COMPANION MEETING

BREAST

Bayside Room 104
Time: 9:00 – 10:45

Convenor: A/Prof. Gelareh Farshid, Consultant Pathologist, SA Pathology and Clinical Director, BreastScreen SA, Adelaide, SA

- Dr Oana Crainic, Canterbury Health Laboratories, Christchurch, NZ
- Dr Gavin Harris, Canterbury Health Laboratories, Christchurch, NZ
- A/Prof. Sandra O’Toole, Consultant Pathologist, Royal Prince Alfred Hospital, Sydney, NSW
- A/Prof. Gelareh Farshid, SA Pathology and BreastScreen, SA
Primary angiosarcoma of the breast is a rare malignancy which accounts for less than 0.05% of primary breast tumours and 8% of all sarcomas of the breast. It is a highly aggressive tumour, most commonly seen in premenopausal patients (third and fourth decades), with a tendency to recur locally and to metastasise mainly haematogenously to bone, lungs, skin, liver, CNS. Primary angiosarcoma usually presents as a palpable mass. Angiosarcomas require complete excision and are more often treated by mastectomy. Adjuvant radiotherapy and chemotherapy have been used with varied results.

Our patient is a postmenopausal 50-year-old woman without a previous history of breast malignancy or irradiation who presented with an area of nonspecific density within the upper inner quadrant of the right breast on screening. This area corresponded to an area of thickening clinically. The ultrasound there was a poorly defined area of increased echogenicity extending over at least 5cm. The core biopsies from the lesion showed a low grade vascular proliferation interpreted as a haemangioma or an angiolipoma, but a low grade angiosarcoma could not be excluded. Consequently she underwent an open excision biopsy with hookwire localisation. Histologic examination showed abnormally anastomosing vascular channels lined by atypical endothelial cells and a low to intermediate grade angiosarcoma was diagnosed. Surgical margins were positive and she required a wider reexcision eventually proceeding to a complete mastectomy. Sentinel node biopsy was performed at this time with 0/5 lymph nodes involved by tumour. She was consequently treated with local radiotherapy. There has been no recurrences of local or distant disease at this time.

This case opens the discussion on vascular lesions of the breast with emphasis on primary angiosarcoma of the breast and radiation-associated vascular lesions following breast conserving surgery and radiotherapy.

Angiosarcomas are the most frequent primary sarcomas of the breast but are still very uncommon, accounting for less than 0.05% of breast malignancies (1, 2, 3, 4). Angiosarcomas may be primary or they may arise secondary to radiation therapy for breast cancer or other chest irradiation (5, 6). Angiosarcomas may also arise in the arm following radical mastectomy as a result of chronic lymphedema (Stewart-Treves syndrome); however this is rarely seen now with the marked decline in the number of the radical mastectomies performed (7). In contrast, the number of post-radiation angiosarcomas is on the rise given the routine use of breast-conserving surgery and radiation therapy in the management of breast cancer today (8, 9).

Postradiation angiosarcoma is a well known rare complication of radiotherapy following breast conserving therapy for breast carcinoma with a latency of 5-7 years (8,9). Secondary angiosarcomas more frequently present with violaceous
discoloration of the skin, due to cutaneous involvement. Compared to the primary angiosarcomas they arise in a cutaneous location rather than in the breast parenchyma, in older female group and are higher grade usually. Histologically the angiosarcomas are characterised by interanastomosing vascular spaces that dissect through the breast stroma and adipose tissue and surround and invade lobules, disrupting the normal lobular architecture. The vascular spaces are lined by endothelial cells that show nuclear hyperchromasia. Variable degrees of stromal erythrocyte extravasation are present. In many angiosarcomas, the vascular spaces at the invasive edge of the tumour appear extremely bland and may be difficult to distinguish from benign vascular proliferation. Angiosarcomas have been divided into low, intermediate and high grade based on a combination of histological findings. In the most cited study to address the relationship between grade and outcome, the 5-year survival was 76%, 70% and 15% for low, intermediate and high grade angiosarcomas respectively (10). In contrast, a more recent study failed to demonstrate a relationship between grade of angiosarcoma and outcome, similar to other angiosarcomas at other sites (11). Comparing primary and secondary angiosarcomas, a recent Mayo clinic report showed the same overall survival in primary angiosarcomas and postradiation angiosarcoma groups (8), while an European study from Italy showed poorer prognosis in postradiation angiosarcomas (9).

