Breast cancer pathology and molecular biology

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Department of Pathology
Conflict of Interest

• I have no financial relationships to disclose
Topics

- Pathological features of breast carcinoma
- Standard prognostic and predictive factors of invasive breast carcinoma
- Molecular classification of breast carcinoma
- Molecular markers of invasive breast carcinoma
Invasive breast carcinoma

• BC is a heterogeneous disease
• Tumours with similar morphology show variable behaviour, outcome and response to therapy
Why do we need a classification?

Aim 1: Diagnosis

Aim 2: Prognosis

Aim 3: Prediction

Prediction is difficult, especially about the future
Niels Bohr, 1885-1962
## Summary of prognostic and predictive factors for invasive breast cancer

<table>
<thead>
<tr>
<th>Prognostic</th>
<th>Predictive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient age</td>
<td>√√</td>
</tr>
<tr>
<td>Nodal status</td>
<td>√√√</td>
</tr>
<tr>
<td>Tumor size</td>
<td>√√√</td>
</tr>
<tr>
<td>Lymphovascular invasion</td>
<td>√√</td>
</tr>
<tr>
<td>Histological grade</td>
<td>√√</td>
</tr>
<tr>
<td>Histologic type</td>
<td>√</td>
</tr>
<tr>
<td>Steroid receptors</td>
<td>√</td>
</tr>
<tr>
<td>Her2/neu</td>
<td>√√√</td>
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</tbody>
</table>

Nodal status

The Effect of Tumor Size and Lymph Node Status on Breast Carcinoma Lethality


For women with equivalent lymph node status, tumor size was associated with increased lethality, such that each millimeter of tumor diameter was associated with an additional 1% chance of death.

For women with tumors of equivalent size, lethality increased with increasing number of positive lymph nodes, such that there was an extra 6% chance of death associated with each positive lymph node.
Nodal status
Lymph node involvement

• **pN1**
  MACROMETASTASIS
  size >2 mm

• **pN1mic**
  MICROMETASTASIS
  size >0.2 mm and <2 mm
  >200 cells in one LN section

• **pN0**
  pN0(i-)
  pN0(i+)

  ISOLATED TUMOR CELLS (ITCs)
  single cells and clusters <0.2 mm, even in H/E-stained slides
  pN0(mol-) and pN0(mol+)

AJCC 2010
SEER micrometastasis study

209,720 patients (SEER)
1992-2003 pN0
pN1mi (0.3-2 mm)
pN1 (>2 mm)

• N1mi significant at multivariate analysis (p<0.0001) vs N0 (HR 1.35)
  vs N1 (HR 0.82)

Chen SL et al Ann Surg Oncol. 2007, 12:3378-84
Sentinel lymph node (SLN) biopsy

- 1st LN draining tumor bed → 1st site of local mets
- Pathologically negative SN have been shown to predict negative axillary status with a 98% degree of accuracy
- Standard method in breast cancer patients cN0

Tumor size

Invasive carcinoma with surrounding intraductal carcinoma

Multiple invasive carcinomas size of the largest is used for T-staging
Tumor grade

- Different grading systems
- Nottingham combined histologic grade (the Elston-Ellis modification of the Scarff-Bloom-Richardson grading system)
- Subjectivity
- Adherence to strict criteria is necessary for reproducibility so that grading can be used as a prognostic marker

Breast cancer grade scoring Nottingham combined histologic grade (the Elston-Ellis modification of the Scarff-Bloom-Richardson grading system)

<table>
<thead>
<tr>
<th>Tubuli</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; 75 %</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>10 – 75 %</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>&lt; 10 %</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>Nuclei</td>
<td></td>
<td></td>
</tr>
<tr>
<td>small monomorphous</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>intermediate size and variability</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>large and polymorphous</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Mitosis</td>
<td>number in 10 HPF</td>
<td>1 - 3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Grade 1</th>
<th>total score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 2</td>
<td>total score</td>
</tr>
<tr>
<td>Grade 3</td>
<td>total score</td>
</tr>
</tbody>
</table>

3 – 5
6 – 7
8 – 9

HPF high-power field

Elston CW and Ellis IO The Breast, Churchill Livingstone 1998
Histologic grade and survival

![Graph showing survival rates by histologic grade.](image)

