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### Acinic Cell Carcinoma of Breast: Morphologic and Immunohistochemical Review of a Rare Breast Cancer Subtype

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**TITLE PAGE**

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Acinic cell carcinoma of breast; triple negative breast cancer; morphology; immunohistochemistry

# Acinic Cell Carcinoma of Breast: Morphologic and Immunohistochemical Review of a Rare Breast Cancer Subtype

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## ABSTRACT

Acinic cell carcinoma of breast is a rare subtype of triple negative breast carcinoma and demonstrates extensive morphologic overlap with acinic cell carcinoma of the salivary gland. In this study, we perform a detailed morphologic and immunohistochemical description of two cases of this rare entity, and undertake a comprehensive review of all reported cases of breast acinic cell carcinoma in the English language literature to date. One third of reported cases of breast acinic cell carcinoma have been associated with the presence of a ductal carcinoma not otherwise specified (NOS) component, which is frequently poorly differentiated. Breast acinic cell carcinoma can demonstrate focal morphologic features similar to microglandular adenosis; these areas are frequently negative for collagen IV and laminin on immunohistochemistry. The true relationship between these two entities remains unclear, but we advocate that microglandular adenosis-like areas at the periphery of a breast acinic cell carcinoma should be considered part of the carcinomatous process and re-excised if this process extends to the initial surgical margins.

## 1. INTRODUCTION

Acinic cell carcinoma (AcCC) of breast was first described by Roncaroli [1] in 1996, and is recognized as a subtype of triple negative breast carcinoma (TNBC) in the current WHO classification [2]. It is one of several rare subtypes of breast carcinoma which demonstrate morphologic overlap with the repertoire of tumors seen in the salivary glands [3]. While most breast carcinomas are “ductal” in appearance and show no evidence of acinar or “secretory”-type differentiation, rare cases of invasive ductal carcinoma can demonstrate S100- or lysozyme-positive cells with granular cytoplasm [4], including carcinomas with apocrine morphology, or those that arise in microglandular adenosis (MGA) [5]. While the term “secretory carcinoma” is currently used exclusively to describe breast carcinomas associated with the presence of the ETV6-NTRK3 translocation, it may be said that there is a larger subcategory of breast carcinomas, including rare entities such as AcCC and cystic hypersecretory carcinoma, which recapitulate the pro-secretory phenotype of the lactating breast. In this paper, we report two recent cases of breast AcCC diagnosed at our institution and we review what is currently known about this rare entity in terms of morphology, immunohistochemistry and molecular pathology.

## 2. METHODS

Two cases of breast AcCC were identified from the departmental pathologic database, and the clinical, radiologic and pathologic details of both cases were reviewed. Immunohistochemistry was undertaken as part of the diagnostic work-up in both cases and the antibodies and dilutions used are summarized in Table 1. One of the cases underwent molecular analysis using the Memorial Sloan Kettering Integrated Mutation Profiling for Actionable Cancer Targets (MSK-IMPACT) platform, a next generation sequencing (NGS) bait-capture platform which assesses for mutations, copy number variations and fusions in 341

genes that are known to be oncogenic drivers [6]. A literature review of breast AcCC was also undertaken and included all prior reports of breast AcCC in the English language literature to date. This study was conducted in accordance with institutional research board guidelines.

### **3. RESULTS**

#### **3.1 Case Report 1**

##### 3.1.1 Clinical History

A 47 year old woman presented with a palpable mass in the lower outer quadrant (LOQ) of right breast, which was 2.8 cm in maximum dimension on sonographic examination, and mammographically occult. The patient had no personal or family history of breast cancer. Core needle biopsy (CNB) of the mass was diagnosed as “poorly differentiated carcinoma with apocrine features”. A separate area of microcalcifications in the right upper outer quadrant (UOQ) was also biopsied, and showed sclerosing adenosis with apocrine change and microcalcifications. At lumpectomy, a 2.3 cm “poorly differentiated carcinoma with apocrine features” was diagnosed. The tumor was estrogen receptor negative (ER-) and HER2-negative, and showed focal weak progesterone receptor (PR) positivity (<5% of cells). A separate 0.5 cm ER-positive, PR-positive, HER2-negative moderately differentiated carcinoma was identified in one of the extra margins excised around the main tumor. Final surgical margins were negative. One of eighteen right axillary lymph nodes contained metastatic poorly differentiated carcinoma. The patient underwent systemic chemotherapy, followed by radiotherapy and hormone therapy. Four years later, a 0.7 cm sonographically-detected mass in the right UOQ was identified and bilateral mastectomy was performed after the presence of recurrent carcinoma was confirmed on biopsy. A diagnosis of multifocal invasive carcinoma with AcCC-like features was made on the right mastectomy. Carcinoma

involved all four quadrants including post-surgical scar tissue in the UOQ and LOQ; the largest single tumor mass was 3.2 cm. Retrospective review of the prior lumpectomy and preceding CNB lead to the recognition that all of the tumors shared features of breast AcCC. The contralateral mastectomy was entirely benign, but one of two left axillary lymph nodes showed metastatic carcinoma. The patient underwent further chemotherapy but developed another metastasis involving a right internal mammary lymph node within one year. She is currently alive with disease, six years after her initial diagnosis.