Angiosarcomas require complete excision and are most often treated by mastectomy. Axillary lymph node involvement is rare although a recent publication found 25% of patients to have lymph node involvement at the time of recurrence (9). The most common sites of metastatic spread are the lungs, liver, contralateral breast, other skin and soft tissue locations and bone (1). Recent therapeutic efforts have shown promising results with hyperfractionated radiation therapy and radiation therapy with hyperthermia (12, 13) as well as encouraging results with taxane therapy (14) in a tumor for which the diagnosis portends a grave prognosis.

Atypical vascular lesions originally described by Fineberg and Rosen in 1994 are also a well documented rare complication in the setting of breast conserving surgery for breast carcinoma (15). They develop as one or more, small colorless to slightly erythematous papules usually within a period of 3 to 6 years after radiotherapy, and their behaviour was considered initially benign being treated safely by local excision. Some recent series showed histologic heterogeneity among AVLs separating them into those composed of lymphatic vessels (LT AVL) or capillary blood vessels (VT AVL) (16). The larger group, LT AVL, conforms to the classic description of AVL, being circumscribed proliferations arranged as ectatic, tightly clustered back-to-back vessels within the superficial dermis consisting of thin-walled, variably anastomosing lymphatic vessels lined by attenuated or slightly protuberant (hobnail) endothelial cells, with no significant nuclear enlargement, angulated borders, or prominent nucleoli. The second but far less common lesion, VT AVL, resembles a capillary hemangioma, except the vessels are not arranged in lobules. Rather, the round to linear, capillary-sized vessels grow irregularly within the superficial or deep dermis. Unlike angiosarcomas, the vessels do not intercommunicate nor do they display multilayering of the endothelium. It was originally thought that AVLs did not
represent a risk factor for the development of angiosarcoma, being able neither to recur nor to develop metastasis (15). However some studies have reported occasional patients with AVLs who subsequently developed angiosarcoma, raising the possibility that in some cases AVLs may represent a precursor to angiosarcoma (16, 17). VT AVLs have a higher risk than the superficially located LT AVLs (13). In addition AVLs can have areas indistinguishable from angiosarcoma histologically and therefore the histological interpretation of some cases due to inadequate biopsy or sampling bias can be difficult.

In current clinical practice, there are no ancillary tests that can aid in the distinction of atypical vascular lesion from angiosarcoma. Recent literature has shown that at the molecular level postradiation cutaneous angiosarcomas are characterised by expression and amplification of MYC, whereas reactive and AVLs after radiotherapy do not show alterations in MYC (18, 19). Whether this alteration can be used reliably to distinguish these two lesions remains to be determined. Treatment for atypical vascular lesions is by complete excision with careful clinical follow-up.

References:

Wide local excisions of the breast and the pathologist – are we missing something?

Gavin Harris, Anatomical Pathology, Canterbury Health Laboratories, Christchurch, New Zealand

The margin status of breast wide local excision specimens is one of the most important predictors of ipsilateral recurrence following breast conserving surgery\(^1,2,3,4\), which in turn is associated with an increased risk of distant metastasis and survival\(^5,6\).

Whilst there are a number of international guidelines advising pathologists on how best to sample wide local excision specimens, the evidence base is limited.

The UK, NHS breast screening program pathology guidelines\(^7\) suggest 3 possible methods for approaching therapeutic wide local excisions. Methods 1 and 2 involve serial slicing of the specimen in a medial to lateral or superior inferior plane respectively. Method 3, “Radial block examination with or without shave margins” is illustrated below. The guidelines state “The circumferential edge of the sample can be shaved to allow more extensive examination of relevant surgical resection margins”.

*Figure 1* Radial block examination with shave margins
However, the Royal College of Pathologists of Australasia in the 2010 Breast Cancer Structured Reporting Protocol, actively recommend against the use of shave margins, stating:

“G2.05 For wide local excision specimens, shaved margins immediately adjacent to the tumour should not be examined. CG2.05a The clinical value of findings for shaved margins is limited by a low level of concordance with inked margins”.

