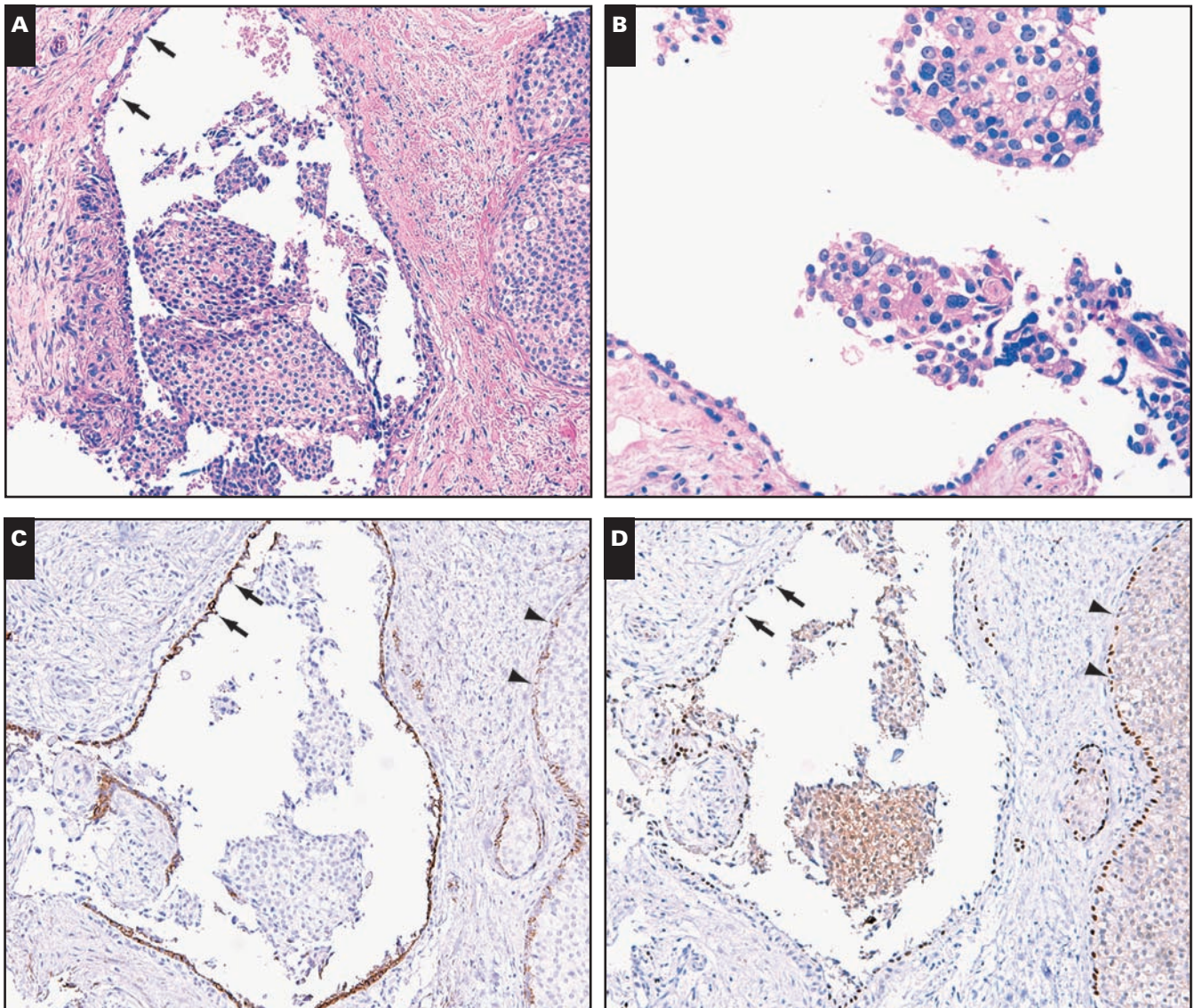


Image 2 Detached clusters of ductal carcinoma in situ (DCIS) from preexisting ducts. A space containing clusters of neoplastic cells is lined by D2-40+ (**C**, $\times 200$) and p63+ (**D**, $\times 200$) cells with hobnail patterns (arrows), similar to the staining patterns of the myoepithelial layer of the adjacent DCIS lesion (arrowheads) (**A** and **B**, H&E, $\times 200$).