### 3.1.2 Morphology

Histological analysis of the first right breast lumpectomy specimen demonstrated a spectrum of architectural appearances, ranging from large nodular areas of carcinoma with a solid and nested appearance, through acinar-like areas with a discrete pseudo-lobular growth pattern, to scattered tubular or microglandular structures and single cells that infiltrated widely into adjacent fibroadipose tissue (Figure 1A-C, F). Microglandular structures frequently contained a central globule of colloid-like eosinophilic material, similar in appearance to microglandular adenosis (MGA) (Figure 1C inset). A multifocal lymphoplasmacytic infiltrate was present, particularly in association with areas of acinar-like architecture (Figure 1b). This spectrum of architectural features was associated with a parallel cytological spectrum, where the solid, nested carcinoma was composed of mitotically-active cells with high grade nuclei of moderate-to-severe pleomorphism, coarse chromatin and a prominent single nucleolus (Figure 1F), while the well-differentiated acinar-like structures demonstrated smaller, monomorphic eccentric nuclei with fine chromatin, a single small nucleolus and less mitotic activity (Figure 1C). In the middle of the cytological spectrum, infiltrative, less-well-developed tubules and scattered cell nests demonstrated intermediate-type nuclei (Figure 1B). The constituent cells of the acinar-type structures contained finely-granular cytoplasm which looked “clear” at low power, while the most poorly differentiated component of the carcinoma showed more uniform basophilic granular

cytoplasm, typical of high grade invasive ductal carcinoma not otherwise specified (NOS). Areas of the tumor which occupied the center of the morphologic and cytological spectrum showed much more variation in the tinctorial qualities of their cytoplasm, ranging from finely granular to coarsely granular, and including the presence of large, brightly eosinophilic globules. Other focal features identified included the involvement of a normal breast terminal duct-lobular unit (TDLU) by an AcCC-like process, which may represent either lobular cancerization or an intraepithelial form of AcCC (AcCC DCIS) (Figure 1 G-H). Pseudo-lactational change was also seen focally within the tumor. Lymphovascular invasion (LVI) by pleomorphic high grade carcinoma cells was readily identifiable.

The recurrent carcinoma present in the mastectomy specimen shared many of the same features; however the most striking difference was the relative absence of the differentiated “clear” acinar-like component. The recurrent tumor was predominantly composed of solid areas, nests and tubules composed of a mixed cell population of brightly eosinophilic and basophilic cells, often within the same tubular structure, with fine-to-coarse cytoplasmic granularity (Figure 2). Of note, scattered rounded solid nests of the AcCC-like cells were present throughout the recurrent tumor, simulating the appearance of a solid DCIS component.

### 3.1.3 Immunohistochemistry

Immunohistochemical (IHC) staining of the original tumor demonstrated that it was focally progesterone receptor (PR) positive (< 5% of cells) and negative for estrogen receptor (ER) and human epidermal growth factor receptor 2 (HER2). The MGA-like tubular areas were positive for PR (focal), EMA, CK7 and S100. Both tubular and solid nested areas of the tumor showed variable positivity for the basement membrane proteins collagen IV and laminin (Figure 1 D-E). Both the original and recurrent tumors showed positivity for alpha-1 anti-chymotrypsin (A1-ACT), S100, EMA, lysozyme and gross cystic disease fluid protein (GCDFP). Very focal synaptophysin and mammaglobin positivity were also seen, while

alpha-1- antitrypsin (A1-AT) and chromogranin were negative. Cells with eosinophilic granules and globules were strongly PAS (diastase-resistant) positive. The recurrent tumor was negative for ER, PR and HER2 and also negative for androgen receptor (AR). The presence of a focus of intraepithelial AcCC was confirmed with p63 and calponin IHC, which demonstrated the presence of a myoepithelial cell layer in the involved lobule (Figure 1H). Myoepithelial markers (p63, calponin) were entirely negative in the remainder of the tumor.

#### 3.1.4 Molecular Analysis

Targeted next-generational sequencing of the recurrent carcinoma demonstrated the presence of a *TP53* frameshift mutation, a *MLL3* splicing mutation, and a *TSC2* missense mutation (A289V). All three mutations were found at similar allelic frequencies. There were also missense mutations with lower allelic frequencies found in *MED12* (D1204E), *MLL* (K1225N), *MLL2* (T2017S), and *TP63* (R594L). Copy number analysis revealed a two-fold increase in *FOXA1*.

### **3.2 Case Report 2**

#### 3.2.1 Clinical History

A 49 year old woman presented with a palpable mass in the right breast. On radiologic assessment, a right UOQ ill-defined solid mass associated with innumerable anechoic cysts was seen. Ultrasound-guided core biopsy showed poorly differentiated carcinoma (Figure 3A). The patient underwent neoadjuvant chemotherapy and subsequently underwent lumpectomy with additional margins. The pathologic diagnosis was well differentiated breast AcCC which was extensively infiltrative, and involved multiple margins. LVI was identified, but two sentinel lymph nodes were benign. No residual solid

poorly differentiated carcinoma was identified. At mastectomy, a residual 1.1 cm focus of breast AcCC was identified. The patient is alive with no evidence of disease eighteen months post mastectomy.

