

Application of Immunohistochemistry to Breast Lesions

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• **Context.**—Immunohistochemistry has an expanding role in mammary pathology that has been facilitated by a growing list of available antibodies and a better understanding of biology.

Objective.—To explore the key role of immunohistochemistry in guiding adjuvant therapy decisions and sentinel node staging in breast cancer, as well as the role of immunohistochemistry as an aid to distinguishing usual ductal hyperplasia from atypical ductal hyperplasia/low-grade carcinoma in situ; subtyping a carcinoma as ductal

or lobular, basal or luminal; ruling out microinvasion in extensive intraductal carcinoma; distinguishing invasive carcinoma from mimics; and establishing that a metastatic carcinoma of unknown primary site has originated in the breast.

Data Sources.—Current literature is reviewed, including clinical and pathologic journals.

Conclusions.—As new, targeted treatments for breast cancer are developed, pathologists can expect additional immunohistochemistry applications in the future.

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Immunohistochemistry (IHC) has an expanding role in the diagnosis and management of mammary disease. A growing list of available antibodies, improved antigen retrieval techniques, and a better understanding of biology have all contributed to the broader utility of IHC for solving everyday diagnostic problems in breast pathology. These include distinguishing usual ductal hyperplasia (UDH) from atypical ductal hyperplasia (ADH)/low-grade carcinoma in situ, subtyping a lesion as ductal versus lobular or basal versus luminal, helping to distinguish true microinvasion from mimics (“pseudoinvasion”), predicting the likelihood of response to antihormonal and other therapeutic agents, improving sentinel node staging, and finally, helping to recognize metastatic carcinoma of unknown primary site as originating in the breast.

UDH VERSUS ADH/DUCTAL CARCINOMA IN SITU

Usual ductal hyperplasia can generally be distinguished from ADH and low-grade ductal carcinoma in situ (LG-DCIS) on hematoxylin-eosin-stained sections by the classical morphologic findings of cellular heterogeneity and an architectural pattern of irregularly interspersed, slitlike spaces that contrast with the more rigid, “punched out” spaces of ADH and LG-DCIS. Usual ductal hyperplasia comprises an admixture of cell types, whereas ADH/LG-DCIS has a monomorphic, “clonal” appearance. Immunohistochemical staining can be used to highlight these distinguishing features.

Normal breast glands and ducts are composed of 3 cell

types that express different subsets of proteins: luminal, basal, and myoepithelial.^{1,2} The luminal and basal cell types express different cytokeratins (CKs); myoepithelial cells (MECs) express basal cell-type CKs and other more specific markers, such as smooth muscle actin, calponin, and p63 (Table 1). ADH/LG-DCIS, consistent with a clonal derivation, expresses markers of only 1 cell type, usually luminal, although a small percentage show basal cell differentiation, a phenotype more typical of high-grade DCIS.^{3,4}

High-molecular-weight CKs (CK34βE12, CK903) can help to distinguish UDH from ADH/LG-DCIS because they mark the myoepithelial and basal cells that mingle with luminal cells in this polymorphic nonneoplastic proliferation. Essentially all examples of UDH have some cells that express high-molecular-weight keratins, whereas only 10% of ADH/LG-DCIS do (Figure 1, A through D). Similarly, the basal cell-type keratins, CK5/6, will mark the basal cells in UDH, whereas ADH/LG-DCIS is negative in most cases, because it preferentially expresses luminal cell type CKs.^{3,5,6} Most meaningful in this diagnostic context is the restricted expression of a single CK class, either luminal or basal, which supports a diagnosis of ADH/LG-DCIS rather than UDH.⁷ This is consistent with an admixture of cell types in UDH compared with the monomorphic cells in ADH/LG-DCIS.

DUCTAL VERSUS LOBULAR CARCINOMA

Although both ductal and lobular carcinomas arise in the terminal duct-lobular unit, characterizing a neoplastic process as one or the other has important clinical implications. This is most important for carcinoma in situ (CIS) because current treatment differs for the 2 major phenotypes. Because DCIS is thought to be a nonobligate, but direct, precursor of invasive carcinoma, its eradication by surgery and/or radiation is the therapeutic goal. Evidence is accruing to support that lobular carcinoma in situ (LCIS) is also a nonobligate, direct precursor to invasive carcinoma but is not yet convincing.^{8–10} Because its signifi-

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Table 1. Immunoreactivity of Normal Breast Epithelia*

Luminal cells	CK7, CK8, CK18, CK19
Basal cells	CK5/6, CK14, CK17
Myoepithelial cells	CK5, CK14, CK17, SMA, calponin, p63

* CK indicates cytokeratin; SMA, smooth muscle actin.

ificance is still ambiguous, LCIS in a core biopsy does not necessarily trigger an excisional biopsy of the site. Close follow-up and chemoprevention with tamoxifen is recommended for some patients.

In contrast to CIS, invasive ductal and lobular carcinomas are treated in the same manner, based on stage and predictive factor profile (see later). Because the clinical presentation and pattern of disease spread may differ between lobular and ductal carcinoma, knowing the histologic subtype can help clinicians detect and better manage disease. For example, if a core biopsy shows invasive lobular carcinoma, which is often more extensive than imaging studies or palpation suggests, a surgeon can plan a wider excision to achieve complete surgical removal.

Most ductal and lobular carcinomas, both in situ and invasive, are readily distinguished on hematoxylin-eosin-stained sections, particularly if there is an adequate sample. However, in cases with intermediate or ambiguous morphologic characteristics, IHC may aid categorization. Examples include low-grade solid in situ proliferations, particularly when identified in a core biopsy, or invasive high-grade carcinomas in which pleomorphic lobular carcinoma is a consideration.

