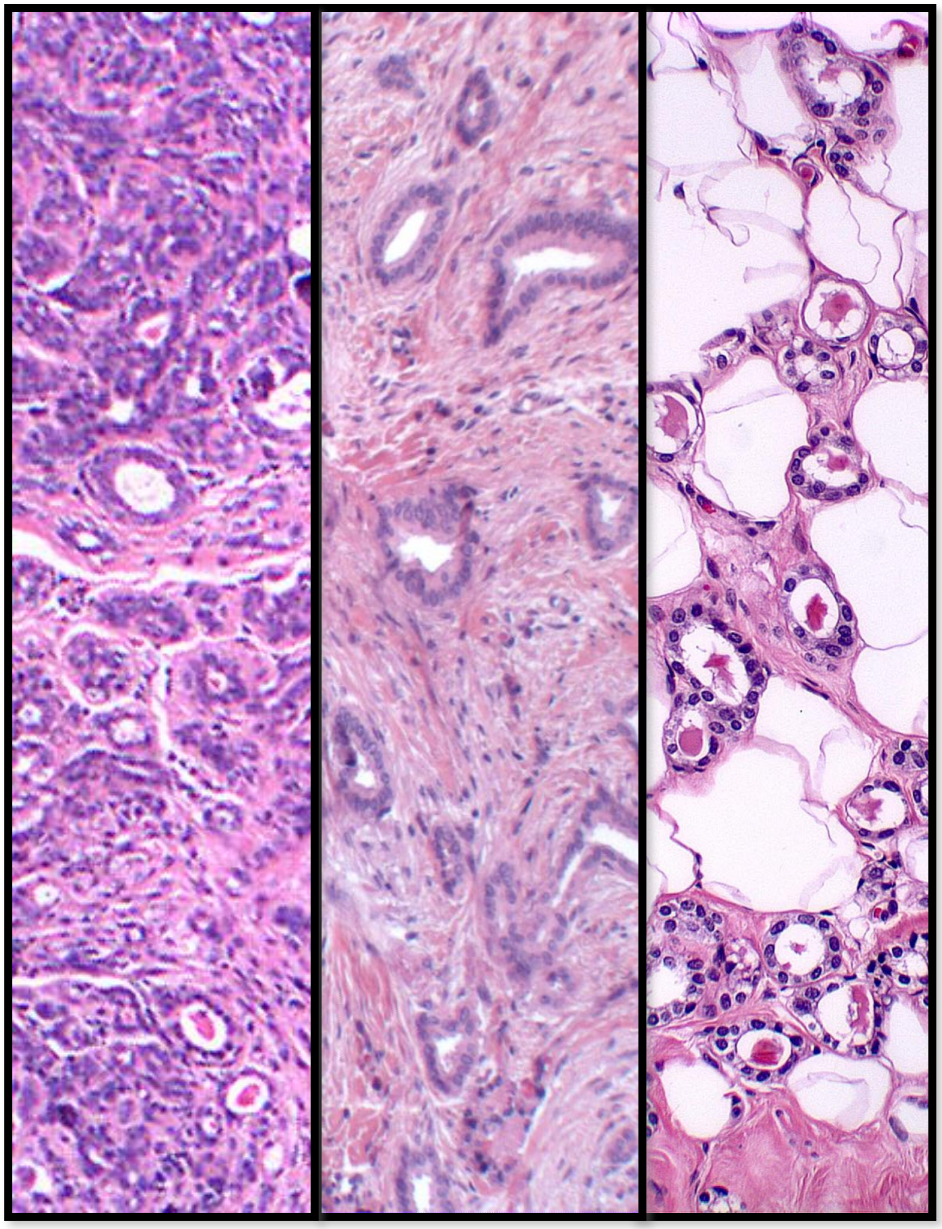
Common Errors in Breast Pathology and How to Avoid Them

33rd Annual Park City Anatomic Pathology Update

February 10, 2020

Susan C. Lester, M.D., Ph.D.
Breast Pathology Services
Brigham and Women's Hospital
Dana Farber Cancer Institute
Harvard Medical School



Errors in Breast Pathology

Fortunately, serious diagnostic errors in breast pathology are uncommon.

Many perceived “errors” are due to being forced to place lesions along a biologic spectrum (e.g. from FEA to ADH to DCIS) into discrete categories.

“A consultation occurs when a pathologist who knows a lesion has a differential diagnosis sends it to a consultant who does not.” Author?

Right and Wrong Errors in Breast Pathology

Sclerosing lesion mistaken for invasive carcinoma

Metastatic estrogen receptor carcinoma mistaken for neuroendocrine carcinoma

False negative estrogen receptor results

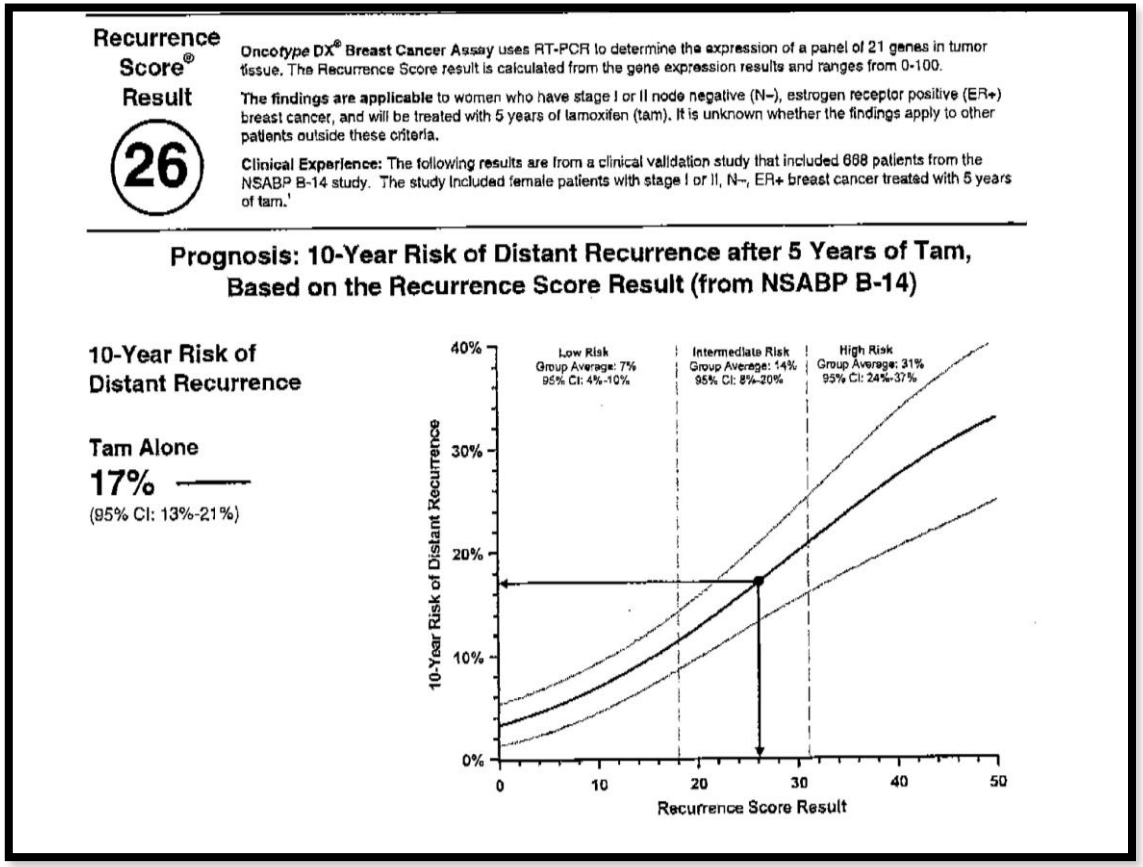
False positive HER2 results

Women #1

A 60 year old woman was found to have a 0.7 cm mass with ill-defined margins and calcifications on mammographic screening.

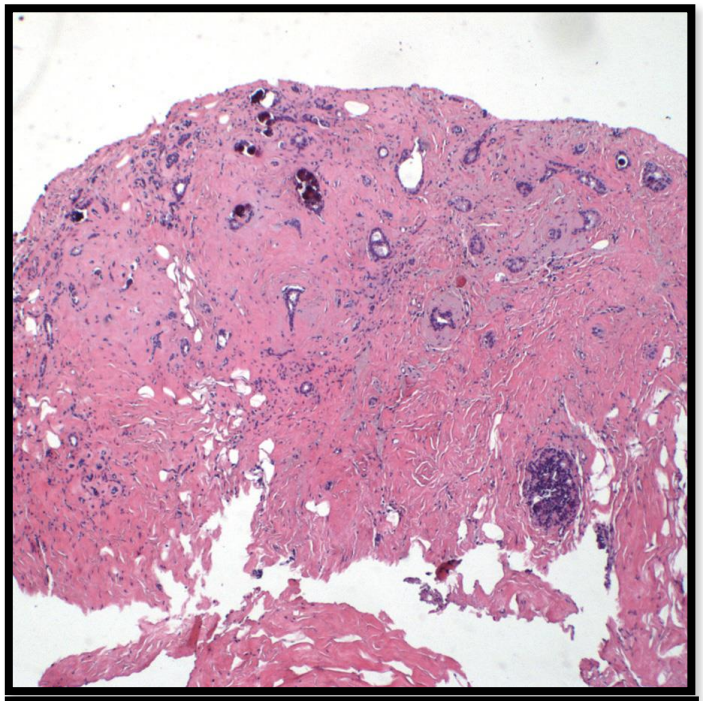
She was diagnosed with a 0.9 cm well differentiated invasive carcinoma on both the core needle biopsy and the subsequent excision. A sentinel lymph node was free of carcinoma.

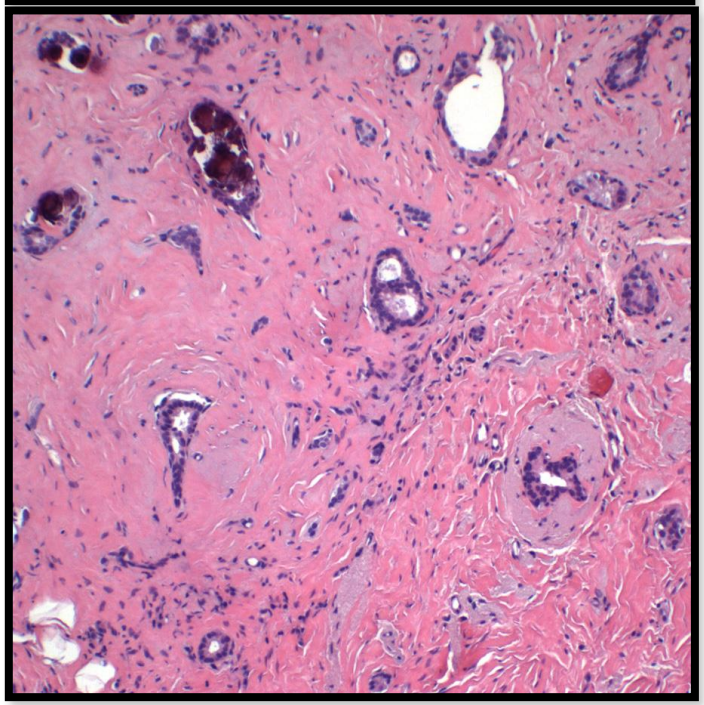
An Oncotype DX assay was requested.



The Oncotype DX recurrence score was 26 (mid range).

She requested a second opinion at Dana Farber Cancer Institute as to whether or not she should receive chemotherapy in addition to endocrine therapy.

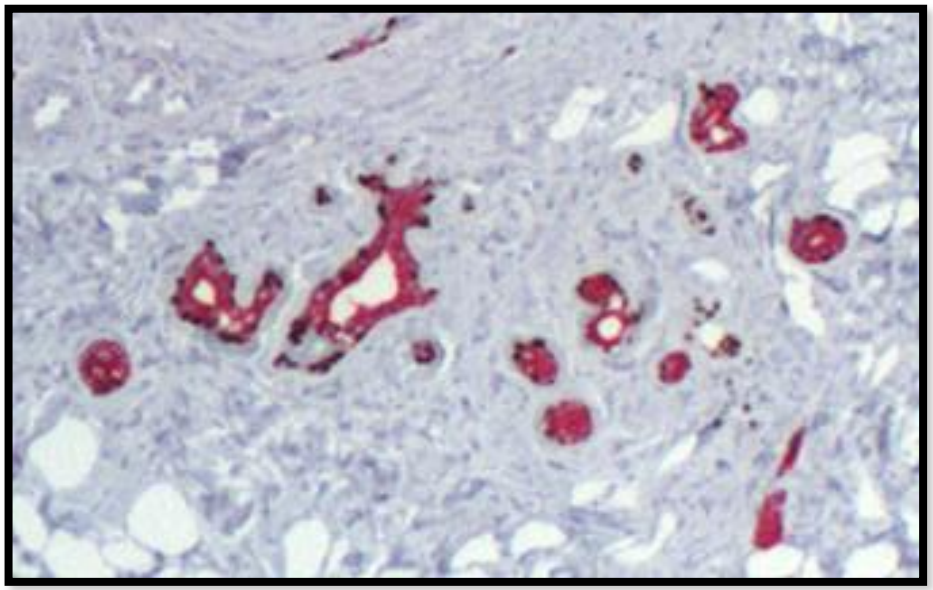


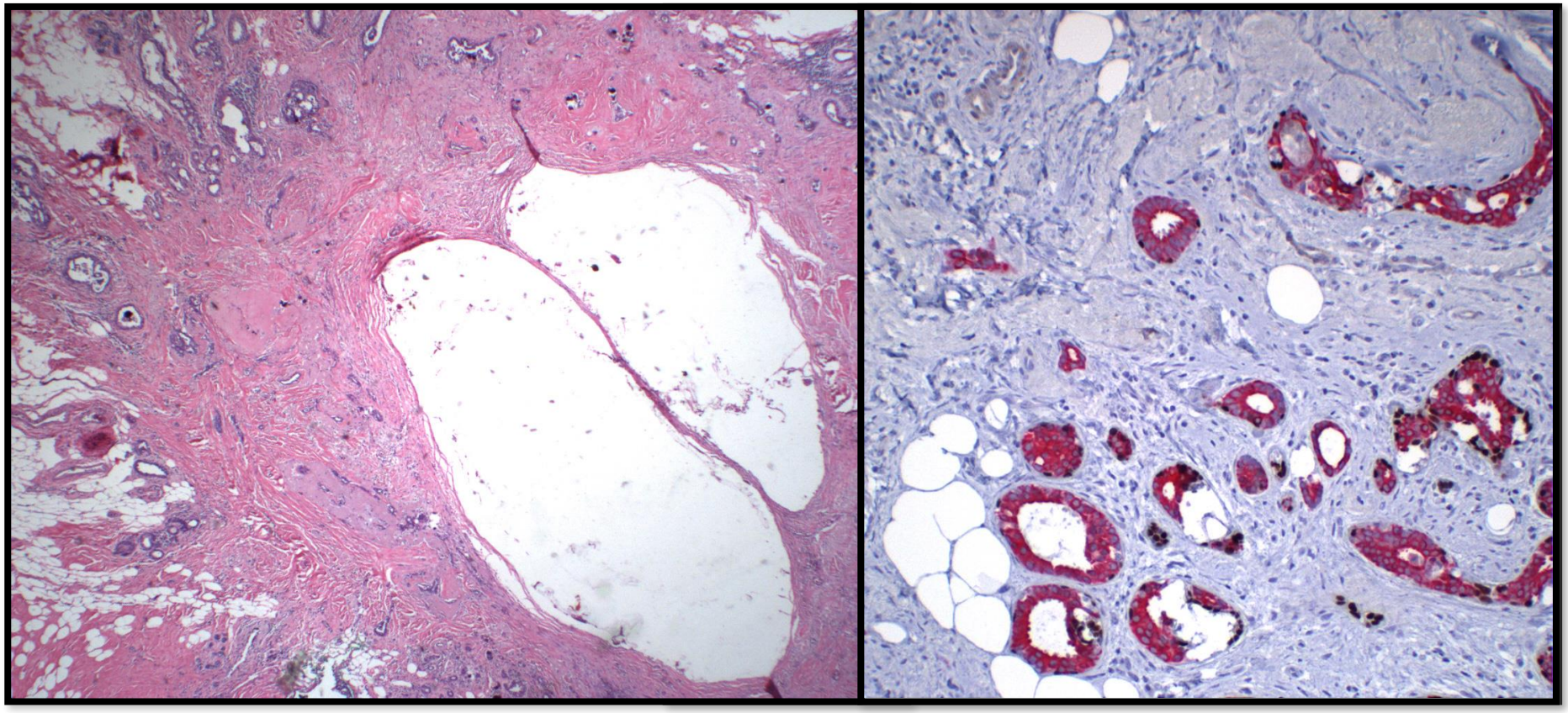


On review, the core biopsy showed small compressed tubules in a very dense sclerotic stroma.

The tubules did not involve adipose tissue or infiltrate around normal epithelium.

Immunoperoxidase studies confirmed myoepithelial cells.





The excision showed the core site in the center of an area of sclerosing adenosis.

Immunoperoxidase studies again demonstrated myoepithelial cells.

This patient's sclerosing adenosis had been misdiagnosed as invasive carcinoma.

Consequences:

The patient underwent unnecessary surgery and lymph node biopsy – with the additional stress of thinking she had cancer.

Due to the Oncotype DX score, she might have received unnecessary chemotherapy.

Of note, this is a *prognostic test* and not a *diagnostic test*. Benign lesions can have moderately high scores because the level of ER and PR expression is generally not very high.

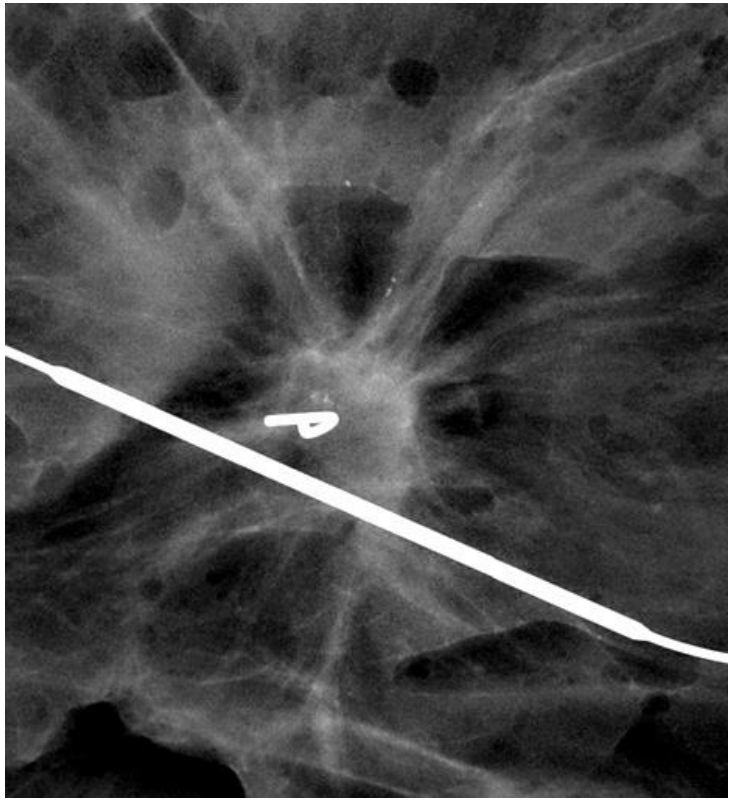
She could have received unnecessary radiation.