### 3.2.2 Morphology

The original biopsy was predominantly composed of a solid, poorly differentiated, triple negative IDC NOS. However at the periphery of the solid tumor, small moderately-to-poorly-formed microglandular structures composed of cells with variably eosinophilic and amphophilic cytoplasm were identified, infiltrating normal breast stroma in a haphazard manner and associated with a lymphocytic infiltrate (Figure 3A inset). Post-neoadjuvant chemotherapy, no residual poorly differentiated IDC NOS was identified, and the predominant carcinoma cell type was a relatively monomorphic cell with abundant amphophilic, finely granular cytoplasm which was dispersed in single cells, clusters, tubules and sheets (Figure 3B-C). In sheet-like areas, the tumor developed a histiocytoid appearance, with particularly voluminous cytoplasm. Focally, a component of high grade DCIS NOS was identified (Figure 3D). A scattered secondary population of tubules and solid nodules composed of cells with brightly eosinophilic, coarsely granular cytoplasm was also present, very similar in appearance to the tumor morphology seen in the first case (Figure 4A). Rare foci of LVI were seen.

### 3.2.3 Immunohistochemistry

IHC demonstrated that tumor cells were positive for lysozyme, A1-ACT (Figure 4C-D) and S100, and negative for amylase, A1-AT, synaptophysin, chromogranin, ER, PR and HER2. p63 staining confirmed the presence of a residual solid poorly differentiated in-situ component. Areas with eosinophilic granules and globules were strongly PAS-D positive (Figure 4B).

## **3.3 Literature review of breast AcCC**

There have been forty seven cases of breast AcCC reported in the English language literature, including the two new cases reported in this paper [1, 4, 7-27]. A detailed morphologic description was provided in thirty one of these cases [1, 4, 7-25]. Shimao et al [9] reported the only case of breast AcCC to date occurring in a male patient. The mean patient age at diagnosis was 51 years (median age 49; range 23-80 years) while the mean tumor size was 3.2 cm (median 3 cm; range 1.3-5.5 cm). Follow-up data was available on 25 cases, with a median follow-up duration of 28 months (range 6-185 months); 19 of the 25 cases had experienced no tumor recurrence at last review (76%). Six patients developed metastasis to sites such as bone, liver and lung [4, 7, 8, 13] and two died of their disease [4, 8].

Review of prior reports demonstrates the remarkable consistency in the morphologic appearance of this tumor type. Table 2 summarizes the reported relative frequency of each of the individual characteristic morphologic features of breast AcCC in the subset of reported cases with detailed morphologic review (n=31), while Table 3 is a summary of the immunohistochemical profile of the breast AcCCs reported to date. Most reported cases of breast AcCC describe variation in the tumor architecture, with solid and nested components admixed with areas of tubular and microglandular morphology. The phenomenon of tubules with central luminal extracellular “colloid-like” material was described as “microglandular-adenosis (MGA)-like” in several papers [4, 7, 13], while other papers interpreted the appearance as suggestive of true MGA [22]. Interestingly, in eleven cases where collagen IV and laminin immunohistochemistry was performed, these MGA- or MGA-like areas were entirely negative for these basement membrane markers in 8 cases [7, 13, 19, 24]. In the remaining 3 cases [12] (including the two cases reported in this paper), very focal positivity was noted. The cytologic appearance of tumor cells, with coarse eosinophilic granules and globules, was labelled “Paneth-cell-like” in eleven cases [4, 7, 16, 19, 22, 24]. Seven cases also reported a population of cells with basophilic cytoplasm [4, 11, 19]; in four of these cases [4] (including case 2 from this paper), cells with basophilic and eosinophilic cytoplasmic

granules were located together within the same tubular structure, giving a variegated appearance (Figure 2).

Of the forty seven breast AcCC cases reported, fifteen patients (32%) had an associated IDC NOS; of these, ten had a solid, poorly differentiated carcinoma, two had a well differentiated tubular/microglandular carcinoma, one had a moderately differentiated carcinoma while one metaplastic carcinoma has also been reported [4, 8, 11, 23, 27]. Tumor grade was not detailed in the remaining cases. In one case [4], the poorly differentiated IDC NOS component was ER positive, while in the first case described in this paper, the original ER-negative tumor showed weak PR positivity in less than 5% of cells. The remaining cases with an IDC NOS component were reportedly triple negative (ER/PR/HER2 negative). Three cases (10%) had solid high grade DCIS NOS identified in association with breast AcCC [4, 15] including the second case reported in this paper. LVI was identified in 7 of 13 cases where that parameter was explicitly assessed, and lymph node metastases were identified in 8 of 27 cases, ranging from one to ten positive lymph nodes. Four of the eight cases with lymph node metastasis had a poorly differentiated IDC NOS component to their main tumor [4, 7].