<table>
<thead>
<tr>
<th>Years</th>
<th>Grade 1</th>
<th>Grade 2</th>
<th>Grade 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>399</td>
<td>686</td>
<td>920</td>
</tr>
<tr>
<td>4</td>
<td>356</td>
<td>529</td>
<td>520</td>
</tr>
<tr>
<td>6</td>
<td>179</td>
<td>241</td>
<td>219</td>
</tr>
<tr>
<td>8</td>
<td>76</td>
<td>100</td>
<td>95</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>14</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Elston CW and Ellis IO The Breast, Churchill Livingstone 1998
Histologic type

- Invasive ductal carcinoma of no special type - 75%

Histologic appearance

Gross Features
20 Histological types: morphology matters!

• The identification of special histologic types enables further refinement of the prediction of clinical outcome
Special histological types of breast carcinoma

1. Invasive lobular carcinoma
2. Tubular carcinoma
3. Cribriform carcinoma
4. Carcinoma with medullary features
5. Metaplastic carcinoma
6. Carcinoma with apocrine differentiation
7. Salivary gland/skin adnexal-type tumors
8. Adenoid cystic carcinoma
9. Mucoepidermoid carcinoma
10. Polymorphous carcinoma
11. Mucinous carcinoma and carcinoma with signet ring cell differentiation
12. Carcinoma with neuroendocrine features
13. Invasive papillary carcinoma
14. Invasive micropapillary carcinoma
15. Secretary carcinoma
16. Oncocytic carcinoma
17. Sebaceous carcinoma
18. Lipid-rich carcinoma
19. Glycogen-rich clear cell carcinoma
20. Acinic cell carcinoma
Strict diagnostic criteria must be used to ensure the accuracy of diagnosis and, consequently, the prediction of outcome.
Invasive lobular carcinoma

- bilateral and multifocal
- older patients
- larger in size
- positive for steroid receptors and negative for Her2/neu
- E-cadherin negative
Hormone Receptors

- Weak prognostic factors
- Predictive factors of the response to hormonal therapy
- Evaluation of ER and PR - a mandatory component of the pathologic evaluation of breast carcinomas
Hormone Receptors

- IHC evaluation - standard of practice
- Most guidelines recommend reporting both the proportion of positively stained nuclei and the intensity of nuclear staining
The interlaboratory variance in ER and PR data is as high as 30%.
Clinical data indicate that ER positivity as low as 1% can identify patients who would benefit from hormonal therapy.
Immunohistochemistry of Estrogen and Progesterone Receptors Reconsidered

Experience With 5,993 Breast Cancers

Mehrdad Nadji, MD, Carmen Gomez-Fernandez, MD, Parvin Ganjej-Azar, MD, and Azorides R. Morales, MD

Status of ER and PR in 5,497 Cases of Infiltrating Mammary Carcinoma in Histologic Specimens

<table>
<thead>
<tr>
<th>Receptor</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER+</td>
<td>4,100 (75)</td>
</tr>
<tr>
<td>PR+</td>
<td>3,016 (55)</td>
</tr>
<tr>
<td>ER+/PR+</td>
<td>3,016 (55)</td>
</tr>
<tr>
<td>ER+/PR−</td>
<td>1,084 (20)</td>
</tr>
<tr>
<td>ER−/PR−</td>
<td>1,397 (25)</td>
</tr>
<tr>
<td>ER−/PR+</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

ER, estrogen receptor; PR, progesterone receptor; +, positive; −, negative.

Relationship of ER and PR to Histologic Subtypes of Mammary Carcinoma*

<table>
<thead>
<tr>
<th>Type of Carcinoma</th>
<th>ER+ (%)</th>
<th>PR+ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infiltrating ductal, not otherwise specified (n = 4,396)</td>
<td>3,255 (74)</td>
<td>2,330 (53)</td>
</tr>
<tr>
<td>Tubular (n = 237)</td>
<td>237 (100)</td>
<td>225 (95)</td>
</tr>
<tr>
<td>Colloid (n = 184)</td>
<td>184 (100)</td>
<td>133 (72)</td>
</tr>
<tr>
<td>Papillary (n = 44)</td>
<td>44 (100)</td>
<td>35 (80)</td>
</tr>
<tr>
<td>Apocrine (n = 40)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Medullary (n = 96)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Metaplastic (n = 120)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Infiltrating lobular (n = 380)</td>
<td>380 (100)</td>
<td>293 (77)</td>
</tr>
</tbody>
</table>