Sclerosing Adenosis

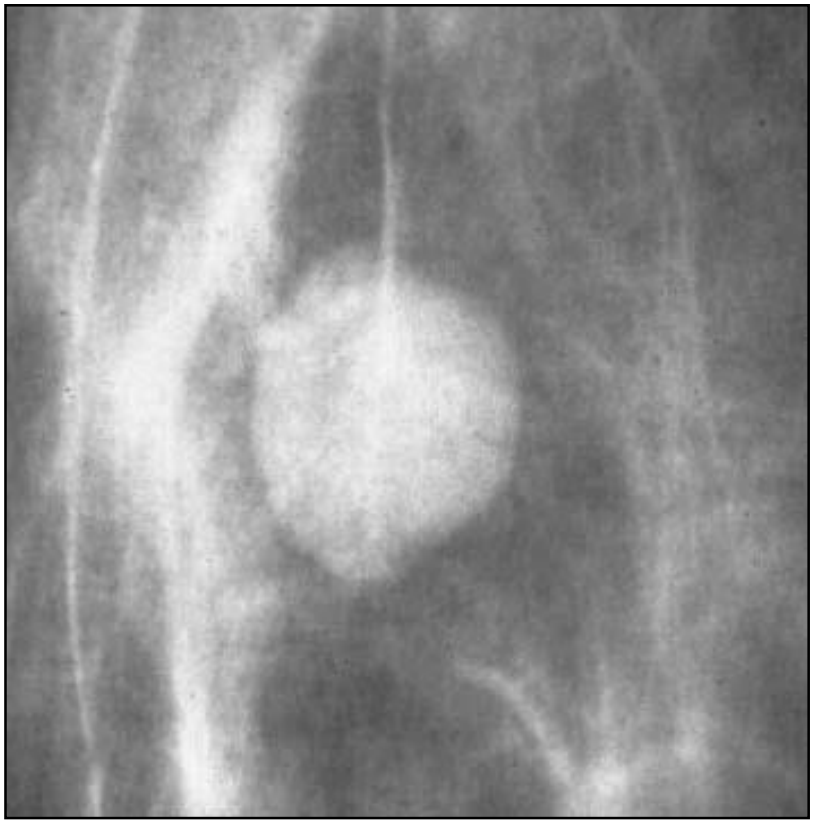
Sclerosing adenosis is the most common lesion mistaken for invasive carcinoma.

Distinguishing sclerosing adenosis from invasive carcinoma is particularly difficult when the lesion is involved by apocrine metaplasia, ductal carcinoma in situ, or lobular carcinoma in situ.

Invasive Carcinoma vs Sclerosing Lesion

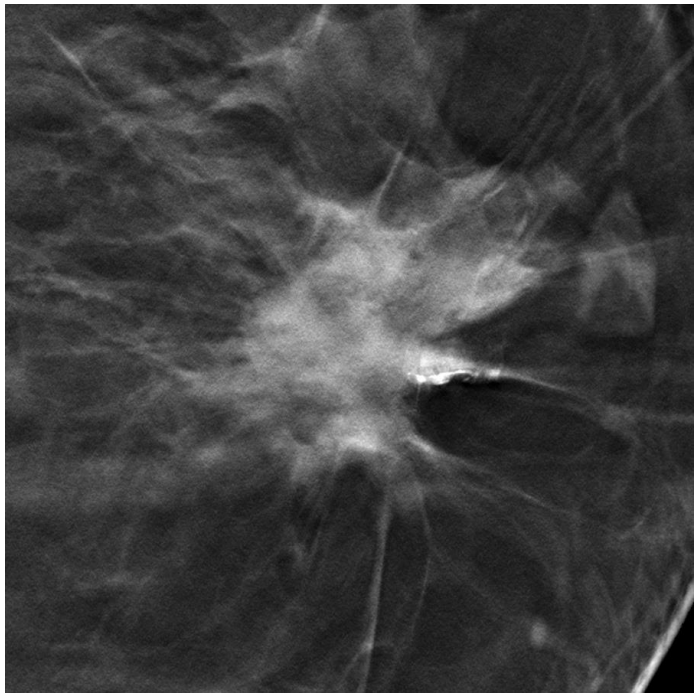


Usually forms a mass with spiculated margins.
Masses with circumscribed margins are less common.

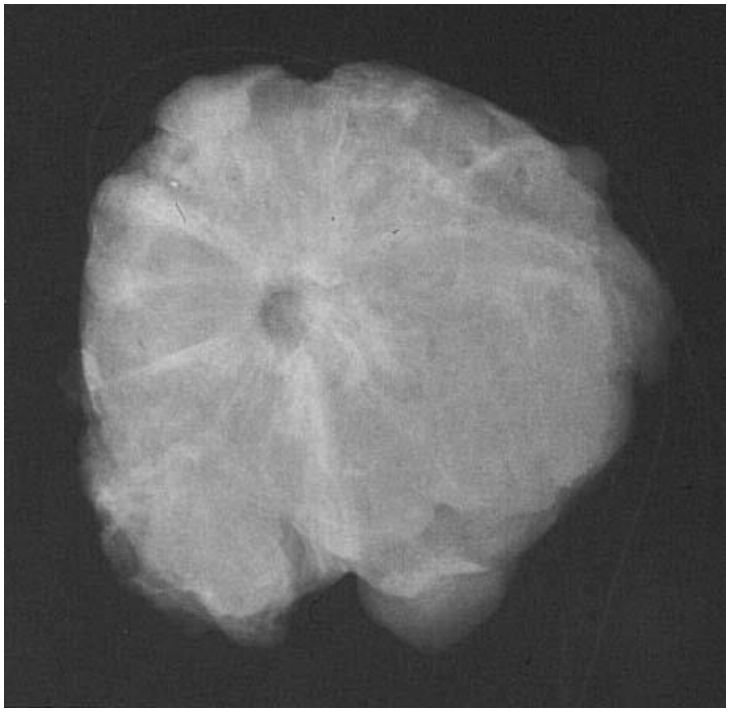


Often forms a mass with circumscribed margins – with the exception of . . .

Invasive Carcinoma vs Radial Sclerosing Lesion

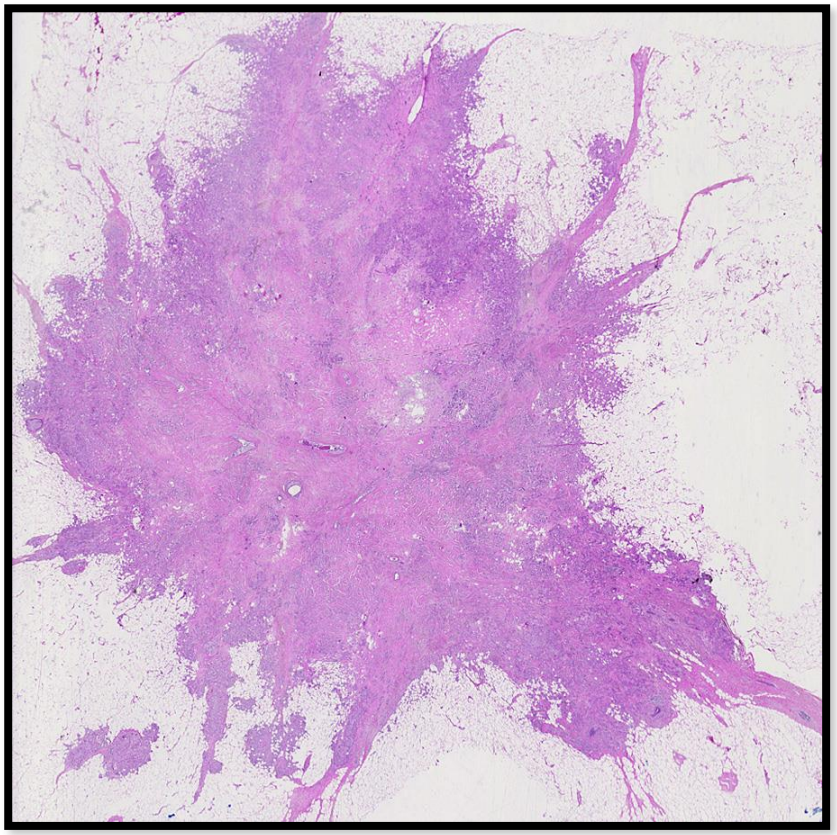


Invasive carcinoma: The central area of the irregular mass is larger than the length of the spicules.

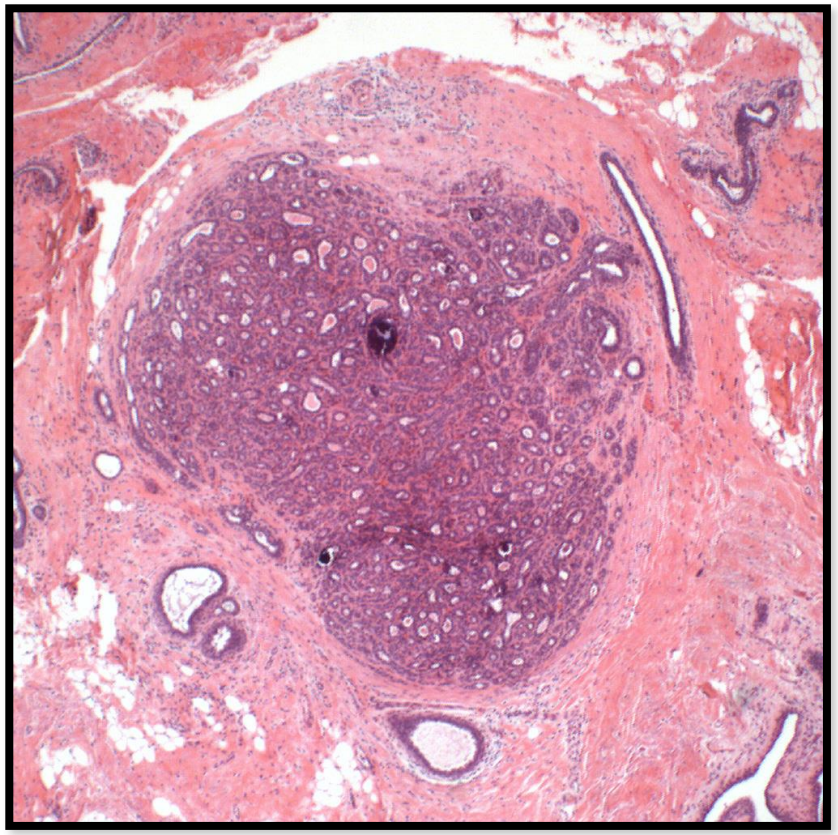


Radial sclerosing lesion: The central area of the irregular mass is smaller than the length of the spicules.

Invasive Carcinoma vs Sclerosing Lesion

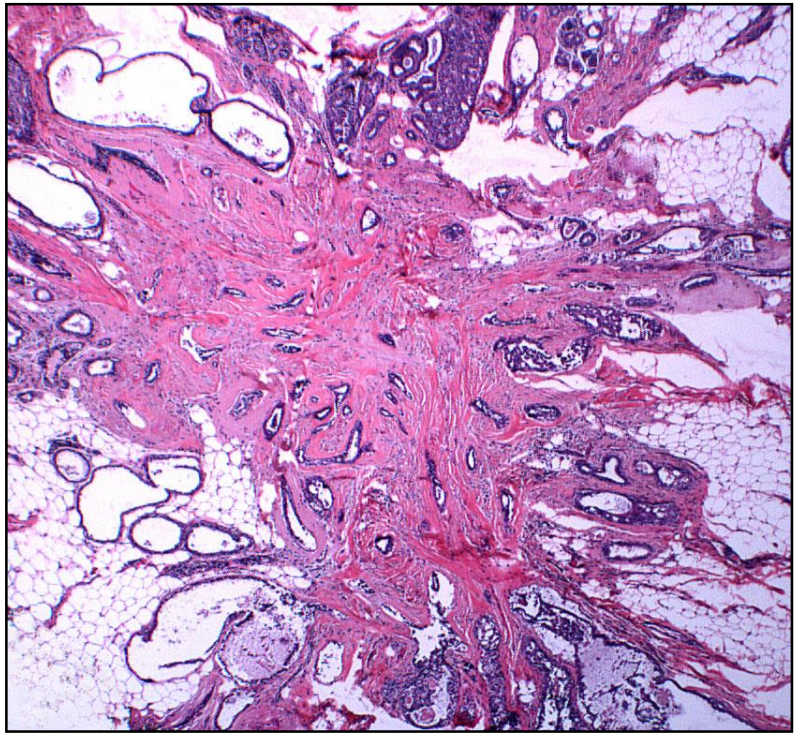


The majority of invasive carcinomas infiltrate into the surrounding tissue.

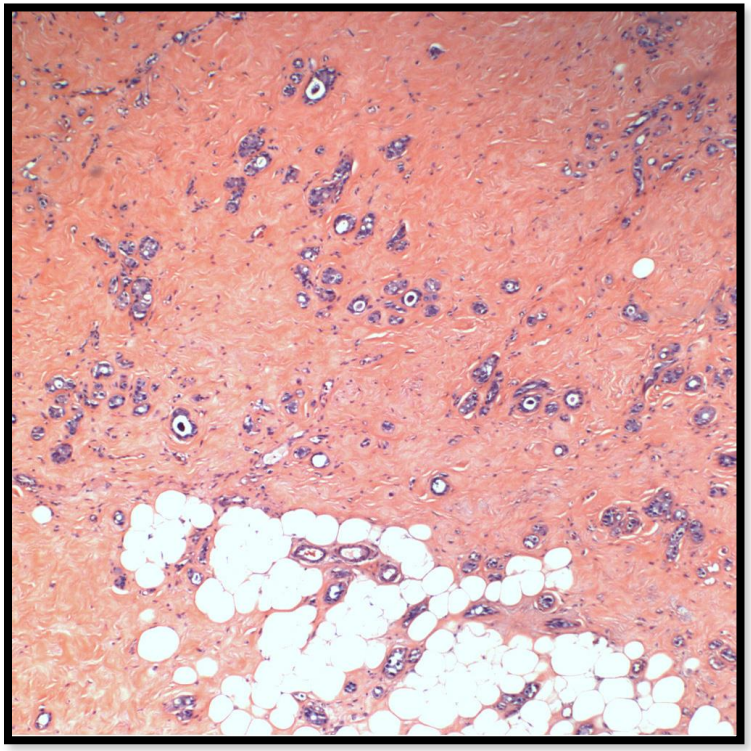


The majority of sclerosing lesions have circumscribed margins, except . . .

Radial Sclerosing Lesion & "Wandering Adenosis"

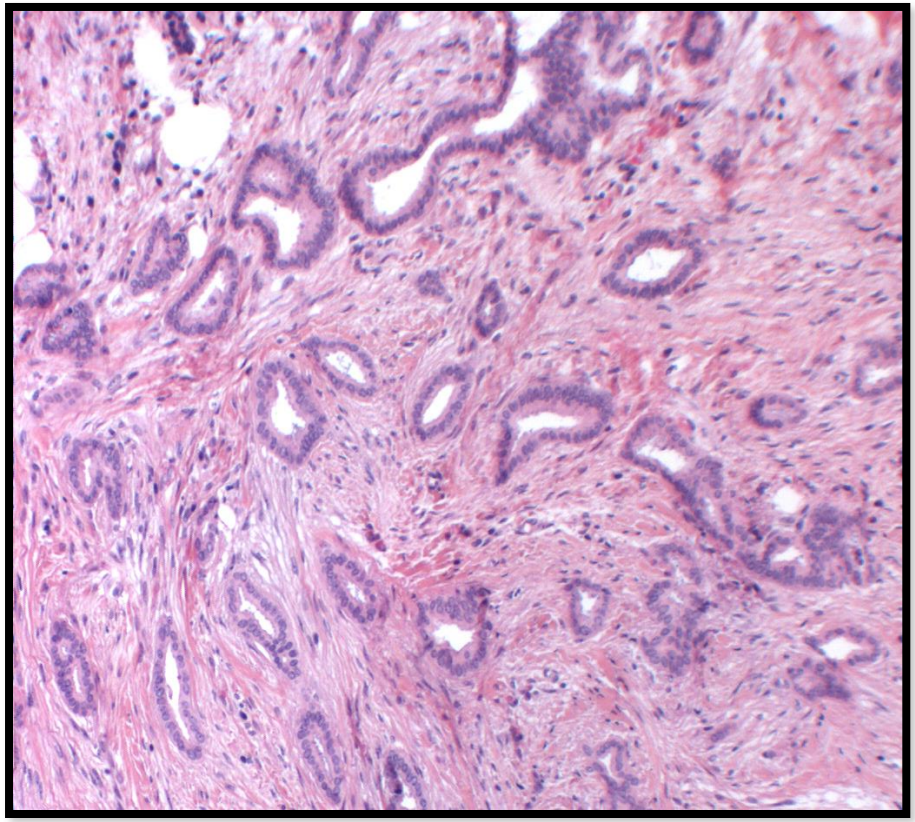


Radial sclerosing lesions (= radial scars) can have an irregular shape.

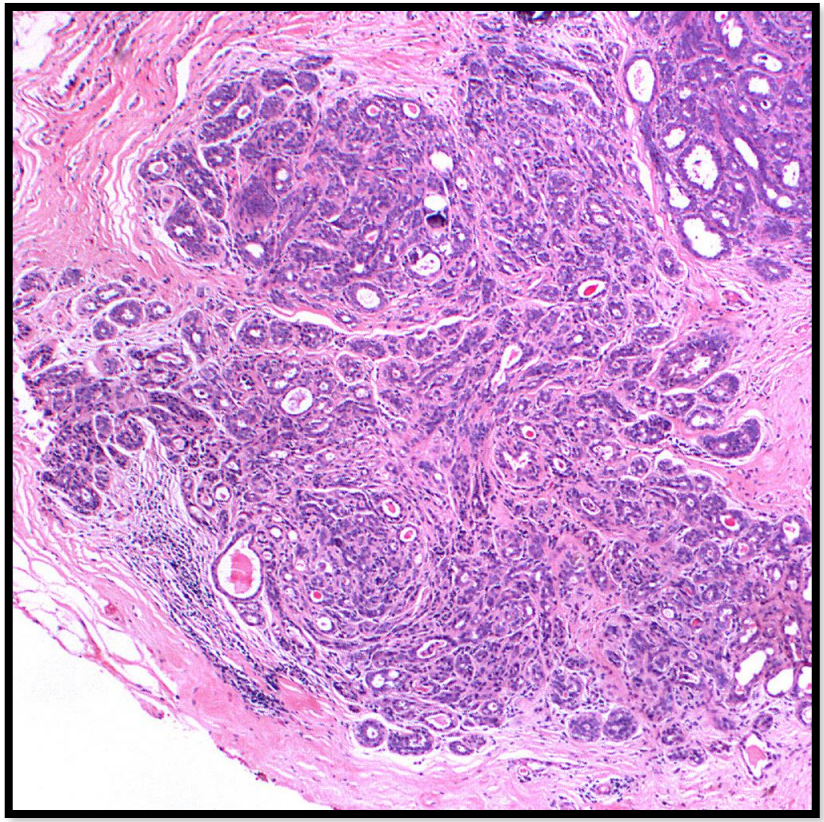


In "wandering adenosis" small tubules are scattered in breast tissue.

