

Immunohistochemical and morphologic evaluation of primary cutaneous apocrine carcinomas and cutaneous metastases from ductal breast carcinoma

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Abstract

The differential diagnosis between a primary cutaneous apocrine carcinoma (CAC) and a cutaneous metastasis from a breast carcinoma can be a very difficult task if it is only made on morphologic bases. Concerning adnexal tumors (in general), there have been many attempts to define an immunohistochemical panel, and while a definition is useful in certain respects, the series presented often times does not include examples of CAC. Other times, CAC seems to behave in an odd way in an immunohistochemical context; they behave differently than other adnexal tumors, and this in turn adds a grade of confusion to the differential diagnosis of a cutaneous metastasis. In the current study, we include seven cases of primary cutaneous apocrine tumors, including one carcinoma *in situ*, five infiltrating carcinomas, and one adenoma. Additionally, we examine the expression of estrogen receptors (ER), progesterone receptors (PR), and c-erbB-2. We also study myoepithelial markers, such as p63, D2-40, and SMA in them, as well as the pattern of expression of the following cytokeratins: CK7, CK8, CK18, CK19, CK5/6 and 34betaE12. On the other hand, we examine the expression of six immunohistochemical markers (ER, PR, p63, mammaglobin, CK5/6 and D2-40) in 30 cases of cutaneous metastases from breast carcinoma, ductal type. None of our infiltrating primary CAC expressed ER or PR, while the cutaneous metastasis expressed the markers in 90% of the cases. D2-40 was expressed in 60% of the infiltrating CACs, while the metastases were either negative (93.33% of the cases) or positive with luminal reinforcement. Mammaglobin was a very useful marker, expressed by 66.66% of the metastases, and by only one CAC (and in less than 10% of the cells). None of the metastases were positive for p63, while 60% of the CAC expressed the marker. CK 5/6 was also expressed by a high percentage of our CACs (80%), while it was seen in only 6.66% of the metastases. We found SMA as a very useful tool in diagnosing an invasion in CAC. Regarding the expression of c-erbB-2, all of our cases had a value of either 0 or 1.

Keywords: cutaneous apocrine carcinoma, p63, cribriform carcinoma, cutaneous metastasis, mammaglobin, D2-40.

Introduction

Difficulties in the differential diagnosis of the cutaneous apocrine carcinoma (CAC) on morphologic bases

Apocrine cutaneous carcinoma is not a frequent neoplasia [1]. Most publications are single cases [2–6], although some long series have been presented [1, 5]. Studies of the immunophenotype of these neoplasias are even rarer, and are mainly focused on a select group of antibodies.

There are two main problems in cutaneous pathology regarding CACs. First, the differential diagnosis with a metastasis from a breast carcinoma is not an easy task. Such a diagnosis is so difficult when based only on morphology, that some have claimed, “*apocrine carcinoma is otherwise indistinguishable from apocrine mammary carcinoma metastatic to the skin or apocrine carcinomas arising in ectopic breast tissue in the axilla*” [7]. The criterion that the patient does not have breast cancer is many times a requisite to consider an apocrine carcinoma as primary cutaneous [1]. Since one of the main locations of CAC is the axilla [1], the differential diagnosis with a “*carcinoma developed from an axillary extension from the breast*” [8], or with a carcinoma

originating from ectopic mammary tissue [9–14] should also be considered. In this sense, several authors have pointed out the importance of clinical information [9–14].

The second problem is to determine if an apocrine cutaneous tumor is benign or malignant. An infiltrative margin and/or cytologic pleomorphism are unacceptable for benignancy [1], and are considered as a sign of malignancy but diagnosing malignancy vs. benignancy is not always easy.

Immunohistochemical tools in the differential diagnosis between primary CAC and a cutaneous metastasis

There are certain morphologic clues in the differential diagnosis between a primary tumor and a metastasis. For instance, evidence of an *in situ* carcinoma of the sweat gland would support diagnosis of a primary cutaneous tumor [14–16]. In immunohistochemistry, no marker has been categorized as “determinant” in a differential diagnosis. Although several markers have been proven to be useful when facing adnexal tumors, they have not been so useful when applied to CAC. That happens partly because CAC is so rare, and not many studies have been performed in this area. In the

following section, we comment upon some of the previous studies.

Carcinoembryogenic antigen (CEA) and gross cystic disease fluid protein (GCDFP)-15

GCDFP-15 usually stains apocrine adnexal glands in axillary and anogenital skin [14, 17, 18]. However, it is also a good marker of breast cancer [19, 20]. GCDFP-15 has demonstrated a high specificity for mammary origins in studies of tumors with unknown sites (98–99%) (as long as skin adnexal and salivary gland cancers can be excluded on clinical grounds) [17], although their sensitivity is not that high (50–74%) [21, 22]. In general, a phenotype CEA+ (moreover if GCDFP-15-) favors a metastasis [1–3].

There are examples in literature that follow this rule, of CAC with weak positivity for GCDFP-15 [11], or even GCDFP-15-negative [9, 18, 23–25]. However, there are also examples that break this rule. For example, in one study, GCDFP-15 failed to mark four ductal breast carcinomas, while it was expressed by the only CAC being studied [26]. Other authors demonstrated that GCDFP-15 was positive in less than half of the cases studied of cutaneous metastasis of breast carcinoma [27].

Hormonal receptors

Some hormonal receptors have been considered as good markers of primary tumors in the breast [19, 20]. In breast carcinomas, estrogen receptors (ER), progesterone receptors (PR), and androgen receptors (AR) are expressed in 70–75%, 54–59% and 60–70% of the cases, respectively [28–31]. It has also been suggested that an immunophenotype AR+, ER-, PR- would support an apocrine origin [32, 33]. Such is the phenotype of normal apocrine cells, and of extra-mammary Paget's disease, which is alleged by some to have an apocrine origin [32]. In a study of cutaneous metastases, most cases from the breast (82%) expressed AR [34]. Nevertheless, certain examples in literature do not follow these rules. For instance, cases of cribriform carcinoma are ER+ [35], and in one study, 62% of the CACs were ER+ [1]. There are also cases of papillary CAC with an expression of PR [18] and in one study, 60% of CACs were PR+ [1]. Expression of AR has been found in up to 36% of the cases studied [1].

Protein p63

The protein p63 has been considered as a useful tool in the differential diagnosis between several adnexal tumors and cutaneous metastases: the expression of the marker would favor a primary cutaneous tumor [36–38]. However, the results seem not to have been as spectacular when applied to CAC. In one study, Ivan *et al.* included two cases of CAC and two cutaneous metastases mimicking CAC [37]. In one of these cases, the staining was not available, and in the other, only 5 to 25% of the tumoral nuclei were stained [37]. From the two metastases, one stained in a similar way to the CAC (5 to 25% of the tumoral nuclei) and the other was negative [37].

A recent study on 113 cases (59 primary adnexal carcinomas and 54 cutaneous metastases) concluded that

an immunophenotype p63+, CK15+, nestin+ and D2-40+ favored a primary cutaneous origin vs. a metastasis [39]. However, the two cases of CAC that were included in the study were negative for the four markers [39].

Cytokeratins (CK)

There are studies that suggest the use of CK7 in the differential diagnosis between cutaneous metastases and primary adnexal tumors. While a focal expression of CK7 would suggest a primary cutaneous tumor [23], a diffuse staining would favor a metastasis [23]. Again, there are examples in large series in which CAC do not accomplish this rule [23], and there are also cases of CAC that show a diffuse staining [35].