To clarify the utility of radial margin shaves performed by the pathologist we undertook an audit of 300 consecutive wide local excision specimens in our laboratory reported by a single pathologist (GH). Ninety one specimens were blocked in total leaving 209 where radial margin shavings were used. Of these 209 cases, a positive perpendicular margin was defined as positive if invasive carcinoma, DCIS or pleomorphic LCIS were present less than 2mm from the inked surgical margin. The shave margins were embedded with the inked surface towards the base of the cassette. The section was taken at a 1mm depth from the inked margin without any additional trimming. If invasive carcinoma, DCIS or pleomorphic LCIS were identified within the shave margin, the report stated that the abnormality was “1mm or less” from that margin.

Seventy-seven of the 209 cases had a positive perpendicular radial and/or shave margin. Of these, 22 (10.5%) cases had positive perpendicular radial margins only, 28 cases (13.3%) had positive shave margin(s) only and 27 (12.9%) had both positive perpendicular radial and shave margins. Seventeen of the latter group had a positive shave margin which was different to the positive radial margin. In our unit therefore, in the setting of a delayed re-excision, the shave margin qualified the patient either for a re-excision or more extensive re-excision in 43 of the 209 cases (20.6%).

The RCPA breast cancer structured reporting protocol guideline is based on the paper by Guidi et al who studied 22 breast re-excision specimens, in which the margins were shaved and tumour was present in at least one of the shaved margins. The shaved margin was embedded so that the section was taken from opposite the inked surgical margin (ie opposite to the approach in our audit). Once this section had been taken, the tissue was then sectioned perpendicular to the inked margin and a further section taken. This allowed comparison of the presence or absence of malignant disease in the shaved margin section, which represented tissue away from the inked margin equal to the thickness of the tissue removed, and the inked surgical margin section. For 69 positive shave margins, the corresponding inked surgical margin was positive in only 42 (61%). The authors concluded that the clinical implications of a positive shave margin may not be the same as a positive inked margin.
We would argue that the shave margin in this study represented tissue which was approximately 2-3mm (the thickness of the shaved margin) from the inked surgical margin. A positive inked margin required DCIS or invasive carcinoma to extend to the surgical margin. Certainly in our unit, where a 2mm margin is considered adequate, positive shave margins sectioned in this way would not lead to a re-excision. It would seem likely that if the shave margins had been embedded as in our unit (i.e. with the inked surface down in the cassette), the correlation with the perpendicular inked surgical margins would have been higher. Given the variation in what is considered an adequate margin for invasive and in situ disease in the setting of breast conserving surgery, with cut-offs often less then 3mm, it would appear that this particular study may in fact not be relevant to the debate on the utility of shave margins.

In a review of 471 wide local excision specimens for invasive carcinoma at the Nottingham Breast Pathology Unit, UK, 10% of cases had positive shave margins only, 16% had positive radial margins only (defined as less than 5mm) and 12% had both positive shave and radial margins. Shave margins were either the only involved margin or differed from the involved radial margin in 14% of patients (compared to 20.6% in our audit). If any shave margin was positive, residual disease was identified in 44% of re-excision specimens. They noted that the local recurrence rate had dropped from 1% to 0.4% per annum following the introduction of shave margin assessment.

Interestingly Pass et al reviewed 607 patients who had undergone breast conserving-therapy with axillary staging and postoperative radiotherapy over a 15 year period at a single institution. In addition to changes in surgical and adjuvant therapy (local and systemic), there was a doubling in the mean number of slides examined (10.6 slides versus 21.1 slides) per case. They credited the “emphasis on optimizing complete pathologic documentation of complete tumour excision”, at least in part for the improvement in 5-year (8% versus 1% respectively) and 12-year (21% versus 9% respectively) actuarial ipsilateral disease recurrence rates with breast conserving surgery.