Invasive Carcinoma vs Sclerosing Lesion

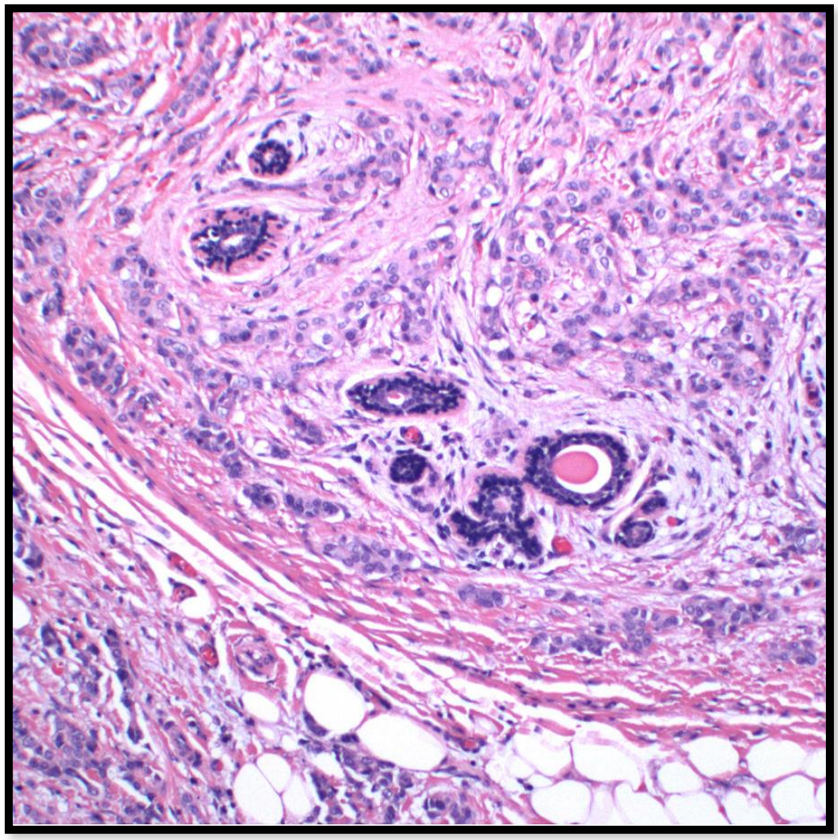


The tubules of invasive cancers are haphazardly distributed in a cellular desmoplastic stroma.

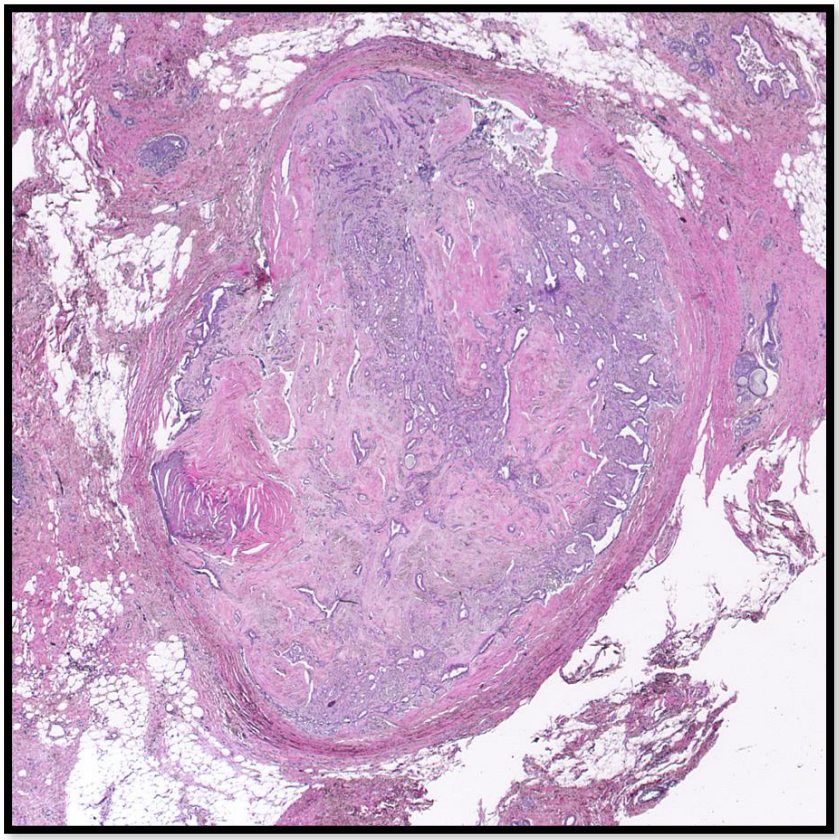


The tubules of sclerosing lesions are usually back-to-back and in a swirling pattern. The stroma is dense.

Invasive Carcinoma vs Sclerosing Lesion

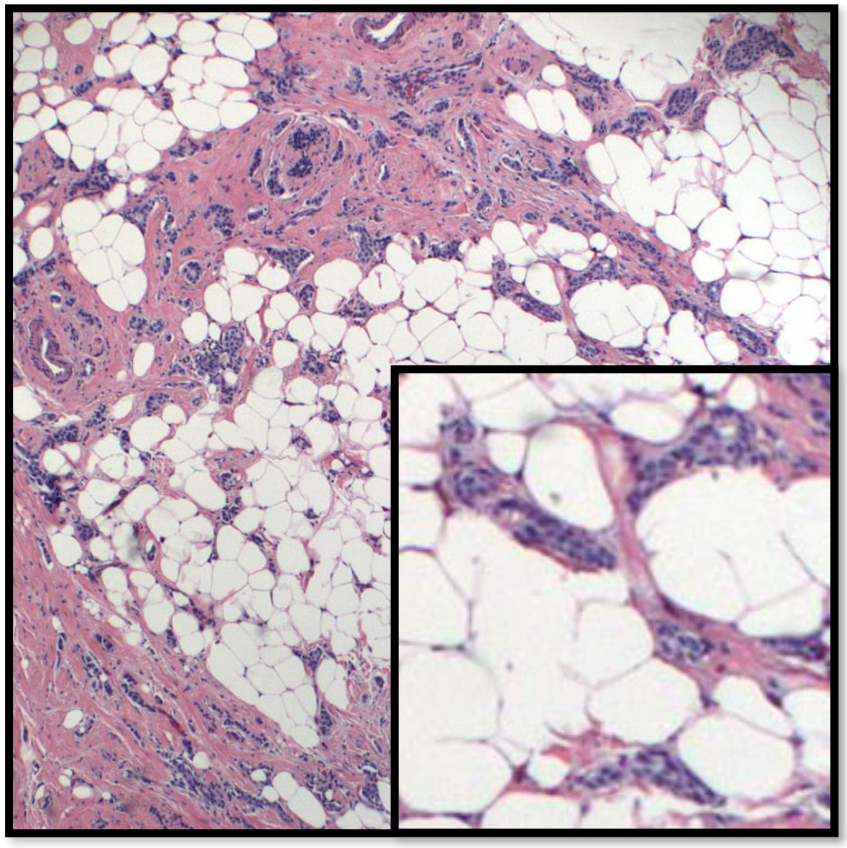


Invasive carcinomas typically invade around normal epithelium.

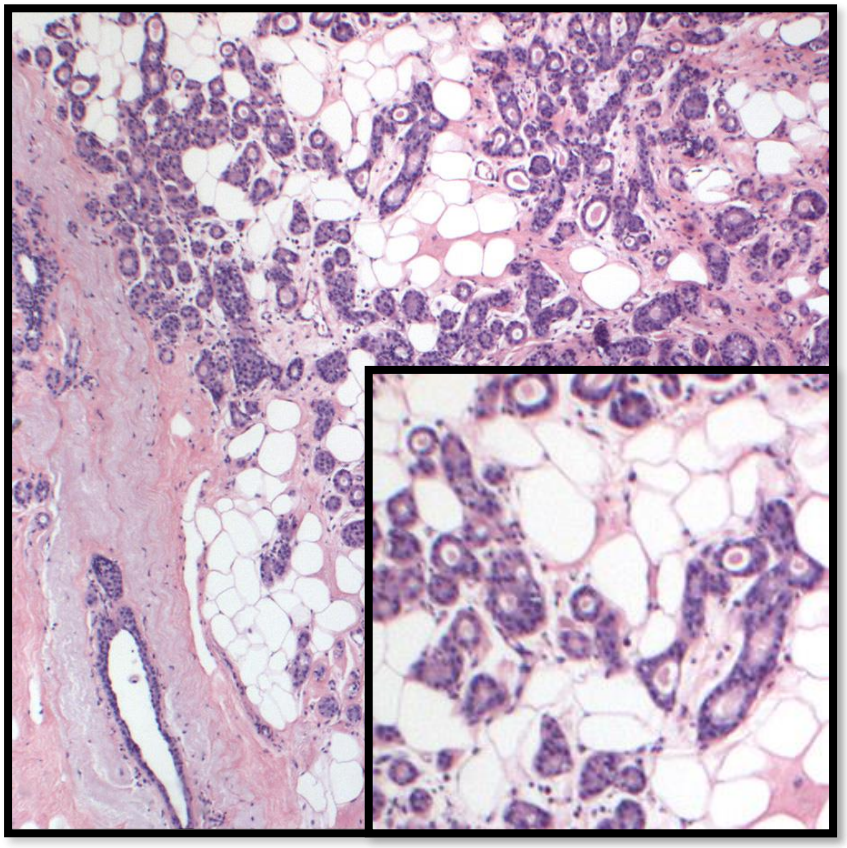


Sclerosing lesions consist of similar appearing tubules – invasion around distinct normal tissue is not seen.

Invasive Carcinoma vs Sclerosing Lesion

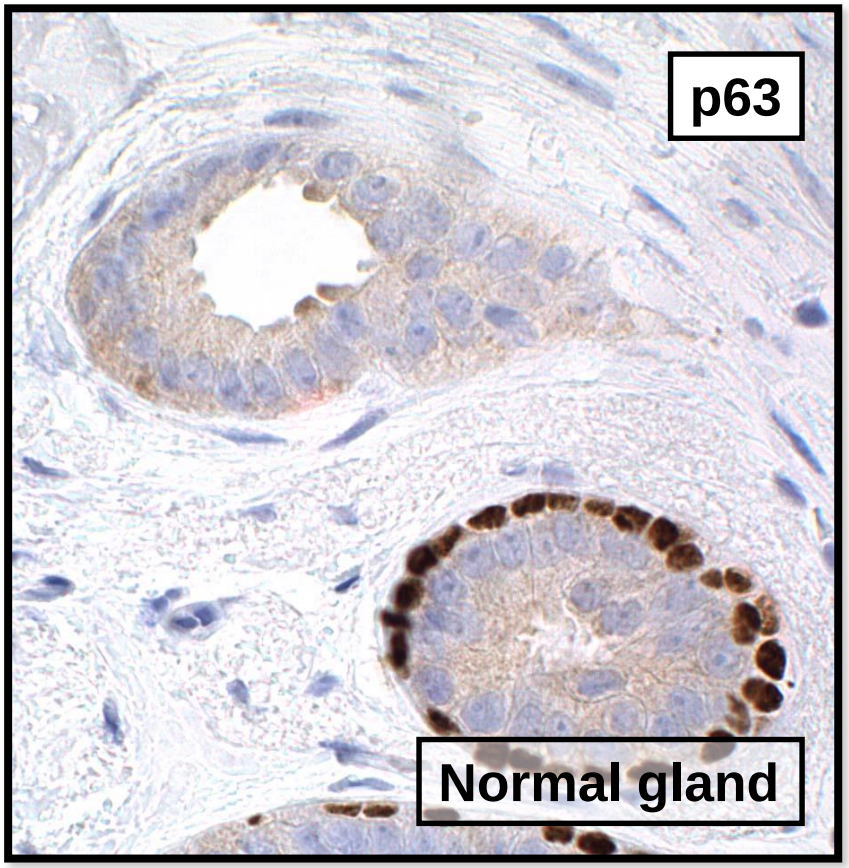


Invasive carcinomas often invade into adipose tissue and disrupt the adipocytes.

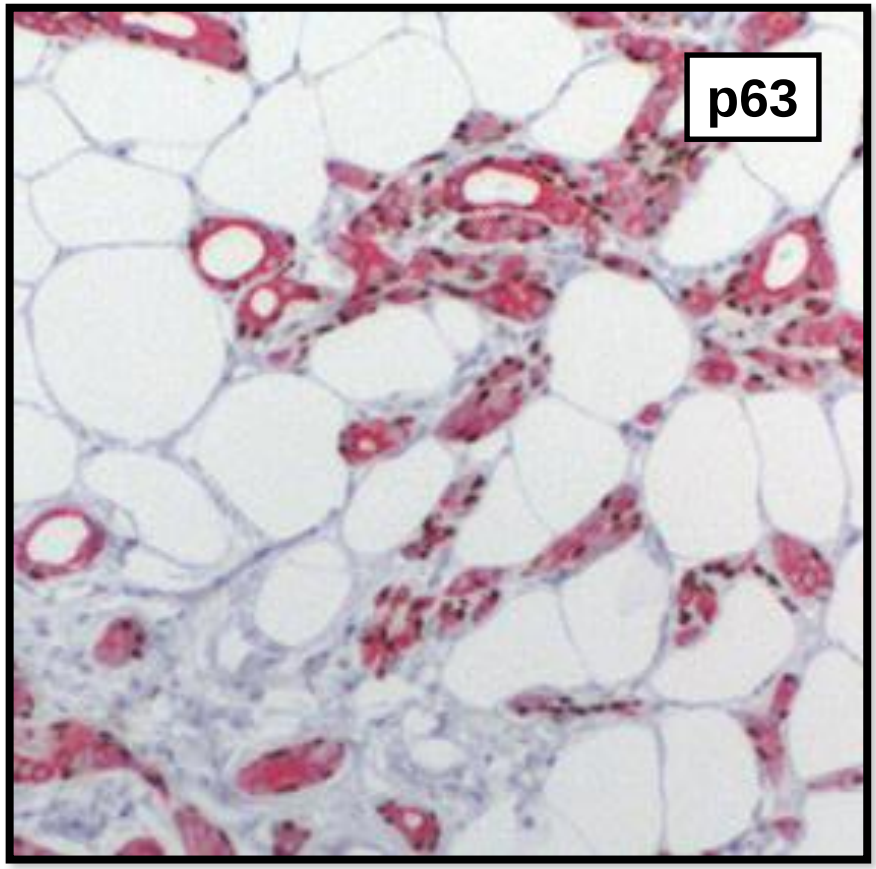


Sclerosing lesions can involve adipose tissue, but the adipocytes do not appear to be disrupted by the tubules.

Invasive Carcinoma vs Sclerosing Lesion

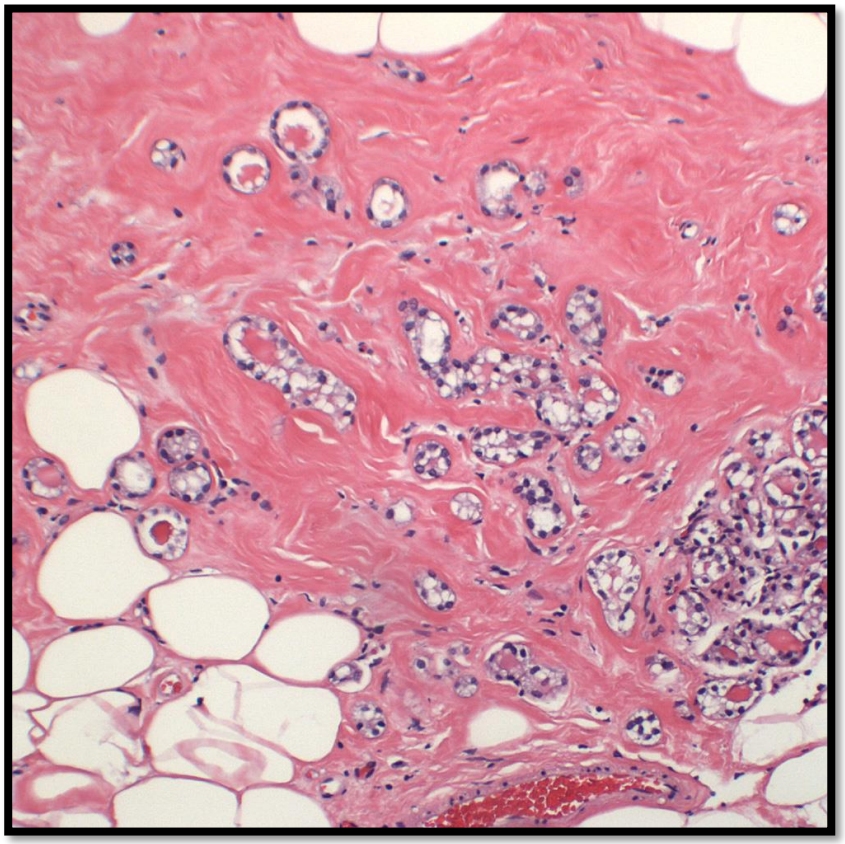


Immunoperoxidase studies can confirm the absence of myoepithelial cells in cancers.

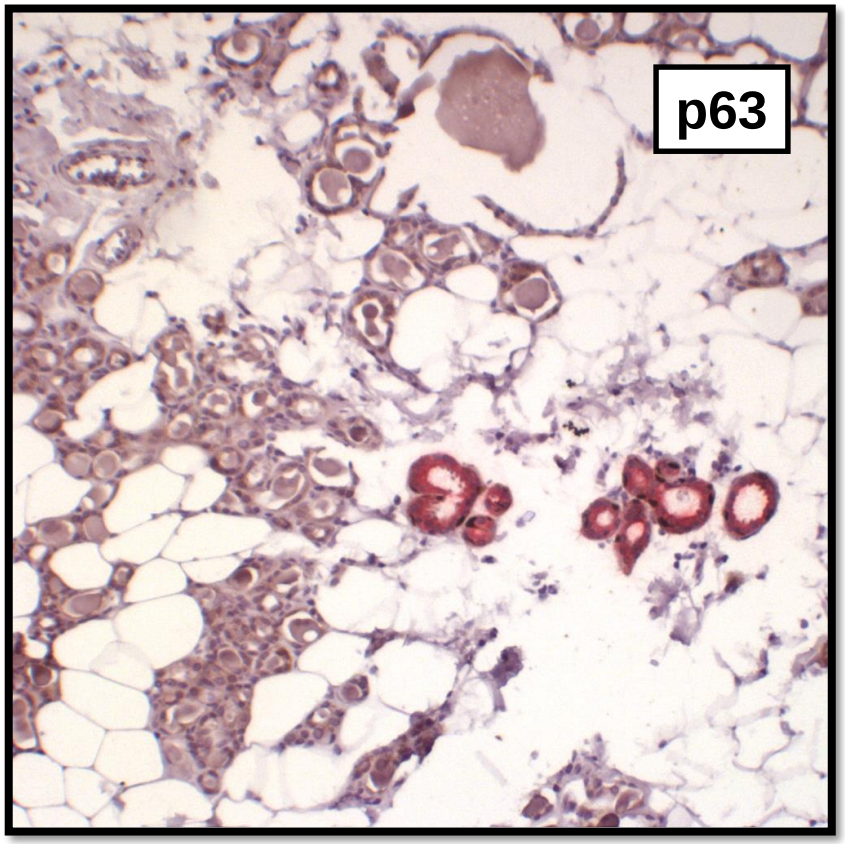


Immunoperoxidase studies can confirm the presence of myoepithelial cells in sclerosing lesions.