Seven cases of breast AcCC have had ultrastructural analysis [1, 7-10, 22]. In all seven cases the dominant feature was the presence of multiple, variably-sized cytoplasmic granules, while ribosomal endoplasmic reticulum was prominent in five cases and abundant mitochondria were noted in three cases.

#### 4. DISCUSSION

The breast is embryologically, morphologically and functionally related to secretory glands of other sites, including the salivary glands. It is therefore unsurprising that breast carcinomas may recapitulate the

appearance of tumors more commonly seen in the salivary glands, including adenoid cystic carcinoma, pleomorphic adenoma, adenomyoepithelioma, myoepithelioma, oncocytic carcinoma and mucoepidermoid carcinoma [3, 28]. Conversely, ETV6-NTKR3 translocation-associated secretory carcinoma of the breast (T-SC) has a rare analogous counterpart in the salivary gland, mammary analogue salivary gland carcinoma, which bears the same translocation [29]. However, T-SC is only one of a number of breast carcinoma subtypes that can demonstrate a “secretion-rich” phenotype, with variably-sized cysts and pools of extracellular secretions and/or prominent cytoplasmic zymogen-like granules. These entities may together constitute a spectrum of morphologies in which the degree of development of the “secretory” phenotype varies from focal to predominant. Cystic hypersecretory carcinoma, with its dominant secretion-rich morphology, may be thought of as representing one end of this spectrum, while ductal carcinoma with focal eosinophilic granular cells [4] or pseudo-lactational features may represent the opposite pole of this morphologic range. The middle ground in this morphologic spectrum is represented by AcCC of breast.

It is clear from the forty seven cases of breast AcCC that have now been described that this entity has a wide morphologic spectrum of appearances in terms of architectural and cytologic features. Extensive intratumoral morphologic variation can be seen in a single tumor, highlighting the diagnostic challenges associated with core biopsy of these lesions.

The immunohistochemical profile of breast AcCC shares many features with AcCC of salivary gland, with frequent expression of S100, lysozyme, amylase and alpha-1-antichymotrypsin, and PAS positivity in addition. While the majority of cases have been negative for hormone receptors (including AR) and all have been negative for HER2, it is useful to note that rare cases have shown some expression of ER and PR. Where myoepithelial markers such as calponin and p63 have been tested in breast AcCC, they are consistently negative, confirming the true invasiveness of this tumor type. Interestingly, the basement

membrane-associated proteins collagen IV and laminin have also been negative in most breast AcCC cases where they have been reported (Table 3). However, as demonstrated in the first case described in this paper, these markers are not always entirely negative in breast AcCC.

The true nature of the relationship between MGA and breast AcCC is unclear. There is extensive morphologic overlap between the well differentiated tubular component of breast AcCC and MGA, while both entities are characterized by a widely infiltrative growth pattern. Furthermore, both express S100 and are negative for myoepithelial markers and hormone receptors. The question of whether the two lesions are related has been previously highlighted [12], and some authors have interpreted the presence of infiltrative microglandular structures at the periphery of a breast AcCC tumor as evidence that the carcinoma arose on the background of an MGA substrate. Since IHC for laminin and collagen IV is thought to be positive in most MGA [5], in contrast to the results of such IHC in the majority of tested cases of breast AcCC, it might appear that these markers could be used to differentiate these lesions. However, the first case reported in this paper highlights the need for caution in adopting this strategy, as this tumor showed significant variation in basement membrane protein expression on IHC, ranging from entirely negative to strongly positive. Therefore, a core needle biopsy of this lesion may demonstrate variable collagen IV and laminin immunohistochemical results, depending on the area sampled. At present, one can suggest that the absence of collagen IV and laminin expression may represent evidence against the presence of true MGA, but this cannot be stated definitively due to known variation in staining patterns. When the MGA-like process extends to surgical margins in the setting of breast AcCC, re-excision is advised.

The presence of a *TP53* mutation in the molecular analysis of the first case described in this paper is in keeping with the findings of Ripamonti et al [19], who reported the presence of a *TP53* mutation in a breast AcCC which occurred in a BRCA1 mutation carrier and Piscuoglio et al [27], who recently

described *TP53* mutations in 80% (8 of 10) breast AcCCs tested. *TP53* mutation was found in 37% of all breast carcinomas tested in The Cancer Genome Atlas (TCGA) breast carcinoma study [30], and is the most common mutation in breast cancer. In contrast, Piscuoglio et al [27] did not detect any mutations in *TP53* in 20 salivary AcCCs, leading those authors to conclude that on from a molecular pathology standpoint, breast AcCCs have far more in common with TNBC than salivary AcCCs. In keeping with these results, Guerini-Rocco et al [31] recently performed massively parallel sequencing analysis of the same cohort of breast AcCCs as Piscuoglio et al [27], and demonstrated a pattern of complex copy number aberrations (CNAs) that also mirrors that seen in TNBC. *MLL3*, another likely oncogenic driver in our case, was also mutated in 7% cases in the breast carcinoma TCGA study [30], making it one of the top five most frequently mutated genes. Finally, the amplification of *FOXA1*, a transcription factor important for estrogen receptor (ER) binding to DNA, is predicted to lead to cellular reprogramming to establish an estrogen-dependent cell phenotype [30]. *FOXA1* has also been shown to repress the transcription of a subset of basal-type breast cancer-associated genes [32], and therefore may play a role in the maintenance of a luminal phenotype in breast AcCC tumor cells.