E-cadherin, a cell-cohesion protein encoded by a gene on chromosome 16q22.1, is the current marker of choice to help discriminate between lobular and ductal carcinoma. The majority of usual ductal carcinomas express cytoplasmic E-cadherin, whereas most in situ and invasive lobular carcinomas, both classic and pleomorphic types, lack expression (Figure 2, A through D).¹¹⁻¹⁴

Differences in CK expression may also serve to distinguish ductal from lobular lesions. High-molecular-weight CKs, specifically clone 34 β E12, also known as CK903, (CK1, CK5, CK10, CK14), are commonly expressed by lobular carcinomas but are absent or expressed at low levels in most examples of DCIS (Table 2).^{15,16} Because studies

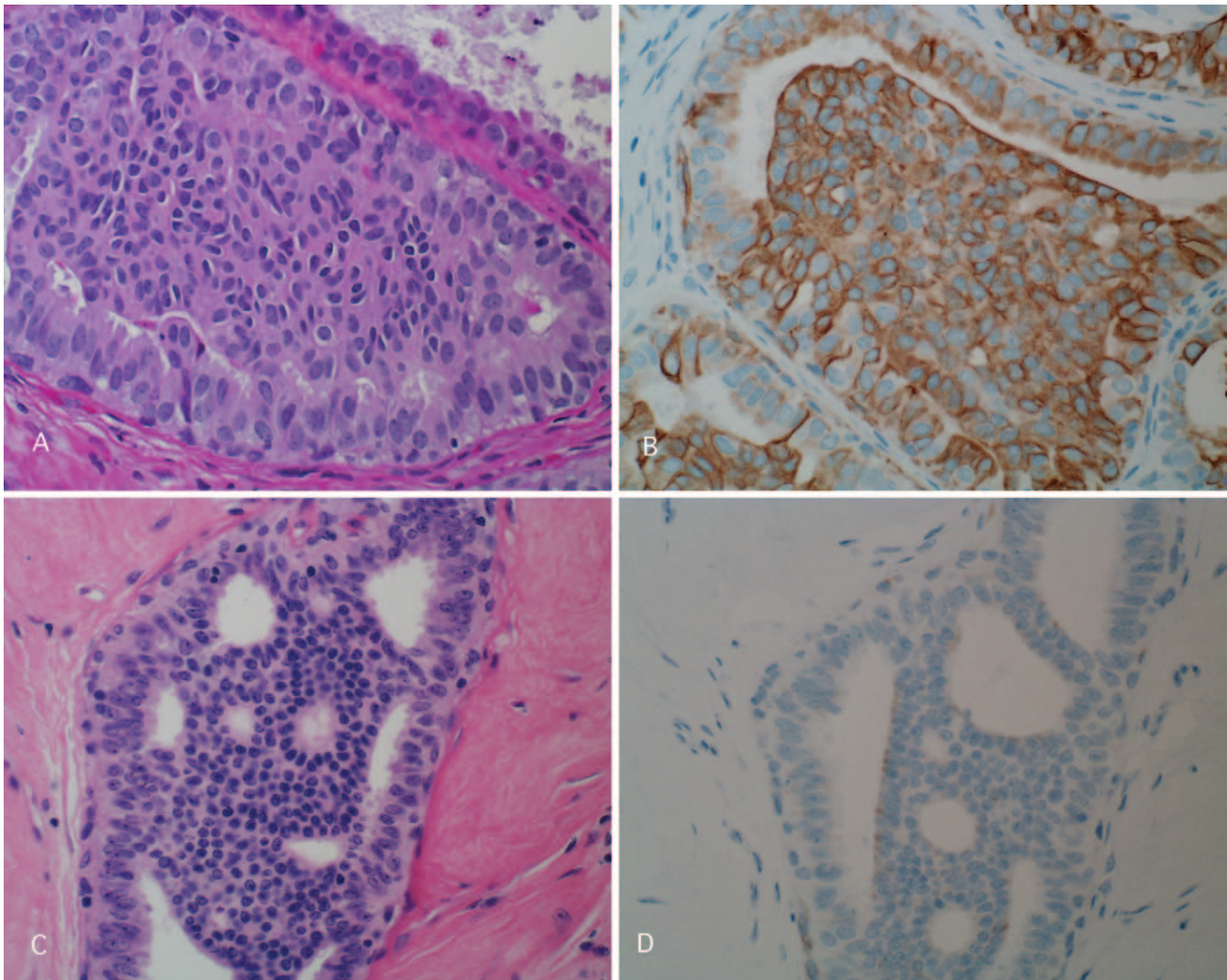


Figure 1. Comparison of usual ductal hyperplasia (UDH) with atypical ductal hyperplasia (ADH) with immunostains. A, UDH (hematoxylin-eosin, original magnification $\times 200$). B, UDH, keratin 903, note strong cytoplasmic staining of cells (original magnification $\times 200$). C, ADH (hematoxylin-eosin, original magnification $\times 200$). D, ADH, keratin 903, note minimal to no staining of cells (original magnification $\times 200$).

