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Sarcomatoid carcinoma of the breast: an immunohistochemical study of six cases

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Summary. Six cases of sarcomatoid carcinoma of the breast (SCB) were studied with a panel of antibodies directed against epithelial and sarcomatoid components. The monoclonal antibodies (MoAb) AE-1/3, CAM 5.2, and CEA were used to detect epithelial differentiation; polyclonal antibodies against S-100 protein and MoAb against the intermediate filaments desmin and vimentin were used to detect mesenchymal differentiation in the sarcomatoid component. Six cases of invasive duct carcinoma (IDC) and two cases of cystosarcoma phyllodes (CP) were compared to SCB using the same panel of antibodies. In all three groups studied, the epithelial component in the majority of cases stained with anti-cytokeratin antibodies. S-100 protein antibodies stained the epithelial and sarcomatoid components in four cases of SCB; vimentin MoAb stained the epithelium in two cases and the sarcomatoid component in four cases of SCB, while MoAb CEA failed to stain any component of SCB. In contrast, the epithelium in five of six cases of IDC stained with CEA MoAb and only one of six stained for S-100 protein. Possible reasons for the discrepant immunohistochemical staining patterns among SCB, IDC and CP are discussed, in addition to the limitations and pitfalls of immunohistochemistry in diagnostic surgical pathology.

Key words: Sarcoma – Metaplastic breast carcinoma – Immunoperoxidase

Introduction

Sarcomatoid carcinoma of the breast (SCB), also known as a type of metaplastic breast carcinoma, is a malignant neoplasm containing both epithelial and sarcomatoid elements. The sarcomatoid component may include neoplastic cartilage, bone, adipose tissue, skeletal muscle or an undifferentiated spindle cell component. Although the sarcomatoid component may predominate, a malignant epithelial component must be present to distinguish this lesion from sarcoma (McDivitt et al. 1968; Smith and Taylor 1969; Huvos et al. 1973; Harris and Persand 1974; Kahn et al. 1978; Kaufman et al. 1984).

There has been controversy whether carcinoma induces sarcoma in stroma or whether the epithelial component becomes pseudosarcomatous through a metaplastic process. It is now generally accepted that the sarcomatoid component is metaplastic rather than a sarcoma (Gonzalez-Licea et al. 1967; McDivitt et al. 1968; Huvos et al. 1973; Battifora 1976; Gersell and Katzenstein 1981; Bauer et al. 1984; Kaufman et al. 1984).

An occasional breast carcinoma is so poorly differentiated it is difficult to distinguish from a sarcoma. In at least some of these cases, the issue of poorly differentiated SCB arises.

In view of the diagnostic dilemmas that can occur with such lesions and recognition of pseudosarcomatous metaplasia in carcinomas, an immunohistochemical study was undertaken to determine if epithelial differentiation can be detected in the sarcomatoid component of SCB. Various mesenchymal markers were also used to detect evidence of stromal differentiation (Osborn and Weber 1983) in epithelial cells and to see if mesenchymal markers can reliably distinguish the epithelial and sarcomatoid components of SCB. To our knowledge, this entity has not been previously described utilizing immunohistochemical studies.

Study of two other breast lesions with either similar epithelial or stromal components, invasive duct carcinoma (IDC) and cystosarcoma phyllodes (CP), respectively, was also undertaken to compare

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the immunohistochemical staining pattern of these entities with SCB.

Material and methods

Six cases of SCB, reported previously (Kaufman et al. 1984) formalin-fixed and paraffin-embedded, were available for immunohistochemical study. In all cases, a malignant epithelial component was present in conjunction with a sarcomatoid component. No squamous differentiation was present in any of the neoplasms. The sarcomatoid component was purely chondroid in three cases, a mixture of chondroid and undifferentiated uniform spindle cells in one case, purely undifferentiated uniform spindle cells in one case, and pleomorphic spindle cells with osteoclast-like giant cells in the remaining case. For comparison, six cases of invasive duct carcinoma (IDC) and two cases of cystosarcoma phyllodes (CP) were also studied with the same panel of antibodies. These cases were also formalin-fixed and paraffin-embedded. One case of CP had also been fixed in 95% ethanol.