Regarding CK5/6, it has been suggested that a diffuse staining would support a primary cutaneous neoplasia [40], while negativity or a weak expression of the marker would favor a metastasis [40].

Epidermal growth factor receptor (EGFR)

The expression of EGFR by the tumor has been suggested as another clue more indicative of sweat gland carcinoma than of breast carcinoma [41]. However, there are examples breaking these rules [42]. Moreover, 22% of breast carcinomas are EGFR+ [41, 43].

Combined use of mammaglobin and podoplanin

Mammaglobin is expressed by normal tumoral cells and by breast carcinomas [14, 44–47]. The latter commonly expresses it in a diffuse way [48]. Mammaglobin A seems to be more specific of breast and gynecologic organs, whereas mammaglobin B is found in many tumors, such as some gastrointestinal carcinomas [49].

In one study, it was suggested that although negativity for mammaglobin did not help in the differential diagnosis between a primary cutaneous carcinoma and a metastasis, the expression of mammaglobin in more than 10% of the cells would suggest a metastasis [50, 51].

While negativity for D2-40 (which marks podoplanin) is not considered to be contributive, a basilar staining would suggest a CAC, while luminal reinforcement would suggest a metastasis [51].

In a recent study, Plaza JA *et al.* demonstrated the use of p63 and podoplanin in the differential diagnosis between several primary cutaneous tumors and cutaneous metastases [52]. However, they did not have a case with a cutaneous apocrine carcinoma. Liang H *et al.* did not observe any CAC in their series on podoplanin, which included 78 cutaneous tumors and 15 metastases [53].

Markers to determine malignancy in a CAC

Diagnosing a cutaneous apocrine tumor as “malignant” is not always straightforward. Finding an infiltrative margin and/or cytologic pleomorphism unacceptable for benignancy [1] are always very useful clues.

In the field of immunohistochemistry, there are many lessons that we can learn from the literature on apocrine lesions in breast pathology, a field that has been more widely investigated than the equivalent malignancy in

skin: several myoepithelial markers have been used to confirm that a certain tumor is “non-invasive”. Some examples are p63, alpha-smooth muscle actin (SMA), or smooth muscle myosin heavy chain. Also, several types of keratins have been used with the same goal [54]. Such is the case of high molecular weight keratins (CK5/6, CK14, 34betaE12), which usually show a mosaic-like pattern of expression in benign hyperplastic lesions, due to the fact that myoepithelial cells, as well as basal cells, mingle with luminal cells. Only a minority of atypical ductal hyperplasias of the breast, show cells that express high molecular weight keratins, and such markers apparently do not mark the basal cells in atypical ductal hyperplasia, while they do mark common hyperplasias and are negative in intraductal carcinoma [54]. In general, the restrictive expression of one only type of keratins has an interpretative value of malignancy or atypia, since it is found in atypical ductal hyperplasias or in low-grade *in situ* carcinomas. For instance, Shamloula MM *et al.* found that p63 was expressed in the peripheral rim of the myoepithelial cell layer in cases of atypical ductal hyperplasia and ductal carcinoma *in situ* [55], while the invasive ductal carcinoma showed occasional gabs. Occasional malignant cells expressed the marker in cases of invasive ductal carcinoma. Similar results have been found for SMA, although this marker, contrary to what is seen with p63, reacts with fibroblasts and with the vessel walls [56]. Some isolated cases of apocrine carcinomas of the breast have shown an absence of SMA [57].

Regarding CK5/6, for instance, Ding Y *et al.* found that in cases of benign breast lesions, the positive rate of CK5/6 expression was 100% [56]. In cases of atypical ductal hyperplasia, there were few positive cells in the ducts. When the lesion was a carcinoma (*in situ* or infiltrative), there was no expression of CK5/6 [56].

Myoepithelial markers have also been found as valuable in the confirmation of tumoral infiltration. Markers for basement membranes have failed in several of these cases, since some invasive tumors produce basement membrane components [58, 59]. However, in cases of *in situ* carcinoma, myoepithelial markers are useful to demonstrate the basilar layer and to determine the duct integrity and the absence of invasiveness. There are several useful markers for such matter, such as CK5, CK14, CK17, CD10, S100, SMA, smooth muscle myosin heavy chain and p63 [35, 42, 54, 60–69]. Out of them, the most sensitive and specific are smooth muscle myosin heavy chain, calponin, and p63 [17]. In breast pathology, it is advised to combine two markers, and calponin and p63 are the most recommended [70]. Recent work in breast pathology has demonstrated that benign and noninvasive apocrine lesions can show reduction and occasional complete loss of ME cells [70].

Markers of subtyping breast tumors, applied to CAC

The mammary gland is made up of three main types of cells: the luminal, basal and myoepithelial cell. While luminal cells express CK7, CK8, CK18 and CK19, basal

cells express CK5/6, CK14 and CK17. Myoepithelial cells, on the contrary, express CK5, CK14, CK17, SMA, calponin, and p63. Mammary carcinomas have been categorized according to their molecular features, mainly in six subtypes [71]. On the other hand, in practical terms, a simple immunohistochemical study is able to help in the subcategorization of such subtypes (Figure 1) [72].

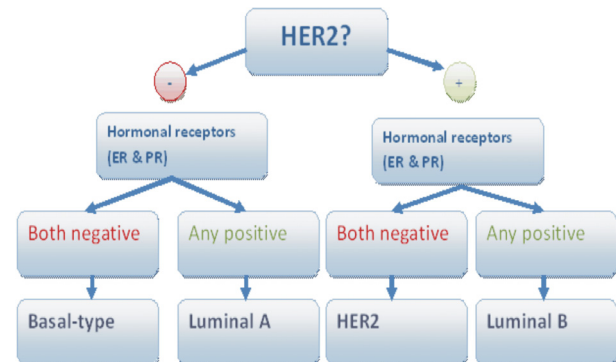


Figure 1 – Immunophenotyping of ductal carcinomas of the breast.

However, it was suggested that apocrine carcinomas of the breast could represent a different group on their own: Farmer P *et al.* demonstrated that there was an “apocrine” subtype, with increased androgen signaling and frequent HER2 amplification [73]. Gene expression microarray studies demonstrated that the molecular pattern of apocrine carcinomas would be different from common luminal and basal cell breast carcinoma subtypes [73–76]. Among other peculiarities, mammary apocrine carcinomas show expression of AR along with increased human epidermal growth factor receptor 2 (*HER-2/neu*) gene signaling [73, 76]. There is also a study on apocrine carcinoma of the breast in which the authors concluded the existence of two subtypes [77]: (A) the pure apocrine carcinomas (ER-, AR+); and (B) the apocrine-like carcinomas, with morphologic apocrine appearance, but with an immunohistochemical pattern that is different from those previously mentioned [73, 78–80]. Pure apocrine carcinomas show consistent over-expression of either EGFR or *HER-2/neu* [77]. These authors also demonstrated that breast apocrine carcinomas would be included in the categories of *HER-2*-over-expressing or triple negative types [77]. Contrary to this finding, apocrine-like carcinomas predominantly belong to the luminal molecular phenotype (A and B) [77].