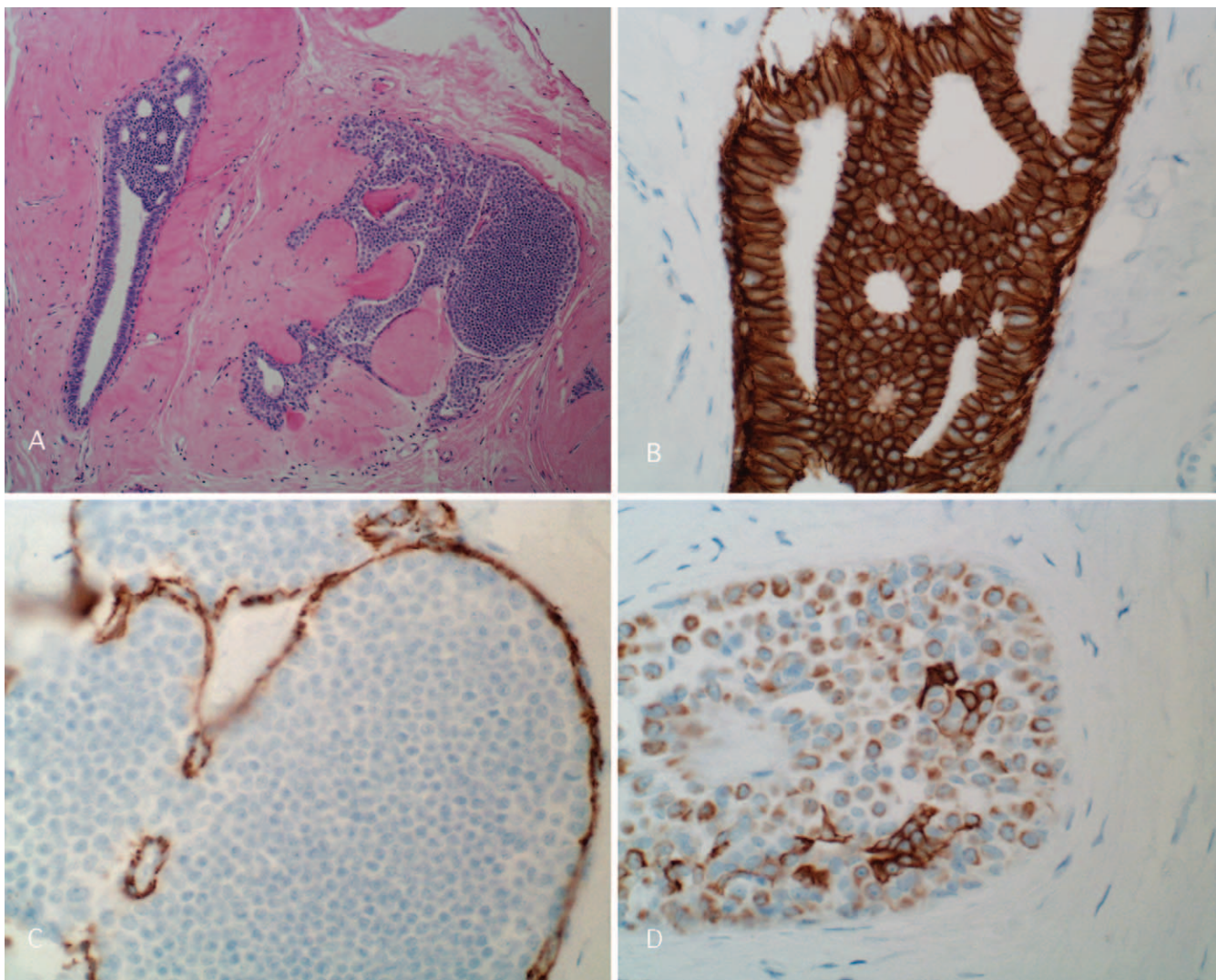


Figure 2. A, Two abnormal proliferations: a cribriform pattern on the left and a solid proliferation on the right (hematoxylin-eosin, original magnification $\times 100$). The submitting pathologist called the lesion ductal carcinoma in situ, with no mention of a lobular process; presumably he thought the solid proliferation characteristic of a ductal lesion. B, E-cadherin, diffuse, strong staining, typical of ductal lesions (original magnification $\times 200$). C, Loss of E-cadherin in gland to the right, characteristic of a lobular proliferation (original magnification $\times 200$). D, Retention of keratin 903 staining in the right gland, characteristic of lobular lesions (original magnification $\times 200$). Final diagnosis: Atypical ductal hyperplasia (single focus) and lobular carcinoma in situ (multiple foci).

Table 2. Immunohistochemical Stains That Differentiate Ductal and Lobular Carcinoma*

	E-Cadherin	CK34 β E12 (Keratin 903)	CK8 (CAM 5.2)
Ductal	Positive	Negative (most)	Peripheral
Lobular	Negative	Positive (most)	Perinuclear

* CK indicates cytokeratin.

show an intermediate group with overlapping staining characteristics, as is true for E-cadherin, it is important to interpret findings in the context of morphology.

Other CKs such as CK5/6 and CK8 (CAM 5.2) are less informative because the former is negative in most ductal and lobular lesions and the latter is positive in both.^{3,16} On the other hand, there are some differences between ductal and lobular carcinomas in CK8 staining pattern that may be useful. Cytokeratin 8 stains ductal carcinoma cells with a peripheral cytoplasmic accentuation with molding of

cells to each other; perinuclear staining is characteristic of lobular carcinoma.¹⁷ This perinuclear concentration may be because of the noncohesiveness of lobular carcinoma cells, which make them look rather rounded, whereas staining in the cohesive ductal carcinoma cells appears more peripheral (I.Y., personal observation, 2006).