Immunohistochemical staining was performed on tissue sections using the avidin-biotin-peroxidase complex (ABC) method of Hsu et al. (1981). Trypsinization for 20 min at 37° C (0.1% trypsin in 0.134% calcium chloride, pH 7.8) was performed to enhance immunohistochemical staining. Tissue sections were stained with AE-1/3, a mixture of two mouse monoclonal antibodies (MoAb), AE-1 and AE-3, directed against human epidermal keratin (Hybritech, Inc., San Diego, CA, USA; 1:200 dilution) and a mouse monoclonal keratin antibody, CAM 5.2 (Becton-Dickinson, Mountainview, CA, USA; 1:300 dilution), raised against a colon carcinoma cell line. The AE-1 keratin antibody reacts with 40 kDa, 50 kDa and 56.6 kDa classes of keratin proteins. The AE-3 keratin antibody reacts with 46 kDa, 52 kDa, 58 kDa, and 65–67 kDa classes of keratin (Nelson and Sun 1983; Sun et al. 1983; Cooper et al. 1985). The CAM 5.2 MoAb reacts with 39 kDa, 43 kDa and 50 kDa keratin proteins (Makin et al. 1984). In addition, tissue sections were stained with the following: mouse MoAb to CEA (Hybritech, Inc.; 1:1,000 dilution), mouse MoAb to vimentin (Lab Systems, Chicago, Il. USA; 1:25 dilution), mouse MoAb to desmin (Dako Corp., Santa Barbara, CA, USA; 1:1,000 dilution), and rabbit anti-ox polyclonal antibody to S-100 protein (Dako Corp., Santa Barbara, CA, USA; 1:700 dilution).

The specificity of the immunologic reactions and preservation of antigens were verified by the use of the following internal positive controls: normal breast epithelial cells staining for keratin proteins; myoepithelium and dendritic histiocytes staining for S-100 protein; and blood vessel walls staining for desmin and vimentin. Normal breast epithelium failed to stain with anti-CEA. Known positive control tissue sections were used as extrinsic controls. The primary antibody was deleted in negative controls.

The staining patterns in both the epithelial and sarcomatoid or stromal components of SCB, IDC and CP were tabulated.

Results

In all the cases of SCB, the staining patterns with anti-AE-1/3 and CAM 5.2 were generally parallel (Table 1), with a total of five cases staining for cytokeratin. In four of six cases the malignant epithelium stained diffusely for AE-1/3 (Fig. 1). Four of six cases stained similarly with anti-CAM 5.2. One case that failed to stain for AE-1/3 stained with anti-CAM 5.2. One of six cases failed to stain for either AE-1/3 or CAM 5.2. None of the sarcomatoid components stained for either AE-1/3 or CAM 5.2.

MoAb CEA failed to stain either the epithelial or sarcomatoid component in any of the six cases of SCB.

Anti-S-100 protein antibodies stained both the malignant epithelial and sarcomatoid components in four of six cases of SCB. In all four cases the sarcomatoid component was at least partially chondroid (Figs. 2–4). Normal breast epithelium failed to stain for S-100 protein, but the surrounding myoepithelial cells did. Positive staining was seen in both the cell nuclei and cytoplasm.

Anti-vimentin MoAb stained the epithelial component in two of six cases of SCB, both of which contained chondroid stroma. Staining for vimentin was seen in the sarcomatoid component of four of six cases, all of which demonstrated chondroid features (Fig. 5).

