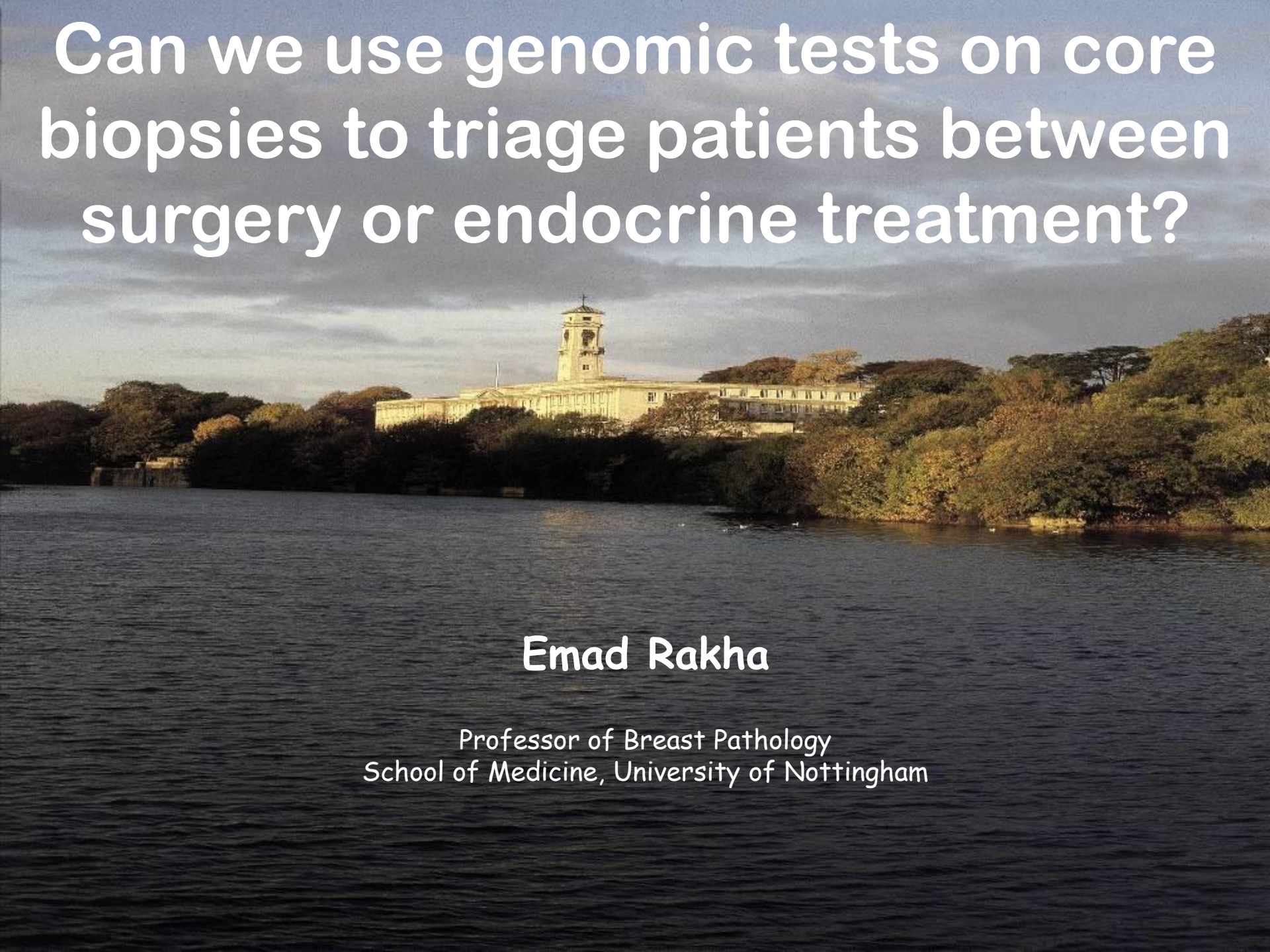
Can we use genomic tests on core biopsies to triage patients between surgery or endocrine treatment?



Emad Rakha

Professor of Breast Pathology
School of Medicine, University of Nottingham

COVID-19 Guidelines for Triage of Breast Cancer Patients

Online March 24, 2020

Download a print-friendly version

Developed by the COVID 19 Pandemic Breast Cancer Consortium (this consortium is made up of representatives from the NAPBC, CoC, ASBrS, and NCCN)

Phase I. Semi-Urgent Setting (Preparation Phase)

Few COVID 19 patients, hospital resources not exhausted, institution still has ICU vent capacity, and COVID trajectory not in rapid escalation phase

Surgery restricted to patients likely to have survivorship compromised if surgery not performed within next 3 months

Cases that need to be done as soon as feasible (recognizing status of hospital likely to progress over next few weeks):

- Neoadjuvant patients finishing treatment
- Clinical Stage T2 or N1 ERpos/PRpos/HER2 negative tumors*†

The Breast 52 (2020) 8–16

Contents lists available at ScienceDirect

The Breast

journal homepage: www.elsevier.com/brst

Original article

Recommendations for triage, prioritization and treatment of breast cancer patients during the COVID-19 pandemic

Giuseppe Curigliano [a, b, 1, *], Maria Joao Cardoso [c, d], Philip Poortmans [e, f], Oreste Gentilini [g], Gabriella Pravettoni [a, b], Ketti Mazzocco [a, b], Nehmat Houssami [h], Olivia Pagani [i], Elzbieta Senkus [j], Fatima Cardoso [c], on behalf of the editorial board of The Breast

As breast pathologists, our main goal is to take care of BC patients according to established quality indicators

However, the current extraordinary situation may require an urgent re-organisation and adapted new protocols without compromising patients' outcomes

This includes selection criteria to service provision and prioritization of treatments

Surgery should be postponed and NAET should be offered if there is evidence that NAET is effective

Overall response rates to hormone manipulation in ER+ HER2- BC is >50% in a neoadjuvant setting (though the rate of pCR is much less)

Therefore: we need to identify patients whose BC is likely to respond to NAET and those who are not likely to respond to NAET so surgery is indicated

NAET for BC

Preoperative NCB recommendations

*** Pre treatment breast core biopsy must be adequate for:**

- Unequivocal diagnosis of invasive carcinoma & Assessment of key prognostic/ predictive factors:

- **Tumour grade and Histological type**
- **Hormone receptor (ER & PR) and HER2 status**
- **Other biomarkers such as KI67**
- **Additional genomic tests e.g. multigene assays**

*** If there is an inadequate amount of invasive carcinoma in the core, repeat biopsy**

NAET for BC

Preoperative NCB recommendations

*** Make sure tumour type and grade are correct (E-cadherin, mitotic counts), receptor assessment is accurate**

*** If multiple lesions, biopsy of at least 2 foci is advised to confirm multifocality and look for heterogeneity to make sure that all are ER+ HER2-**

- **Turnaround time for reporting including receptors and Multigene assays**

- *** Not sure about invasion *(such as in papillary carcinomas) or* DCIS with focal invasion *:* Perform ER and if + advise for excision after NAET to confirm diagnosis**

Predictors of NAET response / eligibility

Clinical:

Menopausal status, age, tumour stage, nodal stage, mammographic density, presentation

Pathological:

Grade, **Tumour type**, mitotic activity, necrosis and fibrosis, TILs,

Molecular:

ER, PR, HER2, **KI67**, **Multigene assay**, intrinsic molecular classes, ……..etc

Cortazar et al., Lancet 2014;384(9938):164-72

Tumour grade on core biopsy

Nottingham Grade

- Grade 1-2: Low response to NACT BUT better response to NAET

- Grade 3: higher response to NACT but variable response to NAET

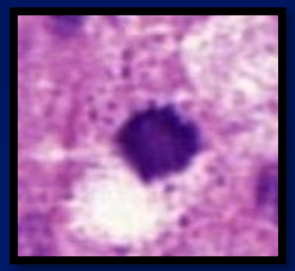
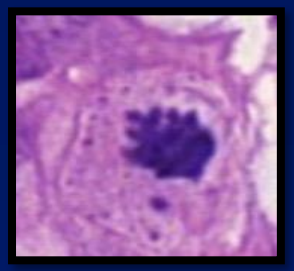
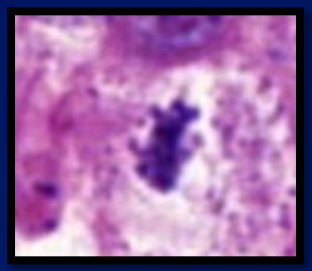
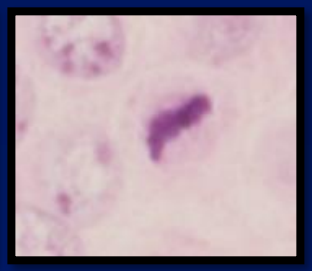
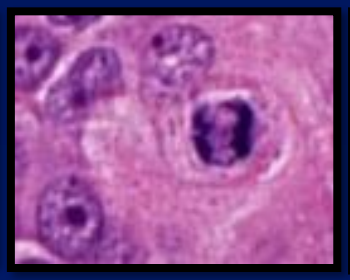
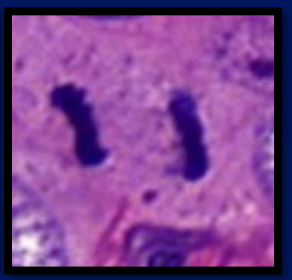
*** The gold standard tumour grade is based on assessment of the whole tumour following excision**

