Modern classification of breast cancer-should we stick with morphology or convert to molecular profiles?

Ian Ellis  
Professor of Cancer Pathology  
Molecular Medical Sciences  
University of Nottingham  
Dept Histopathology  
City Hospital Campus  
Nottingham University Hospitals  
Hucknall Rd  
Nottingham  
NG5 1PB

Tel: +44 115 9691169 ext 56875  
email: ian.ellis@nottingham.ac.uk

Introduction

The future role of Pathology will to improve personalisation of care for breast cancer patients. In the past the importance of the Pathology Laboratory in the field of tumour pathology has been under-estimated and under-utilised, the clinician wanting (and receiving) only a one line report stating the diagnosis. It has, however, now become widely accepted that more information can be gleaned from the histopathological appearance of a tumour and that this may be used to predict the biological behaviour and clinical outcome in many malignancies, including breast cancer. In addition, the use of other techniques, such as immunohistochemistry and molecular examination, has been expanded in the search for features of the tumour which can predict reliably not only the prognosis of the patient but also the potential for response to a given treatment.

PROGNOSTIC FACTORS

Prognostic factors, although not specific predictors of response to a therapy, can be used for appropriate treatment selection of patients with malignancies; those patients who have an extremely good prognosis after tumour excision may not warrant toxic adjuvant therapies which themselves carry a significant morbidity, but those with a poor prognosis may benefit from an aggressive adjuvant approach. Identifying the prognostic features of an invasive breast carcinoma for these reasons is particularly important as the disease has a markedly variable course; a group of women with “curable” carcinomas who do not receive significant benefit from adjuvant therapy can be identified, whilst others will succumb relatively rapidly.
to the disease. Because of this widely differing clinical outcome and because breast carcinoma is common, prognostic factors, and more recently predictive factors, in this malignant disease are probably the most widely studied.

**PREDICTIVE FACTORS**

In breast cancer oestrogen receptor (ER) and HER2 are the best, and at present the only widely applied example of specific tests used to predict response to a specific therapy in breast cancer management. They are not in our hands of independent significance in predicting prognosis in breast cancer patients due to their close relationship with histological grade. We nevertheless assess ER and HER2 status routinely on patients with invasive breast carcinoma as a predictive factor to assist in selection of appropriate treatment.

**FUTURE PREDICTIVE MARKERS**

With expanding knowledge of molecular biology of cancer and the availability of the Human Genome Project we are now experiencing a rapid increase in potential molecular targets for drug development. It is inevitable that pathology laboratories will play a pivotal role in developing methods to assess 1) potential efficacy based on signalling pathway changes, 2) identify surrogate endpoints for early clinical trials based on biomarker change, 3) identify markers predictive of response, 4) identify and understand mechanisms of response and failure to respond and 5) integrate diagnostic and predictive testing into routine cancer management.

**FUTURE CLASSIFICATION**

Recent high-throughput genomic studies have offered the opportunity to challenge the molecular complexity of breast cancer and have provided evidence for an alternative method for classifying breast cancer into biologically and clinically distinct groups based on gene expression patterns. Such new molecular taxonomies have identified many genes, some of which are being proposed as candidate genes for sub-grouping breast cancer. Such studies have been applied on a relatively small number of tumours and require validation in large series and comparison with traditional classification systems prior to acceptance in clinical practice. This has partly been achieved using using high throughput tissue screening tissue microarray (TMA) technology. These studies have examined expression of proteins known
to be of relevance in breast cancer and have resulted in recognition of classes of breast
cancer broadly similar to those identified by gene expression studies. Of note is the
recognition of the importance of luminal or basal epithelial differentiation as well as hormone
receptor and HER 2 expression. Basal epithelial differentiation or HER 2 expression being
associated with an adverse outcome when compared to tumours showing luminal epithelial
differentiation and hormone receptor expression
Modern Classification of Breast Cancer
Should we stick with morphology or convert to molecular profiles?

Ian Ellis
Molecular Medical Sciences, University of Nottingham
Department of Histopathology, Nottingham University Hospitals NHS Trust

Time --- Prognosis --- Intrinsic

How long the tumour has been there
Stage

The nature of the tumour
Biology

Traditional Prognostic Factors

- Histological grade
- Histological type
- Lymph node stage
- Tumour size
- Vascular invasion

Nottingham Prognostic Index

Grade + LN Stage + (0.2 x Size)

1 - 3
cm

What Would Your Treatment Strategy Be For This Patient?