Table 2
Clinicopathologic Features of Cases Showing EDLS in Breast Core Needle Biopsy Specimens

| Case No./Age (y) | No. of EDLS Foci | Architecture | Nuclear Grade | Comedo Necrosis | Final Diagnosis* | ER | PR | HER-2 |
|------------------|------------------|--------------|---------------|-----------------|------------------|----|----|-------|
| 1/60 | 1 | Solid | Low | – | DCIS | + | + | 3+ |
| 2/45 | 1 | Solid | Low | – | DCIS | + | + | – |
| 3/51 | 1 | Comedo | High | + | DCIS | – | – | 1+ |
| 4/63 | 1 | Solid | Intermediate | + | DCIS | + | + | – |
| 5/70 | 1 | Solid | Low | – | DCIS | + | + | – |
| 6/51 | 3 | Solid | Intermediate | + | DCIS | + | + | 2+ |
| 7/45 | 2 | Cribriform | Low | – | DCIS | + | + | – |

EDLS, epithelial displacement into lymphovascular spaces; ER, estrogen receptor; PR, progesterone receptor; +, positive or present; –, negative or absent; 1+, equivocal; 3+, positive.

* Diagnosis of the subsequent surgically excised specimen.

can result in the misinterpretation of tumor size, presence of stromal invasion, presence of lymphovascular invasion, and also surgical margin involvement status.^{7,8} In such cases, the recognition of displaced epithelial cells is assisted by awareness of a previous BCNB procedure and appreciation of the typical post-BCNB changes including hemorrhage, granulation tissue, fat necrosis, and hemosiderin-laden macrophages.

A few previous studies on post-BCNB lesions demonstrated that EDLS is recognized in subsequent surgically excised specimens,⁷ but the presence of EDLS in initial BCNB tissues has not been hitherto reported. In this study, we examined initial BCNB specimens for the presence of EDLS. EDLS was recognized in 7 (3.2%) of 218 BCNB tissues with DCIS, which is a lower frequency than that reported for subsequent surgical excision specimens after needling procedures (16%-28%),^{10,12} but still surprisingly high. The recognition of EDLS in initial BCNB specimens can be more problematic compared with surgically excised specimens because post-BCNB changes such as granulation tissue formation, stromal fibrosis, fat necrosis, inflammation, and hemosiderin-laden macrophage infiltration that serve as important clues in surgically resected specimens are unfortunately not present.

A few explanations could be suggested for the presence of EDLS in initial BCNB tissues. First, and most important, we should consider the possibility of EDLS actually being true lymphovascular invasion by invasive cancer cells. The possibility of an occult microinvasive focus within the tumor could be raised. However, extensive sampling is always performed for resected cases of DCIS in our surgical practice to exclude the possibility of occult microinvasive foci. Therefore, the probability that occult microinvasive components invaded the lymphatic spaces and were also sampled incidentally during BCNB would be extremely small. In fact, our clinical follow-up data (mean duration, 53 months; range, 24-84 months) demonstrate that the 7 patients with DCIS with EDLS in BCNB specimens did not have subsequent recurrences or metastases.