Benign Lesions Lacking Myoepithelial Cells

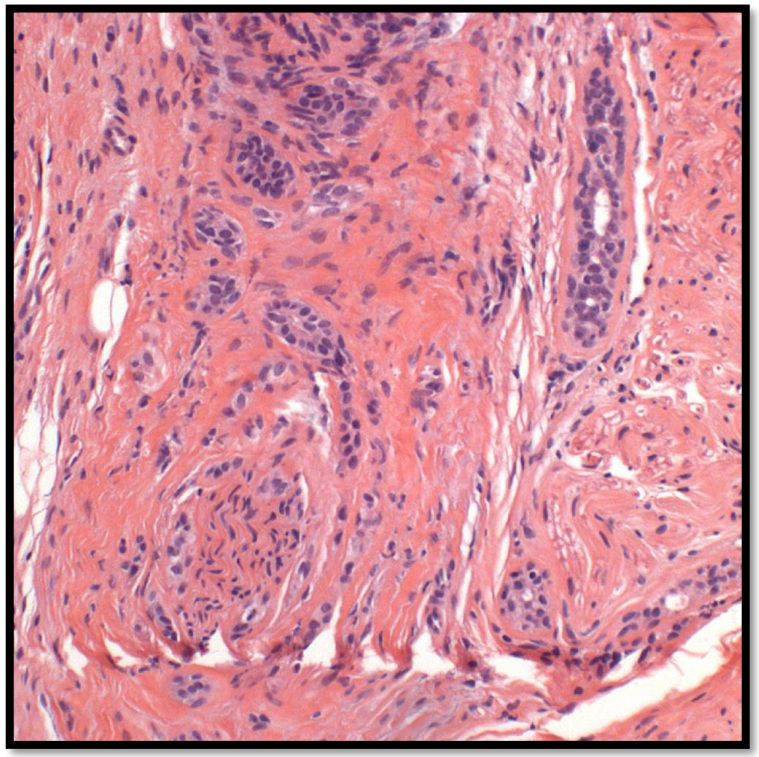


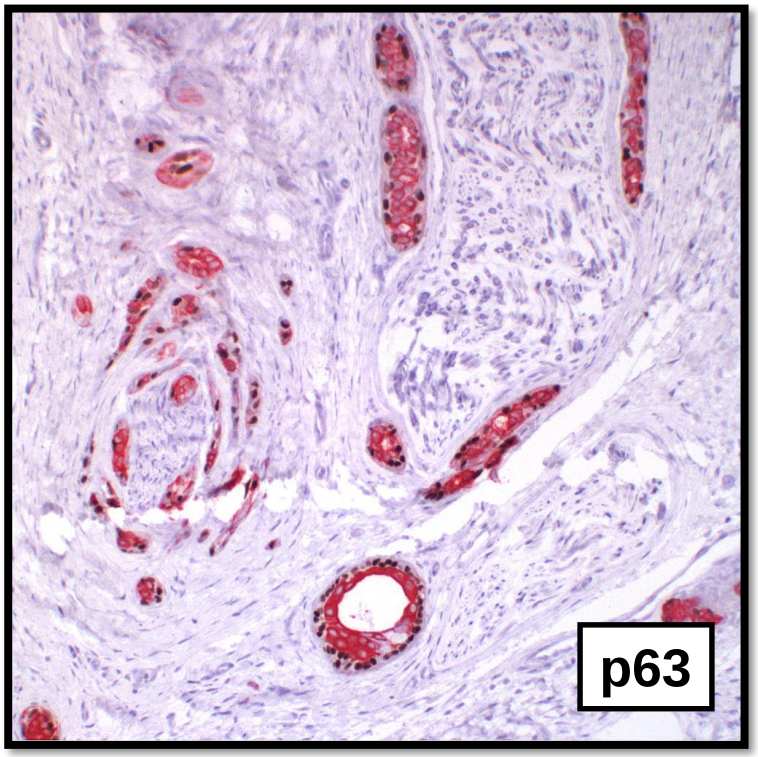
Microglandular adenosis is also a small glandular proliferation that infiltrates in breast tissue.



No myoepithelial cells are present. Other rare benign glandular lesions (some apocrine) can also lack myoepithelial cells.

Sclerosing Lesion – “pseudo perineural invasion”





Benign sclerosing lesions can involve nerves.

Artifactual displacement can mimic lymphovascular invasion.

Immunoperoxidase studies demonstrate that the tubules in nerves have myoepithelial cells.

Myoepithelial Markers

Antibody	Location	Myoepithelial cells	Myofibroblasts	Vessels	Tumor Cells
P63	Nucleus	Strong	Negative	Negative	Occasional
SMA	Cytoplasm	Strong	Moderate	Strong	Rare
SM-MHC	Cytoplasm	Strong	Rare	Strong	Rare
Calponin	Cytoplasm	Strong	Moderate	Strong	Rare
CD10	Cytoplasm	Strong	Moderate	Variable	Occasional

P63 is very specific, but less “sensitive” than muscle markers as the nucleus is smaller than the cell body (cytoplasm).

Muscle markers are more “sensitive” (larger area to stain), but less specific as stromal cells and blood vessels stain.

Myoepithelial cells may be reduced in number and may fail to express some proteins when associated with some types of lesions . . .

Myoepithelial Markers – Reduced Immunoreactivity

Marker	% of lesions with reduced staining	
	Sclerosing lesions	DCIS
Cytokeratin 5/6	32%	30%
SM-MHC	21%	77%
CD10	15%	34%
P63	9%	13%
Calponin	6%	17%
Smooth muscle actin	0%	1%

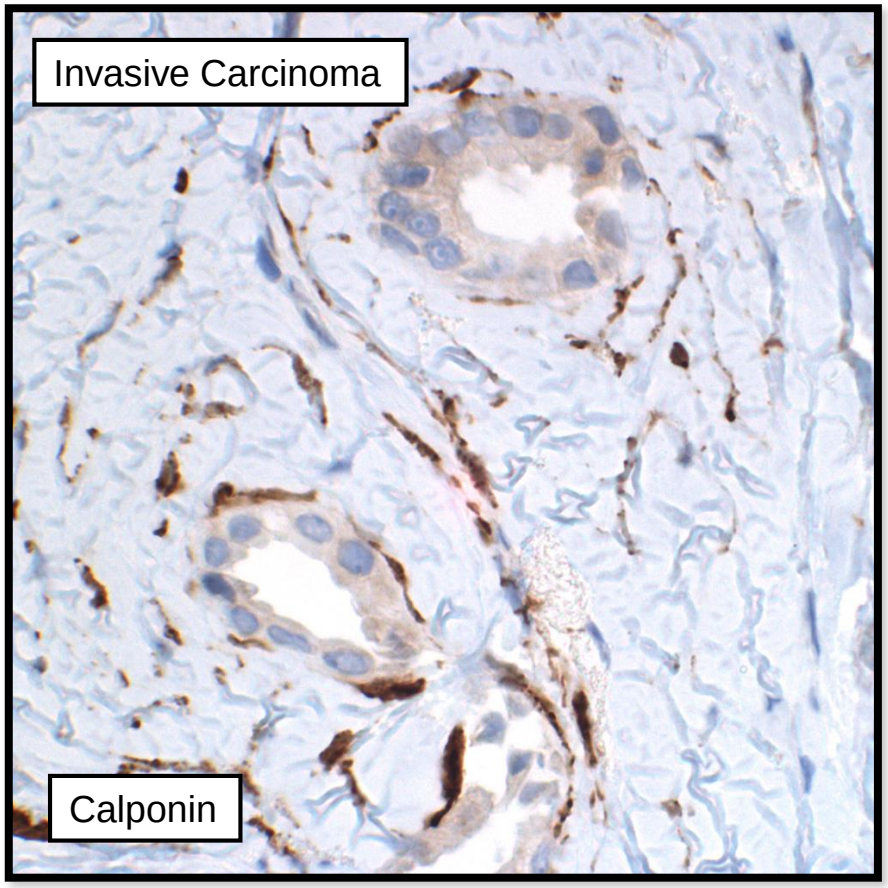
Use multiple markers before concluding myoepithelial cells are not present. Use more if initial results not clear.

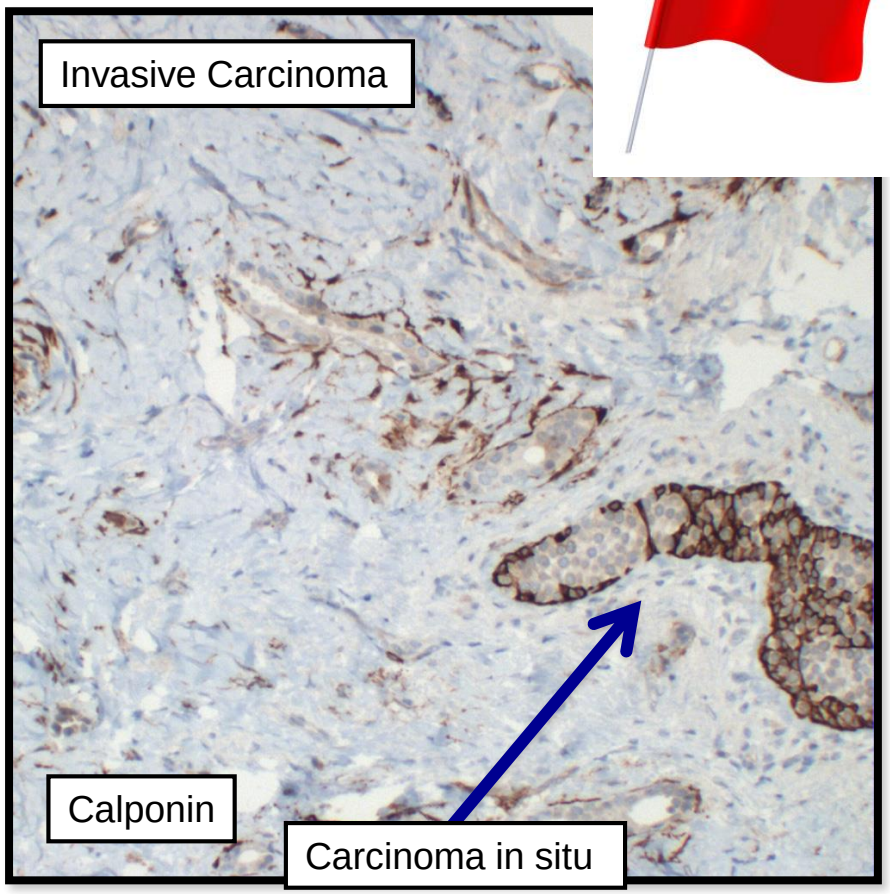
A conservative approach is advised – don't base a diagnosis of invasive carcinoma on IHC alone!

Hilson, JB, Schnitt SJ, Collins LC, Phenotypic alterations in myoepithelial cells associated with benign sclerosing lesions of the breast, Am J Surg Pathol 34:896-900, 2010.

Hilson JB, Schnitt SJ, Collins LC, Phenotypic alterations in ductal carcinoma in situ-associated myoepithelial cells, Am J Surg Pathol 33:227-232, 2009.

Myofibroblasts Mimicking Myoepithelial Cells



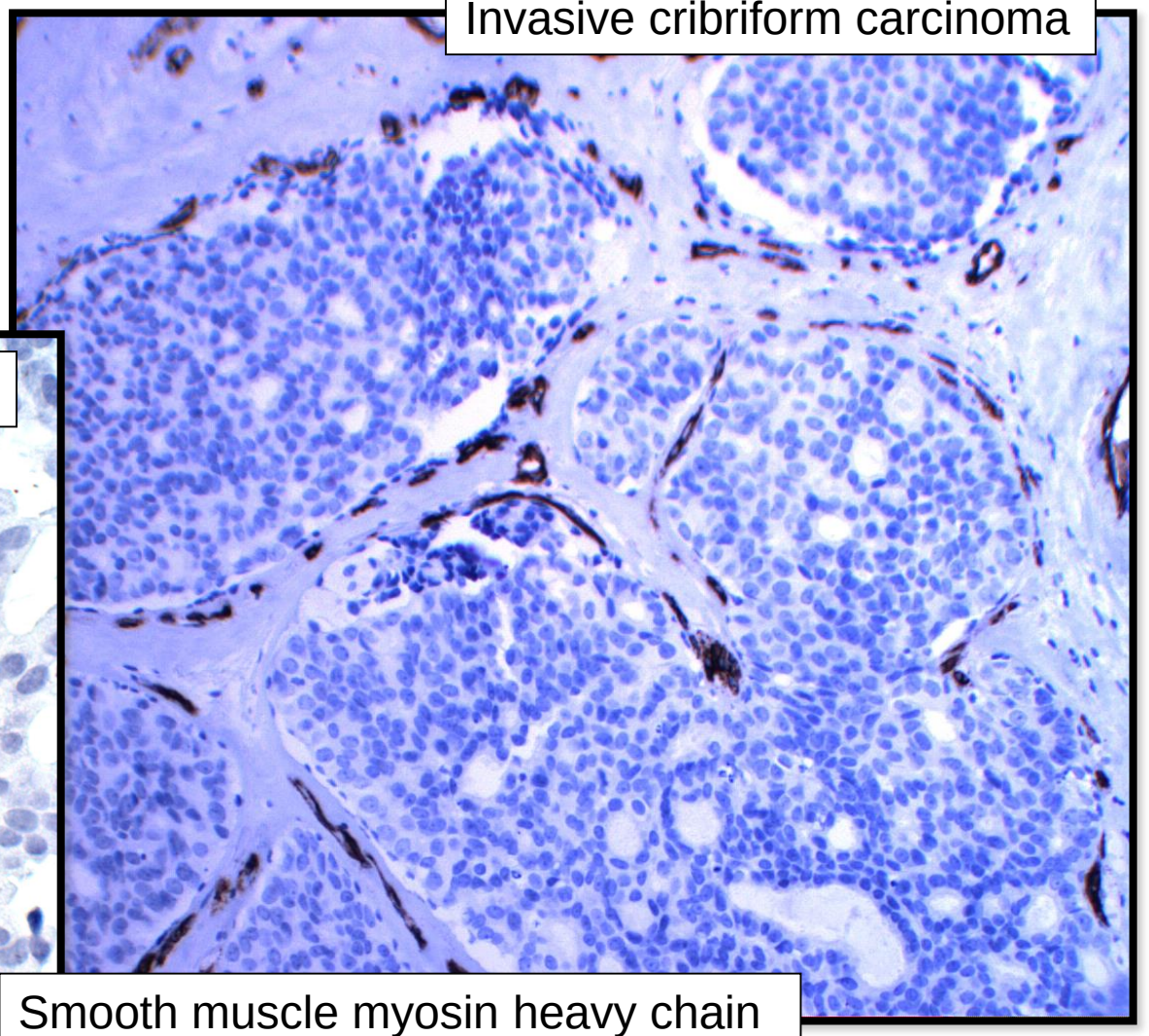


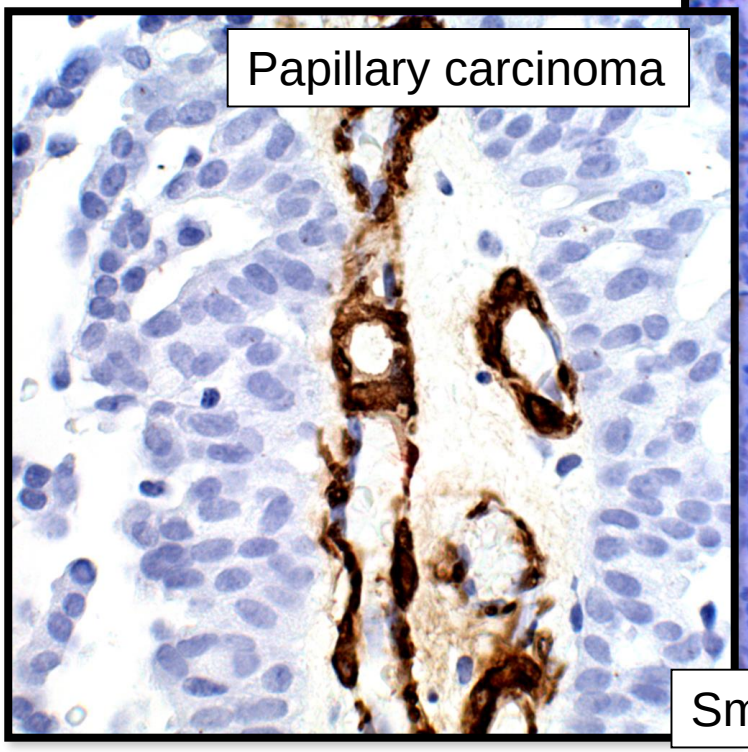
Myofibroblasts adjacent to tumor cell nests can mimic a myoepithelial cell layer.

True myoepithelial cells should be located above the basement membrane. P63 can be helpful to confirm their presence.

Blood Vessels Mimicking Myoepithelial Cells

Small blood vessels positive for muscle markers closely apposed to tumor cells can mimic myoepithelial cells.