Many early case reports suggested, based on very small case series, that breast AcCC is likely to have a good prognosis [1, 7]. However, it is clear that poorly differentiated TNBC can frequently be a component of breast AcCC, and that recurrences and death from this disease do occur. It seems probable that prognosis is largely driven by the presence of the poorly differentiated component. Furthermore, there is limited evidence from cases involving surgical excision following neoadjuvant chemotherapy (including case 2 of this study) that while the poorly differentiated tumor component may be comparatively chemosensitive, the better differentiated acinar and microglandular carcinoma may be more chemoresistant [8, 23]. However, this data analysis is provisional, pending future larger studies of this rare tumor entity.

In summary, breast AcCC is a rare subtype of breast carcinoma that is usually triple negative on immunohistochemistry and has a wide morphologic spectrum that can include a high grade, poorly differentiated component. The relationship between breast AcCC and MGA remains unclear but it is apparent that MGA-like areas at the periphery of breast AcCC should be considered a part of the carcinomatous process and re-excised if they extend to the initial surgical margins. Recognition of the entity of breast AcCC is also important in avoiding the potential diagnostic pitfall of interpreting well-differentiated areas of AcCC as MGA on biopsy. The key to making the diagnosis is the combination of knowledge of the entity, and recognition of the characteristic morphology and immunohistochemical profile (as detailed in tables 2 and 3). Recent molecular analysis of this tumor subtype suggests that the most common mutations (*TP53*, *PIK3CA*) seen in IDC NOS also occur in breast AcCC. Thus, in contrast to other analogous breast and salivary gland tumor types, such as ETV6-NTRK translocation-related tumors or MYB-NFIB translocation-related adenoid cystic carcinomas of both sites, AcCC of breast and salivary gland appear to be genetically distinct, despite their morphologic similarities.

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## Figure Legends

Figure 1: Representative images of breast acinic cell carcinoma (AcCC) (Case 1). A, infiltrative margin, infiltrating fat (inset)[H&E, x 20]; B, prominent lymphoplasmacytic infiltrate)[H&E, x 100]; C, clear cell morphology, with colloid-like intraluminal material (inset) ) [H&E, x 100]; D, Collagen IV immunohistochemistry (IHC) in tubular areas, ) [collagen IV, x 100]; E, Collagen IV IHC in solid nested areas, [collagen IV, x 100]; F, poorly differentiated tumor component, [H&E, x 400]; G, In-situ AcCC component, [H&E, x 40]; H, Calponin IHC in in-situ component, [Calponin, x 40];

Figure 2: Representative images of the recurrent breast acinic cell carcinoma (case 1). A, solid area, [H&E, x 40]; B, infiltrative tubular areas, [H&E, x 20]; C, subtle infiltration of adipose tissue, [H&E, x 200]; D, variably eosinophilic and basophilic granular cytoplasm in tubular cells, imparting a variegated appearance[H&E, x 400].

Figure 3: Representative images of breast acinic cell carcinoma (Case 2). A, poorly differentiated carcinoma, [H&E, x 40], with scattered adjacent tubules with eosinophilic granular cytoplasm [inset, H&E, x 200]; B, diffuse, infiltrative residual carcinoma, post neoadjuvant chemotherapy [H&E, x 40]; C, tubules and single infiltrative carcinoma cells with clear and basophilic cytoplasm [H&E, x 100]; D, residual in-situ carcinoma [H&E, x 100].

Figure 4: Representative images of breast acinic cell carcinoma (Case 2). Variegated tubules composed of cells with eosinophilic or basophilic granular cytoplasm and focal calcification, positive for PAS-diastase, lysozyme, alpha-1-antitrypsin [A: H&E, x 400, B: PAS-diastase, x 400, C: lysozyme IHC, x 200, D: alpha-1-antichymotrypsin IHC, x 400].