- Age: 61
- ER: 95%
- PR: 95%
- Tumor Type: IDC
- Tumor Size: 0.6 cm
- Tumor Grade: 2
- HER-2 neu Neg (FISH)

*Additional 6 mm on re-excision

### Histological grade

![Graph showing survival percentage by histological grade](image)

- Survival %:
  - Grade 1
  - Grade 2
  - Grade 3

### Time to Death

![Graph showing time to death by grade](image)

- Grade I
- Grade II
- Grade III

### 90% of Events

<table>
<thead>
<tr>
<th></th>
<th>Gr I</th>
<th>Gr II</th>
<th>Gr III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time to LR/RR</td>
<td>100</td>
<td>82</td>
<td>48</td>
</tr>
<tr>
<td>Time to Death</td>
<td>106</td>
<td>88</td>
<td>56</td>
</tr>
<tr>
<td>Time to Death</td>
<td>336</td>
<td>150</td>
<td>90</td>
</tr>
</tbody>
</table>

### Tumour Size

Most studies have found a significant association between tumour size and prognosis:

- Fisher et al, 1969
- Elston et al, 1982
- Haybittle et al, 1982
- Neville et al, 1990

Patients with small tumours have a longer survival than those with large tumours.

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**Time — Prognosis — Intrinsic**

- How long the tumour has been there
- The nature of the tumour

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**Tumour Size**

- 0-9mm
- 10-14mm
- 15-19mm
- 20-24mm
- 25mm or more
Time to death by Size

Lymph Node Stage

Survival by LN Stage

Lymph Node Involvement

Sentinel node biopsy
Vascular Invasion
Prognostic significance

Close correlation with loco-regional lymph node status –
Correlates with early recurrence in lymph node negative patients –
Rosen et al, 1983; Bettelheim et al, 1984; Neville et al, 1992

Predicts for long term survival, independent of nodal status
Rosen et al, 1982; Pinder et al, 1994
Predicts for local recurrence following breast conserving surgery
Fourquet et al, 1989; Borger et al, 1994; Pinder et al, 1994; Sundquist et al, 2000
Predicts for local recurrence after mastectomy
O’Rourke et al, 1994; Sundquist et al, 2000

Progress to systemic metastatic disease

- 173 women who developed metastatic disease after a previous breast cancer.
- 72% had nodal metastases
- 59% had definite vascular invasion
- 84% had either lymph node metastases or vascular invasion or both.
- Consistently present whatever the histological grade of the primary tumour.
- Absence of VI and nodal involvement indicated a low risk of subsequent metastatic disease. (Evans 2001)

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LVI for LN neg

Cumulative Proportion Surviving (Kaplan-Meier)
Complete   Censored
LVInegLNneg
LVIposLNneg
one
two
three
four

LVI contribution and nodal status contribution to hazard
**Merging LVI pos 1 and 2**

Cumulative Proportion Surviving (Kaplan-Meier)
- Complete
- Censored
LVIneg LNneg
- three
- fourplus
LVIpos 1 and 2

Time
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
Cumulative Proportion Surviving

**N P I**

Chi-Square
0
.2
.4
.6
.8
1
Cum. Survival
0 48 96 144 192 240 288
Time

**The Need**

Many Questions
- Major Questions in Breast Medical Problem
- Risk of relapse
- Risk of death due to breast cancer
- Expected relative and absolute benefits of different systemic Therapies

Differing Guidelines
- NICE - UK
- NIH - USA
- ESMO - EU
- ST Gallen - International consensus
- Others - other national and organisations

Changing Guidelines
- Up to 2005 Risk was the major determinant
- “Average” the best for all
- From 2005 The major determinant is the target
- Risk is not a target

**Prognostic Factor:**
Altered natural history

**Predictive Factor:**
Resistance or sensitivity to therapy

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**St. Gallen 2007 Treatment Choice**

<table>
<thead>
<tr>
<th>Highly endocrine responsive</th>
<th>Incompletely endocrine responsive</th>
<th>Endocrine non-responsive</th>
</tr>
</thead>
<tbody>
<tr>
<td>HER2-negative</td>
<td>ET (consider adding CT according to risk)</td>
<td>ET+ (consider adding CT according to risk)</td>
</tr>
<tr>
<td>HER2-positive</td>
<td>ET + Trastuzumab + CT</td>
<td>ET + Trastuzumab + CT</td>
</tr>
</tbody>
</table>