MYOEPITHELIAL MARKERS: IN SITU VERSUS INVASIVE CARCINOMA; INVASION VERSUS PSEUDOINVASION

Broder¹⁸ defined CIS as a proliferation of malignant epithelia confined by the basal lamina, whereas invasive carcinoma penetrates and grows beyond the basement membrane of the microanatomic structure in which it arises. Invasive ductal carcinoma, even the smallest, is treated differently from DCIS. Ruling out small foci of invasion ("microinvasion") is most problematic in cases of extensive high-grade DCIS accompanied by prominent periductal stromal fibrosis and inflammation. Another vexing diagnostic problem in which IHC can help is distinguish-

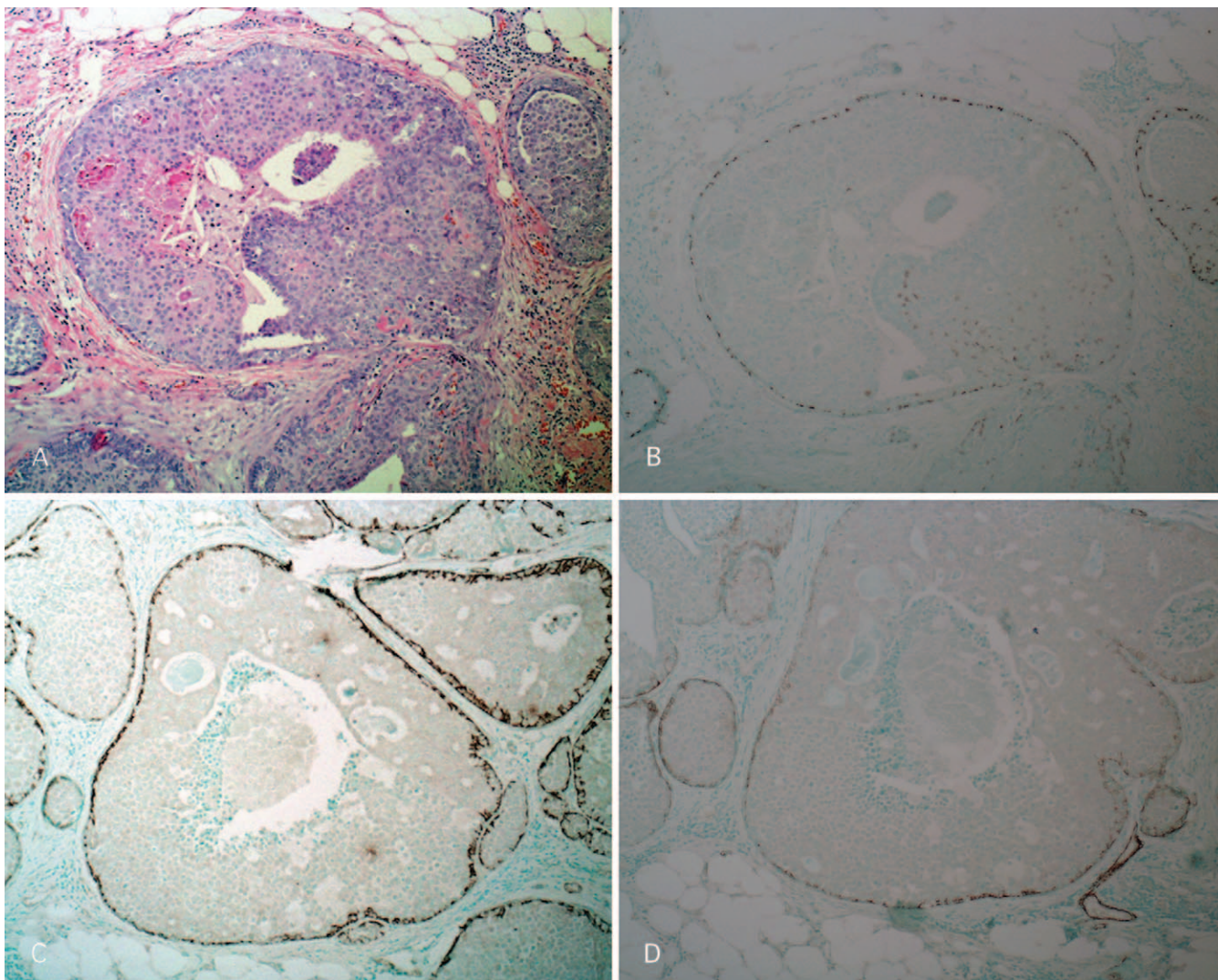


Figure 3. Comparison of commonly used myoepithelial markers in a case of high-grade ductal carcinoma in situ (DCIS), with reactive stroma, questionable invasion. A, Hematoxylin-eosin (original magnification $\times 100$). B, p63 stain shows continuous circle of positive nuclei around duct (original magnification $\times 100$). C, Calponin stain shows cytoplasmic stain on all myoepithelial cells at periphery of duct (original magnification $\times 100$). D, Smooth muscle myosin heavy chain stain also demonstrates the outer rim of myoepithelial cells (original magnification $\times 100$). Final diagnosis: DCIS, no invasion identified.

ing DCIS/LCIS involving sclerosing adenosis or other complex sclerosing lesions from invasive carcinoma.

Earlier investigators approaching this problem stained for basal lamina components but found this could not reliably distinguish in situ from invasive tumors because some invasive carcinomas produce basement membrane components, including laminin, type IV collagen, and type VII collagen.^{19,20}

In cases of DCIS, in which inflammation and reactive fibrosis obscure the interface between involved ducts and adjacent stroma, IHC stains to highlight MECs can help to clarify the integrity of the duct wall by simplifying the low-power microscopic picture and making it easier to spot foci that deviate from the overall normal rounded ductal configuration (Figure 3, A through D). Except in the rare case of myoepithelial carcinoma, usual ductal carcinoma cells are negative for MEC markers.