Desmin MoAb failed to stain either the epithe-

Table 1. Comparative immunohistochemical staining results of SCB, IDC and CP

Antibody to	Epithelial component			Sarcomatoid component or stroma		
	SCB	IDC	CP	SCB	IDC	CP
	(No. staining/Total no. cases)			(No. staining/Total no. cases)		
AE-1/3	4/6	5/6	2/2	0/6	0/6	0/2
CAM 5.2	4/6	6/6	2/2	0/6	0/6	0/2
CEA	0/6	5/6	0/2	0/6	0/6	0/2
S-100	4/6[a]	1/6	0/2	4/6[a]	0/6	0/2
Vimentin	2/6[b]	0/6	1/2	4/6[b]	0/6	2/2
Desmin	0/6	0/6	0/2	0/6	0/6	0/2

E, epithelial component; S, sarcomatoid/stromal component
[a] same four cases staining with anti-S-100 protein
[b] same two cases staining for vimentin

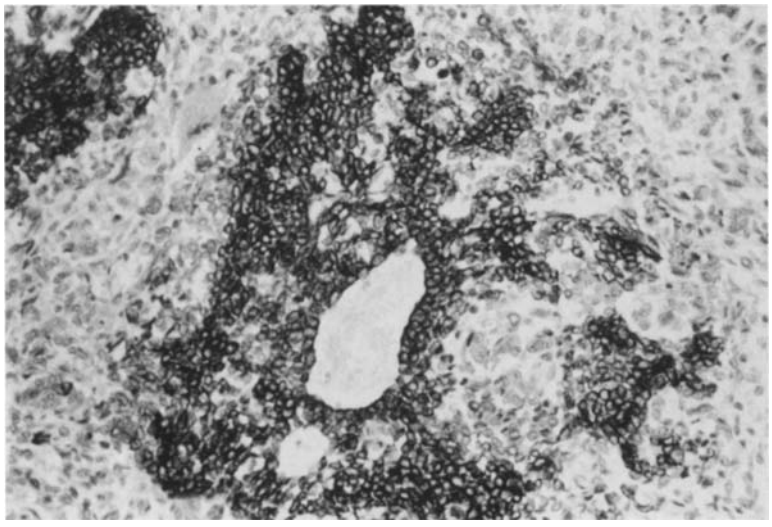

Fig. 1. Epithelial component of SCB staining for keratin (AE-1/3). Note failure of sarcomatoid component to stain. (Immunoperoxidase, ×90)

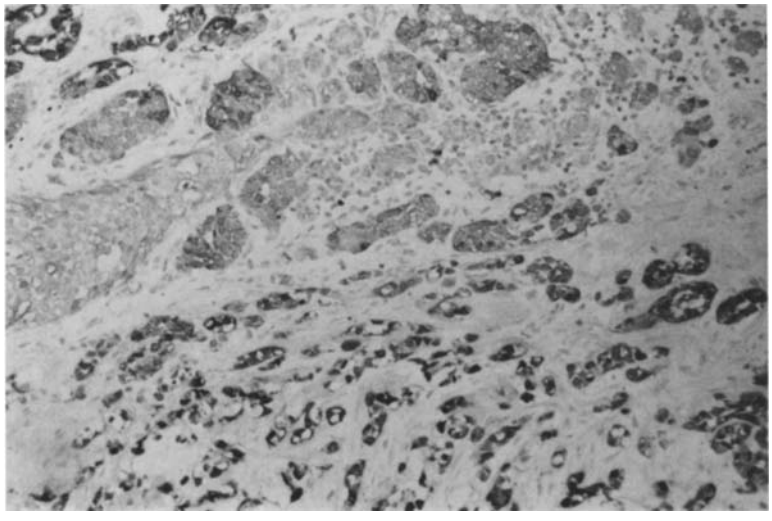

Fig. 2. Epithelial and chondroid components of SCB staining with anti-S-100 protein. (Immunoperoxidase, ×90)