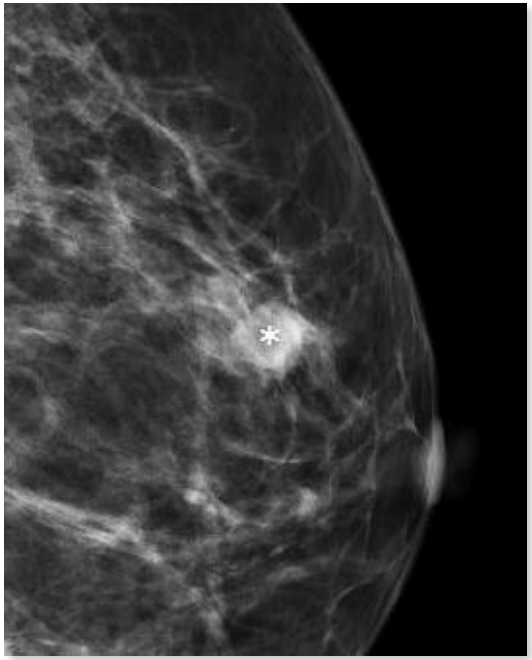


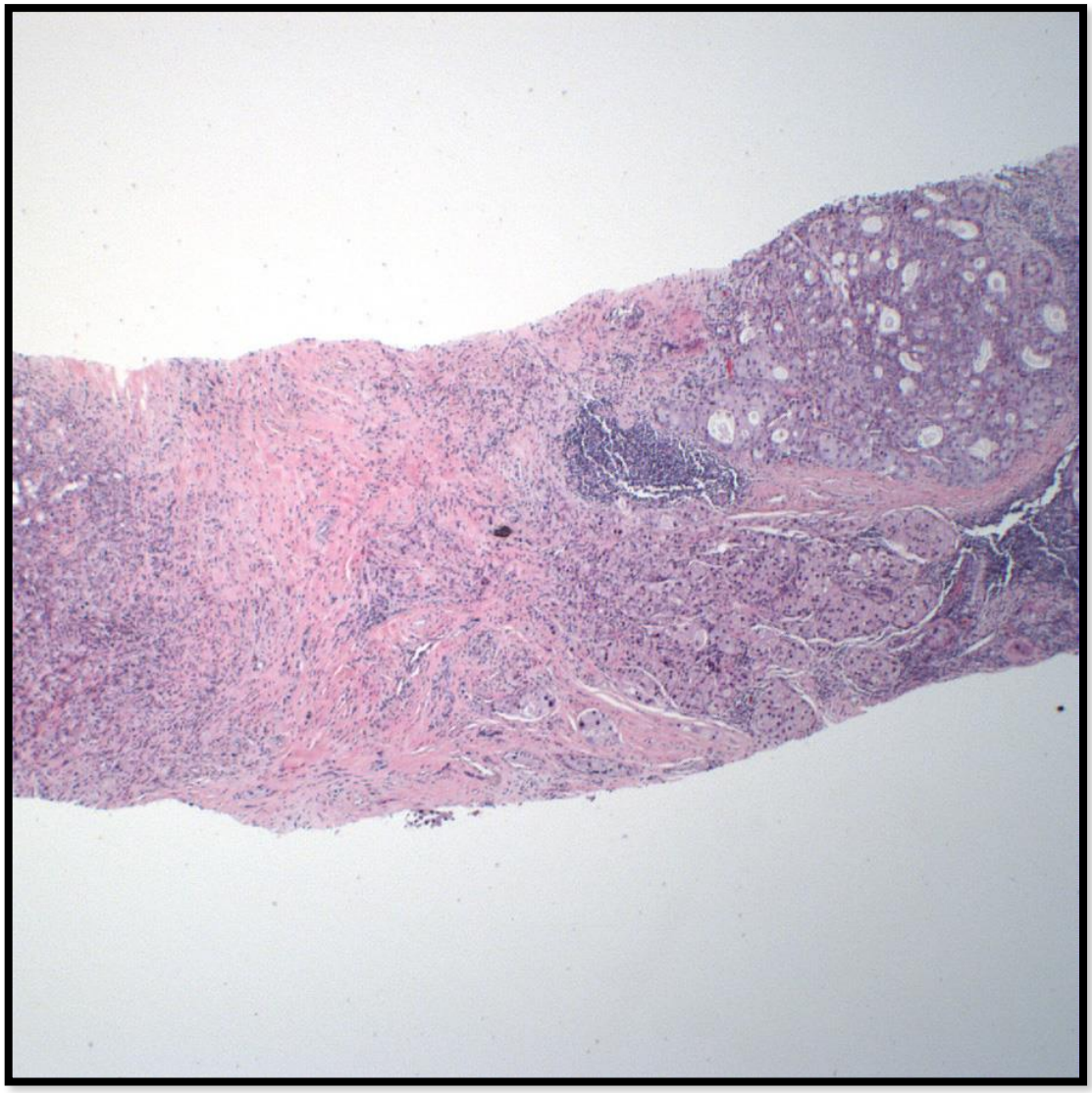


Invasive Carcinoma vs Sclerosing Lesion

Feature	Sclerosing Lesion	Invasive Carcinoma
Margins	Circumscribed margins or irregular margins (RSL)	Irregular margins
Low power pattern	Lobulocentric	Haphazard, infiltrates around normal ducts/lobules
Relationship of glands	Usually back-to-back, swirling	Separated by stroma
Adipose tissue	If involved, maintains tubular pattern	May invade as irregular nests
Stroma	Sclerotic, dense	Loose, desmoplastic
Glands	Distorted, elongated, compressed lumens, no snouts	Angulated, open lumens, apocrine snouts
Myoepithelial cells	Present (may be scant)	Absent
DCIS	Usually absent – but close mimic of invasive carcinoma if present	Usually present
Estrogen receptor	Positive – heterogeneous	Positive – usually strong

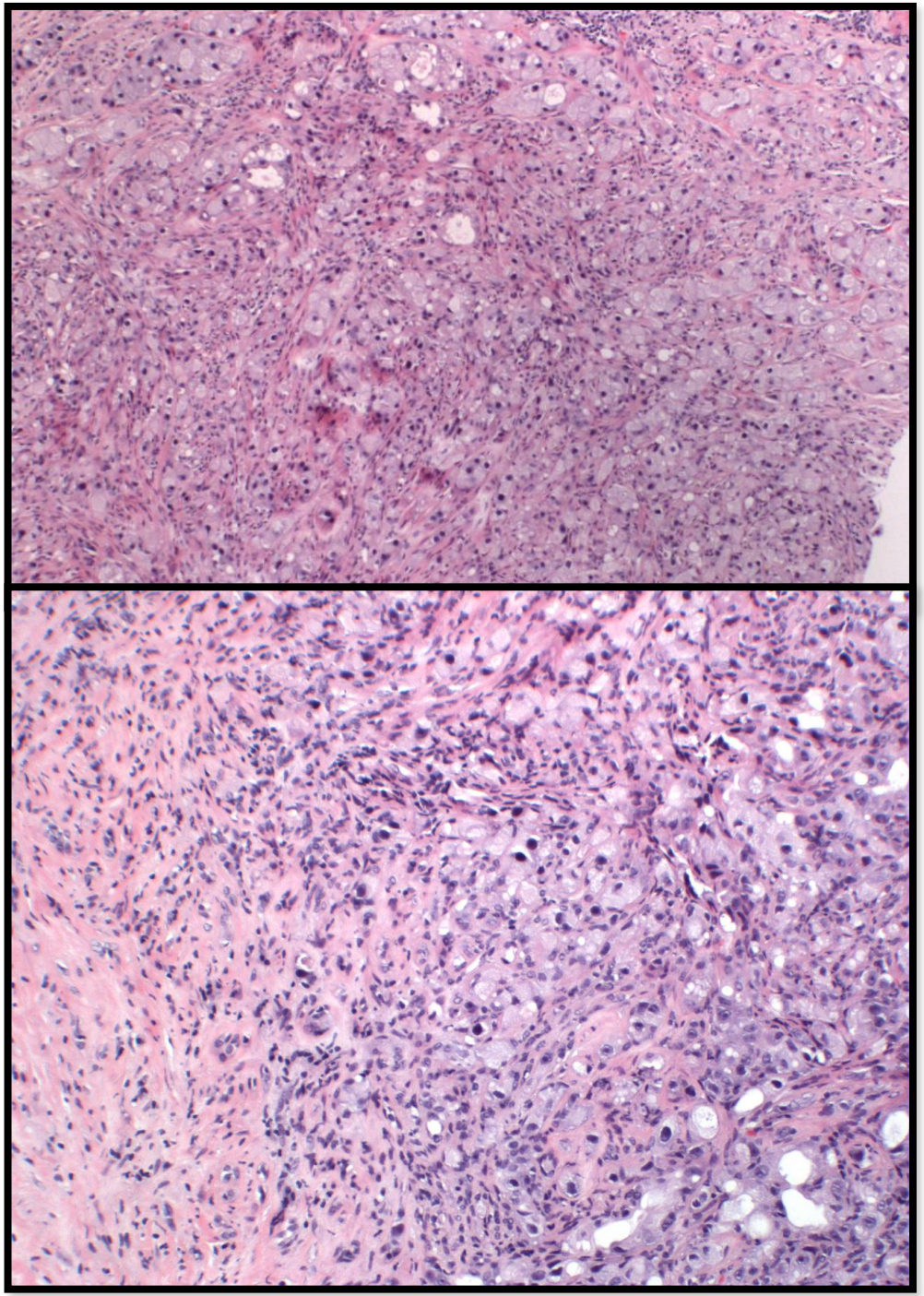
Three cautionary difficult cases: Case 1





A core needle biopsy for a mass with circumscribed margins was performed.

The diagnosis was invasive carcinoma.

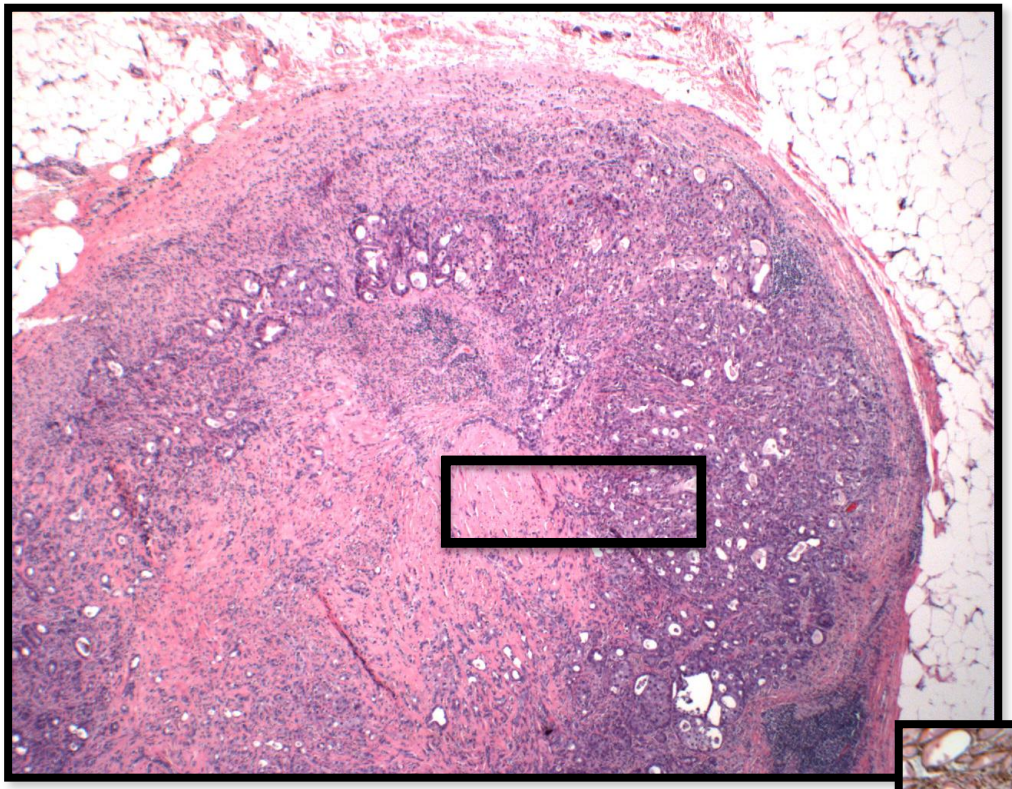


Possible red flags –

Circumscribed borders by imaging – but with appearance of invasion into fibrous tissue.

Back-to-back glands.

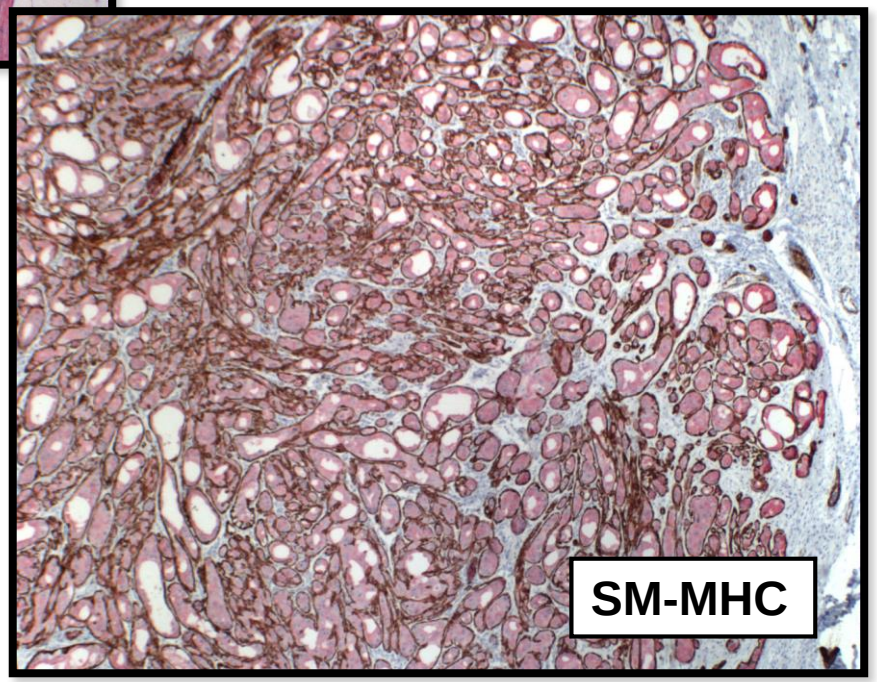
Absence of infiltration around normal ducts or lobules or into fat.

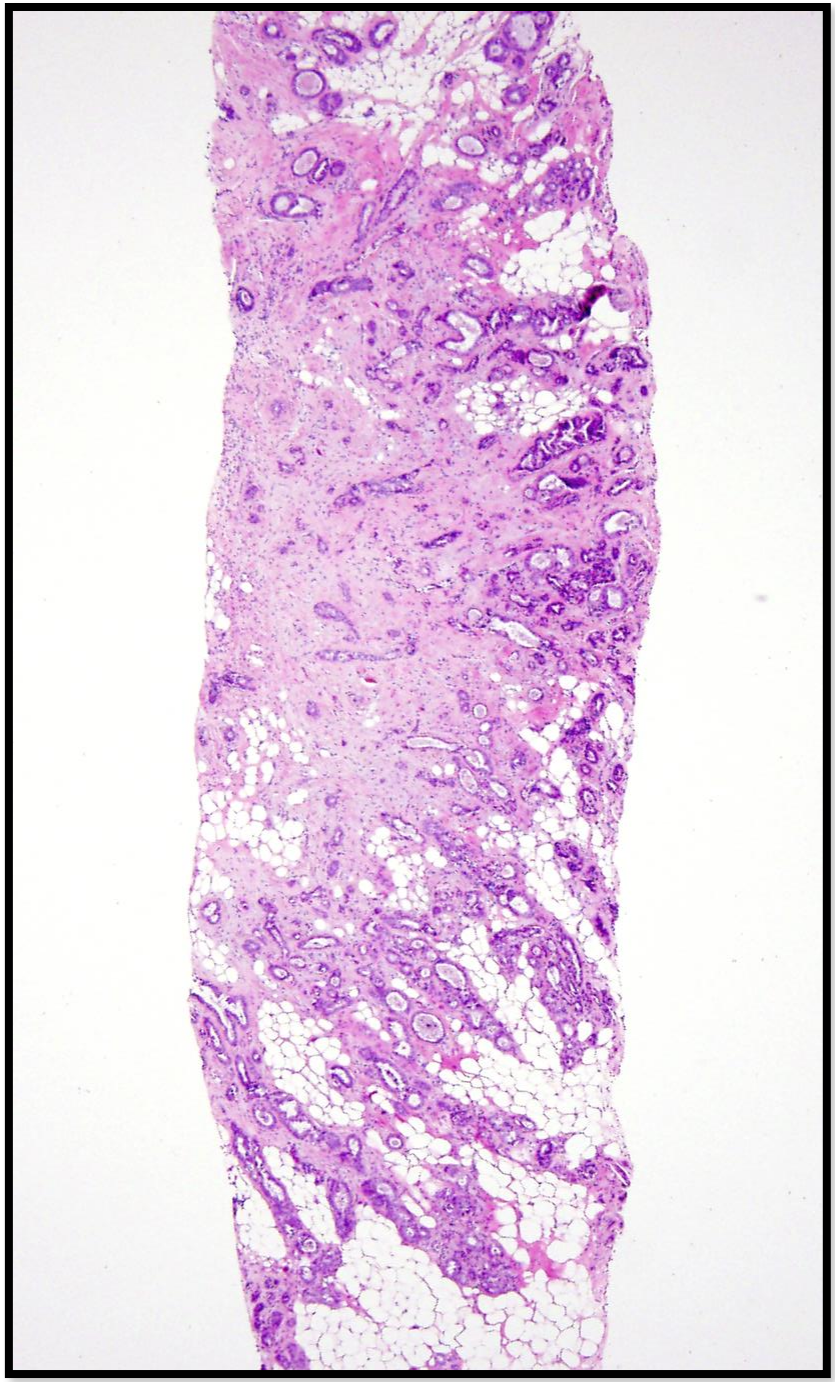


On excision, the lesion was more clearly DCIS involving a sclerosing lesion with a circumscribed border.

The appearance of invasion into adjacent stroma was mimicked by the central area of dense fibrosis.

Subsequent IHC on the core needle biopsy confirmed the presence of a myoepithelial cell layer.



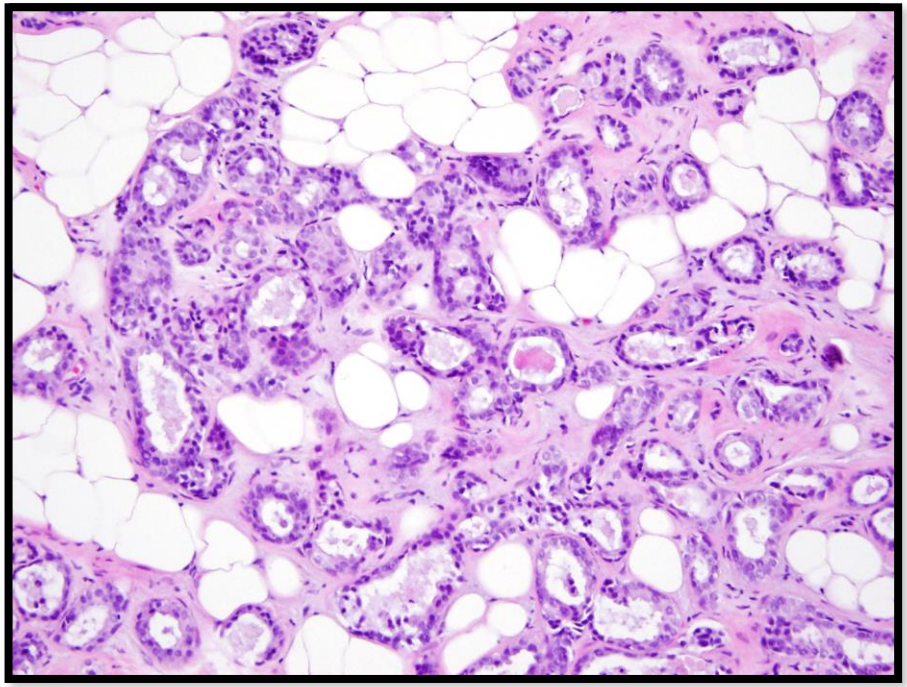


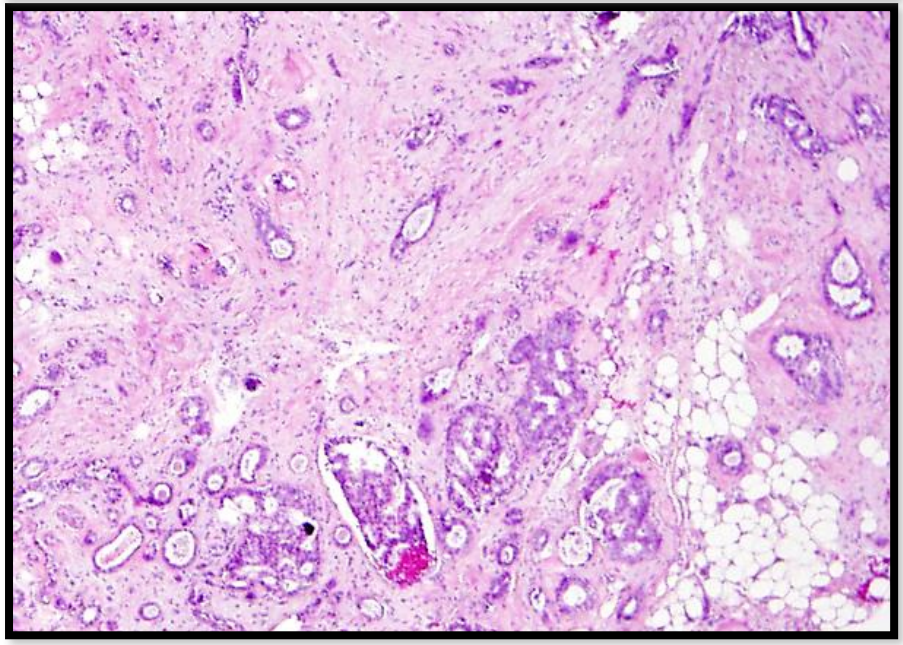
Cautionary Case 2

A core needle biopsy was performed for architectural distortion.

The diagnosis was invasive carcinoma.