ER, estrogen receptor; PR, progesterone receptor; +, positive.
Her2/Neu

- Positive in 15–25 %
- Poor prognostic factor
- Predictive factor of the response to anti-HER2 therapy

- Her2 testing
  - IHC
  - ISH (FISH, CISH, SISH)
IHC scoring: semi-quantitative interpretation of HER2 expression

- IHC 0 (negative)
- IHC 2+ (equivocal)
- IHC 1+ (negative)
- IHC 3+ (positive)
HER2 ISH

<4 Her2/neu gene copies per nucleus, or a ISH gene ratio <2.0

>6 gene copies per nucleus, or a ISH gene ratio (ratio of Her2/neu gene signals to chromosome 17 signals) ≥2

Her2/neu testing

- All primary invasive breast cancers
- All metastasis
- All recurrences
Tumor proliferation: Ki67


PRODUCTION OF A MOUSE MONOCLONAL ANTIBODY REACTIVE WITH A HUMAN NUCLEAR ANTIGEN ASSOCIATED WITH CELL PROLIFERATION

Johannes Gerdes¹, Ulrich Schwab², Hilmar Lemke² and Harald Stein¹,³

¹Institute of Pathology, Christian Albrecht University, Hospitalstrasse 42, D-2300 Kiel; and ²Institute of Biochemistry, Christian Albrecht University, Olshausenstrasse 40-60, D-2300 Kiel, Germany.

MATERIAL AND METHODS

Cells and specimens

Human peripheral blood lymphocytes and mono-
• 17 of the 18 studies that included more than 200 patients showed statistically significant association between Ki67 and prognosis providing compelling evidence for a biological relationship.

• but the cut-offs to distinguish “Ki67 high” from “Ki67 low” varied from 1% to 28.6%, thereby severely limiting its clinical utility.

DowsettM et al; JNCI 2011
Ki-67

- **Limits of procedure**
  - Quantification
  - Interpretation
  - Tumor heterogeneity
  - Tissue fixation
    - Artefacts
    - Staining
  - Reproducibility

Ki67 staining: surgicals vs. TMAs
• Clinical Limits

– Cut points arbitrary
• Various cut points suggested
• Still under debate
• May vary depending on topic (prognostic or predictive)
– For adjuvant treatment choice
• Cut points from 5 - 34%
• Most frequently 10 – 20%
• St.Gallen 2013
  – 20% (Panel decision)
• Proliferation rates are a continuum and are not bimodal
St Gallen 2017

“…when is traditional pathology (stage, grade, LVI, ER/PR/HER2) not informative enough?”

Traditional clinicopathological parameters

Prognosis of patients with breast carcinoma

Biology?
Prognosis

• High risk: Chemotherapy
• Low risk: No chemotherapy
• However, clinically indeterminate groups such as LN-/ER+/HER2- tumours: Additional prognostic tests are needed (Multigene Prognostic Assays)
Microarray-based gene expression analysis

Perou et al. In 2000
>1700 genes

Each row is a gene
Each column is a sample
Green: <median
Black: =median
Red: >median
Rt panel: cell lines
Left panel: tissue

Dendrogram: similarities in the expression patterns
Molecular portraits of human breast tumours

Charles M. Perou† ‡, Therese Sorlie‡ ‡, Michael B. Eisen*, Matt van de Rijn†, Stefanie S. Jeffrey†, Christian A. Rees*, Jonathan R. Pollack‡, Douglas T. Ross‡, Hilde Johnsen†, Lars A. Akslen‡, Øystein Fuge‡ §, Alexander Pergamenschikov*, Cheryl Williams*, Shirley X. Zhu*, Per E. Lønning** ‡, Anne-Lise Børresen-Dale† ‡, Patrick O. Brown‡ ‡ ‡ & David Botstein* 

Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications

Molecular Subtypes and Prognosis

Sorlie T et al, PNAS 2001
Clinicopathologic surrogate definition

- **Luminal A-like**
  ER+, HER2-, Ki67 low, PgR high
  Low-risk molecular signature (if available)