Aims of the study

- To examine the expression of ER, PR and c-erbB-2 in cases of CAC. To perform chromogenic *in situ* hybridization (CISH) to study *HER-2/neu* amplification in those cases with HercepTest™ of 2+.
- To investigate the evidence of myoepithelial markers (p63, D2-40, SMA) in cases of CAC.
- To investigate the pattern of expression of the following types of cytokeratins (CK) in cases of CAC: CK7, CK8, CK18, CK19, CK5/6, and 34betaE12.
- To investigate the expression of ER, PR, p63, mammaglobin, CK5/6, and D2-40 in 30 cases of cutaneous metastases from breast carcinoma, ductal type.

Materials and Methods

We included in the study six cases of CAC (Table 1): five infiltrating CAC (three common ones and two cribriform ones) and one *in situ* CAC. Three of the cases were sent to us as the result of a plea that was published in a journal [81] (see “Acknowledgments”). We did not include cases of the so-called variant signet-ring cell carcinoma of the skin [82–84]. For comparison, we also included an apocrine adenoma in our study.

Table 1 – Cases of apocrine tumors included in the current study

Case No.	Gender	Age [years]	Size of the tumor [cm]	Location	Morphologic diagnosis
1.	M	76	0.5	Eyelid, right upper	“ <i>In situ</i> ” CAC
2.	F	63	0.9	Scalp, left side	Infiltrating CAC
3.	F	38	–	Axillary nodule	Probably metastasis of CAC
4.	F	62	1	Right popliteal fossa	Infiltrating CAC, cribriform variant
5.	F	59	1	Axillary nodule	Apocrine adenoma
6.	F	58	1.2	Right knee	Infiltrating CAC, cribriform variant
7.	M	53	1.3	Axillary tumor	Infiltrating CAC

CAC: Cutaneous apocrine carcinoma.

In our cases of CAC, we studied the following antibodies: gross cystic disease fluid protein-15 (GCDGFP-15) (Dako, clone 23A3), cytokeratin (CK) 5/6 (Dako, clone D5/16 B4), CK8 (Dako, clone 35betaH11), CK7 (Dako, clone OV-TL 12/30), CAM 5.2, 34betaE12 (Dako, clone 34βE12), p63 (Menarini), smooth muscle actin (SMA) (Dako, clone 1A4), estrogen receptors (ER) (Dako, clone 1D5), progesterone receptors (PR) (Dako, clone PgR 636), mammaglobin (Dako, clone 304-1A5), podoplanin (D2-40) (Dako, clone D2-40), S100 protein (Dako), c-erbB-2 oncoprotein (Dako) and androgen receptors (AR) (Dako, clone AR441). In some cases, other additional antibodies were investigated, such as bcl-2 oncoprotein (Dako, clone 124), vimentin (Dako, clone V9), CD117 (Dako), CK AE1/AE3 (Dako clone AE1/AE3), Ki67 (Dako, clone MIB-1), CK20 (Dako clone Ks20.8), epithelial membrane antigen (EMA) (Dako, clone E29), CD15 (Dako, clone Carb-3), p53 (Dako, clone DO-7), and carcinoembryogenic antigen (CEA) (Dako, clone II-7).

We also studied the standardized immunostaining HercepTest™ (Dako, anti-human HER2 protein).

The results of the antibody c-erbB-2 were evaluated with similar criteria as what is admitted by the *American Society of Clinical Oncology/College of American Pathologists* and the *UK Guideline Recommendations* for HER-2 classification for ductal breast carcinoma [85, 86].

From our archives, we recovered 30 cases of cutaneous metastasis of breast carcinoma, ductal type. We specifically excluded cases of lobular breast carcinoma. On such cases of cutaneous metastasis, we performed an immunohistochemical study for the following antibodies (same clones and companies as above): ER, PR, CK5/6, D2-40, and p63.

Results

Table 1 shows the gender and age of the patients with cutaneous apocrine tumors included in this study, as well as the sizes of the lesions, locations of the tumors, and diagnoses rendered. Five of the tumors were infiltrating CAC (two were of the cribriform variant) and one of them was an *in situ* CAC. The other apocrine tumor was an apocrine adenoma.

All CACs were well-differentiated with a predominant glandular pattern. Signs of apocrine differentiation, such as apical snouts, were easily found (Figure 2, top left). All of the carcinomas showed cellular atypia and frequent mitoses (Figure 2, top right) (some of the latter atypical) (Figure 2, medium right). Areas of necrosis and apoptosis were also common features (Figure 2, medium left and bottom left). In all the infiltrating cases, invasive tumoral growths in the stroma were easily found (Figure 2, bottom right).

Case No. 3 was an axillary tumor, and showed a metastatic lymph node deep in the biopsy (Figure 3) in continuity with the superjacent tumor. In this case, we also observed many lymphatic invasions (Figure 3, bottom right). Since a primary breast carcinoma was clinically excluded in the patient (a 38-year-old woman), we decided to include this case in the study, but with the note “suspicious of metastasis”.

Case No. 1 was symmetric, well-limited and with no morphologic signs of infiltration (Figure 4). However, signs of architectural, as well as cytologic atypia were easily found. Therefore, the diagnosis of *in situ* CAC was rendered.

Two of our cases accomplished the criteria of the cribriform variant of CAC, with an obvious cribriform pattern, interconnected tumoral groups that varied in size and shape and had no deposits of basement membrane (Figure 5). Case No. 4 was published in a previous report [35].

Table 2 shows the results of the immunohistochemical study performed on the cutaneous apocrine tumors.

GCDGFP-15 was strong and diffuse in three out of the five infiltrating cases. Moreover, one of the negative cases was Case No. 3 (“suspicious” for metastasis). Estrogen and progesterone receptors were only strong and diffuse in Case No. 1. Since c-erbB-2 was evaluated as either 0 or 1+, no additional studies, with CISH for amplification of HER2-neu gene, were considered. CK7 was expressed in several of our CACs, in spite of previous literature claiming it as a marker suggestive of metastasis. On the contrary, CK5/6 marked three out of five of the infiltrating cases, and again, Case No. 3 was negative for the marker (Figure 6). D2-40 did not show a luminal expression in any of our cases. Two of the infiltrating cases showed basilar expression, as well as the adenoma.

The pattern seen with mammaglobin was considered non-contributing, since neither was persistently negative or was expressed in less than 10% of the cells.

Only two of our cases accomplished the immunohistochemical pattern required to be considered “pure” apocrine carcinomas (ER+, PR-, ER-). One of them was Case No. 3. The other was Case No. 2. Due to the morphologic suspicion of “metastasis” that we had in Case No. 3, we decided to consider Case No. 2 as the only “immunohistochemically pure” apocrine case in our series (Figure 7).

The marker p63 was expressed in two infiltrating

cases, but failed to stain the three other infiltrating cases. One of the cases that expressed the marker was Case No. 2 (the immunohistochemically “pure” apocrine carcinoma).

Regarding the markers intended to be useful in demonstrating infiltration, the results for p63, 34betaE12, CK5/6, and S100 were not distinct in infiltrating vs. non-infiltrating lesions. However, SMA was only expressed in a continuous layer in the adenoma, as well as in the *in situ* carcinoma. In the infiltrating cases, it was either negative, or expressed in a discontinuous pattern (Case No. 2).