*** Correlation between NCB and excision grade is typically between 60-80%**

Consistency of different grading components:

Tubules – 82%, Pleomorphism – 73%, Mitotic count – 58%

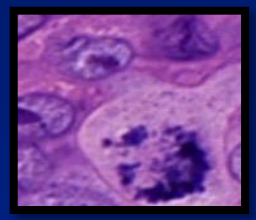
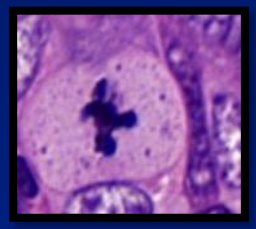
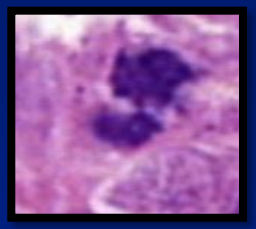
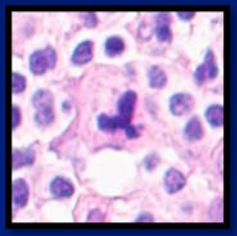
*** Core biopsy tends to under grade tumors mainly due to lower mitotic counts**

Prophase

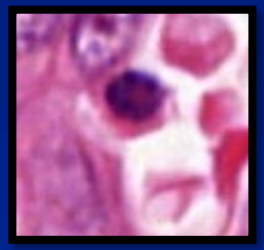
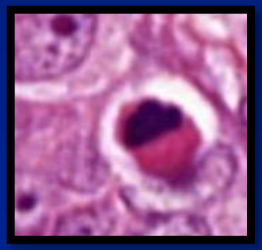
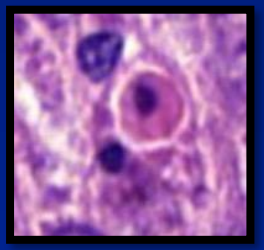
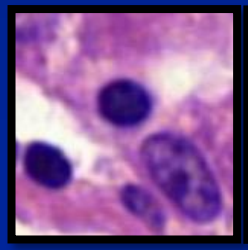
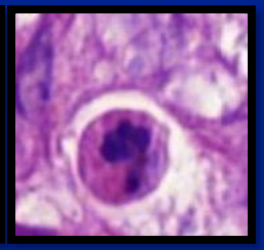
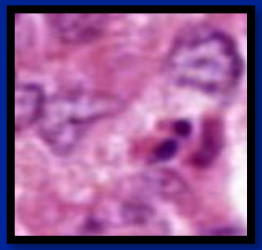
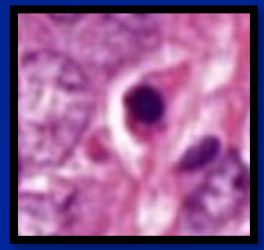
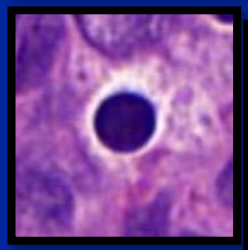
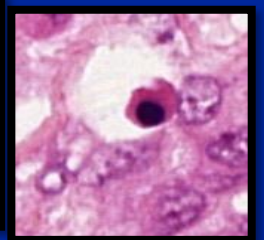
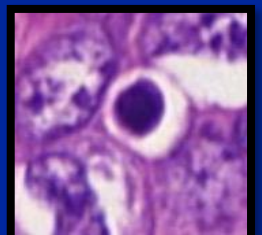
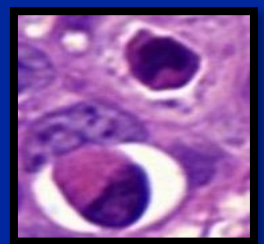
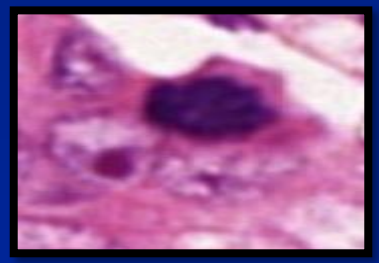
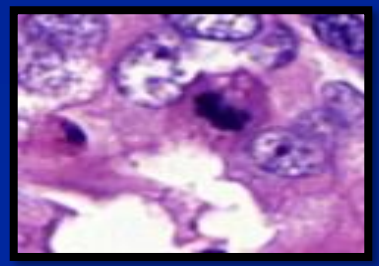
Metaphase