ET= endocrine therapy; CT= chemotherapy
**ER Immunohistology**

Cut off points for treatment

<table>
<thead>
<tr>
<th>Score treatment</th>
<th>Effect of Endocrine</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No effect</td>
</tr>
<tr>
<td>2 - 3</td>
<td>Small (20%) chance</td>
</tr>
<tr>
<td>4 - 6</td>
<td>Even (50%) chance</td>
</tr>
<tr>
<td>7 - 8</td>
<td>Good (75%) chance</td>
</tr>
</tbody>
</table>


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**Her-2 status**

Predictive assays

- **ER & PR** Presence of target protein
- **HER 2** Presence and overexpression TP
  - Gene amplification
- **EGFR**
  - Target protein
  - Gene copy number
  - Gene mutation
A signature to rule them all?

Gene expression profiles to predict distant metastasis of lymph-node-negative primary breast cancer

**Gene Expression Profiling in Breast Cancer: Understanding the Molecular Basis of Histologic Grade to Improve Prognosis**

The Prognostic Role of a Gene Signature from Tumorigenic Breast-Cancer Cells

**OncoType DX™ Clinical Validation:**

RS as Continuous Predictor

**OncoType DX™ 21-Gene Recurrence Score (RS) Assay**

16 Cancer and 5 Reference Genes From 3 Studies

**Gene Expression Signature to Predict Survival in Breast Cancer across Independent Data Sets**

Fan et al. NEJM 2006; Sotiriou et al. JNCI 2006

A signature to rule them all?

Intrinsic properties of the tumour

- Wound signature
- Invasiveness gene signature
- Tumour burden

- 70-gene signature
- High proliferation
- Poor prognosis

Molecular classification
- 76-gene signature
- 21-gene recurrence score

Genomic grade

Nodal status

70% CI

My RS is 30. What is the chance of recurrence within 10 years?

0% 5% 10% 15% 20% 25% 30% 35% 40% 45% 50%

Recurrence Score

Distant Recurrence at 10 Years

Low-Risk Group

Intermediate-Risk Group

High-Risk Group

95% CI


The Prognosis of a Gene Signature from Tumorigenic Breast-Cancer Cells

Bartlett, Ph.D., Seidman Ang, M.D., C. John Chen, M.D., Ph.D., F. Michael, M.D., A. Audrey, M.D., P. Theresa, M.D., J. John, M.D., F. Harlan, M.D., N. Michael, M.D., and F. Michael, M.D., N. Michael, M.D.
Meta-Analysis – Gene signatures

Blue dots: good prognosis
Red dots: poor prognosis

Take home message

• Prognostic gene signatures
  – Correlate with proliferation (and grade!)
    • Ki-67?
  – Good discriminatory power
    • ER positive disease
  – Limited value for ER negative disease
  – Cannot be readily applied to FFPE samples
  – Complementary to histopathology

Breast Cancer is a Family of Diseases

• Convergence of clinical and genomic data
• Unclear how many distinct members of this family
• At a minimum:
  - HER-2+
  - Basal-like or triple negative
  - ER+ (luminal A)
  - ER+ (luminal B)

"Basal-like"
ER/PR-negative
HER2-negative

HER2-positive
ER-positive
Luminal B

ER-positive
Luminal A

"Common Precursor"
LOH 16q

?Common Precursor
LOH 16q

C-erbB-2 & p53

High Grade Carcinoma

Other candidates: BRCA 1 17q
BRCA 2 13q
1q 3p 11q 13q 17q

Medullary
Tub & Lob
Tubular

Lobular
Ductal

Luminal / ER positive/ basal negative group [group 1]

Basal positive luminal /ER negative [group 5]
Luminal Subtypes

- Luminal A/B - generally carry a good prognosis \(^{(1)}\)
- Luminal A better prognosis than B \(^{(1)}\)
- Expect better response to ET in luminal subtypes

\(^{(1)}\) Sorlie et al PNAS 2003: 100: 8418 - 23
\(^{(2)}\) Rouzier et al Human Cancer Biology 2005; 11: 5678-85
A case of tubular carcinoma
A Cases of Invasive Lobular Carcinoma