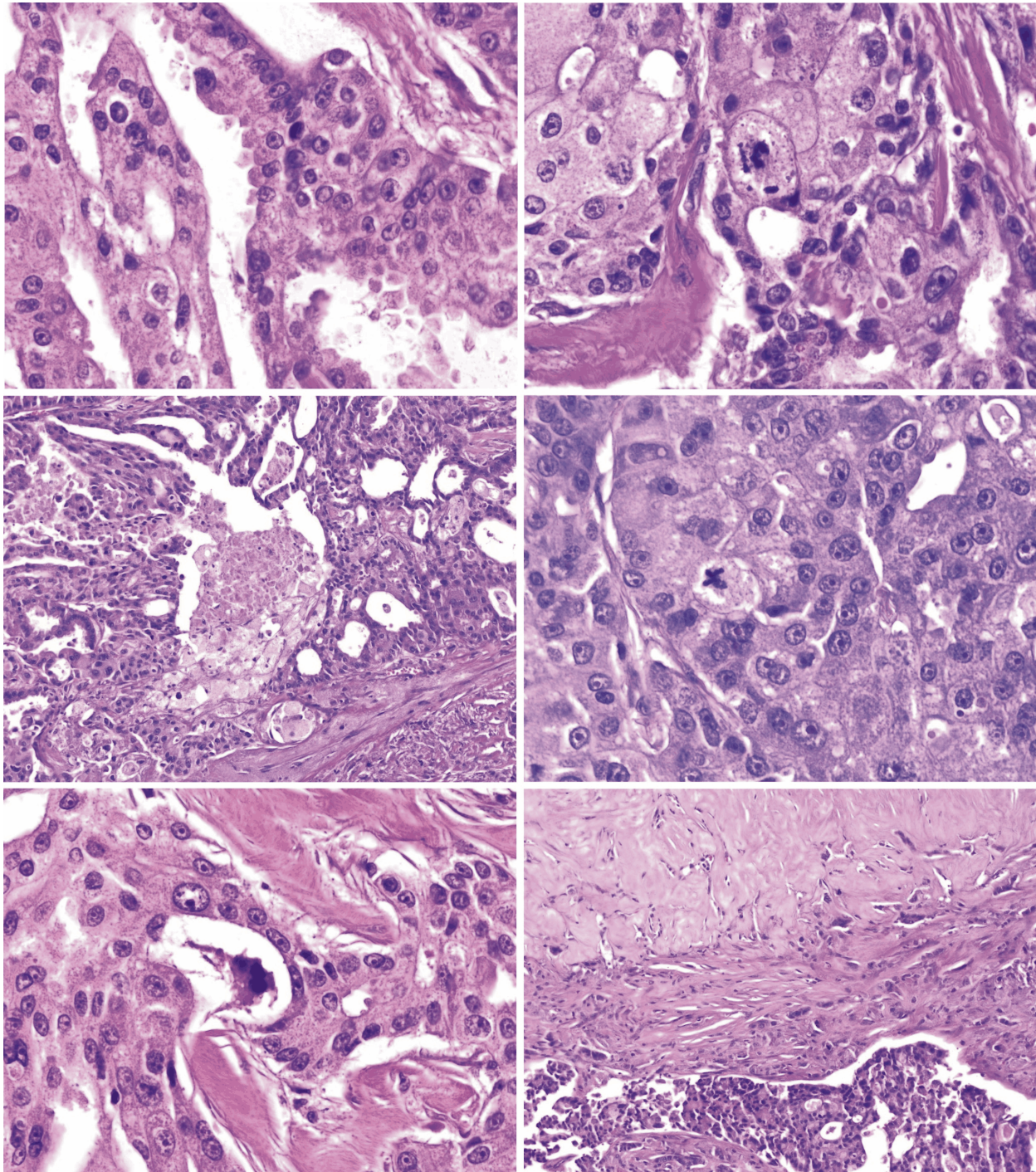


Figure 2 – Typical images of the primary cutaneous apocrine carcinomas in our study. Apocrine differentiation in the form of apical snouts was easily found (top left). Features of malignancy, such as frequent mitoses (top right), necrosis (medium left), atypical mitoses (medium right), apoptosis (bottom left) and invasion in the adjacent stroma (bottom right) were common findings.

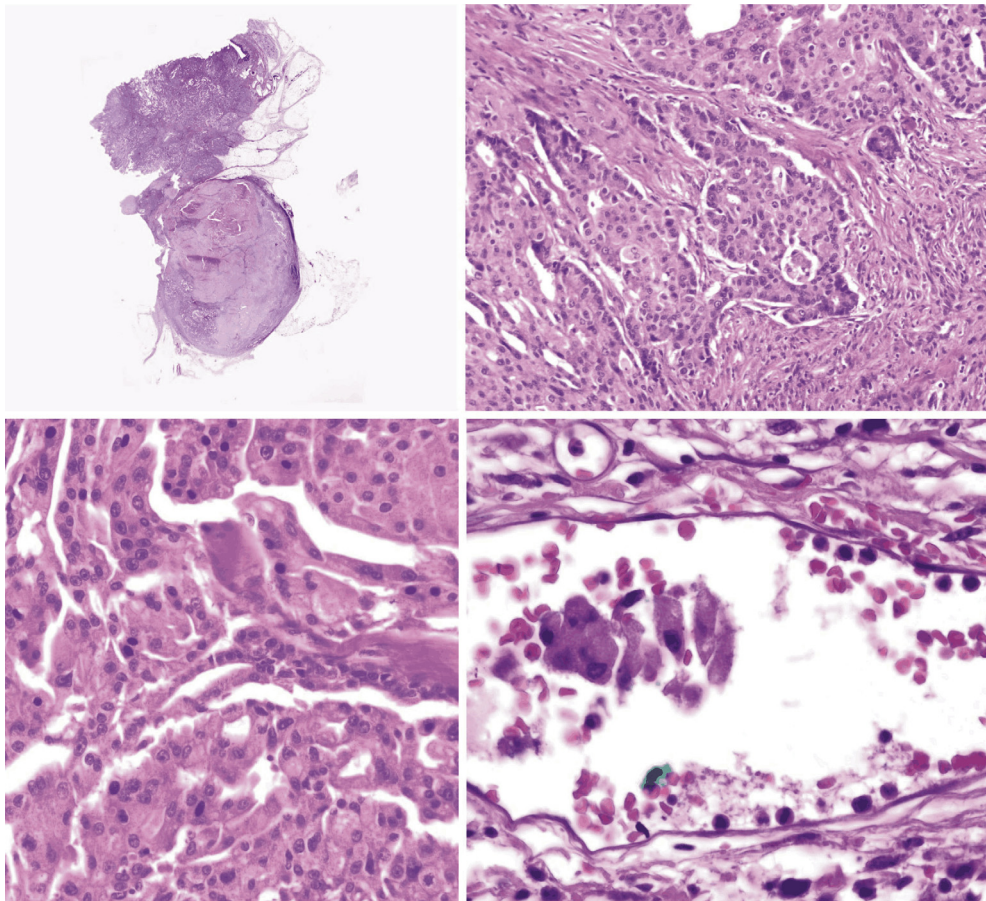


Figure 3 – Case No. 3 was peculiar in many ways. A metastatic lymph node was found in the subcutaneous tissue of the biopsy, and there was a connection between the infiltrating carcinoma and the lymph node. Tumoral invasions of lymph vessels were also common. Therefore, we were suspicious of the possibility that this case could actually be a metastasis, although there was no clinical evidence of this.