Telophase

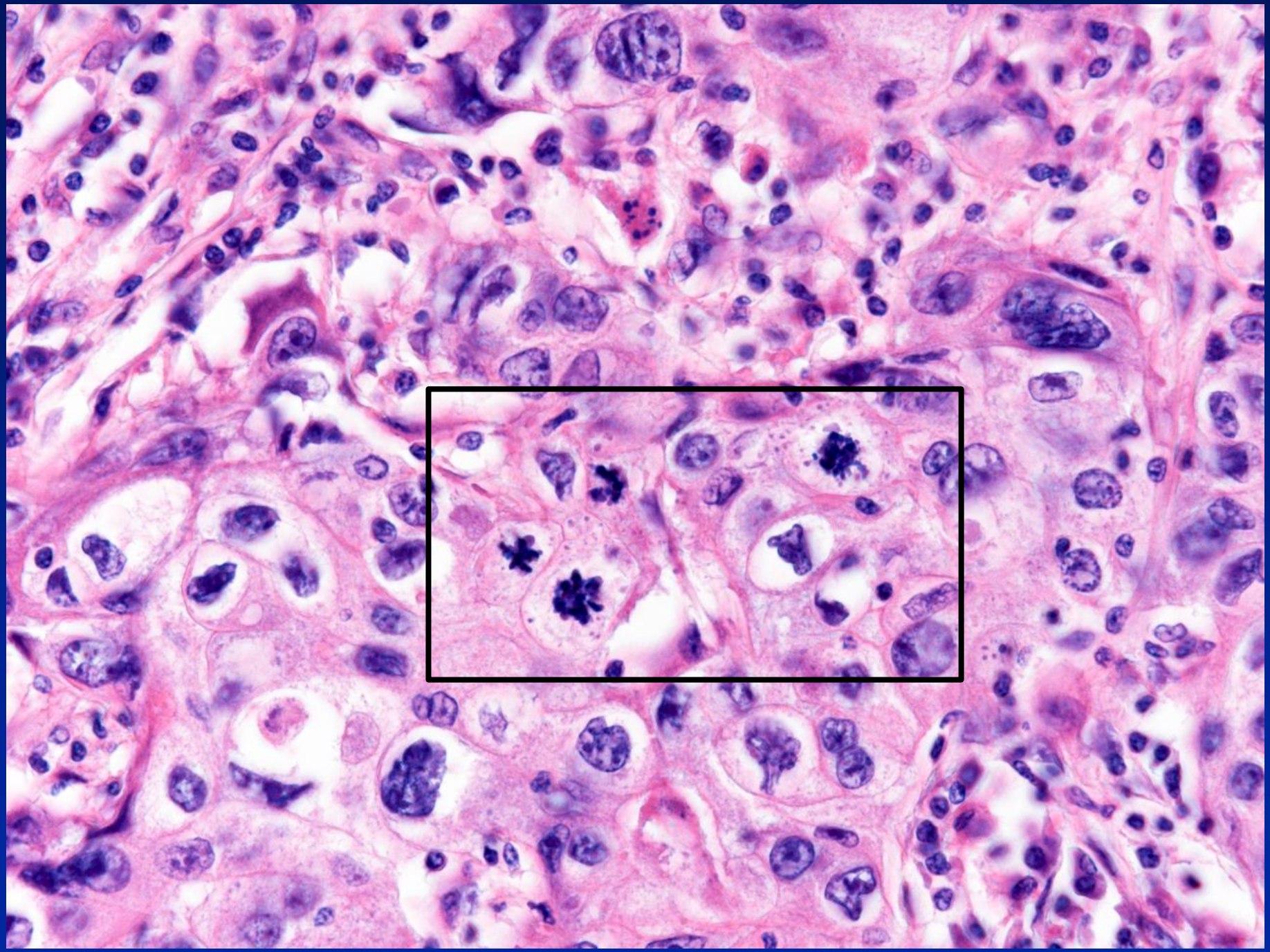





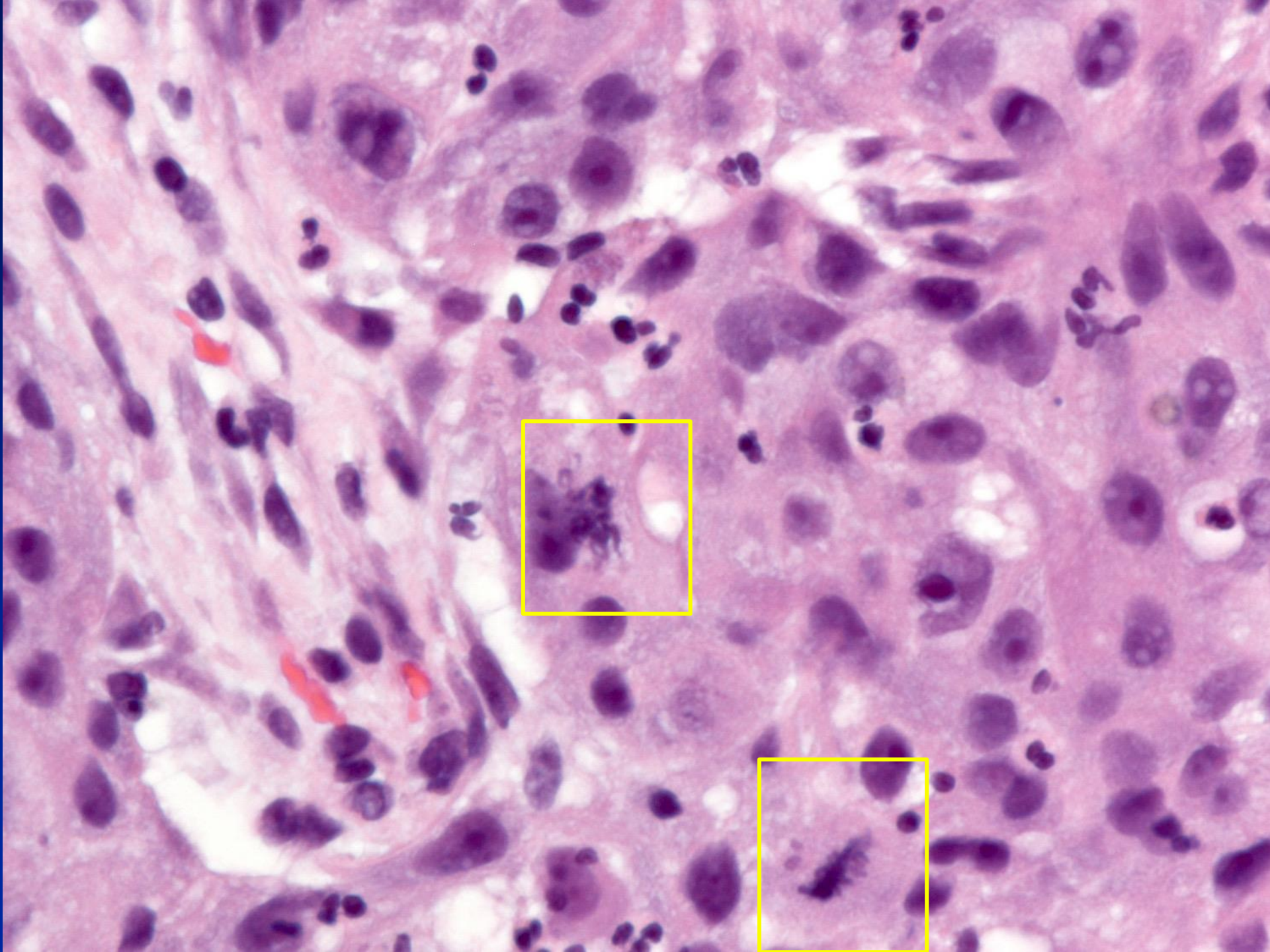
Atypical mitosis

Apoptosis

Mitosis with eosinophilic cytoplasm





Features of mitotic figure

Criteria for mitoses

1-Absence of nuclear membrane

2- Chromatin clumps margin show hairy projection (50%) but almost all show slightly irregular outline

3- Chromatin material is homogeneous

4- No fragmentation of nuclear material

5-Cytoplasm is mainly eosinophilic (80%), but it can be deep eosinophilic similar to apoptotic cell cytoplasm (5%), pale or slightly basophilic cytoplasm

6- The size of the cell is relatively similar in size of the adjacent malignant cells whereas

7-Only 20% are asymmetrical in shape

Criteria for apoptosis

1-Most show deeply eosinophilic cytoplasm

2- Decreased in size & some show fragmentation of nuclei

3- Almost all had regular border

Tumour type on core biopsy

- DCIS versus invasive carcinoma

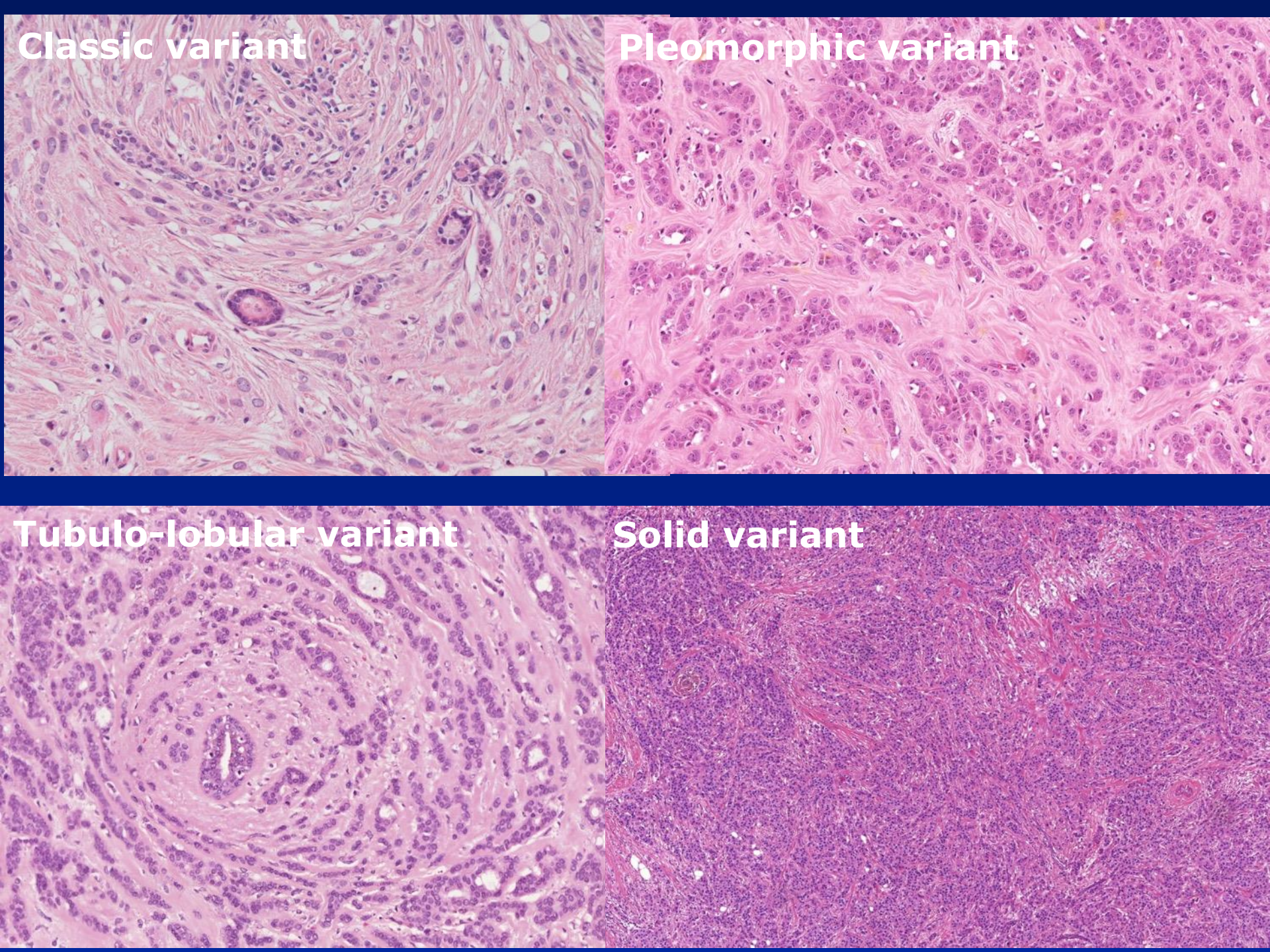
  In doubtful cases: Panel of myoepithelial markers