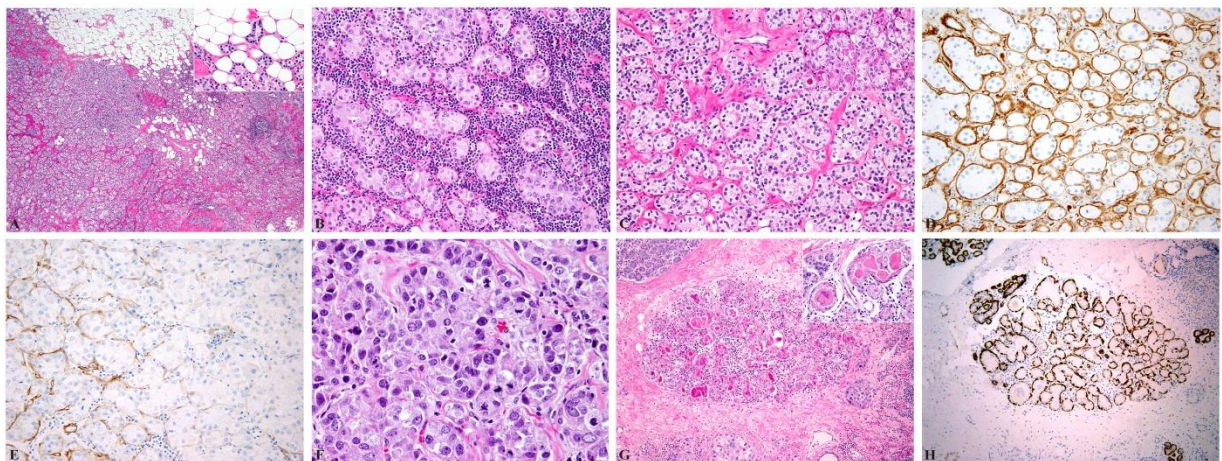


Fig. 1

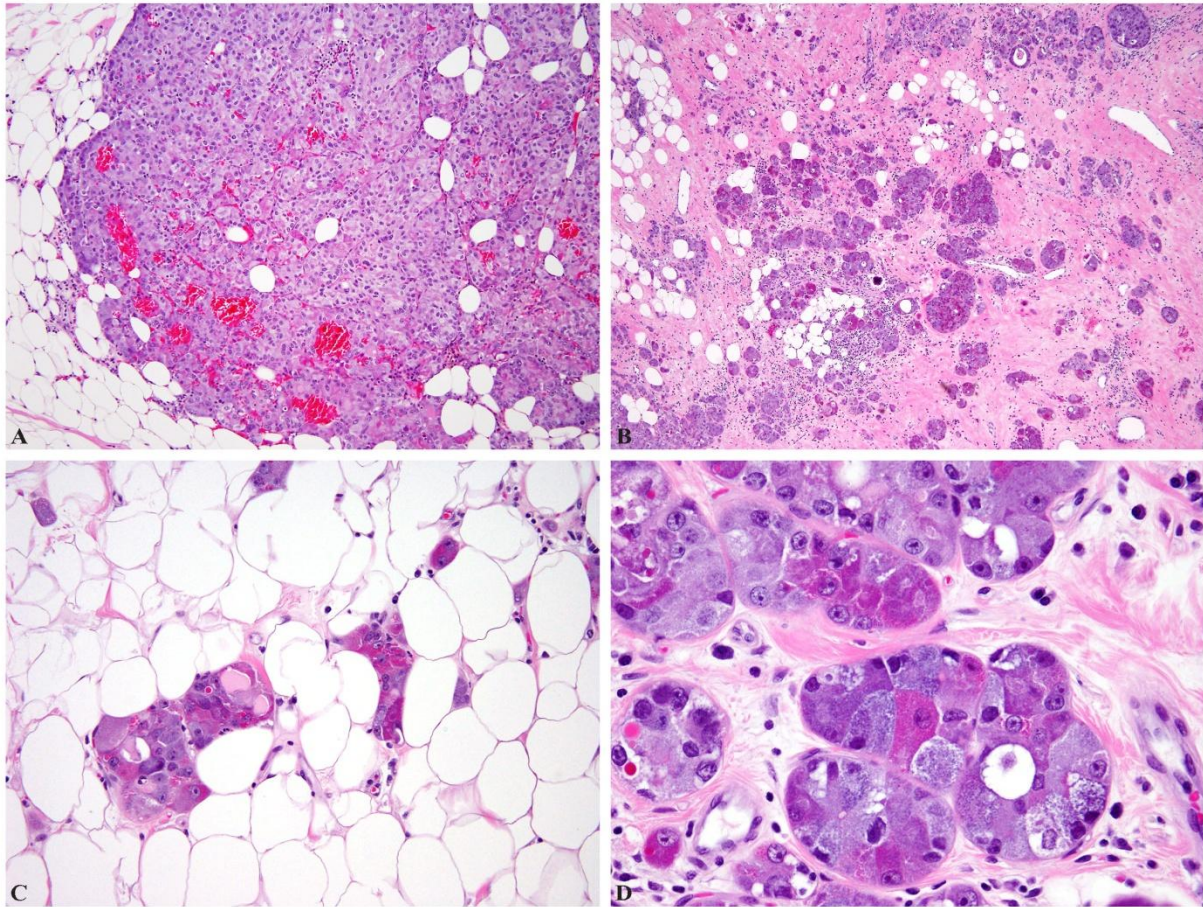


Fig. 2

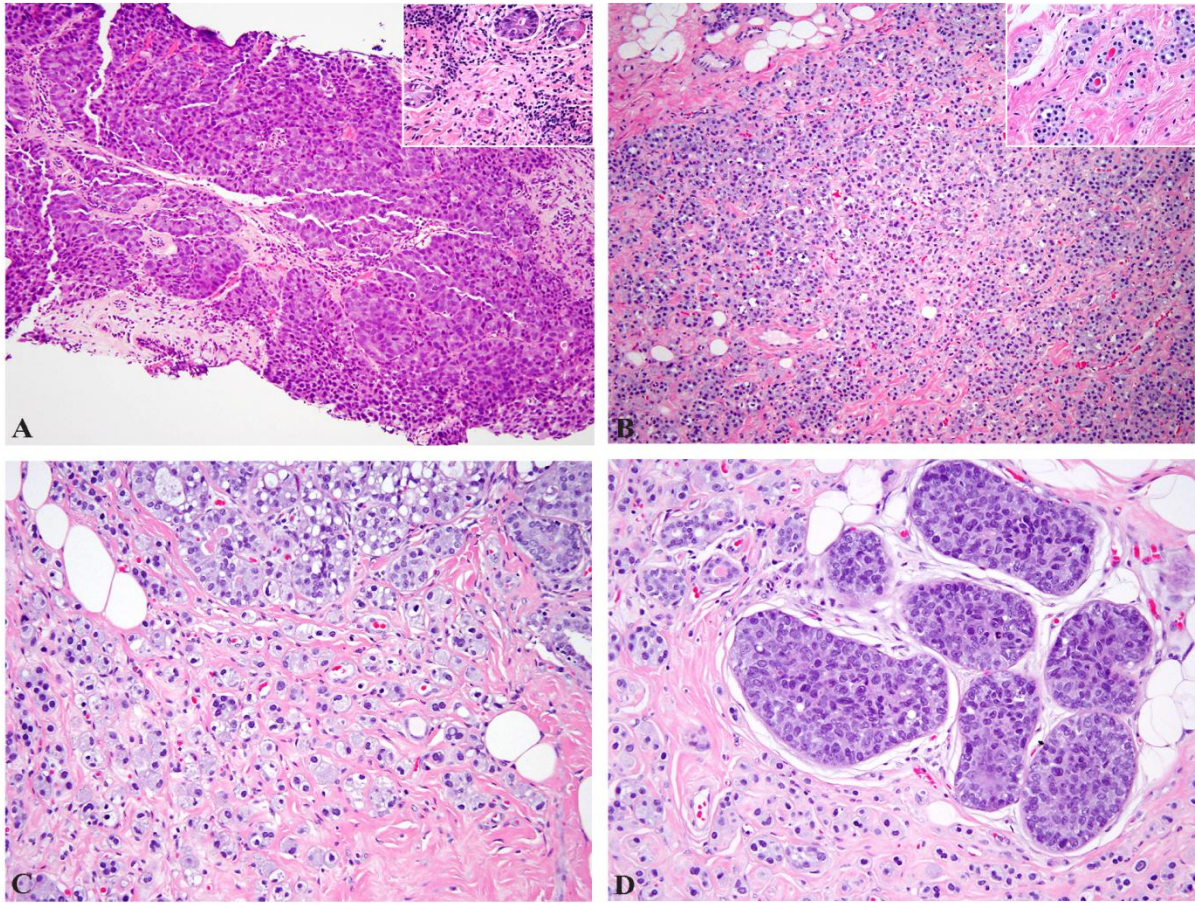


Fig. 3

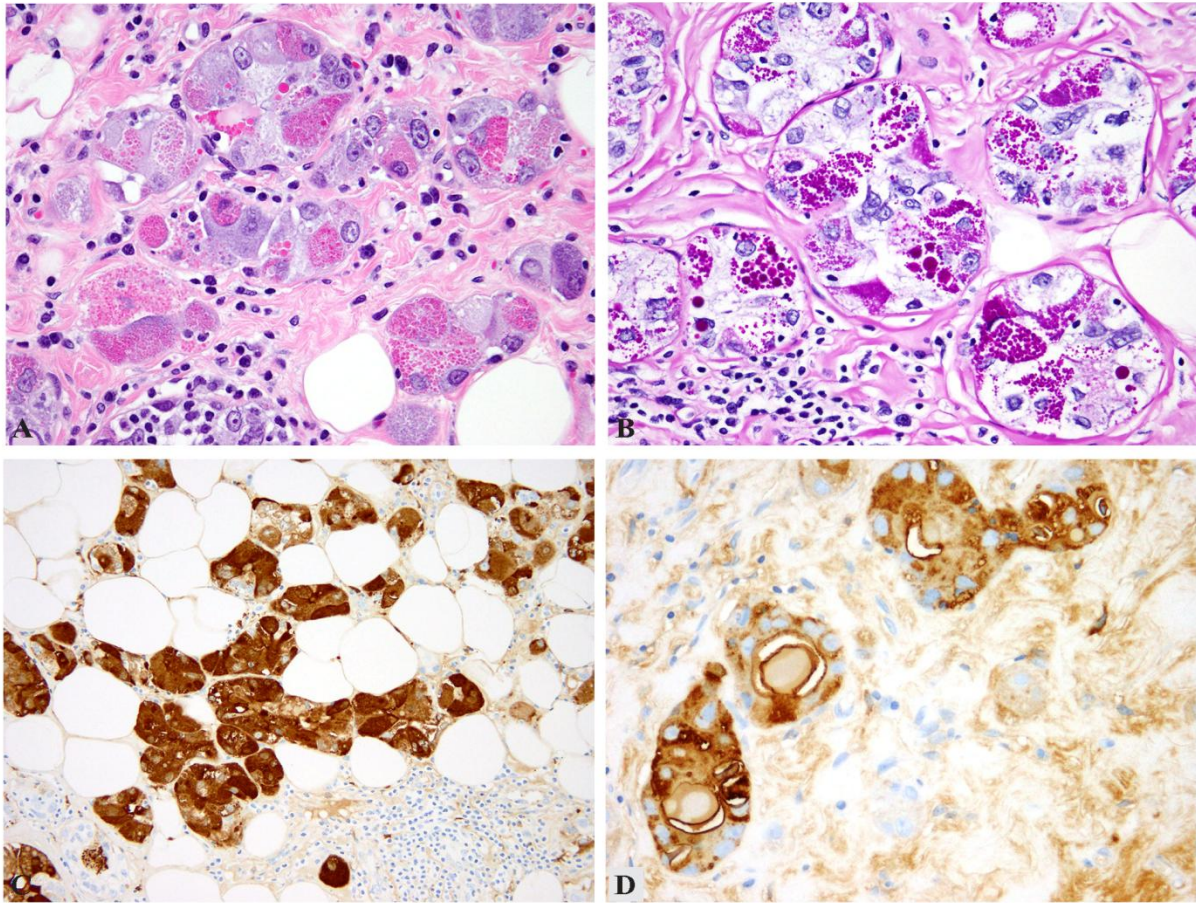


Fig. 4

Table 1: Immunohistochemical Antibodies Utilized

ANTIBODY	CLONE	VENDOR	WORKING DILUTION
ER	SP1	Ventana	RTU
PR	1E2	Ventana	RTU
HER2	4B5	Ventana	RTU
EMA	E29	Ventana	RTU
CK7	OV-TL 12/30	DAKO	1: 1600
S100	pAb (Rabbit)	DAKO	1: 8000
Collagen IV	CIV22	Ventana	RTU
Laminin	pAb (Rabbit)	Biogenex	1: 100
Alpha-1-antitrypsin	pAb (Rabbit)	Ventana	RTU
Alpha-1-antichymotrypsin	pAb (Rabbit)	Ventana	RTU
GCDFP-15	D6	Covance	1: 1000
Synaptophysin	SNP88	Biogenex	1: 2000
Chromogranin	LK2H10	Ventana	RTU
Mammaglobin	31A5	Ventana	RTU
Lysozyme	pAb (Rabbit)	Ventana	RTU

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<b>Amylase</b>	pAb (Rabbit)	Nordic Immunology	1:5000