A variety of antibodies stain MECs, including CK5, CK14, CK17, CD10, S100, smooth muscle actin, smooth muscle myosin heavy chain (SMMHC), calponin, and

p63.^{21–32} The most sensitive/specific markers among these are SMMHC, calponin, and p63, the last having the advantage in this context because it does not stain myofibroblasts; this is also true of some basal CKs. Table 3 lists the staining patterns of these MEC antibodies. Except for p63, a nuclear stain, these antibodies stain MEC cytoplasm. Because absent cytoplasmic staining with p63 can make staining around the outer contours appear incomplete, some may prefer calponin or SMMHC, which gives a more complete, clearer outer rim staining. A combined approach is another option, using calponin or SMMHC with p63 in a complementary pair.

In addition to penetrating basement membrane, invasive carcinomas lack the MEC layer that normally surrounds benign breast glands; microglandular adenosis, a rare lesion, is the only benign proliferative condition that lacks the MEC layer.^{33,34} This observation is the basis of using myoepithelial markers to distinguish invasive carcinoma from infiltrative mimics such as florid sclerosing adenosis, which can be particularly problematic when secondarily

Table 3. Myoepithelial Cell (MEC) Markers, Specificity*

Antibody	MEC	Myofibroblast	Vascular SM	Luminal Cells	Tumor Cells
Calponin	+++	++	+++	—	Rare
SMMHC	+++	+	+++	—	—
p63	+++	—	—	+	Rare
CD10	++	+	—	+	+
S100	+	Variable	—	+	Variable
SMA	+++	++	+++	—	—
Basal CKs	+++	—	—	+	+

* SM indicates smooth muscle; SMMHC, smooth muscle myosin heavy chain; SMA, smooth muscle actin, and CK, cytokeratin.

involved by coexistent DCIS or LCIS (Figure 4, A and B). Highlighting myoepithelium can also help distinguish invasion from “pseudoinvasion” in radial scars and sclerosing papillary lesions.

MARKERS OF PROGNOSIS AND RESPONSE TO THERAPY

Hormone Receptor Analysis in Invasive Carcinoma

It has been standard practice for 25 years to analyze all invasive breast cancers for estrogen receptor (ER) and progesterone receptor (PR) content as a means of estimating prognosis and predicting responsiveness to endocrine treatment should cancer recur after initial treatment.³⁵ As adjuvant chemotherapy and endocrine treatment were found to prevent or delay recurrence, the biomarker role of ER/PR became more salient.

Biochemical ligand-binding assay for ER/PR testing gave way in the early 1990s to IHC when monoclonal antibodies that could reliably stain ER and PR in paraffin-embedded tissues became commercially available. Immunohistochemistry fully replaced the biochemical assay when it was shown that the 2 tests were highly concordant and that IHC was as good or superior in predicting tamoxifen response in both the therapeutic and the adjuvant settings.^{36–38}

What has been less clear for the practicing pathologist is how to evaluate or “score” ER/PR stains in a clinically relevant manner. Both Barnes et al³⁹ and Fisher et al⁴⁰ compared the ability of multiple scoring systems to predict treatment response and found that all gave statistically significant correlations with outcome. Good predictive value and simplicity favors the “any-or-none” approach, which scores the percentage cells positive and ignores staining intensity.^{39,40}

One must still decide the proportion of stained cells that merits the tag “positive” and constitutes a hormonally treatable state. Three staining categories emerged from the International Breast Cancer Study Group, which correlated ER staining with response to adjuvant hormonal treatment in a node-negative cohort: none (0), low (1%–9%), and high ($\geq 10\%$).³⁸ Most tumors were in the none (22%) and high (75%) categories; only 3% were in the low ER category, too few for meaningful statistical analysis.³⁸ Adjuvant endocrine treatment has no role in treating ER/PR-negative cancers.

The International Breast Cancer Study Group scheme is the basis of the most recent St Gallen treatment guidelines, which partition breast cancer into 3 “endocrine” groups: responsive, response uncertain, and nonresponsive, corresponding to high (>10%), low (1%–9%), and none (0) ER staining.^{41,42} These guidelines, widely followed in Europe and the United States, suggest for the first time that

endocrine responsiveness should be a major driver in selecting adjuvant systemic treatment, a paradigm shift that makes standardized, reproducible ER/PR staining very important.

Currently available antibodies for ER/PR testing are listed in Table 4.⁴³ The Food and Drug Administration has recently approved the DakoCytomation (Glostrup, Denmark) ER/PR pharmDX immunohistochemistry kit, a “seal of approval” that will be a powerful incentive to use this product. To facilitate reproducibility, the manufacturer and the Food and Drug Administration require that users report results as an Allred score, which comprises both percentage of cells positive and staining intensity.³⁶ A total score of 3 or more, corresponding to as few as 1% to 10% weakly or intermediate positive cells, defines the lowest “positive” and corresponds to the St Gallen “endocrine response uncertain” category in which adjuvant hormone treatment is recommended but has uncertain benefit.^{41,42}

Hormone Receptor IHC in DCIS

Immunohistochemical staining for ER in DCIS, lacking associated invasive carcinoma, has an emerging role in estimating potential tamoxifen benefit. The impetus comes from the National Surgical Adjuvant Breast and Bowel Project Protocol B-24, which randomized 1804 women with DCIS treated with partial mastectomy and irradiation, to receive placebo or tamoxifen for 5 years. Results showed a conclusive reduction in both ipsilateral and contralateral breast cancer in the adjuvant tamoxifen group (relative risk [RR] = 0.63; $P < .001$).^{44,45}

In 2003, Baylor University and the National Surgical Adjuvant Breast and Bowel Project investigators reported preliminary findings of a retrospective study that correlated ER staining with the outcome of the B-24 trial. Of 628 DCIS cases (327 placebo, 301 tamoxifen) stained for ER at a central core facility or using reported data, most (77%) were ER positive and these patients clearly benefited from adjuvant tamoxifen, which reduced both ipsilateral recurrence and new contralateral cancers (RR = 0.41; $P < .001$). ER-negative patients showed little benefit (RR = 0.80; $P = .51$), but the number of endpoint events ($n = 36$) was too small to rule out a small but clinically significant advantage.⁴⁶ Concerns about false-negative ER staining were being more fully investigated; however, the final results have not yet been published. Although it is still unclear if the data support omitting adjuvant tamoxifen for ER-negative DCIS, the preliminary findings found their way onto many breast cancer Web sites, prompting many DCIS patients and clinicians to insist on ER staining to guide therapy.