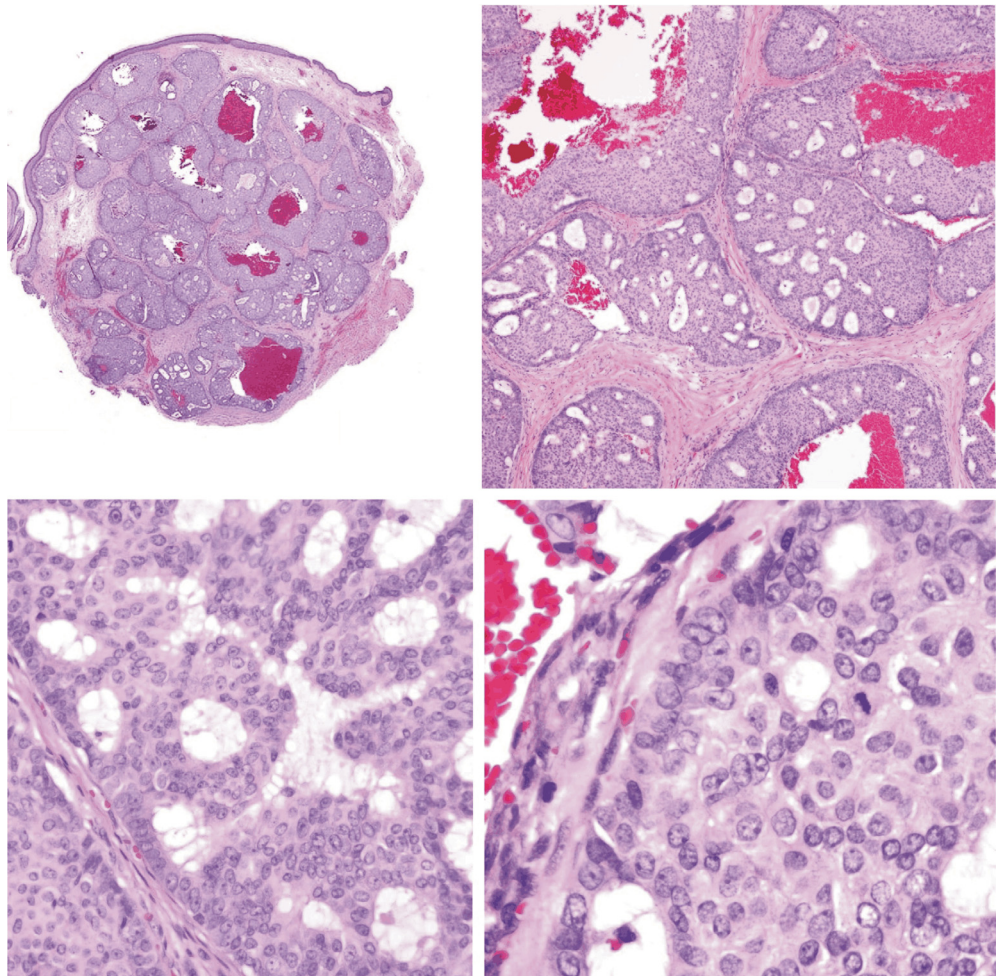


Figure 4 – Case No. 1 accomplished all the criteria of what has been considered as a primary cutaneous apocrine carcinoma in situ, such as being a well-delimited lesion (top left), and having architectural as well and cytologic atypia.

Figure 5 – Two of our cases belonged to the cribriform variant of CAC, with a cribriform pattern all over the tumor.

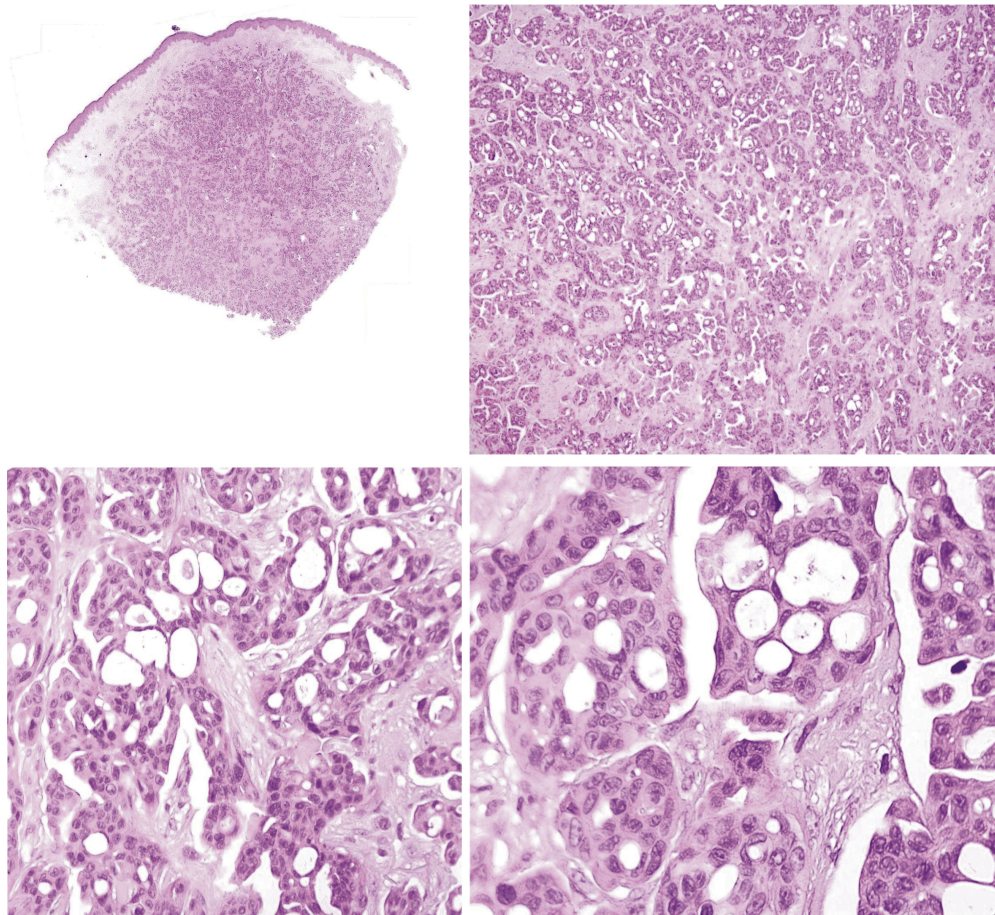


Table 2 – Immunohistochemistry performed in our seven apocrine tumors

Antibody	Case No.						
	1	2	3	4	5	6	7
GCDPF-15	Strong in scattered cells	Strong and diffuse	Negative	Negative	Strong and diffuse	Strong and diffuse	Strong and diffuse
CK5/6	Strong and diffuse	Strong and diffuse	Negative	Negative	Strong and diffuse	Strong and diffuse	Strong and diffuse
CK8	Strong and diffuse	Moderate and diffuse	Strong and diffuse	Negative	Negative	Strong and diffuse	Negative
CK7	Patchy and strong	Strong and diffuse	Strong and diffuse	Negative	Strong and diffuse (mainly luminal)	Strong and diffuse	Strong and diffuse
CAM 5.2	Strong and diffuse	Strong and diffuse	Strong and diffuse	Strong and diffuse	Strong and diffuse (mainly luminal)	Strong and diffuse	Scattered cells
34betaE12	Patchy and strong	Strong and diffuse	Strong and diffuse	Negative	Strong and diffuse	Strong and diffuse	Strong and diffuse
P63	Scattered cells, peripheral layer of many of the lobules	Strong (aprox. 40% of the cells), only basilar cells	Negative	Negative	Strong (basilar layer)	Negative	Strong (basilar layer)
SMA	Strong; peripheral cells, completely surrounding the tumoral nodules	Strong; peripheral cells, incompletely surrounding many of the tumoral nests	Strong and diffuse	Negative	Strong; only surrounding most of the nests	Negative	Negative
ER	3; strong (aprox. 100% of the tumoral cells)	0	0	0; strong (aprox. 10% of the tumoral cells)	0	0	0
PR	3; strong (aprox. 100% of the tumoral cells)	0; patchy but moderately to strong; aprox. 10% of the cells	0	0	0	0	0