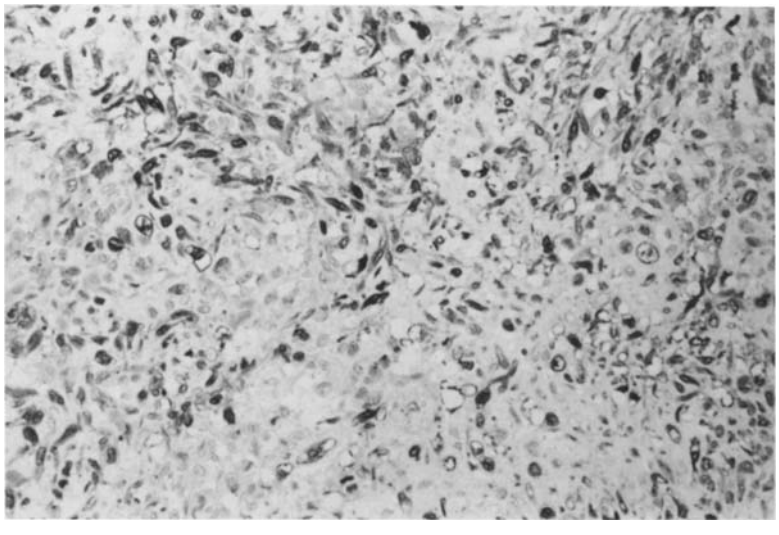

Fig. 3. Spindle and chondroid components of SCB staining for S-100 protein. (Immunoperoxidase, ×225)

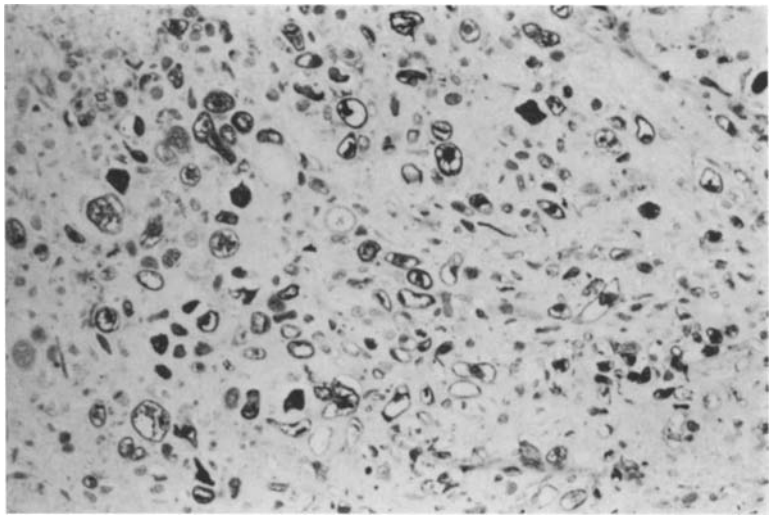

Fig. 4. Chondroid component (same case as Fig. 3) staining with anti-S-100 protein. (Immunoperoxidase, ×225)

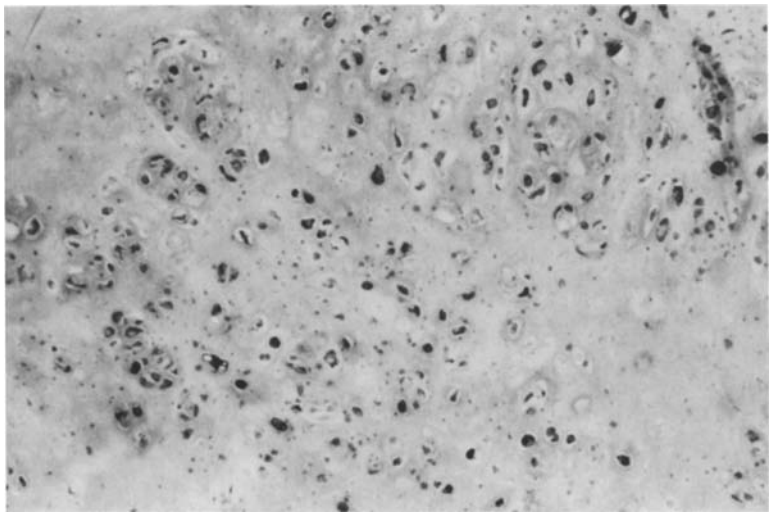

Fig. 5. Vimentin MoAb staining chondroid component of another case of SCB. (Immunoperoxidase, ×90)