- Lobular versus ductal carcinoma

  In doubtful cases: E-cadherin & Beta catenin

- Discordance between tumour type and receptor

  Further work up


Classic variant
Pleomorphic variant
Tubulo-lobular variant
Solid variant

Molecular predictor of response to NAET

* **Hormone receptors:** ER & PR

ER is the most powerful predictor of response to NAET

ER 1% positive, (1-9% be careful or suggest repeat)

>10% Positive with positive correlation between ER score and level of response to NAET

PR status is a also a good predictor with double positive respond better

* **HER2:** If HER2 is positive: No NAET

* **Ki67** (negative correlation with NAET)

* **Multigene prognostic tests:** Oncotype Dx

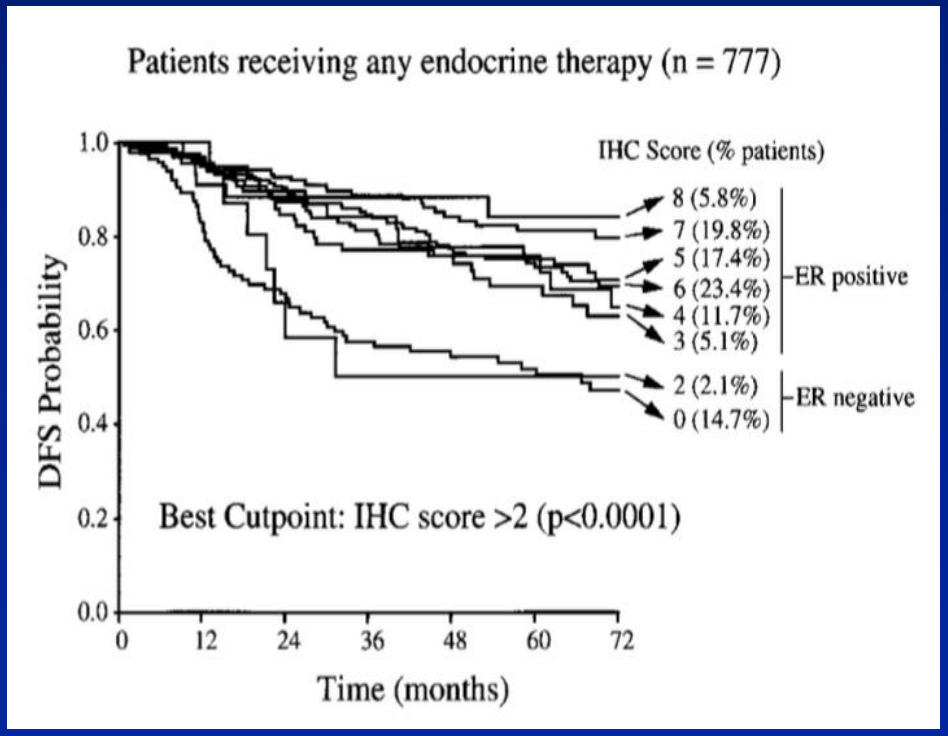
Estrogen Receptor Status by Immunohistochemistry Is Superior to the Ligand-Binding Assay for Predicting Response to Adjuvant Endocrine Therapy in Breast Cancer

J Clin Oncol 17:1474-1481. © 1999

Jennet M. Harvey, Gary M. Clark, C. Kent Osborne, and D. Craig Allred

Table 1. Comparison of ER Status Results, as Determined by IHC and LBA in 1,982 Primary Breast Cancer Cases

IHC Score	Patients		Ligand Binding Results (fmol/mg protein)				
	No.	%	Mean	SD	Median	Minimum	Maximum
0	517	26	10	49	1	0	758
2	67	3	50	100	8	0	548
3	117	6	59	95	23	0	623
4	190	10	67	73	39	0	428
5	320	16	104	139	56	0	1549
6	370	19	141	158	89	0	1181
7	318	16	193	215	142	0	1798
8	83	4	282	312	185	0	1439



Allred 2: <1% cells staining weakly

Positivity cutoff: ≥1%

?? Q Score = Positive (2 or 3 or 4 or more?)

ER status	Allred score	Allred equation	Stained cells (%)	Intensity of immunoreactivity	Equivalent H-score
Negative	0	0 + 0	0	0	0 (−)
	1 (does not exist)				
	2/3/4	1 + 1/2/3	<1	1/2/3	0 (−)
Poor	3/4/5	2 + 1/2/3	1–10	1/2/3	1–30 (+)
	4/5	3 + 1/2	11–33	1/2	11–66 (+)
	5	4 + 1	34–66	1	34–66 (+)
Intermediate	6	5 + 1	67–100	1	67–100 (+)
	6	4 + 2	34–66	2	68–132 (+/++)
	6	3 + 3	11–33	3	33–99 (+)
Rich	7	4 + 3	34–66	3	102–198 (++)
	7/8	5 + 2/3	67–100	2/3	134–300 (++/+++)

A critical review why assessment of steroid hormone receptors in breast cancer should be quantitative

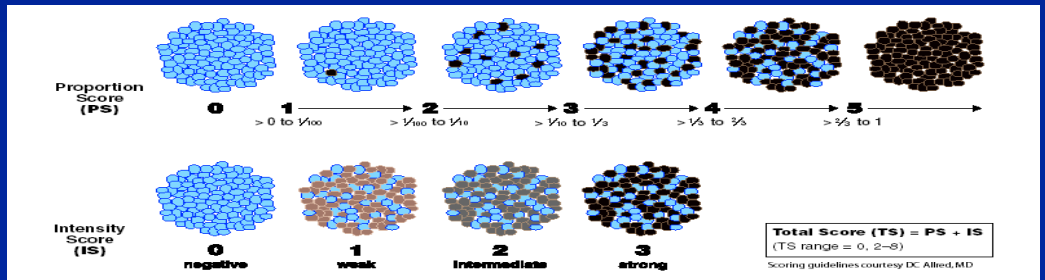
O. Brouckaert[1*], R. Paridaens[1], G. Floris[1], E. Rakha[2], K. Osborne[3] & P. Neven[1]

Annals of Oncology 24: 46–53, 2013

Allred score

<1%=1 + (1, 2 or 3)= 2, 3, 4 (-ve)

1-10%=2 + (1, 2 or 3)= 3, 4, 5 (+ve)



ER Low expresser: 1-9%

PAM50 revealed that tumors with low ER (1-9%) were more like ER-negative tumours than ER-positive tumors, and most such cases should therefore would better be treated as ER negative tumours.

Ohara et al., Br Ca Res Tr 2019 Feb;173(3):533-543.

Among the ER low-positive group (1-9%), 30 of 76 (40%) had a *BRCA* 1/2 mutations

Sanford et al Cancer. 2015 Oct 1;121(19):3422-7

Viale Breast. 2017 Aug;34 Suppl 1:S61-S63

Histopathology 2016 DOI: 10.1111/his.13089

Further evidence to support bimodality of oestrogen receptor expression in breast cancer

Abir A Muftah,[1,2] Mohammed Aleskandarany,[1] Sultan N Sonbul,[1] Christopher C Nolan,[1] Maria Diez Rodriguez,[1] Carlos Caldas,[3] Ian O Ellis,[1] Andrew R Green[1] & Emad A Rakha[1]

Analysis of the 10,550 unselected BC assessed on CNBs:

Weakly positive (**1–9%**) cases were infrequent (178; 2.2%) whereas intermediately + (10–69%) were 5.1%.