Antibody	Case No.						
	1	2	3	4	5	6	7
<i>HercepTest™</i>	0	1+	1+	1+	0	0	0
<i>c-erbB-2</i>	0	0	0	0	0	0	0
<i>Mammagobin</i>	Negative	Negative	Negative	Negative	Negative	Negative	Positive in less than 10% of the cells
<i>D2-40</i>	Scattered cells in the basilar layer	Basilar cells of most of the tumoral structures	Negative	Negative	Strong, basal layer	Negative	Strong, basal layer
<i>S100</i>	Negative	Negative	Negative	Strong, basal layer	Scattered cells in basal layer	Scattered cells in basal layer	Scattered cells in basal layer
<i>AR</i>	Negative	Strong and diffuse	Strong and diffuse	Negative	Negative	Negative	Negative
<i>Other antibodies performed</i>	Bcl-2: Strong and diffuse	Vimentin: Strong and diffuse CD117: Moderate to strong	–	AE1–AE3: Strong and diffuse MIB1: 10% CK20: Negative EMA: Positive diffuse and strong basilar diffuse CD15: Negative P53: 2% CEA: Negative	–	–	–

GCDFP-15: Gross cystic disease fluid protein; CK: Cytokeratin; SMA: Smooth muscle actin; ER: Estrogen receptors; PR: Progesterone receptors; AR: Androgen receptors; EMA: Epithelial membrane antigen; CEA: Carcinoembryonic antigen.

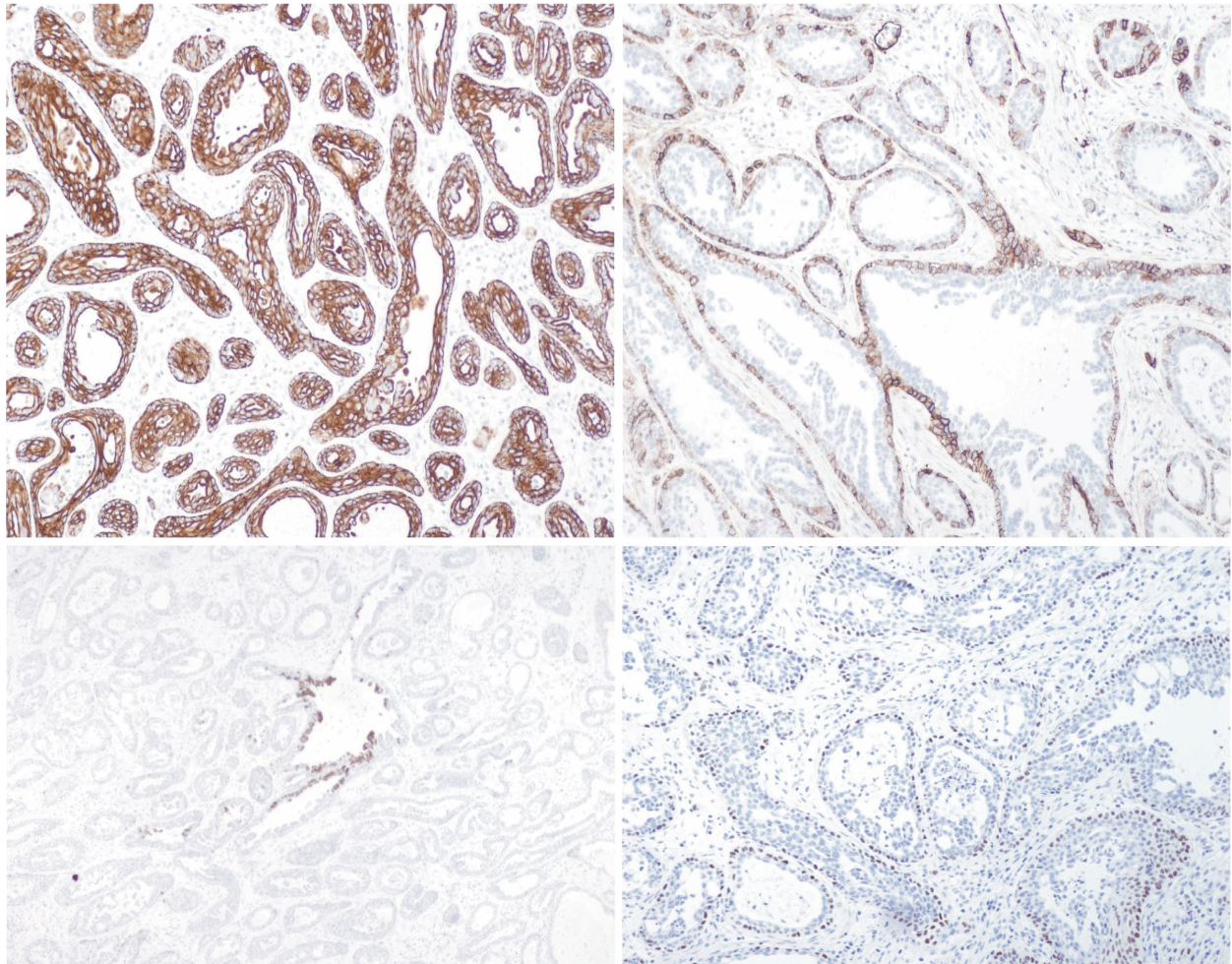


Figure 6 – Some of the immunohistochemical findings in our cases of CAC. CK5/6 in Case No. 5 (top left); D2-40 in Case No. 7 (top right); mammaglobin in Case No. 7 (bottom left); and p63 in Case No. 7 (bottom right).