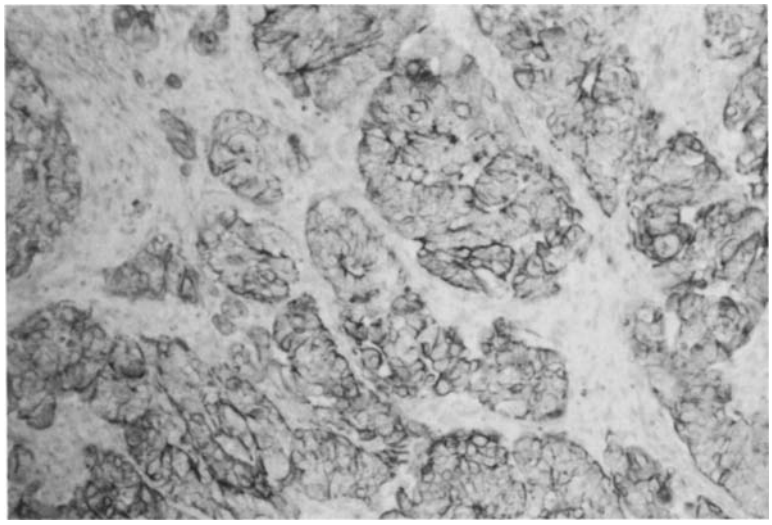

Fig. 6. IDC staining for keratin (CAM 5.2) (Immunoperoxidase, ×90)

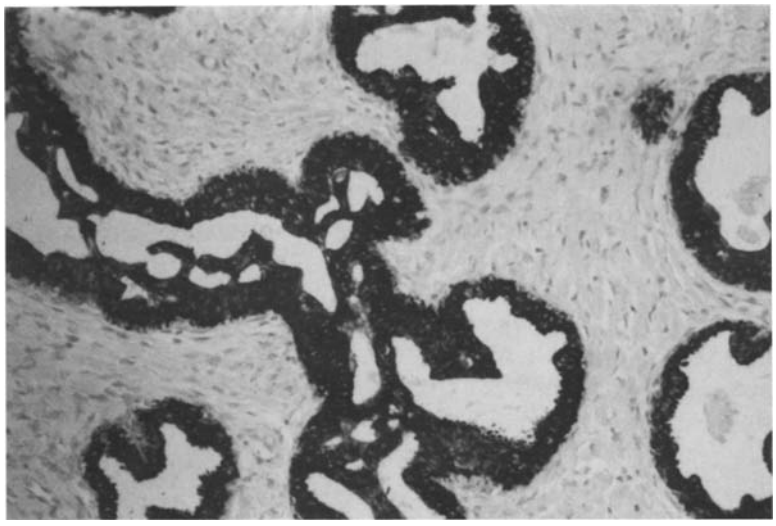

Fig. 7. Keratin MoAb (CAM 5.2) staining epithelium of CP. (Immunoperoxidase, ×90)