- **Luminal B-like**
  HER2-negative:
  ER+, HER2- and either Ki67 high or PgR low
  High-risk molecular signature (if available)
  HER2-positive:
  ER-positive, HER2-positive, any Ki67, any PgR

- **HER2-positive** (non-luminal):
  HER2+, ER and PgR absent

- **Basal-like/Triple-negative**
  ER and PgR absent, HER2-negative
Basal like carcinomas

- Cluster genes characteristically expressed in normal breast basal/myoepithelial cells
- IHC: The basal type of tumors frequently does not express ER, PR, and HER2/neu but also expresses basal cytokeratins 5/6 and 17
- They tend to recur during the first 3 years after diagnosis, and currently there are no specific targeted therapies for them
- Strong association between basal-like carcinomas and BRCA1 mutations carriers
PATHOLOGIC FEATURES OF BASAL-LIKE TUMORS

- High-histologic grade, NOS (75%-100%)

The basal-like breast carcinomas and TNBC do not represent a single uniform group of tumors but a spectrum of tumors from low-grade to high-grade with different morphology.
TN is not a synonym for basal-like phenotype!
• There is heterogeneity within the molecular subtypes: EVEN THE SUBTYPES HAVE SUBTYPES

Comprehensive molecular portraits of human breast tumours

The Cancer Genome Atlas Network*
Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies

Brian D. Lehmann, ... Yu Shyr, Jennifer A. Pietenpol
Published July 1, 2011
Citation Information: J Clin Invest. 2011;121(7):2750-2767. doi:10.1172/JCI45014.
Prognostic 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™)
  - 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)
• In addition to ER, PR and HER2, there is sufficient evidence of clinical utility for the biomarker assays [Oncotype DX, EndoPredict, PAM50, Breast Cancer Index, and urokinase plasminogen activator and plasminogen activator inhibitor type 1 in HR+/HER2- Ln-. groups and can be used.

• These assays should not be used to guide treatment decision in LN+, HER2+ or triple negative cancer (No other molecular test (including ki67) should be used to direct treatment decision)
Oncotype DX™ 21-Gene Recurrence Score (RS) Assay

• Based on the expression levels of 21 genes, a recurrence score (RS) is generated.

\[ RS = 1.04 \times \text{Proliferation Group Score} + 0.47 \times \text{HER2 Group Score} - 0.34 \times \text{ER Group Score} + 0.10 \times \text{Invasion Group Score} + 0.05 \times \text{CD68} - 0.08 \times \text{GSTM1} - 0.07 \times \text{BAG1} \]
• The test is specifically applied to HR+ breast cancers with 0–3 positive nodes that are to be treated with hormonal therapy

• The general consensus is that hormonal therapy without systemic chemotherapy is sufficient for patients with a low RS.


MammaPrint assay

- 70-gene expression assay developed by The Netherlands Cancer Institute
- It is prognostic for early distant recurrence within the first 5 year after diagnosis and predictive for chemoresponse in poor prognostic patients
**Prosigna test**

- PAM50-based assay offered by NanoString Technologies (Seattle, WA)
- Based on the expression levels of 50 genes and clinical variables, a risk of recurrence (ROR) score is generated that correlates to one of the four molecular subtypes (lum A, lum B, HER2-enriched, and basal-like)
Multigene Prognostic Tests: Unresolved Issues

Is this approach really better than using a combination of clinical and pathologic factors supplemented by appropriate biomarkers detected by IHC (e.g., ER, PR, HER2 and Ki67)?
Take Home Messages

• The accurate diagnosis of breast cancer is a critical prerequisite to the therapy decision-making process
• Most of the prognostic factors currently used in clinical practice are based on pathologic evaluation of the primary tumor and lymph nodes (the LN status are more and more detroned)
• ER, PR, and HER2 testing using ASCO/CAP guidelines remain the most important ancillary tests in the management of patients with breast cancer
Take Home Messages

• Among patients with ER+/HER2- (“luminal”) disease, multigene prognostic tests are of value in further defining risk of recurrence and potential benefit from chemotherapy in addition to endocrine therapy.

• Ki67 is not highly predictive for utilisation of adjuvant chemotherapy.

• New technologies and genomewide approaches have the potential to identify additional prognostic and predictive markers for invasive breast cancer.

• The role of the pathologist has changed from that of descriptive pathology of Virchow, to an important team player in the age of personalised medicine.