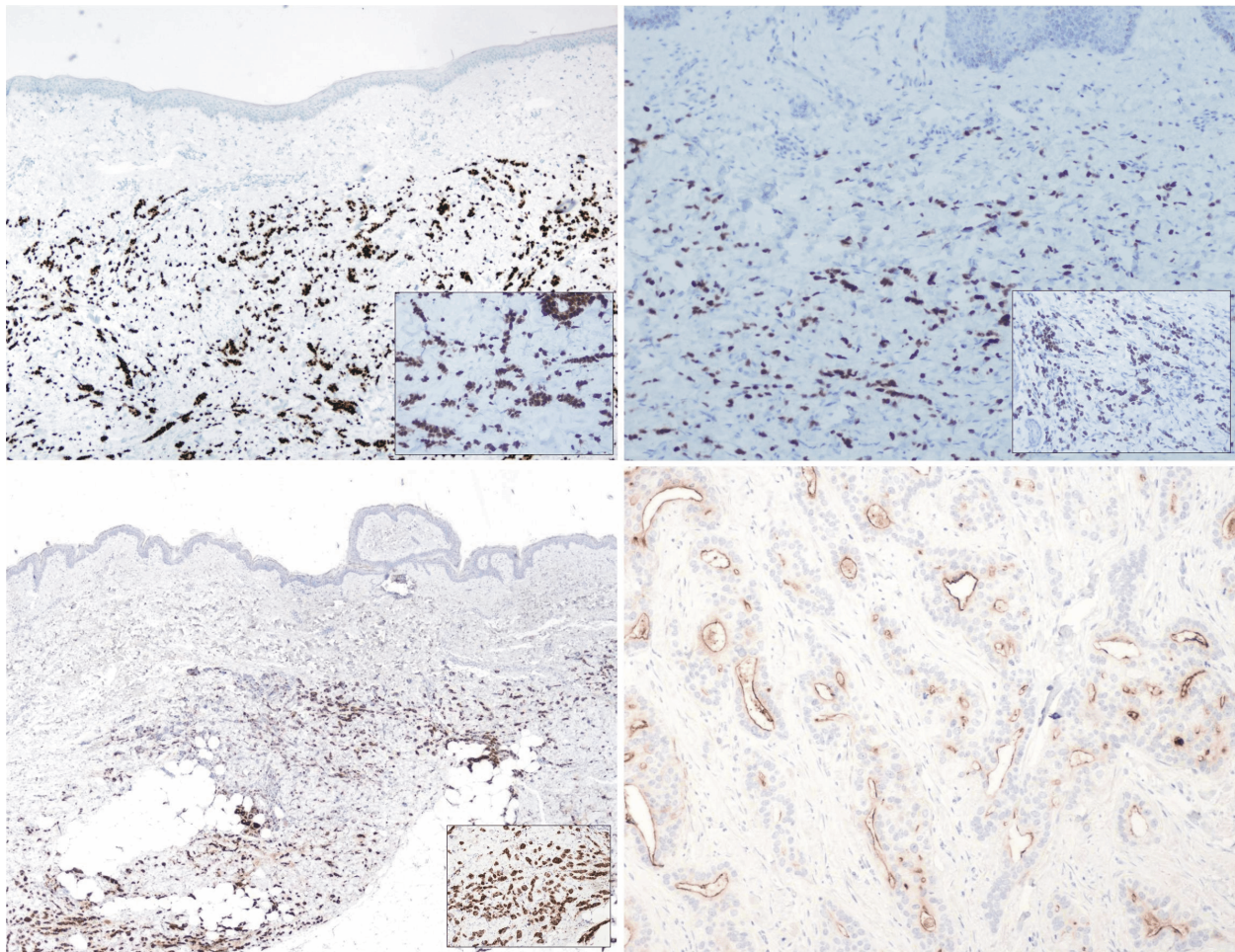


Figure 7 – Some of the immunohistochemical findings in our cases of cutaneous metastases. ER in Case No. 2 (top left); PR in Case No. 6 (top right); mammaglobin in Case No. 18 (bottom left); and D2-40 in Case No. 29 (bottom right).

Table 3 shows the ages of all the patients as well as the immunohistochemical results in the second part of the study (the 30 cases of cutaneous metastases of breast carcinoma).

Mammaglobin expression was strong in 20 (66.66%) cases and in 16 of the cases the marker was expressed

by more than 10% of the tumoral cells. D2-40 was only expressed by two of the metastases and the expression was luminal. CK5/6 was only expressed by two cases.

Regarding the hormonal receptors, ER and PR were each expressed by 90% of the cases.

Table 3 – Cases of cutaneous metastasis from ductal carcinoma of the breast. All patients were females

Case No.	Age [years]	Estrogen receptors	Progesterone receptors	Cytokeratin 5/6	Podoplanin (D2-40)	P63	Mammaglobin
1.	85	1	2	Negative	Negative	Negative	Strong, 5% of the cells
2.	77	3	2	Negative	Negative	Negative	Negative
3.	45	2	2	Negative	Negative	Negative	Strong, 10% of the cells
4.	66	2	1	Strong, 80% of the cells	Negative	Negative	Negative
5.	49	3	2	Negative	Negative	Negative	Strong, 60% of the cells
6.	45	3	3	Negative	Negative	Negative	Strong, 80% of the cells
7.	25	3	3	Negative	Negative	Negative	Negative
8.	41	2	3	Negative	Negative	Negative	Strong, 30% of the cells
9.	52	0	1	Negative	Negative	Negative	Strong, 95% of the cells
10.	49	3	3	Positive	Negative	Negative	Negative
11.	79	0	0	Negative	Negative	Negative	Strong, 100% of the cells
12.	68	2	2	Negative	Negative	Negative	Strong, 100% of the cells
13.	69	3	3	Negative	Negative	Negative	Strong, 30% of the cells
14.	57	0	1	Negative	Negative	Negative	Strong, less than 10%
15.	56	3	2	Negative	Negative	Negative	Negative
16.	46	3	3	Negative	Negative	Negative	Strong, 98% of the cells
17.	83	2	3	Negative	Negative	Negative	Strong, 90% of the cells
18.	47	3	2	Negative	Negative	Negative	Strong, 100% of the cells

Case No.	Age [years]	Estrogen receptors	Progesterone receptors	Cytokeratin 5/6	Podoplanin (D2-40)	P63	Mammaglobin
19.	50	3	2	Negative	Negative	Negative	Strong, less than 10%
20.	51	3	2	Negative	Negative	Negative	Strong, 70% of the cells
21.	56	1	0	Negative	Negative	Negative	Strong, 50% of the cells
22.	86	1	0	Negative	Negative	Negative	Strong, 90% of the cells
23.	37	3	3	Negative	Negative	Negative	Strong, 90% of the cells
24.	64	1	2	Negative	Negative	Negative	Negative
25.	74	3	3	Negative	Negative	Negative	Strong, 50% of the cells
26.	77	3	3	Negative	Negative	Negative	Negative
27.	73	3	2	Negative	Negative	Negative	Negative
28.	64	2	2	Negative	Strong, luminal, 10% of the tumoral cells	Negative	Strong, 50% of the cells
29.	58	3	3	Negative	Strong, luminal, 100% of the tumoral cells	Negative	Negative
30.	–	3	3	Negative	Negative	Negative	Negative

Table 4 shows the comparison between the cases of CAC, which were positive for the six immunohistochemical markers that were also investigated in the cutaneous metastases, and the results observed in the cases of metastases into the skin.

Table 4 – The percentages of cases that were positive for the six immunohistochemical markers studied in cutaneous apocrine carcinomas (CAC) and in cutaneous metastases from breast carcinoma. In cases of CAC, two values are given (A & B), depending if Case No. 3 (morphologically suspicious of metastasis) was considered (B) or not (A)

Immunohistochemical marker	CAC (see legend for explanation on A & B)	Cutaneous metastases (see legend for explanation on C & D)
ER	A: 0/4 B: 0/5	C: 23/30 (76.66%) D: 27/30 (90%)
PR	A: 0/4 B: 0/5	C: 24/30 (80%) D: 27/30 (90%)
Podoplanin	A: 2/4 (50%) B: 3/5 (60%)	2/30 (6.66%) Luminal staining in both cases.
Mammaglobin	A: 1/4 (25%) B: 1/5 (20%) (Immunoreexpression in less than 10% of the tumoral cells in the only positive case).	20/30 (66.66%) In 16 cases, the staining was expressed by more than 10% of the cells.
P63	A: 2/4 (50%) B: 3/5 (60%)	0/30
CK5/6	A: 3/4 (75%) B: 4/5 (80%)	2/30 (6.66%)

ER: Estrogen receptors; PR: Progesterone receptors. In the evaluation of ER and PR in the cutaneous metastases, "C" indicates if values 2 and 3 are considered as positive and "D" if values 1, 2, and 3 are considered positive.