- Haphazard arrangement
- Tubules separated by stroma
- Irregular border



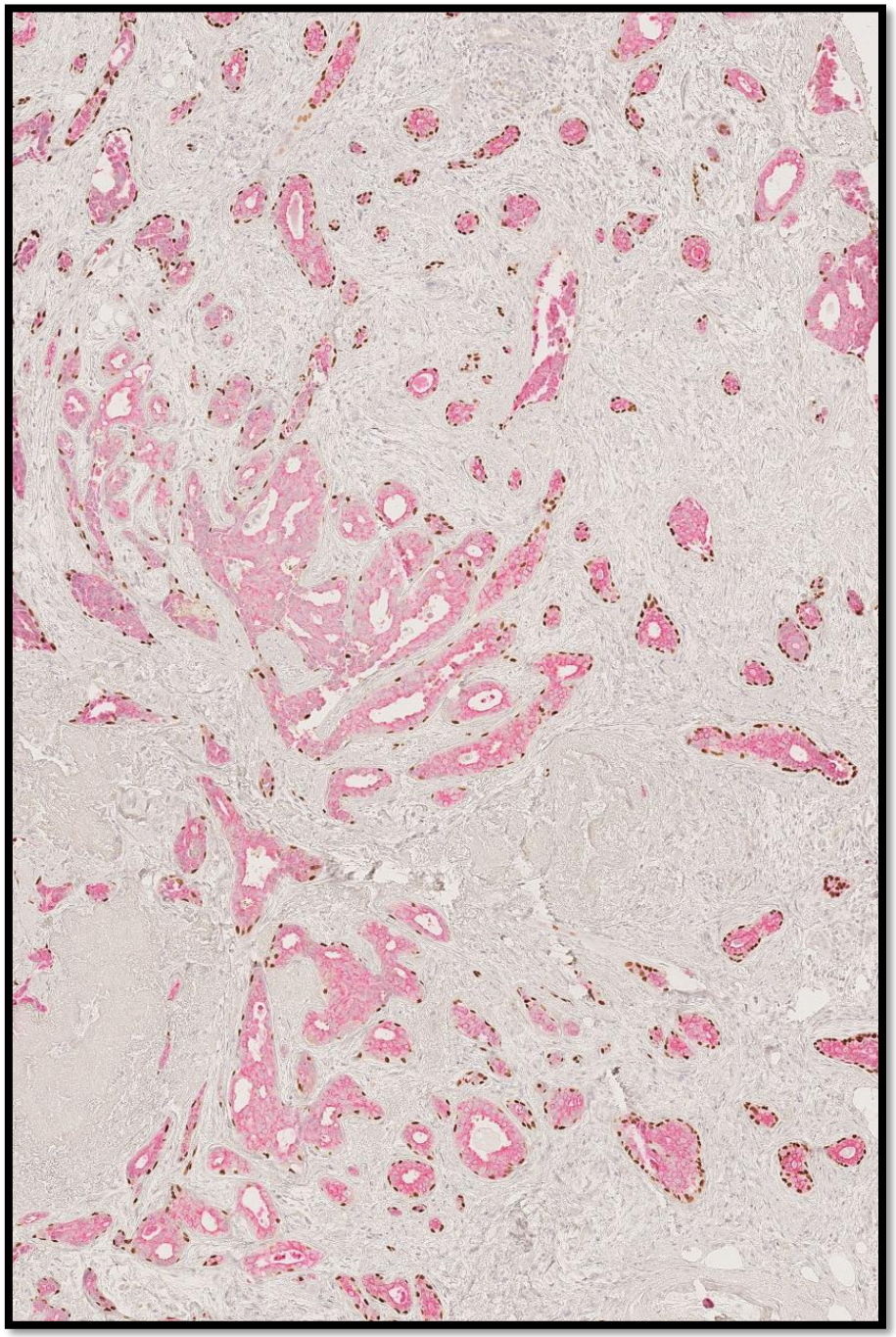


Difficult features –

Infiltration into fat – However, tubules “respect the fat.”

Fibrotic background (perhaps denser than typical for carcinoma).

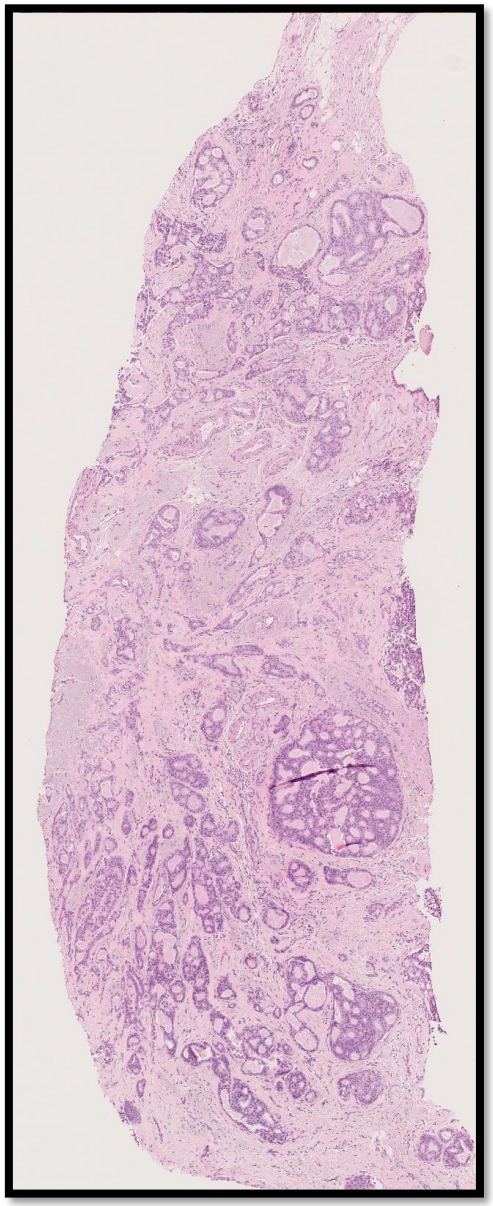
DCIS present in irregular spaces resemble invasive foci.



The error was discovered when the excisional specimen showed DCIS and LCIS involving sclerosing adenosis.

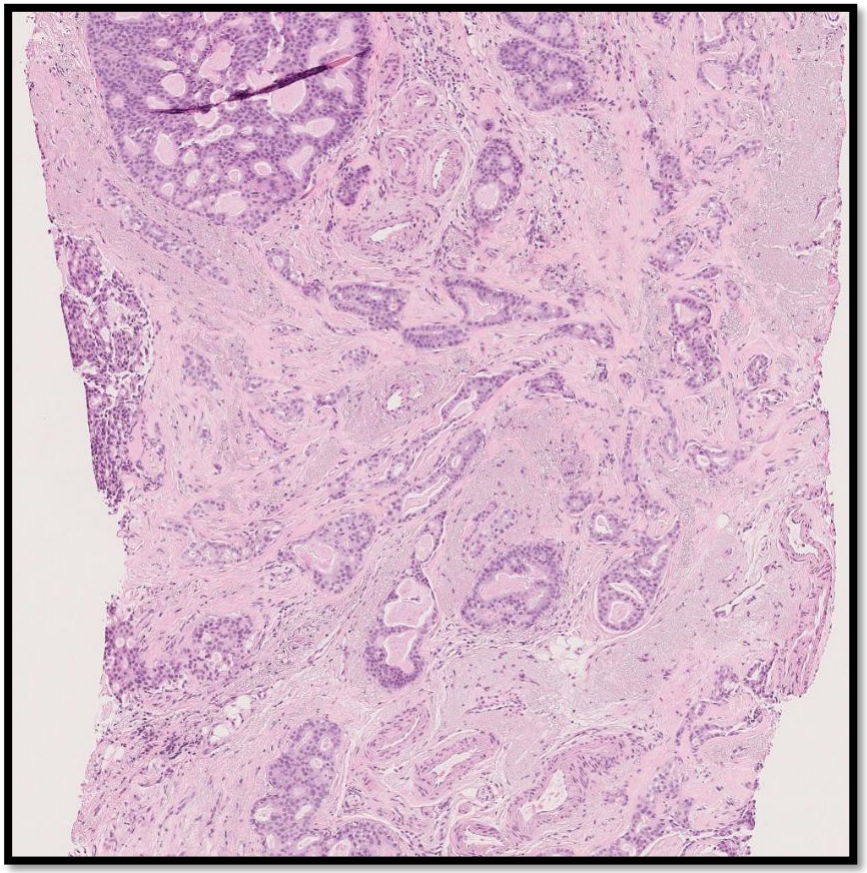
Immunoperoxidase studies on the core needle biopsy confirmed the presence of myoepithelial cells.

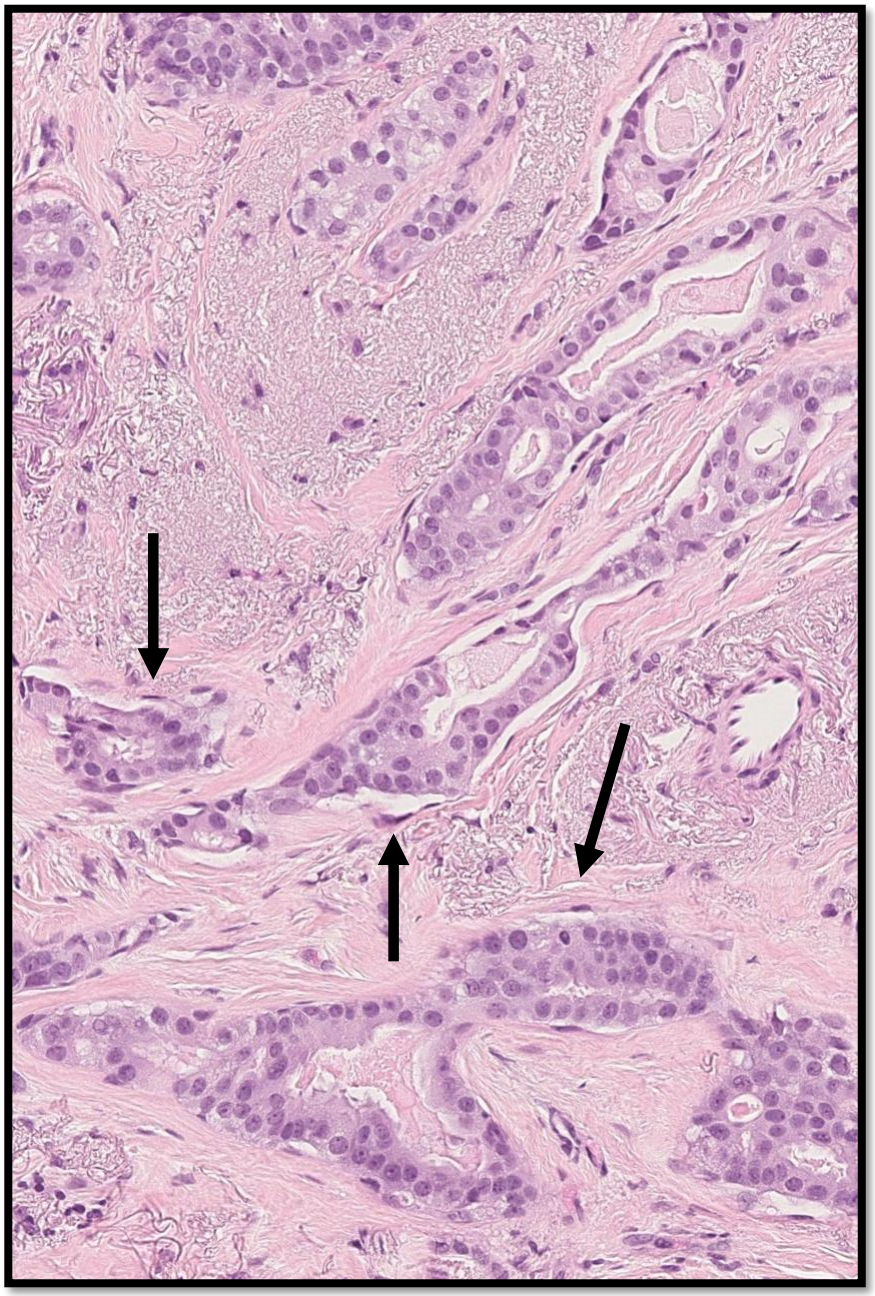
Cautionary Case #3



A woman was found to have a 0.8 cm irregular mass with indistinct margins.

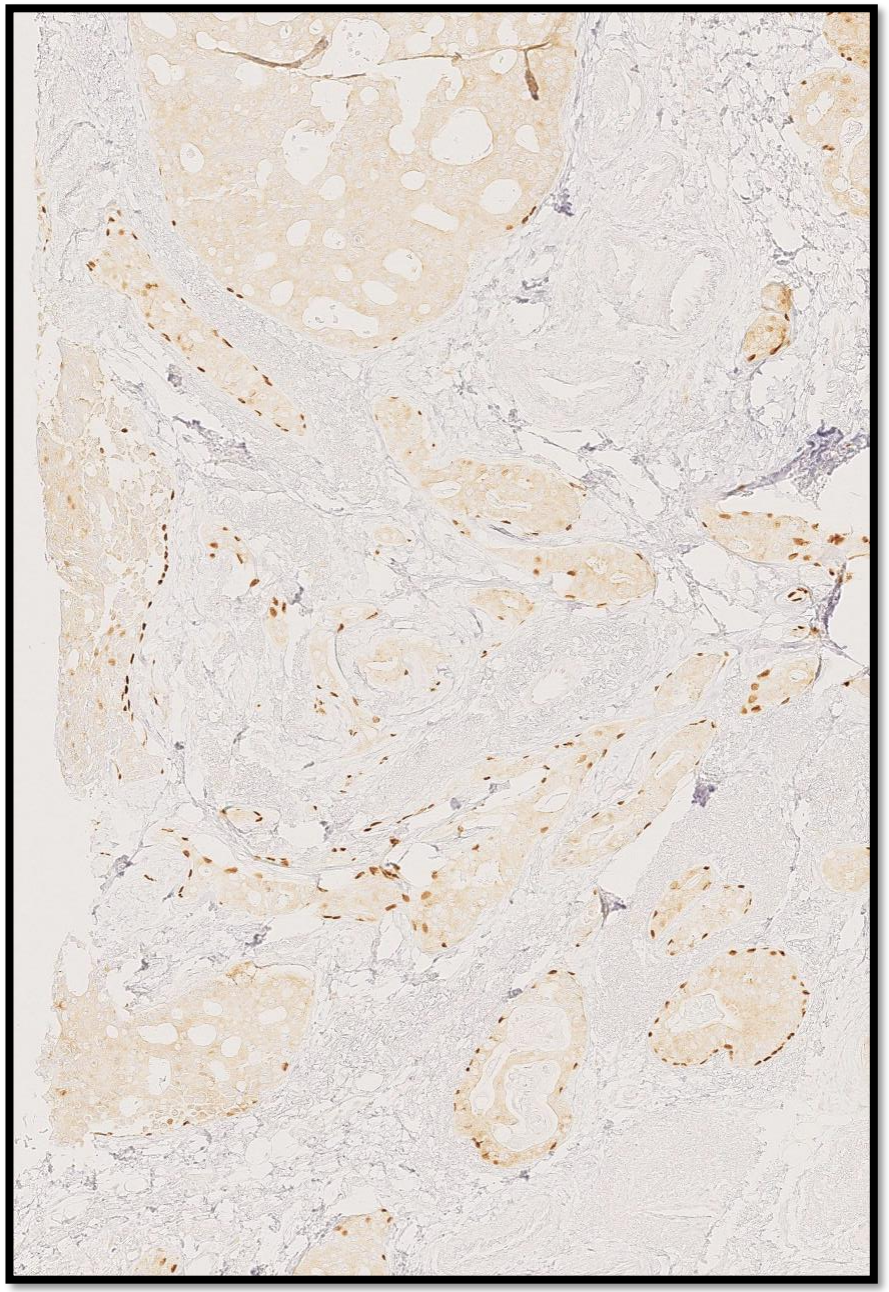
The core was signed out as invasive carcinoma and DCIS.





A second pathologist received the ER, PR, HER2 slides for review and noted the possible presence of myoepithelial cells.

Harrison B, et al. Quality assurance in breast pathology: lessons learned from a review of amended reports. Arch Pathol Lab Med 141:260-266, 2017.



Immunoperoxidase studies confirmed the presence of myoepithelial cells.

The diagnosis was corrected to DCIS involving sclerosing adenosis before she underwent excision.

Invasive Carcinoma vs Sclerosing Lesion

Take Home Points:

Always ask yourself before diagnosing invasive cancer if there is any possibility it is a sclerosing lesion (possibly involved by DCIS or LCIS).

Use 2 or more myoepithelial cells markers if a sclerosing lesion is possible.

However, if a benign appearing lesion lacks myoepithelial cells, use additional markers, consider other diagnoses (e.g. microglandular adenosis), and seek other opinions.

Ginter PS, Shin SJ, D'Alfonso TM. Small glandular proliferations of the breast with absent or attenuated myoepithelial reactivity by immunohistochemistry. Arch Pathol Lab Med. 2016;140:651-664. Good review of other rare glandular proliferative lesions.

Invasive Carcinoma vs Sclerosing Lesion

Take Home Points:

Evaluation of ER/PR/HER2 is an opportunity to re-consider the original diagnosis.

In 9 cases, diagnostic errors were detected when a second pathologist reviewed these studies (e.g. microinvasion missed, DCIS involving sclerosing adenosis interpreted as invasive carcinoma).

In 3 cases, the same pathologist reviewed the studies and the error was not detected until excision.

It can be of value to have a different pathologist review these studies.

Harrison B, et al. Quality assurance in breast pathology: lessons learned from a review of amended reports. Arch Pathol Lab Med 141:260-266, 2017.

Right and Wrong Errors in Breast Pathology

Sclerosing lesion mistaken for invasive carcinoma

Metastatic estrogen receptor carcinoma mistaken for neuroendocrine carcinoma

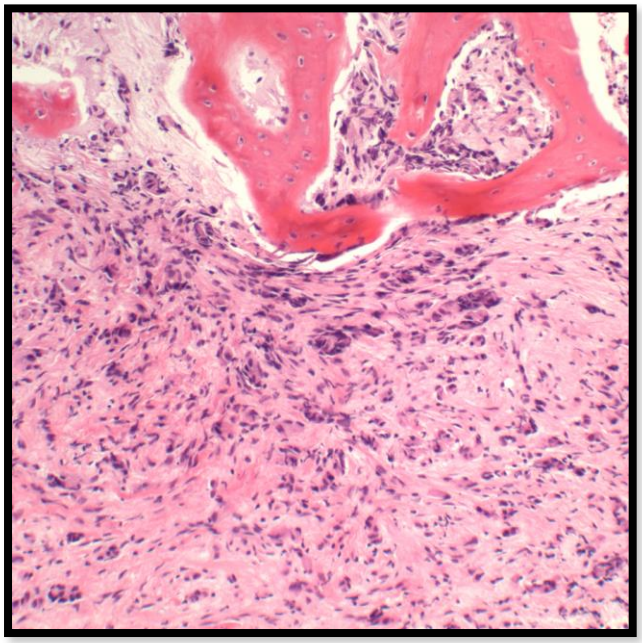
False negative estrogen receptor results

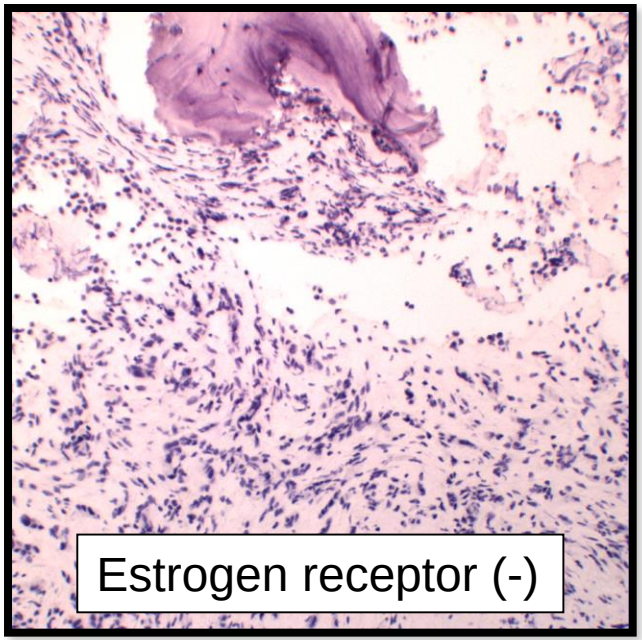
False positive HER2 results

A 65 year old woman was diagnosed with a well differentiated estrogen receptor positive invasive breast cancer.