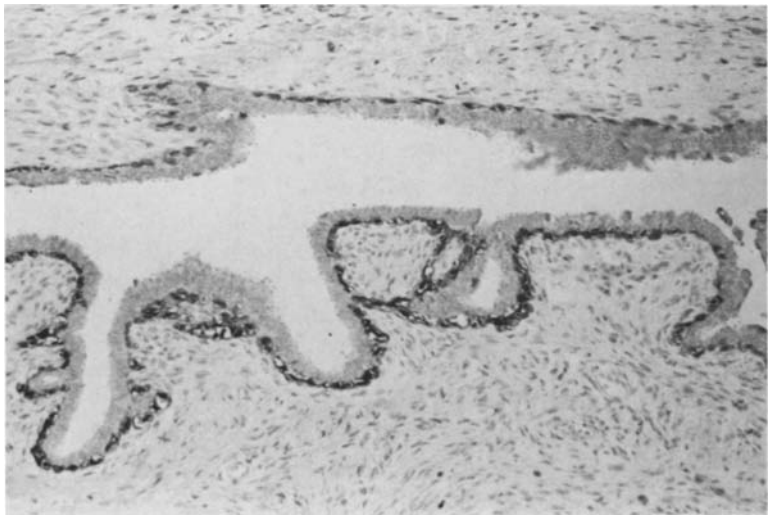

Fig. 8. Anti-S-100 protein staining the myoepithelium in CP (Immunoperoxidase, ×90)

lial or sarcomatoid component in all six cases of SCB.

As with the metaplastic carcinomas, anti-AE-1/3 and CAM 5.2 MoAb stained the malignant epithelium in five and six cases, respectively, of IDC (Fig. 6). The stromal cells failed to stain with cytokeratin markers.

In contrast to SCB, five of six duct carcinomas stained with MoAb to CEA. The staining pattern was weak in most of these cases. The stromal cells did not stain for CEA.

Only one of six duct carcinomas stained for S-100 protein, and the staining was focal and weak. The myoepithelial cells and dendritic macrophages were brightly positive in these cases. The stroma failed to stain for S-100 protein.

Vimentin and desmin MoAb failed to stain either the epithelium or the stroma in all six cases of IDC. Internal positive controls (fibroblasts and smooth muscle) were positive in these cases.

The epithelium in both cases of CP stained positively for cytokeratin with anti-AE-1/3 and CAM 5.2. The cytoplasmic staining pattern was bright and diffuse (Fig. 7). The spindle cell component failed to react with either of these antibodies.

The epithelium in one of two cases of CP stained weakly with antibodies to vimentin, with a diffuse cytoplasmic distribution. The spindle cells in both cases also stained for vimentin. The staining reaction was weak in the formalin-fixed tissue, but bright in the tissue fixed in 95% ethanol.

Anti-CEA, S-100 protein and desmin failed to react with either the epithelial or spindle cell components in both cases of CP. The myoepithelial cells in both cases, however, stained for S-100 protein (Fig. 8).

Discussion

Since the advent of commercially available immunoperoxidase kits, and more recently, MoAb, a plethora of papers has appeared in the medical literature supporting the usefulness of immunohistochemistry in diagnostic surgical pathology. Several theories of histogenesis have also been proposed for various tumors based on immunoperoxidase findings and in an effort to further classify neoplasms. While malignant neoplasms may recapitulate some features of the alleged cell of origin (oncogeny recapitulates ontongeny?), this is not always the case. Tumor cells may differentiate or fail to differentiate (dedifferentiate) in an orderly manner.

Furthermore, variable and/or discrepant reaction patterns have been reported between MoAb raised in one species and polyclonal Ab raised in another species (e.g., S-100 protein, Vanstapel et al. 1986) against the same antigen, implying that different epitopes may be recognized by different antibodies. In addition, the varying reaction patterns of antibodies may reflect a different functional or metabolic state of the proliferating cell and not be indicative of the cell of origin (Vanstapel et al. 1986). Despite these occasional limitations of the immunoperoxidase technique and its ability (or lack thereof) to elucidate a cell of origin in tumors, a comparison between breast neoplasms with certain similar epithelial or stromal features was undertaken.

In four cases of SCB in this study, both the epithelial and sarcomatoid (specifically chondroid) components stained for S-100 protein. The presence of S-100 protein in benign and neoplastic cartilage is well recognized and could explain the staining of the sarcomatoid component in four of the six cases of SCB. However, the epithelial staining for S-100 protein in four cases of SCB and one case of IDC remains unexplained. Since the myoepithelial cell (and possibly terminal duct epithelium) is the only component of normal breast that stains for S-100 protein (Nakajima et al. 1982) it is possible that the cells of both SCB and IDC are recapitulating or expressing evidence of their myoepithelial origin. Alternatively, as suggested by Vanstapel et al., this variable staining pattern may reflect a varying functional state of the neoplasm.