Discussion

CAC is a rare tumor. Because of that, most series of cutaneous adnexal malignancies include few cases of apocrine carcinomas. However, distinguishing a primary CAC from a metastasis from the breast is such a difficult task that it has been claimed that clinical information excluding a breast carcinoma is crucially required. Although there are several reports investigating some panels of antibodies useful in distinguishing a metastasis from a primary cutaneous carcinoma, cases of CAC are either not part of such studies or only a few anecdotic cases are included in them [1, 23, 36–38, 52, 87, 88]. Even in large studies of CAC, not many immunohisto-

chemical markers are studied [1]. To start with, several cases of CAC were reported even before immunohistochemistry was even available [3, 4, 89, 90]. Other cases were published when the panel of antibodies for the differential between a primary tumor and a metastasis was not so well defined. For instance, the work by Paties C *et al.* is exhaustive, and includes 18 antibodies studied in six cases of CAC [5]. However, since the report was published nearly one decade ago, most of these antibodies are not related to the differential diagnosis with a metastasis [5].

As discussed in the introduction, the immunohistochemical markers mainly found useful in adnexal tumors are GCDP-15, D2-40, CK7, CK5/6, ER, PR, mammaglobin, and p63. We tested these markers in four cases of CAC and found that most of them followed the pattern of expression that was expected for a primary cutaneous tumor in many of our cases.

GCDP-15 stained our two cases of infiltrating CACs, as well as one of the cribriform carcinomas. It failed to stain Case No. 3, which we suspected might be a metastasis. Therefore, although we considered it a potentially good marker to include in the panel to distinguish an apocrine carcinoma from a metastasis from a breast carcinoma, the antibody was not available in our laboratory, and so it could not be included in this second part of the study.

In the last years, a molecular classification of ductal breast carcinoma has been achieved [91]. This has allowed doctors to distinguish luminal (A, B and C), Her-2+, basal-like and normal breast-like types of cancers [75, 92–96]. Since molecular tools are not available in all laboratories, a simple immunohistochemical panel, including ER, PR, and HER2neu has been recognized as useful in categorizing these breast tumors (Table 1). This is one of the reasons why we decided to test the HER2neu status by HercepTestTM. We found that all the cases were either negative or 1+. Therefore, since no cases were 2+, CISH was not even considered. This has an important therapeutic meaning: since some have suggested that additional therapy should perhaps be considered in aggressive cases of CAC [1], our results speak against Trastuzumab as a useful therapeutic tool.

Similar to the "immunohistochemical" and "molecular" classification of ductal breast carcinoma

not otherwise specified, “pure” apocrine carcinomas of the breast were defined as those with an immunohistochemical pattern AR+, ER-, PR- [78, 97–99]. This has later been demonstrated on molecular bases [73]. From these claims, we found that if such criteria were applied to cutaneous apocrine carcinoma, the number of “pure” apocrine carcinomas would be even smaller in the literature series. In a report by Robson A *et al.* on 24 CACs, they studied GCDPF in 13 cases, ER in 13, PR in 5, and AR in 11 [1]. In only one of their cases were the three markers simultaneously studied (ER+, PR-, AR+), and this case would therefore not achieve the “immunohistochemical” qualification of “pure” apocrine carcinoma. In our study, only Cases No. 2 and No. 3 would meet such requirements. One of them was Case No. 3, in which we looked with certain reticence and considered it “suspicious for a metastasis”, which was not clinically proved.

One of our cases (Case No. 1) accomplished the criteria of what has been considered in literature as *in situ* apocrine carcinoma [100], despite some claims that such an entity has not yet been described in cutaneous pathology [1]. The differences with an apocrine adenoma are cytologic (atypia, necrosis, number of mitoses), as well as architectural (rigid bridges) [100]. It was interesting to see how this case showed a strong expression of ER and PR. In *in situ* breast carcinoma, expression of ER has been shown in most cases, while PR expression is not so common [101].

We found that from all the markers defined in literature used to evaluate invasions in breast carcinomas, the most useful for us in CAC cases was smooth muscle actin (SMA). It was preserved in our cases of *in situ* CAC, as well in the apocrine adenoma case. On the contrary, it was absent (either partially or totally) in all infiltrating CAC cases. Case No. 3 (suspicious for metastasis) surprisingly showed a diffuse strong pattern.

Two of our cases belonged to the cribriform variant, which was described by Requena L *et al.* in 1998 [102]. We had already published an immunohistochemical study on one of these cases [35]. Cribriform carcinoma is a low-grade apocrine carcinoma with many clinical and morphologic peculiarities on its own [103], involving mainly the upper and low extremities.

Regarding the markers we studied in the cutaneous metastases from the breast carcinoma, we found mammaglobin to be especially useful; in a high percentage of metastases, the marker was strongly expressed and was expressed in more than 10% of the tumoral cells. Contrary to this finding, D2-40 was negative (and therefore not contributive) in most cases; only two cases expressed the marker and they did it with a luminal reinforcement, which has been suggested in literature as a favoring feature of cutaneous metastasis [104].

Cytokeratin 5/6 was negative in nearly all our cases, as expected for metastases, but the strong expression of such a marker by two of our cases adds some caution to the interpretation of CK5/6 (and probably of any marker in this list) as the only immunohistochemically reliable tool.

Regarding p63, the marker did not do very well in our series of primary infiltrating apocrine tumors as a discriminating tool with a metastasis: only half of the CAC cases were positive. However, Case No. 2 (the only “immunohistochemically pure” CAC in our series) expressed the marker. Once aware that such a fact represents an isolated finding in only one case, we then wondered if p63 could actually represent a potentially useful immunohistochemical tool to suggest a primary cutaneous CAC, once the “immunohistochemical” apocrine status of the carcinoma has been established.

Conclusions

We found that a panel of mammaglobin, ER, PR, D2-40, and CK5/6 is useful in the differential diagnosis between CAC and cutaneous metastasis from breast carcinoma. If the “immunohistochemical” apocrine status of the cutaneous carcinoma is demonstrated (AR+, ER-, PR-), p63 might be useful as an additional tool. The panel expected for a primary CAC would be ER-, PR-, CK5/6+, and p63+. Regarding mammaglobin and D2-40, the rule that mammaglobin is either negative or positive in scattered cells for primary CAC and D2-40 is either negative or positive with a basilar pattern, seems to be a correct approach thus far. Mammaglobin seemed to be quite a useful marker in our study, namely for cases of metastases from ductal breast carcinoma. In our limited experience, SMA seems to be a reliable tool to diagnose invasion in CAC. CAC seems not to express c-erbB-2. Therefore, if any additional treatment is considered, Trastuzumab does not seem to be a logical option.

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