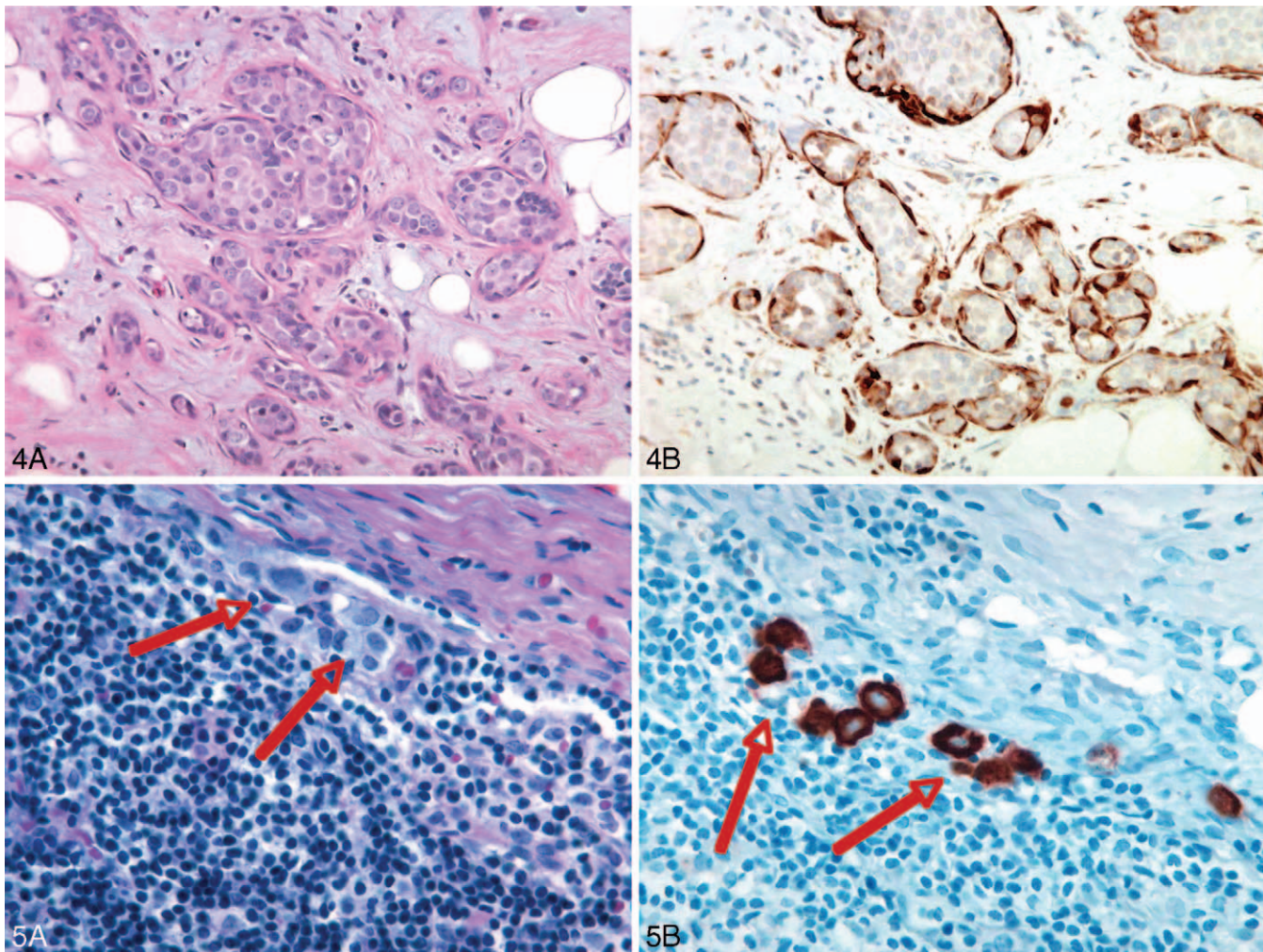


Figure 4. Myoepithelial marker in a case with abnormal tubules, sclerotic stroma, invasive lobular carcinoma (alveolar pattern) versus lobular carcinoma in situ (LCIS) in sclerosing adenosis *A*, Hematoxylin-eosin (original magnification $\times 200$). *B*, Calponin, strong staining at periphery of all cell groups (original magnification $\times 200$). Final diagnosis: LCIS involving sclerosing adenosis.

Figure 5. Cytokeratin staining in sentinel lymph node, individual tumor cells (ITCs). *A*, Arrows show few atypical cells in subcapsular sinus of sentinel lymph node (hematoxylin-eosin, original magnification $\times 200$). *B*, Cytokeratin AE1/AE3 stain confirms the epithelial nature of subcapsular cells (original magnification $\times 200$). Final diagnosis: Sentinel lymph node, positive for ITC, N0(i+).