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Abbreviations: ER estrogen receptor, PR progesterone receptor, HER2 human epidermal growth factor receptor 2, EMA epithelial membrane antigen, GCDP-15 gross cystic disease fluid protein-15, RTU ready to use

Table 2: Summary of Morphologic Features of Breast AcCC

<b>MORPHOLOGIC FEATURES OF BREAST AcCC</b>	
<b>ARCHITECTURE (N=25)</b>	
<b>Solid component</b>	84% (21/25)
<b>Tubular component</b>	92% (23/25)
<b>Colloid-like luminal material in tubules</b>	92% (23/25)
<b>Infiltrative growth pattern</b>	80% (20/25)
<b>CYTOPLASM (N=25)</b>	
<b>Prominent eosinophilic cytoplasmic granularity (fine-to-coarse)</b>	96% (24/25)
<b>Large cytoplasmic eosinophilic globules</b>	68% (17/25)
<b>Cells with clear cytoplasm</b>	52% (13/25)
<b>Cells with basophilic cytoplasm</b>	28% (7/25)
<b>NUCLEI (N=21)</b>	
<b>Round-to-oval, single nucleolus, low-intermediate grade</b>	71% (15/21)
<b>Range of appearance present, from low to high grade</b>	29% (6/21)

Table 3: Summary of Reported Immunohistochemical Results in Breast AcCC

IMMUNOHISTOCHEMICAL FEATURES OF BREAST	% POSITIVITY
<b>AcCC</b>	
<b>PAS (DIASTASE RESISTANT)</b>	100% (19/19)
<b>S100</b>	85% (23/27)
<b>Lysozyme</b>	100% (22/22)
<b>EMA</b>	100% (19/19)
<b>Amylase</b>	94% (17/18)
<b>Alpha-1-antitrypsin (A1AT)</b>	56% (5/9)
<b>Alpha-1-antichymotrypsin (A1ACT)</b>	91% (10/11)
<b>CK7</b>	100% (7/7)
<b>Neuroendocrine markers (Synaptophysin)</b>	16% (2/12)
<b>GCDFP</b>	44% (7/16)
<b>HORMONE MARKERS</b>	
<b>ER</b>	12% (3/25)
<b>PR</b>	20% (5/25)
<b>AR</b>	11% (1/9)

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<b>HER2</b>	<b>0% (0/19)</b>
<b>Triple negative carcinoma (ER/PR/HER2 negative)</b>	<b>74% (14/19)</b>

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Abbreviations: ER estrogen receptor, PR progesterone receptor, AR androgen receptor, HER2 human epidermal growth factor receptor 2, GCDFP-15 gross cystic disease fluid protein-15,