Of **1-9%**; 60 cases were immunostained using full-face sections of excision specimens:

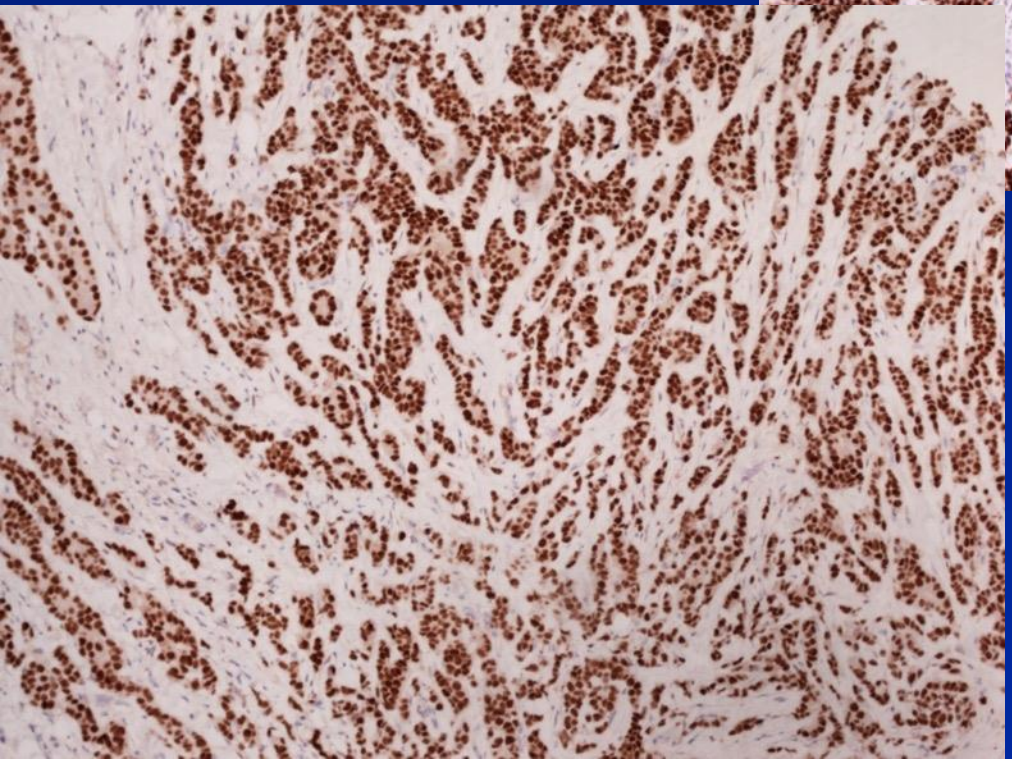
-26 (43%) changed to negative (<1%)

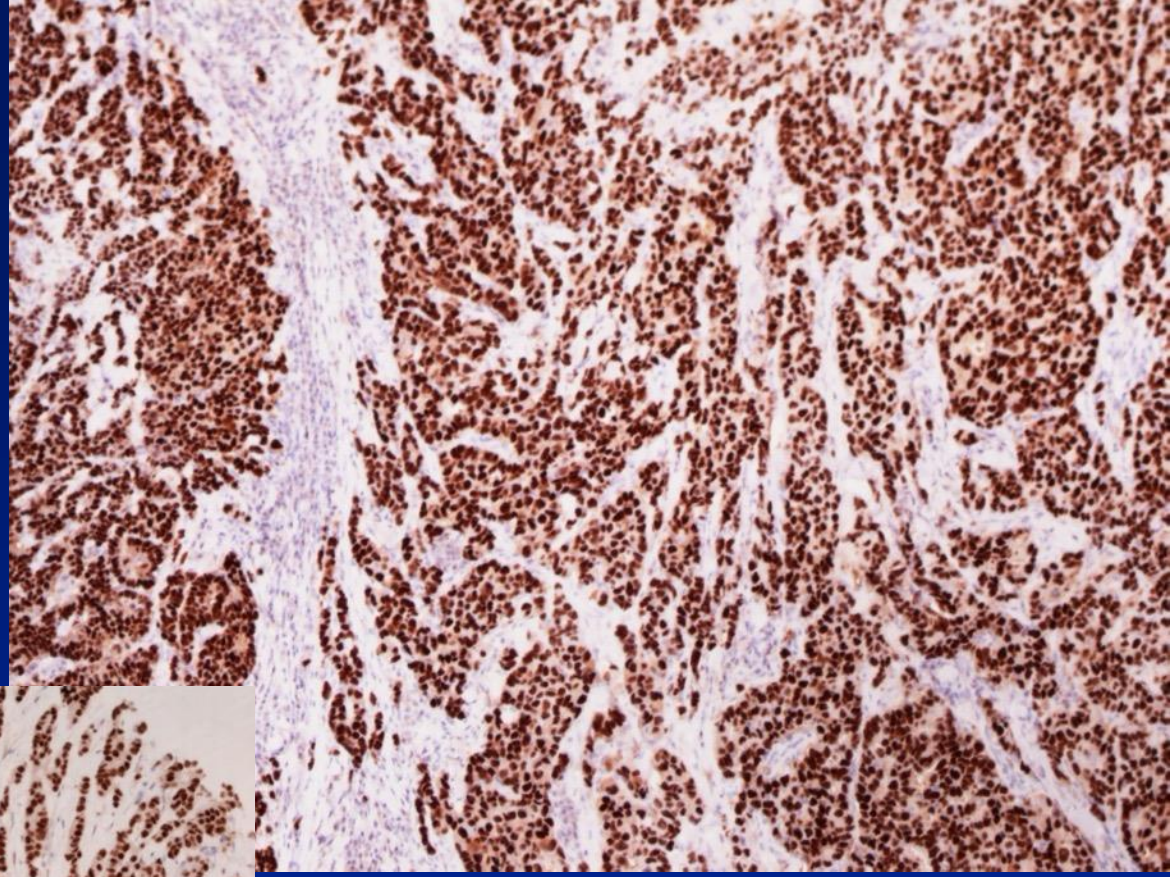
-34 (57%) remained positive (≥1%)