Ten years later she developed bone pain and a bone scan showed multiple metastases. She was started on tamoxifen.

Five years later, she fell and fractured her hip. A pathologic fracture was suspected.





The specimen was decalcified.

Metastatic carcinoma to bone was diagnosed.

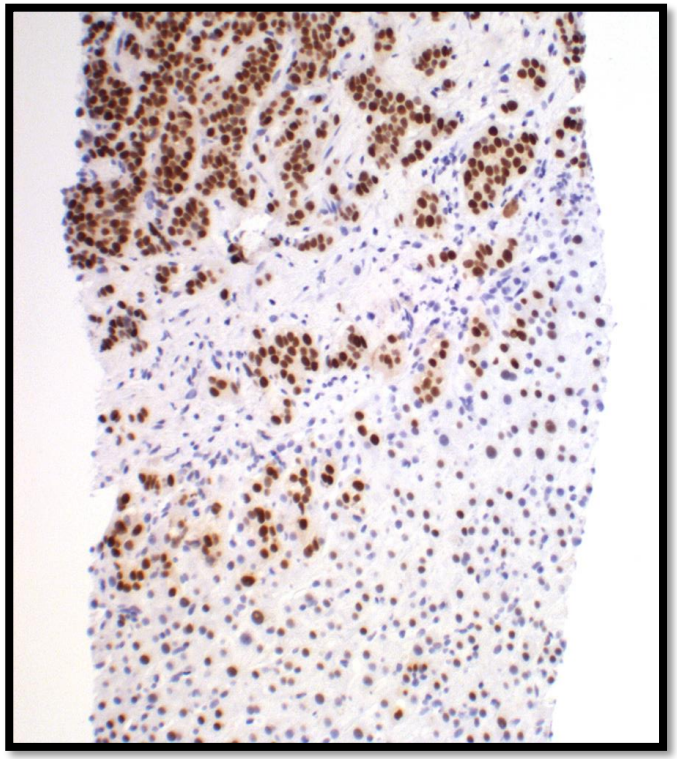
Estrogen receptor was negative.

Other breast markers (mammaglobin and GCDP-15) were positive.

Due to this result, tamoxifen was stopped and instead she was treated with chemotherapy.

She tolerated the chemotherapy poorly and the disease progressed in her bones.

She developed liver metastases and more aggressive chemotherapy was considered.



A liver biopsy showed metastatic cancer that was estrogen receptor positive.

Chemotherapy was stopped and endocrine therapy was re-instituted.

Her disease stabilized and she had minimal side effects.

Could the estrogen receptor result on the bone biopsy have been a false result?

The majority (~85%) of metastases from ER positive cancers are also ER positive.

~15% are truly ER negative – usually after many years of endocrine therapy.

In this setting, additional breast markers are helpful to ensure the metastasis is from the breast cancer and not due to a second clinically occult primary cancer.

When a metastasis from an ER positive cancer is ER negative, the possibility this is a false negative result should be considered.

False Negative Results for Estrogen Receptor

There are many reasons for false estrogen receptor results (especially on a bone biopsy):

Decalcification can diminish immunoreactivity for hormone receptors.

The number of tumor cells available for evaluation can be very limited and low numbers of positive cells can be missed.

Assay failure (antibody and technical issues).

Cautery (heat can destroy hormone receptor antigenicity).

Some non-formalin fixatives.

Faslodex (= fulvestrant) degrades hormone receptors and can cause diminished immunoreactivity.

False Negative Results for Estrogen Receptor

What can a pathologist do to avoid a false negative result?

Avoid decalcification when possible. Many bone marrow biopsies can be processed without decalcification or with minimal decalcification.

If negative results are obtained, repeat assay on a non-decalcified specimen if possible (e.g. a clot section).

Make sure oncologists are cautious in interpreting a negative result – especially when a decalcified specimen was used and/or only a few tumor cells are present.

”Although no immunoreactivity is seen for ER, this result should be interpreted with caution as the number of tumor cells is small (<100) and decalcification can diminish immunoreactivity.”

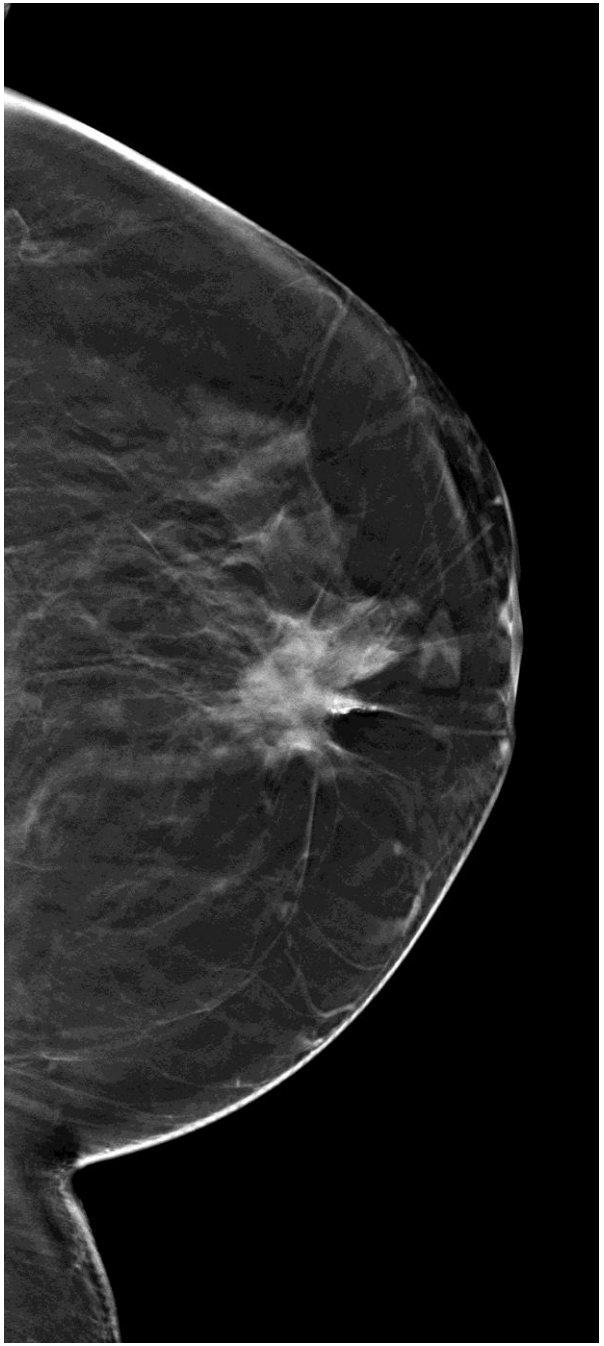
Right and Wrong Errors in Breast Pathology

Sclerosing lesion mistaken for invasive carcinoma

Metastatic estrogen receptor carcinoma mistaken for neuroendocrine carcinoma

False negative estrogen receptor results

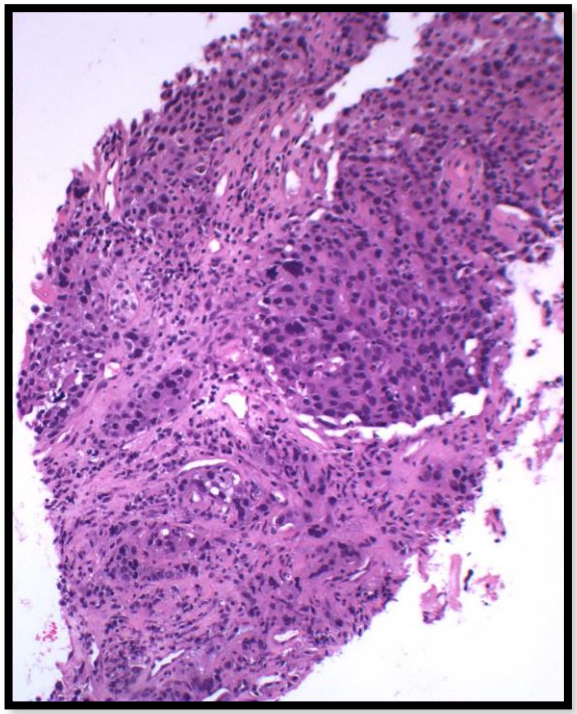
False positive HER2 results

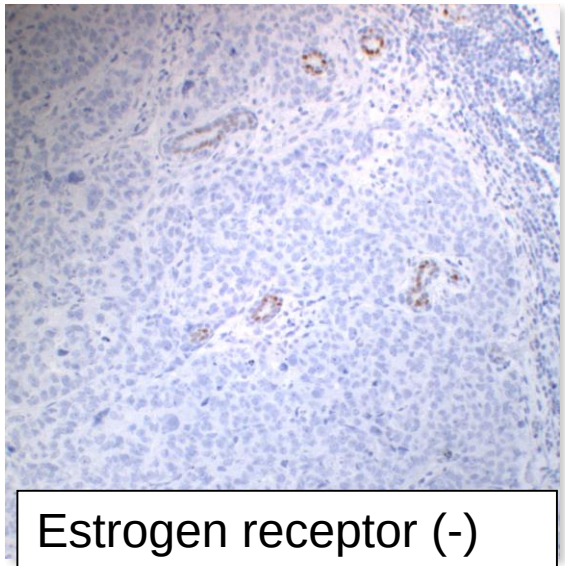


A 45 year old woman presented with a palpable breast mass.

Imaging showed a 3.5 cm mass with irregular margins.

A core needle biopsy was performed.





Estrogen receptor (-)
Positive internal control

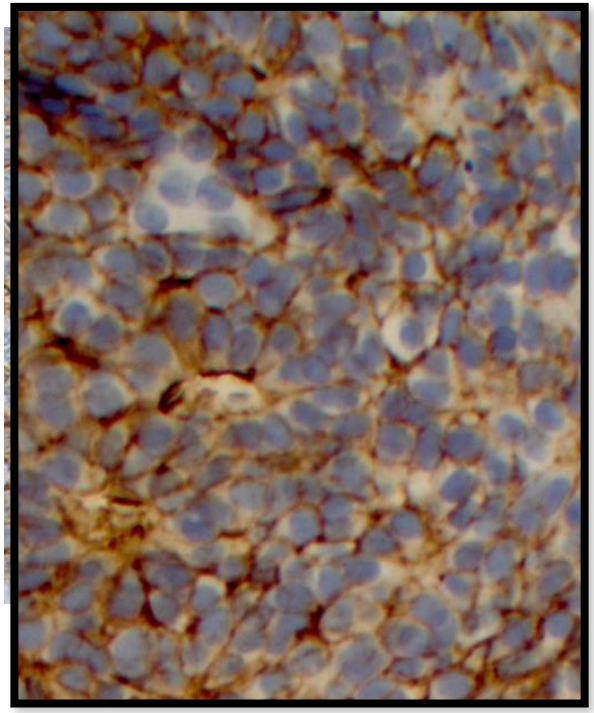
The core needle biopsy showed a poorly differentiated invasive carcinoma.

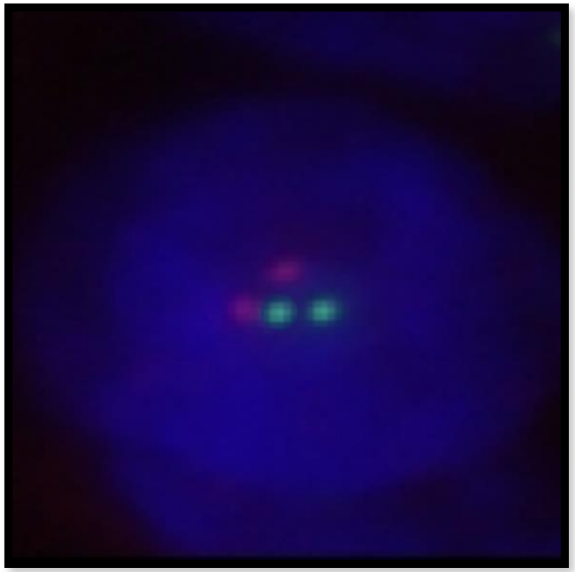
ER and PR were negative (with appropriate positive internal controls).

HER2 was reported to be 3+ positive by immunohistochemistry.

She was enrolled in a study of a new HER2 targeted therapy.

No tumor response was observed and she underwent excision of the cancer.





Repeat HER2 on the excisional specimen showed focal partial membrane immunoreactivity. FISH did not show amplification of HER2.

Review of the prior core needle biopsy also showed only equivocal immunoreactivity with negative FISH results. Therefore, this had been a false positive diagnosis.

The patient was subsequently treated with chemotherapy appropriate for triple negative cancer.

Consequences:

The patient underwent inappropriate therapy.

The patient did not receive the most appropriate therapy for triple negative carcinoma. Appropriate therapy was significantly delayed.

The results of the clinical trial would have been compromised had the error not been discovered.

The patient should have been enrolled on a different trial for triple negative cancers.

False positive HER2 results by immunohistochemistry have become a frequent problem.

False Positive HER2 Immunohistochemical Results

Definition of a false positive result:

Repeat immunohistochemical studies show negative or equivocal results.

In situ hybridization does not show HER2 amplification.

Although a HER2 mutation could theoretically increase protein expression in the absence of gene amplification, this has never been documented in the literature and I have never seen a case of 3+ positivity without gene amplification.

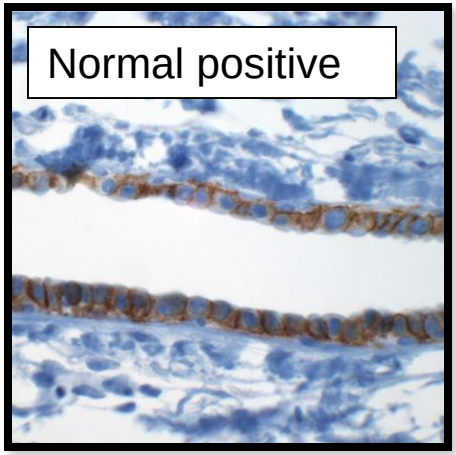
False Positive HER2 Immunohistochemical Results

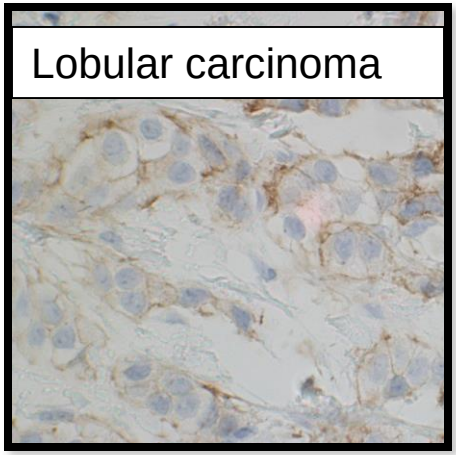
There are several reasons for false positive results:

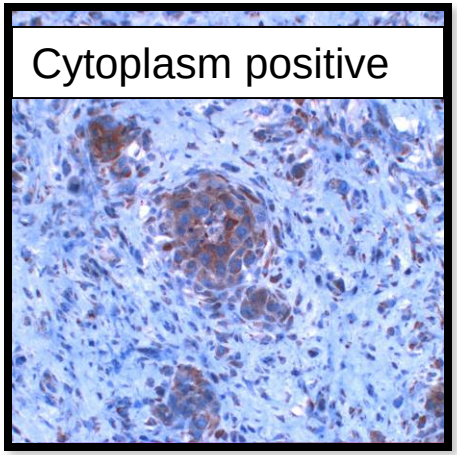
Overstaining – normal breast tissue should be negative

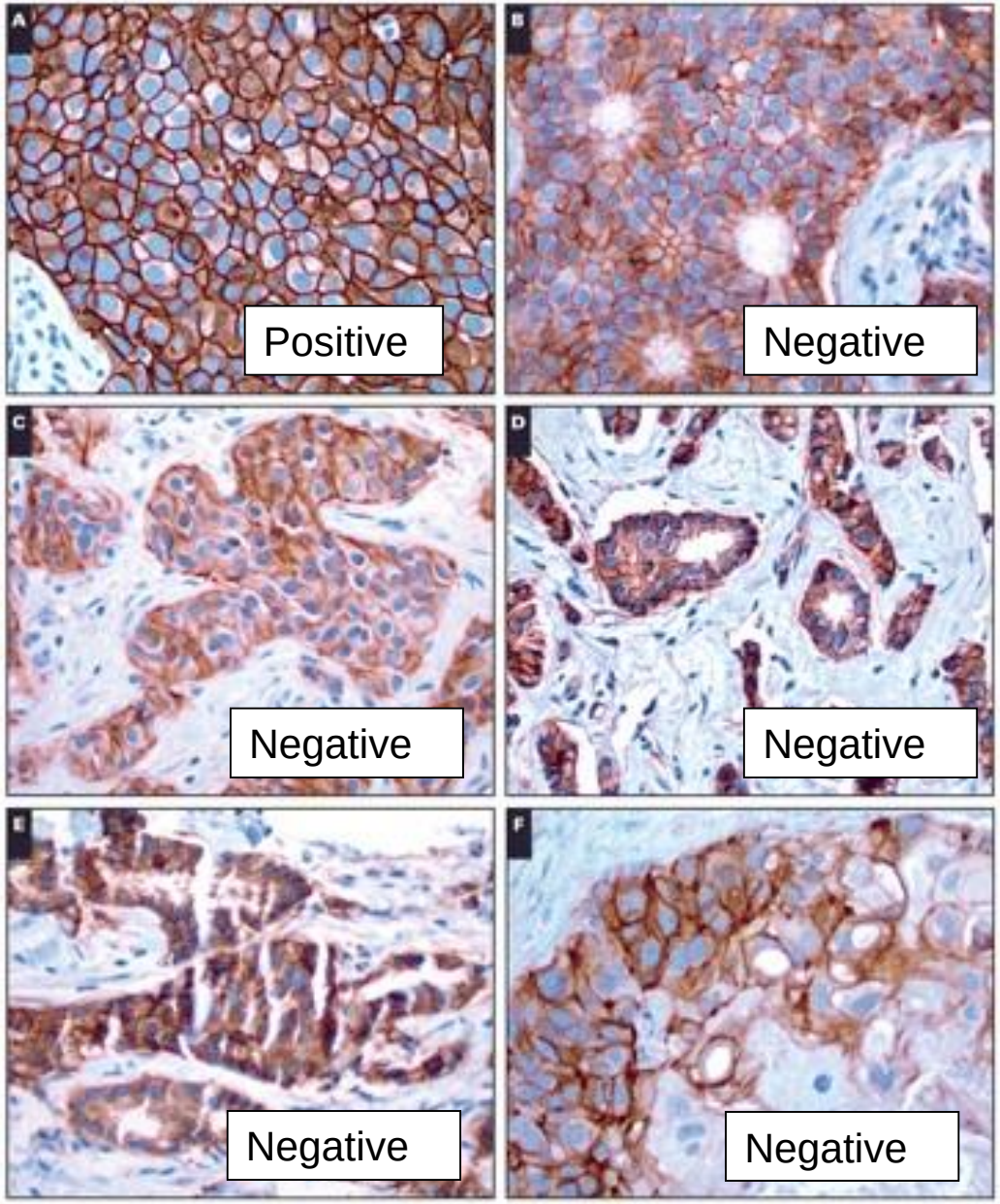
Edge artifact – lobular carcinomas can appear falsely positive in edges or between cells

Cytoplasmic positivity – only membrane positivity should be scored









In this study, 13 IHC (3+) cases had negative FISH results. The false negative result was due to overinterpretation of IHC as 3+ (12 cases) or an assay problem (1 case):

Weak staining – 7 cases

Granular staining – 2 cases

Crush artifact – 3 cases

Technical problem – 1 case

Grimm, EE, et al, Achieving 95% cross-methodological concordance in HER2 testing. Am J Clin Pathol 134:284, 2010.