Table 4. Commonly Used Prognostic/Predictive Antibodies for Breast Cancer

Antibody*	Clone	Type	Source
ER	SP1	Rabbit monoclonal	LabVision, Fremont, Calif
ER	6F11	Mouse monoclonal	Novacastra Laboratories Ltd, Newcastle upon Tyne, United Kingdom
ER	1D5	Mouse monoclonal	DakoCytomation, Glostrup, Denmark
PR	SP2	Rabbit monoclonal	LabVision
PR	636	Mouse monoclonal	DakoCytomation
PR	16	Mouse monoclonal	Ventana, Tucson, Ariz
HER-2	HercepTest	Mouse monoclonal	DakoCytomation
HER-2	CB11	Mouse monoclonal	Ventana

* ER indicates estrogen receptor; PR, progesterone receptor.

HER-2/*neu* IHC in Invasive Breast Cancer

HER-2/neu was one of the first oncogenes studied in clinical samples of invasive breast cancer.^{45,47} Its early significance as a prognostic factor has been surpassed by its key importance as a biomarker for sensitivity to Herceptin (trastuzumab, a monoclonal antibody against the HER-2 receptor) and resistance to tamoxifen.⁴¹

Immunohistochemical staining for HER-2 is currently

the recommended first line of testing for overexpression, which is commonly due to gene amplification.^{35,48} There are currently 2 Food and Drug Administration–approved antibodies for HER-2 testing—the DakoCytomation HercepTest and the Ventana CB11 (Table 4).

The American Society of Clinical Oncology recommends reporting results along with a description of the test method; specific kit or critical reagents; details of the

scoring system; quality controls; and the reproducibility, sensitivity, and specificity of the assay, with correlation or reference to a clinically validated test.³⁵ The National Comprehensive Cancer Network recommends that HER-2 tests be validated by at least 95% concordance in 50 to 100 tumor samples when compared with another validated HER-2 testing method in the same laboratory, a validated HER-2 testing method performed in another laboratory, or validated reference laboratory results.⁴⁸

To improve the accuracy of HER-2 testing, the American Society of Clinical Oncology and the College of American Pathologists set out guidelines for laboratories to follow, including recommendations for specimen handling, assay exclusion, and reporting criteria.⁴⁹ A positive HER-2 result is IHC staining of 3+, defined as uniform, intense membrane staining of more than 30% of invasive tumor cells. No further testing is needed for invasive cancers that stain definitely positive (3+) or negative (0, 1+); however, "equivocal" (2+) cases should be examined for gene amplification by fluorescence in situ hybridization. A positive HER-2 by fluorescence in situ hybridization is defined as more than 6 *HER-2* gene copies per tumor cell nucleus or a *HER-2* gene to chromosome 17 ratio of more than 2.2.^{48,49} Chromogenic in situ hybridization is a promising method for HER-2 testing that has not yet gained widespread acceptance.⁵⁰⁻⁵²

Other Prognostic Assays

Ki-67 (MIB-1), a proliferation marker, stains cells in all cell cycle phases except the resting phase (G0); the percentage of stained nuclei is the proliferation index. A low proliferation index is associated with slower tumor growth and thus better prognosis, whereas the converse is true for a high proliferation index.

Recent studies have shown that gene expression microarray analysis of breast cancers may be of independent prognostic significance.⁵³⁻⁵⁸ Perou et al⁵⁸ identified several genotypes of breast cancers: the normal-like, luminal types A and B, HER-2 overexpressing, and the basal-like types, with the prognosis progressively worse from normal-like to the basal-like types. At this time, gene expression analysis is performed only in a few specialized research laboratories, and there has been interest in finding immunohistochemically demonstrable equivalents, especially because clinical studies have confirmed differences in tumor response to chemotherapy.⁵⁹ HER-2 overexpressing tumors are already being identified through IHC and fluorescence in situ hybridization in most laboratories. Using tissue microarrays, several studies have looked at cohorts of patients using CKs to distinguish basal from luminal types and have confirmed that these phenotypic groups have differing prognoses, that is, tumors expressing a basal phenotype or a mixed basal/luminal phenotype have a worse prognosis⁶⁰⁻⁶³ (Table 5). The basal phenotype correlates with grade 3 invasive carcinomas not otherwise specified, as well as with medullary carcinomas, adenoid cystic carcinomas, and squamous differentiation (metaplastic carcinomas).⁶⁴⁻⁶⁶ Luminal phenotype correlates with lobular, tubular, and invasive cribriform carcinomas.⁶⁰

IMMUNOHISTOCHEMICAL STAINING FOR CYTOKERATINS IN AXILLARY SENTINEL NODES

Sentinel node biopsy is now standard for patients with invasive breast cancer, as well as for some with diffuse

Table 5. Genotypes of Breast Cancer and Corresponding Immunophenotypic Markers

Genotype	Immunophenotypic Marker*
Normal-like	...
Luminal	Cytokeratins 7, 8, 18 (usually ER+, PR+)
HER-2 overexpressing	HER-2
Basal-like	Cytokeratins 5/6, 14, 17 EGFR ("Triple negative": ER, PR, HER-2 negative)

* ER indicates estrogen receptor; PR, progesterone receptor; EGFR, epidermal growth factor receptor; . . . , no immunophenotypic markers.