21 cases <1%: 18 (86%) remained as negative

Rakha et al.. **J Clin Oncol. 2012 Aug 10(23):2929-30**

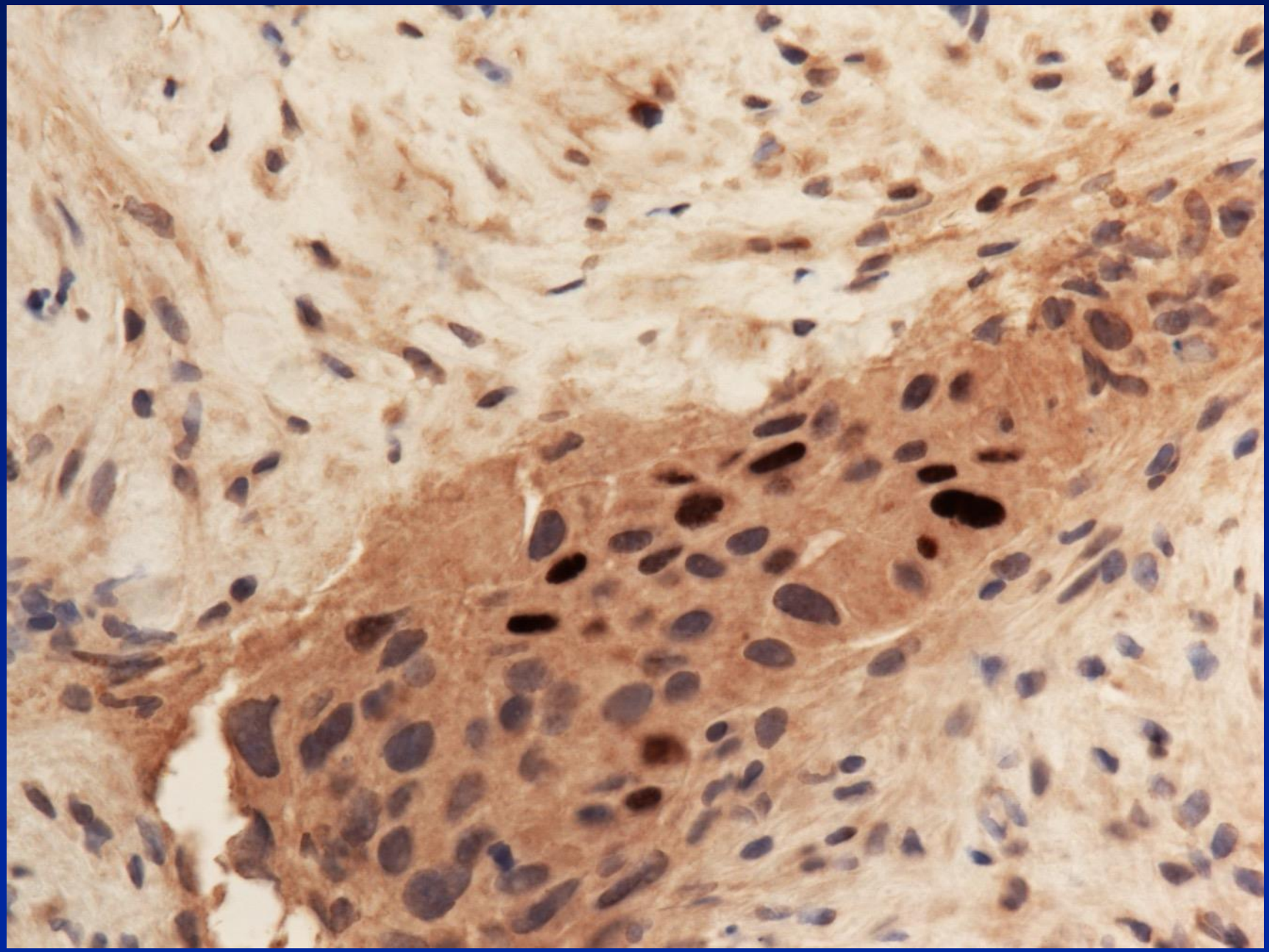
Oestrogen receptor staining pitfalls



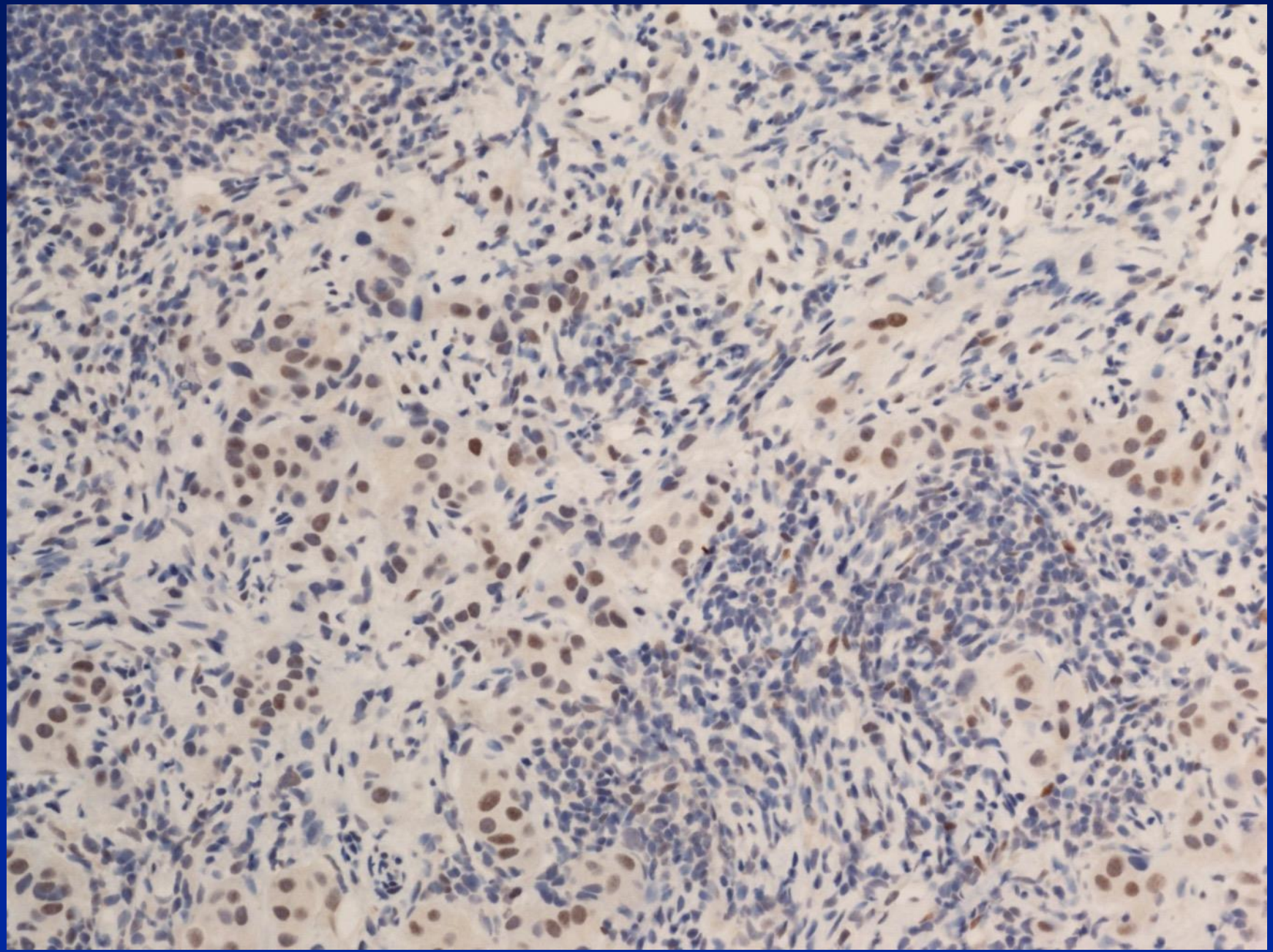


Strong nuclear staining in 100%

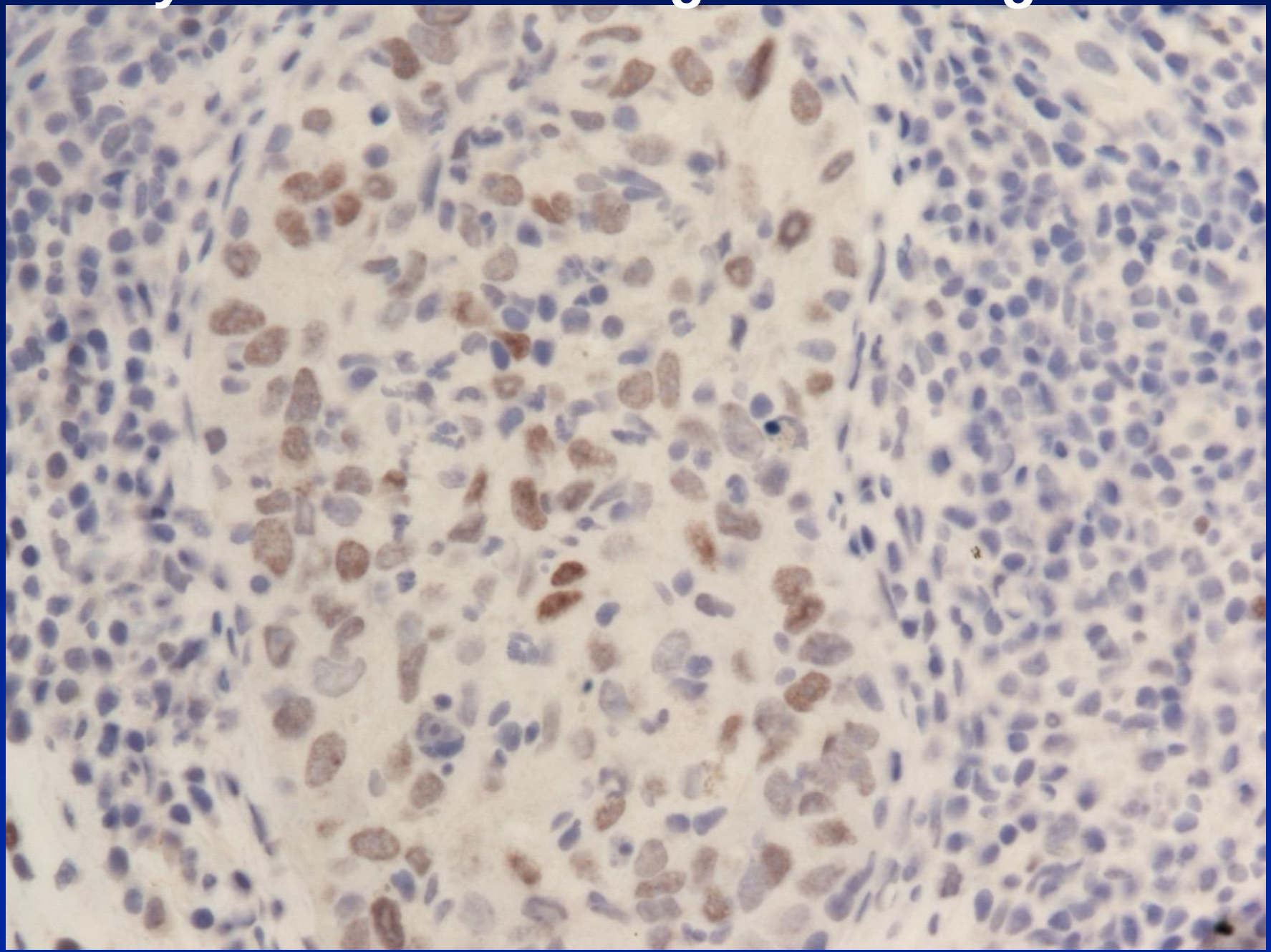
Background staining



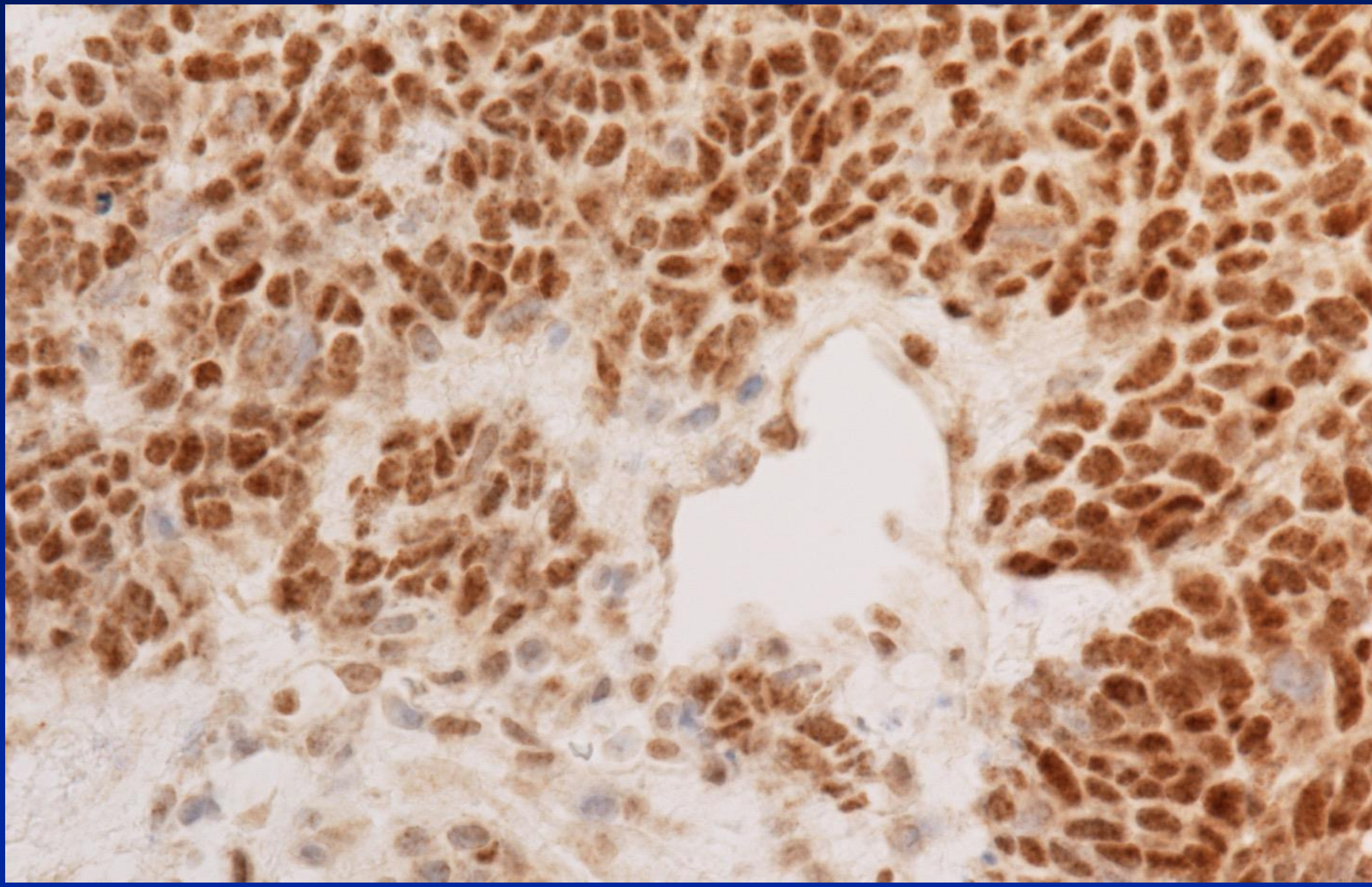
Very weak ER staining with background



Very weak ER staining with background



Strong ER staining but with abnormal pattern



??? False positive ER

*** Unusual staining pattern**

- Granular nuclear with perinuclear cytoplasmic staining

*** Background staining sometimes very obvious**

- Very weak tumour cell staining with background staining

*** Tumour:**

PR is always negative (0%)

Tumour is typically high grade

*** More with certain clones: ie 6F11 mainly on core biopsy**

Rakha et al.. **J Clin Oncol. 2012 Aug 10;30(23):2929-30**

Triage for genomic tests

- **75% of BC cases are ER+ HER2-**

Cases likely to response include:

- **Low grade, strong ER+ and PR+, Low KI67, Lobular subtype**

However, some cases are not definite and need further testing

- **High grade with PR- and or high Ki67**
- **Low expressor of ER**

Prognostic multigene signatures

Several genes used together in a formula to predict outcome and response to endocrine therapy and chemotherapy