Vimentin MoAb stained the epithelial component in two cases and the sarcomatoid component in four cases of SCB. The epithelial component of the same two cases of SCB that stained for vimentin also stained for cytokeratin. The presence of vimentin in the sarcomatoid component of SCB and the co-expression of keratin and vimentin in the epithelial component of SCB support the concept of sarcomatoid (or pseudosarcomatous) metaplasia versus induction of a stromal sarcoma by carcinoma. Co-expression (or simultaneous expression) of intermediate filaments has been observed in sarcomatoid carcinomas, mesotheliomas and some epithelial neoplasms (Holthoeffer et al. 1983; Miettinen et al. 1984; Blobel et al. 1985; Gould 1985; Miettinen et al. 1985).

MoAb CEA stained only the epithelium of IDC while failing to stain that of SCB and CP. It is possible that the malignant epithelial cells of IDC maintain or express glandular differentiation, while the benign epithelium of CP and the less differentiated (or dedifferentiated) epithelium of SCB do not.

Aside from the theoretical and histogenetic implications of immunohistochemistry, there are practical considerations which are perhaps more important from a diagnostic standpoint.

Cytokeratin failed to stain the sarcomatoid component in any case of SCB. The absence of cytokeratin in the sarcomatoid component (while the epithelium stained for cytokeratin) and the presence of vimentin in the sarcomatoid component indicate that the sarcomatoid cells are undifferentiated (or dedifferentiated).

The presence of vimentin in the epithelial component of SCB indicates that either vimentin is not exclusively a marker of mesenchymal cells or that some epithelial cells can have a limited number of mesenchymal characteristics. The presence of cytokeratin in the same cells indicates the necessity of utilizing a panel or battery of MoAb rather than a single MoAb in diagnostic surgical pathology.

Vimentin was detected in the stroma of four cases of SCB and both cases of CP. The brightest staining was seen in alcohol-fixed tissue. Alcohol is reported to preserve the antigenicity of intermediate filaments better than formalin (Gown and Vogel 1984). The failure of more cases of SCB to stain for vimentin may be related to fixation and protein denaturation rather than the absence of vimentin.

The presence of S-100 protein (as detected by polyclonal antibodies) in the malignant epithelial cells of SCB has important implications for the surgical pathologist. Breast carcinoma could be potentially confused with melanoma, particularly if a search for cytokeratin is not conducted at the same time. Those cases of metastatic carcinoma in the axillary lymph node(s) of females for which no primary site is known are particularly prone

to be misdiagnosed as melanoma. We have recently observed two such cases where the axillary lymph node from a female stained for S-100 protein and keratin. The presence of S-100 protein in breast carcinomas has been previously reported, although this is not widely recognized (Nakajima et al. 1982; Kahn et al. 1983; Lee et al. 1986).

SCB has been shown to be more aggressive than IDC of similar stage (Smith and Taylor 1969; Huvos et al. 1973; Kaufman et al. 1984). Those patients with neoplasms that are predominantly pseudosarcomatous have worse prognoses than those with predominantly epithelial neoplasms. In addition, visceral metastases are frequently entirely sarcomatoid in these patients, leading to confusion for the clinician and the pathologist (Kaufman et al. 1984). In view of the clinical implications of this diagnosis, it is important to recognize SCB.

Although immunohistochemistry has contributed greatly to our understanding of the pathogenesis of some neoplasms and diagnostic surgical pathology, its pitfalls and limitations need to be recognized. Most important of all, the immunohistochemical findings must be interpreted in the context of the hematoxylin- and eosin-stained tissue sections and the clinical setting.

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