DCIS who may harbor a small, clinically occult invasive cancer.^{67,68} Immunohistochemical staining for keratins greatly facilitates detecting small carcinoma deposits in lymph nodes. The current American Joint Committee on Cancer (AJCC) staging guidelines incorporate keratin IHC staining results in breast cancer staging.⁶⁹

The keratin cocktail AE1/AE3, which stains predominantly high-molecular-weight keratins, or a wide-spectrum pan-CK are most commonly used.⁷⁰ CAM 5.2 and AE1, which stain only low-molecular-weight keratins, also stain reticular cells in lymph nodes, which is a drawback.^{71,72}

Not all epithelial cells in lymph nodes represent metastatic carcinoma. A biopsy may displace benign breast epithelia, which then drain to the axillary nodes, so-called benign transport.⁷³⁻⁷⁵ Therefore, care must be taken to observe the cytologic characteristics of the cells in question, such as nuclear-cytoplasmic ratio, nuclear size, hyperchromasia, and so forth, and to compare the cells with those of the primary cancer.

Small numbers of tumor cells in lymph nodes, termed *isolated tumor cells*, are defined as "single tumor cells or small cell clusters not greater than 0.2 mm, usually detected only by IHC or molecular methods, but which may be verified on H&E stains"⁷⁶ (Figure 5, A and B). Because we do not yet know the impact on survival of isolated tumor cells, the AJCC currently recommends staging this finding as (N0)(i+).⁷⁶⁻⁷⁸ Follow-up of these N0(i+) patients are in progress and their outcome will help refine AJCC staging. As a practical matter, axillary dissection is recommended when carcinoma, even in the form of isolated tumor cells, is found in a sentinel node biopsy.⁷⁹⁻⁸¹

METASTATIC CARCINOMA, UNKNOWN PRIMARY ORIGIN

In the workup of a tumor of unknown primary origin in a woman, a frequent consideration is breast carcinoma because it is common and may initially present at a metastatic site. A panel of IHC stains, some addressing the site of the metastasis, can help solve this problem.

Markers consistent with mammary origin include receptors for hormones (estrogen, progesterone, androgen) and gross cystic disease fluid protein 15 (GCDFP-15), also known as prolactin-inducing protein,^{82,83} which stains apocrine cells in the breast and axillary glands in axillary and anogenital skin. Gross cystic disease fluid protein 15 staining in a metastatic carcinoma has high specificity (98%-99%) for breast cancer, if skin adnexal and salivary gland cancers can be excluded on clinical grounds, which is usually the case. Although specific, GCDFP-15 is only mod-

Table 6. Tumor of Unknown Primary Origin, Suspect Breast Origin*

Marker	Positive Breast Cancers, %	Other Tissues Often Positive
GCDFP-15	50–74	Ovary, endometrium, pancreas, ampulla, sweat glands, vulva, prostate
ER	70–75	Ovary, uterus, prostate, lung, GI sites, skin
PR	54–59	Ovary, uterus, skin, thyroid, pancreas, CNS
AR	60–70	Prostate, skin, salivary glands
CK7	80+	Lung, mesothelium, esophagus, stomach, gyn sites, many others

* GCDFP-15 indicates gross cystic disease fluid protein 15; ER, estrogen receptor; GI, gastrointestinal; PR, progesterone receptor; CNS, central nervous system; AR, androgen receptor; CK, cytokeratin; and gyn, gynecologic.

erately sensitive (50%–74%) for breast carcinoma, which is why it is important to add other markers to the diagnostic panel.^{84,85}

Receptors for estrogen, progesterone, and androgen have expression rates in breast cancer of 70% to 75%, 54% to 59%, and 60% to 70%, respectively.^{86–89} A significant number of ER-negative high-grade ductal carcinomas express androgen receptors and/or HER-2/*neu*, giving these markers added value in this context.⁸⁶ A study of cutaneous metastases from various primary carcinomas showed that most (82%) metastatic breast cancers expressed androgen receptors.⁹⁰ Thus, if a metastasis originates in the breast, it is likely that a panel including GCDFP-15, ER, PR, androgen receptor, and HER-2/*neu* will point to this site. Positive staining for ER, PR, and HER-2/*neu* can also help determine targeted therapy with tamoxifen or Herceptin (trastuzumab).

The metastatic site also guides the choice of IHC marker. For example, if the site is lung, a positive stain for thyroid transcription factor 1 will exclude breast cancer; a negative stain is uninformative. If stomach is the metastatic site, ER staining would strongly favor breast cancer, whereas staining for Cdx2 would exclude it.⁹¹ CK20 expression in this context would favor primary gastric cancer.

The more sensitive markers, such as CK7 and ER, stain 70% to 80% of breast cancers but lack specificity because CK7 is distributed widely in epithelia and ER is positive in the gynecologic tract tumors.

Mammoglobin A and B have been identified in breast cells and are overexpressed in breast cancer.^{92–96} However, mammoglobin A appears to be more specific for breast and gynecologic organs, whereas mammoglobin B may be found in a number of other tumors, including colon, stomach, and other gastrointestinal malignancies.⁹⁷ The best strategy is to test the tumor for multiple markers to increase sensitivity and specificity (Table 6). The negative expression of breast carcinomas for markers such as HMB-45, CK20, and thyroid transcription factor 1 is also very helpful in the workup.

SUMMARY

Immunohistochemistry is playing an increasing role in the modern pathology of breast disease. Some of the diagnostic uses of IHC include differentiating UDH from ADH/LG-DCIS, ruling out microinvasion, distinguishing invasive carcinoma from pseudoinvasive lesions, identi-

fying breast cancer histologic subtype and molecular phenotype, and confirming the breast as the primary site in metastatic carcinoma. In addition, immunohistochemical markers are useful for estimating prognosis and predicting therapy response. The best approach to the use of immunohistochemical markers is to couple them with standard hematoxylin-eosin histology and to use panels of markers.

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CAP '08 ABSTRACT PROGRAM

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