The second possible reason for EDLS may be the type of core biopsy procedure. The tissue cores in this study were obtained by automated core biopsy, which has been thought to be more frequently associated with epithelial displacement compared with directional vacuum-assisted biopsy. Whereas the vacuum-assisted biopsy acquires the tissue sample by firing the needle once adjacent to the lesion and by suction of the target tissue into the probe, the needle in the automated core biopsy procedure is pushed through the target, and, hence, the lesion is more likely to be displaced along the needle tract during the procedure.¹³ Another possible explanation for epithelial displacement at initial BCNB could be the local anesthesia administered before the core biopsy procedure. Youngson et al⁹ demonstrated displaced carcinomatous epithelium outside the main tumor mass in 12 of 43 cases following needling

procedures and emphasized that the findings may have been influenced by additional needling procedures other than the BCNB itself, including local anesthetic administration with a 25-gauge needle. The BCNB procedures performed in our cases were indeed preceded by local anesthetic infiltration. However, as the needle tip in local anesthesia is usually inserted into the superficial layers such as the dermis or subcutaneous tissue and not into the mammary parenchyma, it is less likely that the local anesthetic infiltration would have caused the epithelial displacement.

The EDLS seen in our BCNB tissues also had to be differentiated from clusters of DCIS cells detached from the surrounding stroma within the preexisting ducts. Detached clusters of DCIS cells within preexisting ducts are larger than EDLS, and the cells lining the clear spaces are more tightly packed together and show a hobnail-like pattern, with the nuclei protruding into the lumen, as seen in 7 of our cases. In addition, simultaneous immunohistochemical stains for D2-40 and p63 can be helpful. Although D2-40 is widely used as a lymphatic endothelial marker, it has also been reported to be expressed in myoepithelial cells.¹⁴ Therefore, use of myoepithelial markers such as p63 in conjunction with D2-40 would be necessary for differentiating lymphovascular spaces and preexisting ducts.

In general, lymphovascular invasion in breast cancer is recognized by the following: (1) Tumor cells are located within endothelial-lined spaces. (2) The tumor cell clusters do not conform to the contour of the lymphovascular spaces. (3) These tumor cell clusters are seen where lymphovascular spaces are normally located, such as periductal and interlobular stroma, and in close association with other vascular structures.¹⁵ These findings were mostly true for our cases of EDLS in BCNB specimens, and, therefore, the distinction between EDLS and true lymphovascular invasion was difficult based solely on the histopathologic features. Immunohistochemical studies for D2-40 and p63 could be helpful in the differential diagnosis in most cases, as the detached cells within the EDLS may partly be composed of myoepithelial cells; however, it should also be borne in mind that p63 may rarely be expressed in epithelial cells,^{16,17} and, therefore, the presence of occasional p63+ cells within the clear spaces should be interpreted with caution.

Previous studies on EDLS in post-BCNB surgically excised specimens consistently demonstrated that this phenomenon was most frequently seen in the whole spectrum of papillary lesions, including intraductal papillomas and intraductal papillary carcinomas.^{6,7} The friable nature of these lesions has been the most plausible explanation for their frequent association with EDLS: the delicate papillary lesions could be easily amputated during needling procedures and subsequently migrate into adjacent tissues or lymphatic spaces. In a study of 53 cases of epithelial displacement by Nagi

et al,⁷ only 3 cases showed no evidence of underlying papillary lesions; however, 2 of these cases were associated with a cystic or mucinous component that could have served as a transport medium for the tumor cells into adjacent tissues. In this study, we excluded all cases containing papillary lesions in the initial BCNB tissues because many of these cases were equivocal, and a definitive diagnosis could not be reached on BCNB findings alone, and we found that EDLS was most frequent in solid-type DCIS (71%) and also seen in cribriform and comedo-type DCIS. None of the micropapillary DCIS cases demonstrated EDLS in our biopsied tissues, which was an unexpected finding; however, this may be explained by the relatively small number of such cases (n = 19). Nevertheless, these findings suggest that EDLS may occur in DCIS without papillary components.

EDLS can occur at initial BCNB, and, therefore, the presence of tumor cell clusters within lymphovascular spaces in a BCNB specimen with DCIS may not represent true lymphovascular invasion.

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