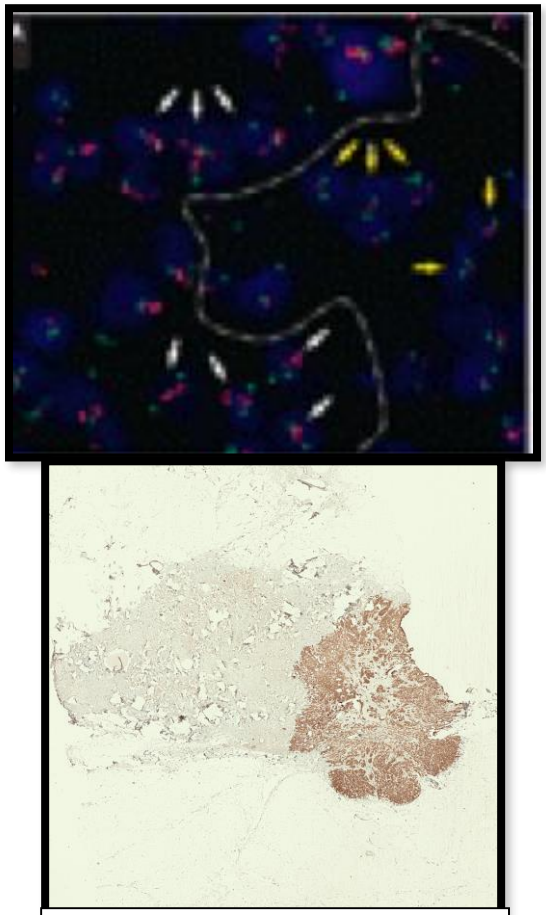
HER2 Immunohistochemical Results

A good 3+ result should be a “shirt sleeve” diagnosis – in general a microscope is not necessary.

If you are hesitating between 2+ and 3+, it is probably best to send for FISH to be certain of amplification.

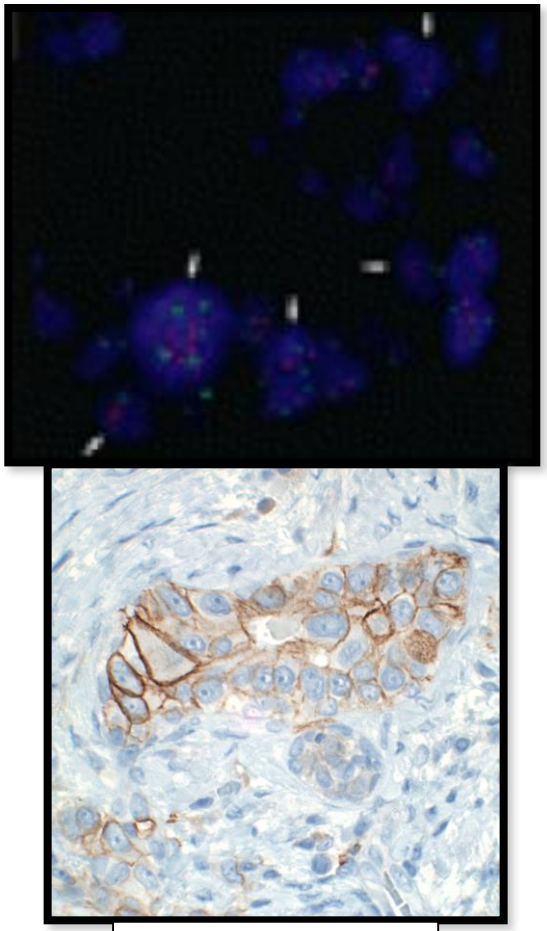
HER2: Heterogeneity - often in ER+ cancer

Separate populations



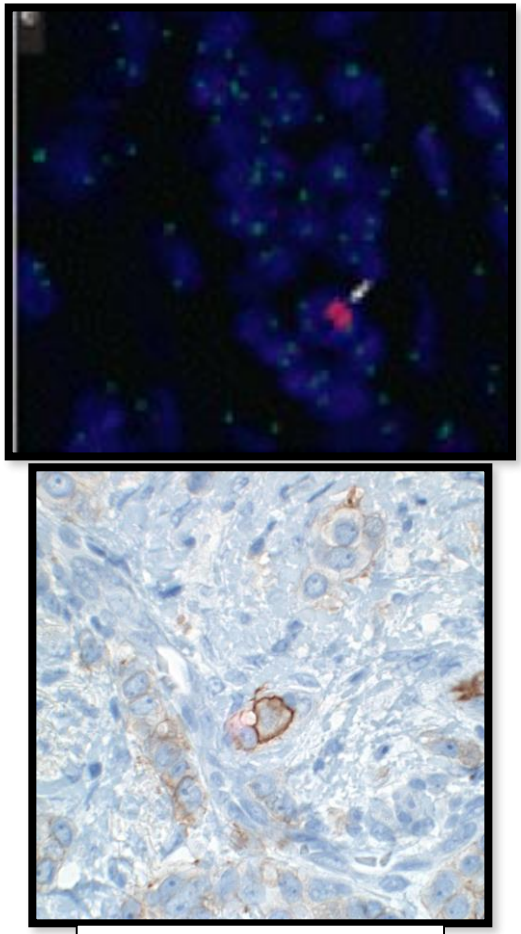
Positive if ≥10%
Very rare

Intermingled clusters



Often FISH negative

Single cells



Usually FISH negative

Starczynski, J, et al, HER2 amplification in breast cancer, Anat Pathol 137:595-605, 2012.

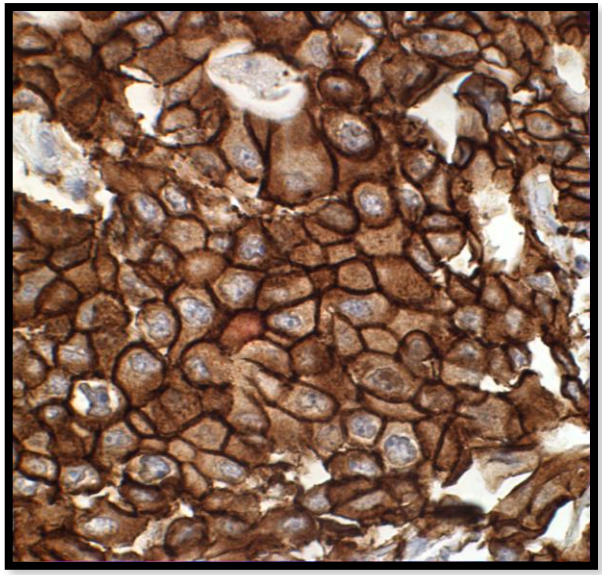
False Positive HER2 Immunohistochemical Results

Take Home Points –

Strongly positive (3+) cancers are usually strongly positive throughout the carcinoma.

The normal breast epithelium should be negative.

If focal, heterogeneous, and/or weak staining is seen (often for ER positive cancers), FISH is advised to support classification as HER2 positive.



Right and Wrong Errors in Breast Pathology

Sclerosing lesion mistaken for invasive carcinoma

Metastatic estrogen receptor carcinoma mistaken for neuroendocrine carcinoma

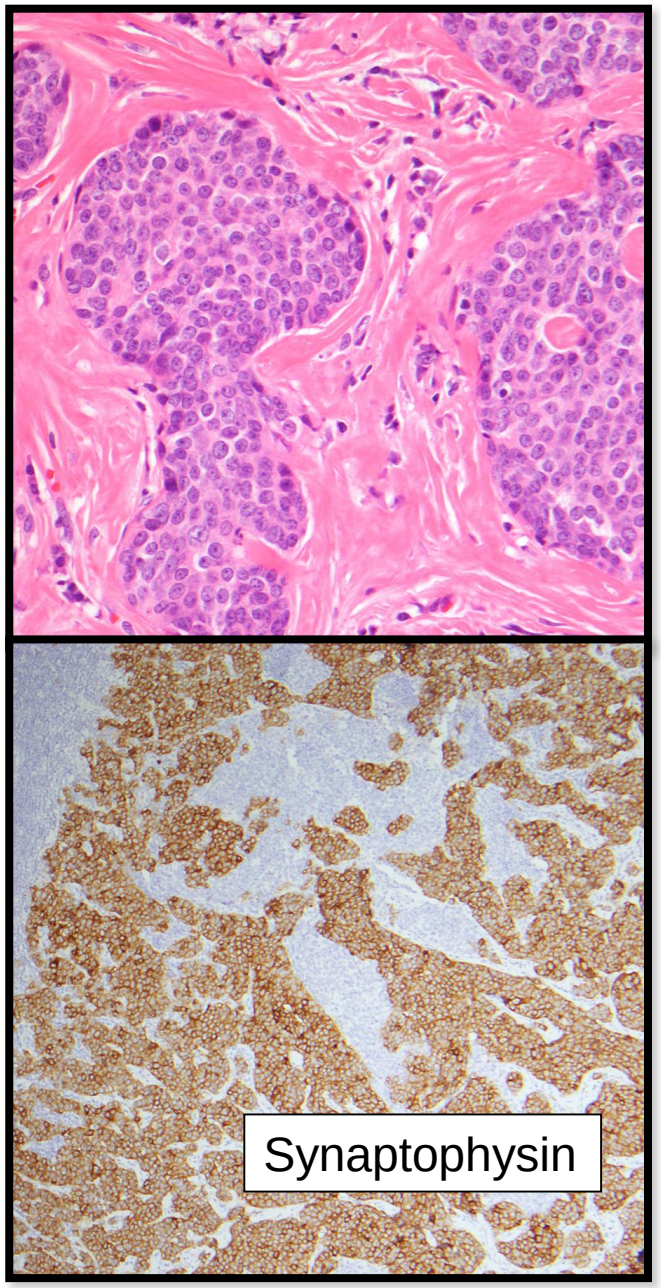
False negative estrogen receptor results

False positive HER2 results

A cautionary case:

A 50 year old woman was diagnosed with right breast invasive carcinoma – ER and PR positive, HER2 negative. Three sentinel nodes were negative.

She underwent chemotherapy, radiation therapy, and received tamoxifen for 5 years followed by an aromatase inhibitor.



Fifteen years later she presented with an enlarged 1.7 cm right supraclavicular lymph node.

A biopsy showed metastatic carcinoma that was positive for chromogranin and synaptophysin. A diagnosis of metastatic neuroendocrine carcinoma was made.

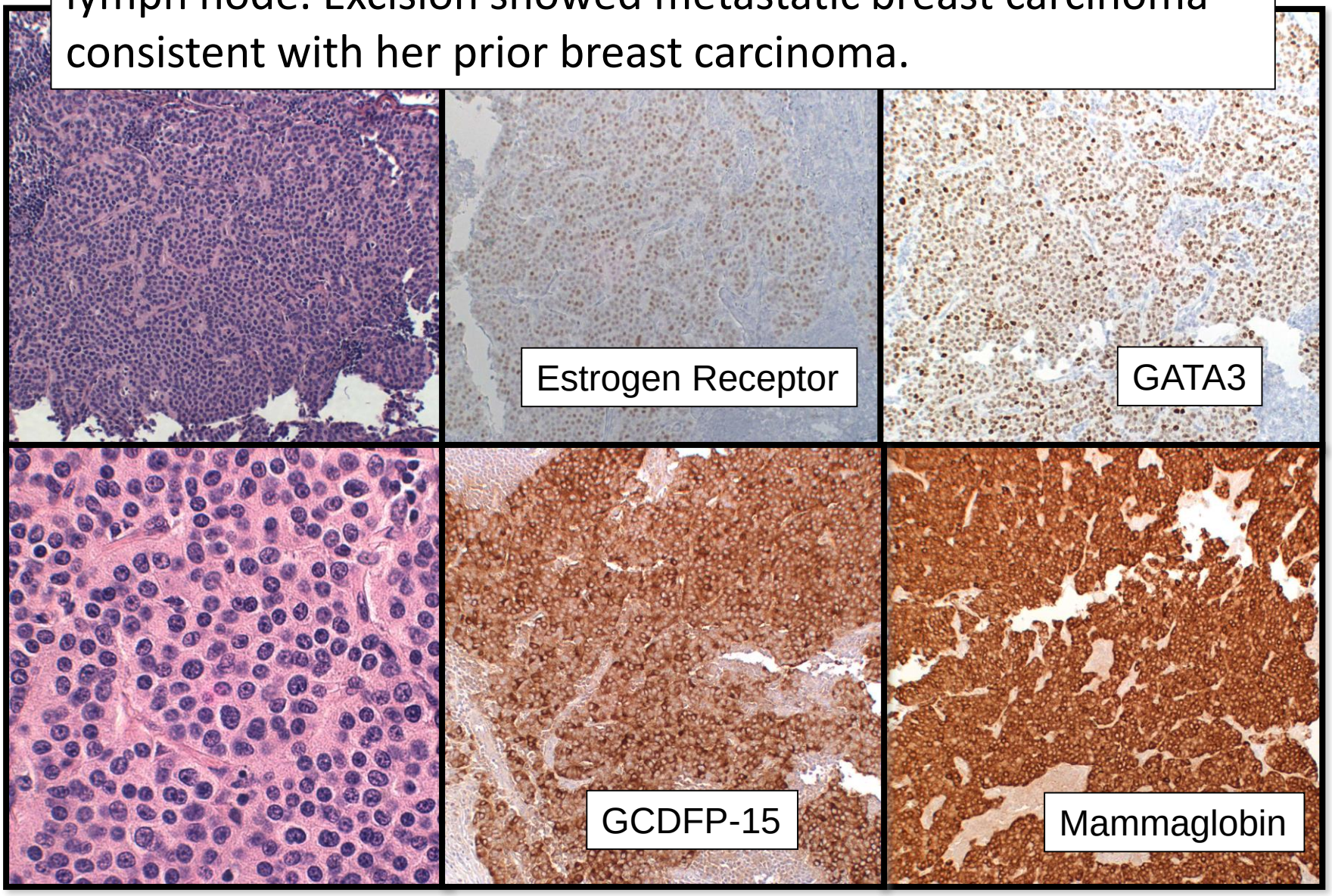
PET scan showed a possible lesion at the base of the tongue. Multiple biopsies of the base of tongue and pyriform sinus did not reveal a primary site.

A neck dissection showed metastases to 3 of 12 lymph nodes.

She was diagnosed with a metastatic neuroendocrine carcinoma of unknown primary.

She received chemotherapy and radiation therapy for this carcinoma.

Two years later she presented with an enlarged right axillary lymph node. Excision showed metastatic breast carcinoma – consistent with her prior breast carcinoma.



The patient was discussed at tumor board for appropriate treatment of her breast recurrence.

At that time, the possibility that the prior “neuroendocrine carcinoma” was metastatic breast carcinoma was suggested.

The prior right supraclavicular lymph node diagnosed as metastatic neuroendocrine carcinoma was retested and was positive for ER, GATA3, GCDFP-15, and mammaglobin.

Therefore, she did not have neuroendocrine carcinoma.

All of her disease was metastatic breast cancer.

Consequences:

She underwent –

Unnecessary head and neck evaluation and biopsies

Unnecessary cervical node dissection

Unnecessary chemotherapy

And – she did not receive appropriate palliative endocrine therapy for an extended period of time.

Misdiagnosis of Metastatic ER Positive Breast Cancer

Mistaking metastatic ER positive breast cancer for another cancer has serious consequences for patients:

The patients fail to receive effective treatment with well tolerated endocrine therapy.

They may receive ineffective and toxic chemotherapy.

Misdiagnosis of ER Positive Metastatic Breast Cancer

	Cloutier	Li
No. patients	5 patients	7 patients
Prior hx breast cancer	4 patients	4 patients
Time to recurrence	0 to 20 years	2 to 10 years
Site of metastasis	Liver, mediastinum	Bone, lung, lymph node, mediastinum
Misdiagnosis	NE (4 cases), small cell (1 case)	NE (4 cases), small cell, thymic, gastric
No. of IHC studies	3 to 9 (average 5)	1 to 12 (average 7)
Delay to diagnosis	0, 2 years (3 unknown)	2 months to 2 years
Incorrect surgery	Not provided	5 patients
Incorrect chemotherapy	3 patients	5 patients

Cloutier J, et al, Metastatic breast cancer simulating well-differentiated neuroendocrine neoplasms of visceral organs. Hum Pathol 82:76-86, 2018.

Li L, et al. Misdiagnosis of metastatic hormone receptor positive breast cancer: Clinical consequences and root cause analysis of the source of errors (in preparation).

Misdiagnosis of Metastatic ER Positive Breast Cancer

These cases involved . .

Well known respected institutions

Experienced very smart pathologists

Experienced very smart oncologists

The problem is that this type of error has not been well described.

There are likely many other cases of misdiagnosis that remain undiscovered.

Misdiagnosis of Metastatic Prostate Cancer

In men, metastatic prostate cancer can be misdiagnosed as a neuroendocrine carcinoma:

Metastases can occur many years after initial diagnosis.

Metastatic prostate cancer can resemble a low grade neuroendocrine carcinoma.

Prostate cancer can be strongly positive for neuroendocrine markers.

Well tolerated endocrine therapy is available.

Misdiagnosis of Metastatic ER Positive Breast Cancer

Take home points

Breast cancer should always be included in the differential diagnosis for metastatic disease in women – especially for women with a history of breast cancer.

Breast cancer can have a neuroendocrine appearance and be strongly positive for neuroendocrine markers – the same is true for prostate cancer in men.

Failure to appropriately identify an ER positive breast metastasis can lead to significant morbidity due to inappropriate surgical procedures and treatment.

Errors in Breast Pathology – Key Points

For breast cancer, it is not only essential to get the diagnosis right, but also the markers that largely determine the treatment the patient will receive (hormone receptors and HER2).

Being aware of the common types of errors helps us avoid making them.

All pathologists make errors – the best we can do is to try to make as few as possible and to learn from them.

Final thoughts -

“There's an old saying in Tennessee — I know it's in Texas, probably in Tennessee — that says, fool me once, shame on — shame on you. Fool me — you can't get fooled again.”

George W. Bush

”It is always good to learn from mistakes – especially when they are someone else’s.”

Stuart J. Schnitt

“ . . . there is no shame in being wrong, only in failing to correct our mistakes.”

George Soros

A hui hou kakou

slester@bwh.Harvard.edu

Nakelele blow hole Maui