- FEA
- DCIS
- FEA with LN
- Lobular neoplasia
- ILC
Genome plots of the previous case

Basal Breast Cancer

- The concept of BP has been known for some time
- First described using electron microscopy >30 years ago
- Its potential poor survival first reported by Dairkee et al in 1987

Conclusion

Luminal Type A lesions
• Luminal ck
• ER rich
• HER2 neg
• 16q del

Basal Breast Cancer

[LR 22.54, p<0.001]
Definition of basal-like

- No internationally accepted definition!
- Triple negative phenotype (ER-ve, PR-ve, HER2-ve)
- Rakha/ Ellis/ Nottingham
  - Expression of high mol wt cytokeratins (Ck 5/6, Ck 14, Ck 17)
- Nielsen
  - ER-ve, HER2-ve
  - Expression of Ck 5/6 and/or EGFR

Breast Cancer Subtypes, Race and Age

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Basal</th>
<th>HER2+</th>
<th>Luminal A</th>
<th>Luminal B</th>
<th>Unclass</th>
</tr>
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<tbody>
<tr>
<td>Premenopausal</td>
<td>97</td>
<td>39%</td>
<td>9%</td>
<td>36%</td>
<td>9%</td>
<td>6%</td>
</tr>
<tr>
<td>African-American</td>
<td>99</td>
<td>14%</td>
<td>7%</td>
<td>59%</td>
<td>16%</td>
<td>4%</td>
</tr>
<tr>
<td>Postmenopausal</td>
<td>164</td>
<td>16%</td>
<td>6%</td>
<td>51%</td>
<td>18%</td>
<td>10%</td>
</tr>
<tr>
<td>African-American</td>
<td>136</td>
<td>16%</td>
<td>6%</td>
<td>58%</td>
<td>16%</td>
<td>4%</td>
</tr>
<tr>
<td>TOTAL</td>
<td>496</td>
<td>20%</td>
<td>7%</td>
<td>51%</td>
<td>16%</td>
<td>6%</td>
</tr>
</tbody>
</table>

Adapted from Carey LA et al, ASCO 04

Pathologic complete response

<table>
<thead>
<tr>
<th>Molecular classification</th>
<th>Residual Disease</th>
<th>Pathologic complete response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luminal</td>
<td>93% [78-99]</td>
<td>7% [1-22]</td>
</tr>
<tr>
<td>Normal breast</td>
<td>100% [29-100]</td>
<td>0% [0-31]</td>
</tr>
<tr>
<td>HER2+</td>
<td>95% [32-77]</td>
<td>45% [23-68]</td>
</tr>
<tr>
<td>Basal subtype</td>
<td>95% [32-76]</td>
<td>45% [24-68]</td>
</tr>
</tbody>
</table>

Chi square: P<0.001

Rouzier R et al, SABCS 2004

Treatment Approaches of Interest for Triple Negative Disease

- Angioinhibitors
- EGFR inhibitors
- PARP inhibitors (particularly in setting of BRCA1 and 2 mutant tumors)
- Platinum salts
  - Take advantage of inability of BLC to repair double strand DNA breaks
  - Similarities between sporadic basal-like cancers and BRCA1 associated tumors

Familial Breast Cancer

- BRCA 1 Prediction
- BRCA 1 - basal phenotype
  - Ck 5/6&14 +ve - 44% of all BRCA 1 carriers
  - Ck 5/6&14 +ve - < 2% sporadic cancers

Lakhani Clin Cancer Res
What Would Your Treatment Strategy Be For This Patient?

- Age: 61
- ER: 95%
- PR: 95%
- Tumor Type: IDC
- Tumor Size: 0.6 cm*
- Tumor Grade: 2
- HER-2 neu Neg (FISH)

*Additional 6 mm on re-excision

Recurrence Score: 36
Average Rate of Distant Recurrence at 10 Yrs: 25%

Results

CLINICAL EXPERIENCE

Future Classification of Breast Cancer

Emerging classification system with clinical relevance based on:
- morphology
- phenotype
- molecular genetics

Routine provision of prognostic and predictive information
Identification of key therapeutic targets
Linked development of theranostics with drug development

Future Classification of Breast Cancer

Translation of research techniques / methods to routine clinical practice
Robust validated & standardised routine methods
Quality assurance integrated into service provision
Pathology has a central role - analytical & coordination
Decision making support systems will be essential