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Utility of cytokeratin 5/6 and high-molecular-weight keratin in evaluation of cauterized surgical margins in excised specimens of breast ductal carcinoma in situ

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Abstract Evaluation of the surgical margins of excision specimens for ductal carcinoma in situ (DCIS) of breast is challenging due to cautery artifact introduced in the specimen at the time of surgery. Cautery destroys the cytoarchitectural features at the tissue margins and makes the distinction between usual ductal hyperplasia (UDH) and DCIS difficult. Previous studies have shown the value of immunohistochemical staining for cytokeratin 5/6 (CK5/6) and high-molecular-weight keratin (HMWK) in distinguishing UDH from DCIS. We hypothesized that staining for CK5/6 and HMWK (34*b*E12) may be helpful in evaluating the cauterized surgical margins, given the 2 antibodies follow the same pattern as described in the preserved foci of the 2 entities. Forty-three excised breast specimens were stained for CK5/6 and HMWK (34*b*E12). Study material was divided into 5 groups: DCIS without cautery artifact, UDH without cautery artifact, UDH with cautery artifact, DCIS with mild-to-moderate cautery artifact morphologically recognizable as involving the surgical margin on hematoxylin and eosin stain, and DCIS with severe cautery artifacts precluding the evaluation of surgical margins on hematoxylin and eosin stain. A comparative evaluation of pattern, extent, and intensity of the 2 immunostains was done. Our results strongly suggest that antibodies for CK5/6 and HMWK (34*b*E12) may be useful in determining the presence of DCIS at surgical margins even in the event of severe cautery artifact.

Keywords: Cautery artifact; Surgical margins; DCIS; CK5/6; HMWK

1. Introduction

The increased use of mammography in breast cancer screening has led to increased incidence of detection of ductal carcinoma in situ (DCIS) and a trend toward conservative excision therapy. Local recurrence of DCIS following excision is a well-observed phenomenon. It is often attributed to the residual disease left behind at the time of initial surgery and hence underscores the importance of disease-free excision margins. Indeed, surgical margin with the width of 10 mm theoretically guarantees clearance in most cases and is considered the best independent prognostic marker for DCIS [1-3].

Ductal carcinoma in situ is distinguished from usual ductal hyperplasia (UDH), based on its cytoarchitectural features; however, in a small percentage of cases, this distinction remains a diagnostic challenge. Identification of DCIS at the surgical margins of excised specimens is sometimes further complicated by the presence of cautery artifact introduced at the time of surgery. Failure to recognize the presence of DCIS at surgical margins may have a great impact on the patient's management and prognosis.

Previous studies have shown the utility of immunostains for cytokeratin (CK) 5/6 and high-molecular-weight keratin (HMWK) in distinguishing DCIS/atypical ductal hyperplasia (ADH) from UDH [4-10]. However, none of the studies in the literature so far have addressed the issue of cautery

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artifact in the evaluation of surgical margins. We hypothesized that immunostaining for CK5/6 and HMWK may be a helpful tool in evaluating the surgical margins if the 2 antibodies follow the same pattern of staining at cauterized margins as described in the preserved foci of the 2 entities.

2. Materials and methods

This study was approved by our institutional review board. A total of 43 cases of surgically excised breast lesions were retrieved from the surgical pathology archives of Long Island Jewish Medical Center, NY. Hematoxylin and eosin (H&E)–stained slides were reviewed by 2 observers jointly (T.B. and A.N.), and a consensus diagnosis was made. To test the validity of immunostains and for comparative evaluation, study cases were divided into 5 groups as follows: group 1: DCIS without cautery artifact, 5 cases; group 2: UDH without cautery artifact, 5 cases; group 3: UDH with cautery artifact, 11 cases; group 4: DCIS with mild-to-moderate cautery artifact morphologically recognizable as DCIS involving the surgical margin on H&E stain, 11 cases; and group 5: DCIS with severe cautery artifact precluding the evaluation of surgical margins on H&E stain, 11 cases. In groups 3, 4, and 5, special care was taken to include sections showing some preserved foci of the lesion. These preserved areas served as an internal control for comparison of staining pattern with the cauterized areas at margins.

Immunohistochemical staining was done for CK5/6 and HMWK on formalin-fixed, paraffin-embedded sections in all the 43 cases. Four-micron-thick sections were taken on poly-l-lysine–coated slides. All the sections were deparaffinized, rehydrated, and treated with 3% hydrogen peroxide for 15 minutes to inhibit endogenous peroxidase. Following heat-induced epitope retrieval in 0.1 mol/L of citrate buffer at pH 6.0 in a microwave for 20 minutes, the slides were incubated with mouse monoclonal antibodies specific for CK5/6 (clone D5/16 B4; Zymed Laboratories Inc; 1:100 dilution) and HMWK (clone 34*b*E12; Cell Marque Corp), respectively. Slides were processed on a DakoCytomation Autostainer Plus S3400 (DakoCytomation, Carpinteria, Calif). Incubation with secondary antibodies was performed, followed by treatment with a 3, 39-diaminobenzidine–containing chromogenic solution and hematoxylin counterstaining. Appropriate positive and negative controls were run with each case.

Immunohistochemically stained slides were jointly evaluated by 2 observers (T.B. and A.N.). Evaluation of immunostaining patterns for CK5/6 and HMWK in the preserved foci of DCIS and UDH was done in each group and compared with the staining pattern in the cauterized areas at the margins of the specimen in groups 3, 4, and 5 cases. Two patterns of staining, luminal or basal, were noted. Stain was interpreted as positive only if more than 10% of luminal cells showed either cytoplasmic or membranous staining. In case of positive luminal staining, extent (percentage positivity) and intensity of stain were recorded as either weak (1+), moderate (2+), or strong (3+). In case of basal staining, pattern of staining whether continuous or discontinuous was recorded. Results of each group were recorded separately and subsequently compared.

3. Results

Results of the 2 immunostains in each group of cases are summarized in Table 1.

Group 1 (DCIS without cautery artifact): none of the 5 cases in this group showed positivity for CK5/6 and HMWK in the luminal epithelial cells (Fig. 1).

Table 1
Summary of the IHC results in various test groups

Diagnostic group	Total cases	Preserved foci			Cauterized margins				Surgical margin status
		Positive (>10% of luminal cells positive)	% Positivity in luminal cells	Intensity of stain in luminal cells	Positive (>10% of luminal cells positive)	Negative (<10% of luminal cells positive or attenuated basal continuous or discontinuous stain)	% Positivity in luminal cells	Intensity of stain in luminal cells	
Group 1	5	0/5	0	–	–	–	–	–	–
Group 2	5	5/5	60-100	3+ (CK5/6) 3+ (HMWK)	–	–	–	–	–
Group 3	11	11/11	60-100	3+ (CK5/6) 3+ (HMWK)	11/11	–	20-100 (CK5/6) 10-50 (HMWK)	2+/3+ (CK5/6) 1+/2+ (HMWK)	Negative
Group 4	11	0/11	0	–	1/11	–	60 (CK5/6) 50 (HMWK)	3+ (CK5/6) 3+ (HMWK)	Positive
						10/11	0 (CK5/6) 0 (HMWK)	–	Positive
Group 5	11	0/11	0	–	9/11 (CK5/6)	–	10-90 (CK5/6)	2+/3+ (CK5/6)	Negative
					8/11 (HMWK)	–	10-80 (HMWK)	2+/3+ (HMWK)	
						2/11	0 (CK5/6) 0 (HMWK)	–	Positive