Multigene signatures

Microarray and RT-PCR based assays

- 21 gene signature (Oncotype Dx)
- 70 gene signature (MammaPrint)
- 76 gene signature (Rotterdam)
- 50 genes: Risk of Recurrence (ROR) score (Prosigna)
- 12 genes (Endopredict) & Epclin
- 5 genes (Molecular grade index)
- 2 gene ratio (*H/I*™)

7 gene assay (THEROS The Breast Cancer Index)

- 97 gene: Genomic grade index (MapQuant Dx)
- 14 genes (BreastOncPx) - 14 gene signature (Celera Metastasis Score™)

Multigene signatures

IHC and ISH based assays

- 4 gene signature (IHC4; ER, PR, HER2 and Ki67)

- 5 gene signature (Mammostrat)

- 9 gene signature (Mammostrat Plus; 5 + ER, PR, HER2 and Ki67)

- 5 gene signature (ProEx™ Br)

- 3 gene signature (*eXagenBC*™)

Signatures based on a biological process

- Wound-response signature (442 genes)
- Immune signatures (14 genes)
- Invasiveness Gene Signature (186 genes)

First generation prognostic signatures

Test	Parameters	1° Validation Population	Location
Oncotype DX	16 +5 genes RT-PCR	ER+ (pN0) +ET	Central: USA
MammaPrint	70 genes array	ER+/- (pN0-1)	Central
Prosigna (PAM50)	50 +5 genes NanoString	ER+ HER2- (pN0-2) +ET	Regional
EndoPredict	8 +4 genes RT-PCR	ER+ HER2- (pN1-3) +ET +CT	Regional/ Central
Breast cancer Index	7 genes	ER+ HER2-	Central

Oncotype DX™ (21-Gene Recurrence Score) Assay

16 Cancer and 5 Reference Genes From 3 Studies

PROLIFERATION
Ki-67
STK15
Survivin
Cyclin B1
MYBL2

INVASION
Stromelysin 3
Cathepsin L2

HER2
GRB7
HER2

ESTROGEN
ER
PR
Bcl2
SCUBE2

GSTM1

BAG1

CD68

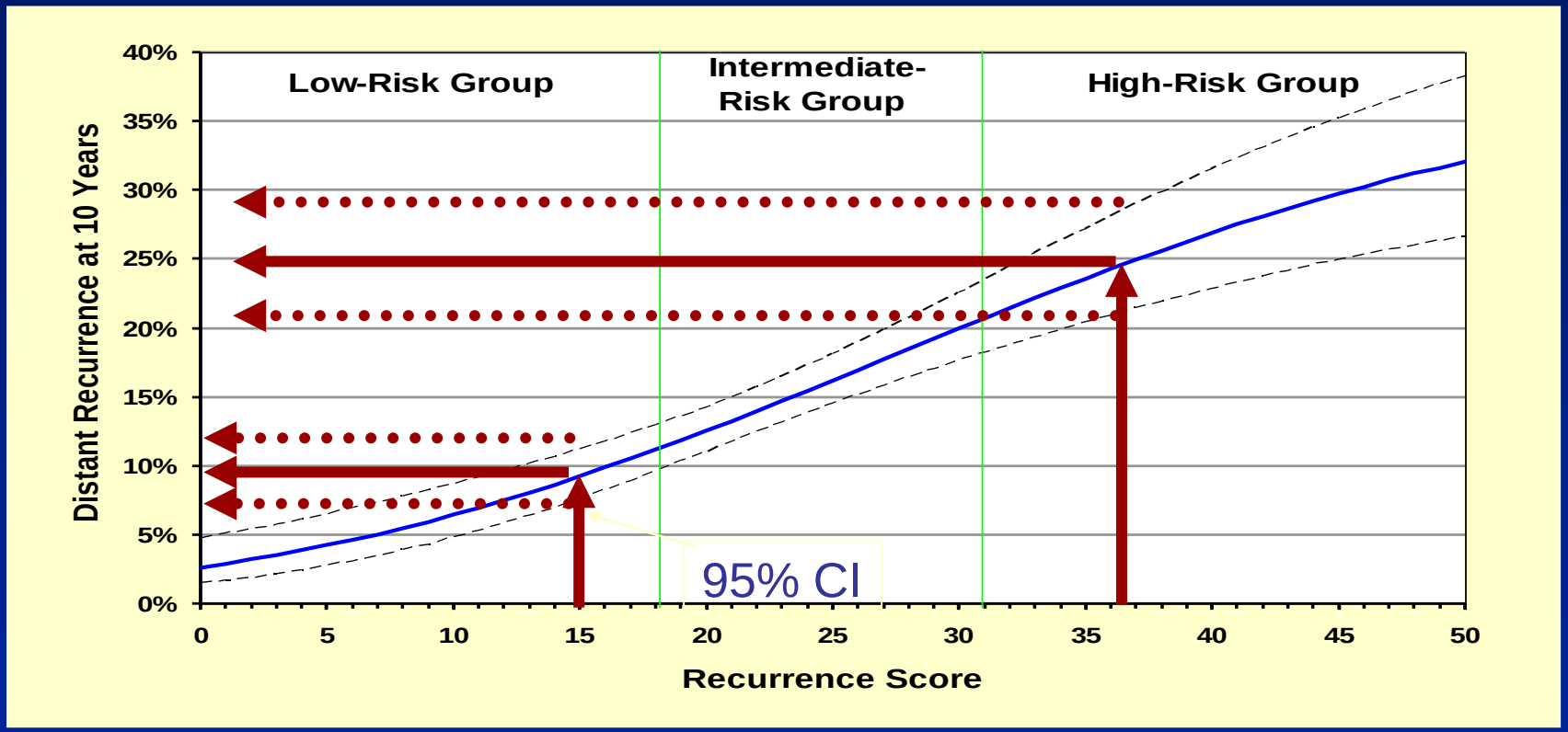
REFERENCE
Beta-actin
GAPDH
RPLPO
GUS
TFRC

RS = + 0.47 x HER2 Group Score
- 0.34 x ER Group Score
+ 1.04 x Proliferation Group Score
+ 0.10 x Invasion Group Score
+ 0.05 x CD68
- 0.08 x GSTM1
- 0.07 x BAG1

Category	RS (0-100)
Low risk	RS <18 (50%; 7%)
Int risk	RS ≥18 - <31 (14%)
High risk	RS ≥31 (27%, 30%

Paik et al. *N Engl J Med.* 2004;351:2817-2826.

Oncotype DX™ Clinical Validation: RS as Continuous Predictor



Breast Cancer Research and Treatment (2019) 173:123–133
https://doi.org/10.1007/s10549-018-4964-y

CLINICAL TRIAL

Validation of the 21-gene test as a predictor of clinical response to neoadjuvant hormonal therapy for ER+, HER2-negative breast cancer: the TransNEOS study

Hiroji Iwata[1] · Norikazu Masuda[2] · Yutaka Yamamoto[3] · Tomomi Fujisawa[4] · Tatsuya Toyama[5] · Masahiro Kashiwaba[6] · Shoichiro Ohtani[7] · Naruto Taira[8] · Takehiko Sakai[9] · Yoshie Hasegawa[10] · Rikiya Nakamura[11] · Hiromitsu Akabane[12] · Yukiko Shibahara[13] · Hironobu Sasano[13] · Takuhiro Yamaguchi[13] · Kentaro Sakamaki[14] · Helen Bailey[15] · Diana B. Cherbavaz[15] · Debbie M. Jakubowski[15] · Naoko Sugiyama[15] · Calvin Chao[15] · Yasuo Ohashi[16]

The Breast 18 (2009) 171–174

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Original article

21-Gene expression profile assay on core needle biopsies predicts responses to neoadjuvant endocrine therapy in breast cancer patients

Sadako Akashi-Tanaka[a,*], Chikako Shimizu[b], Masashi Ando[b], Tatsuhiro Shibata[c], Noriyuki Katsumata[b],

The Breast 18 (2009) 171–174

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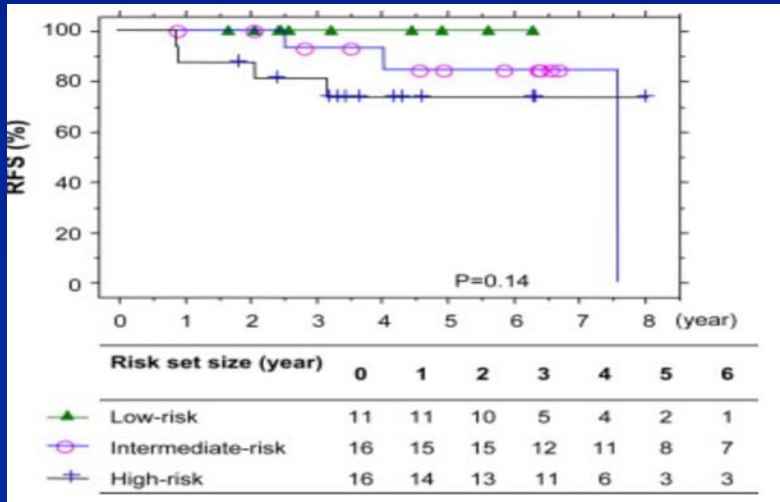
Ten 3-μm unstained sections and two hematoxylin and eosin sections from each core needle biopsy (CNB) paraffin block were

For three patients, the remaining specimen was insufficient to generate unstained slides. The total RNA yields were insufficient to assay (<500 ng) in 29 patients. Therefore, a RS was determined in the remaining 43 patients.

Response rates (cCR + cPR) to NAET for low- (<18, 26%) intermediate- (37%), and high- (37%) risk RS were 64%, 31%, and 31%, respectively (p = 0.11, by trend test)



Pilot study 2009 43 cases

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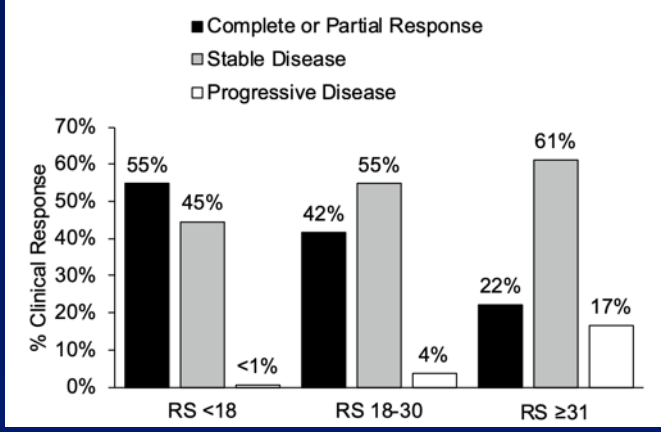

333 NCB, of these, 34 samples were excluded for reasons (10%)

295 BC patients (median age 63 years; median tumor size 25 mm; 66% grade 1),

53% had RS <18

29% had RS18–30

18% had RS ≥31

Clinical response rates were 54% (RS<18), 42% (RS18–30), and 22% (RS≥31).

In multivariable, analyses, continuous Recurrence Score result ($p<0.001$), ESR1 score ($p=0.049$), PGR score ($p<0.001$), and ER gene-groupscore ($p<0.001$) were associated with clinical response.

Summary

* Pathologists can guide management of BC patients in the era of COVID-19

* Core biopsy reporting is important as it can give final information

* Accurate assessment of prognostic variable is crucial
  - Tumour grade, Histological type
  - Receptor status (ER, PR and HER2)
  - Other markers: Ki67 and Genomic tests

Predictor of good response to NAET are
Strong ER expression, positive PR, low Ki67, Low grade, ILC

Indeterminate level of response: Multigene test (e.g., Oncotype Dx)

Low scores : >50% response, High scores: <30% response

Thanks