In summary, negative margins are one of the most important factors for achieving local control in breast conserving surgery. The use of shave margins increases the probability of identifying positive or close margins, allowing re-excision to achieve a negative margin, and therefore reducing the patient’s risk of subsequent recurrence.
References


NHSBSP Publications


The molecular pathology of breast cancer

Sandra O’Toole
Head, Molecular Diagnostic Oncology
Dept of Tissue Pathology and Diagnostic Oncology
Royal Prince Alfred Hospital
Breast Cancer – the current challenges

- Dramatic reduction in mortality in last 2 decades
- 5 year survival now approaching 90%
- Further improvements require “personalised” therapies
- Pathologists play a key role

Screening
Tamoxifen
Trastuzumab
Molecular Classification of Breast Cancer

- Luminal A and B (ER+)
- HER2 (c-erbB2)
- Basal

Sørlie T. et al. PNAS 2003; 100:8418-8423
Strategies for subtypes—dealing with the diversity of breast cancer: highlights of the St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2011

<table>
<thead>
<tr>
<th>'Subtype'</th>
<th>Type of therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>'Luminal A'</td>
<td>Endocrine therapy alone</td>
</tr>
<tr>
<td>'Luminal B (HER2 negative)'</td>
<td>Endocrine ± cytotoxic therapy</td>
</tr>
<tr>
<td>'Luminal B (HER2 positive)'</td>
<td>Cytotoxics + anti-HER2 + endocrine therapy</td>
</tr>
<tr>
<td>'HER2 positive (non luminal)'</td>
<td>Cytotoxics + anti-HER2</td>
</tr>
<tr>
<td>'Triple negative (ductal)'</td>
<td>Cytotoxics</td>
</tr>
</tbody>
</table>
On The Treatment of Inoperable Cases of Carcinoma of the Mamma: Suggestions for a New Method of Treatment, with Illustrative Cases

Lancet 1896

George Thomas Beatson, M.D.

Is cancer of the mamma due to some ovarian irritation, as from some defective steps in the cycle of ovarian changes; and if so, would the cell proliferation be brought to a standstill, or would the cells go on to the fatty degeneration seen in lactation were the ovaries to be removed?
“Targeted” therapy of Breast Cancer

that eight months after my operation all vestiges of her previous cancerous disease had disappeared, and that I am able to show her with a sound cicatrix and healthy thoracic tissues, and that she is apparently in excellent health.
Breast cancer is a heterogeneous disease
Prediction and prognostication in Breast cancer

- Breast cancer divided into ER+ and ER- and therapeutic decisions made on this basis for at least past 3 decades

- NSABP trials B14 and B20: LN- HR+ Rx endocrine therapy alone low recurrence rate of 15% over 5 years

- Chemotherapy overall has resulted in >20% reduction in death but the benefit for an individual patient is small (1-3%)

- 85% of ER positive patients do not require adjuvant chemotherapy – how do we identify them?
Molecular Pathology of Breast Cancer

- Gene expression subtypes
- Multigene assays
- Clinicopathologic features including ER, PR, HER2
- Immunohistochemical biomarkers eg Ki67
Gene expression profiling – defining subtypes

• Measurement of the relative expression/activity of thousands of genes at once
• “DNA microarrays” arrayed series of 1000s microscopic spots of DNA oligonucleotides (primer)
• Reference sample and tumour sample cDNA mixed & hybridized to the microarray
• Relative red or green shows changes in expression
• sophisticated bioinformatics applied to define groups of prognostic significance
DNA microarray studies

• Depend on retrospective analysis of data sets
• capacity to prospectively allocate a particular case to subtype?
• are these signatures stable?
Are microarray signatures useful in practice?

Figure 1: Molecular subtype classification of NKI-295 breast cancers and overall survival of patients assigned to molecular subtypes according to three single sample predictors. ER = oestrogen receptor. SSP = single sample predictor.

Weigelt et al Lancet Oncol 2010
Are microarray signatures useful in practice?

- very poor ability to consistently allocate the same sample using different SSPs except for basal-like carcinoma
- especially poor at differentiating Luminal A from B
- poor allocation of HER2 subtype, poor concordance with HER2 ISH positive
- “normal breast-like” stromal contamination

* Weigelt et al Lancet Oncol 2010*
SECTION INTRODUCTION

Molecular profiling currently offers no more than tumour morphology and basic immunohistochemistry

Britta Weigelt¹ and Jorge S Reis-Filho²*
Multigene assays
Oncotype DX® 21-Gene Recurrence Score (RS) Assay

16 Cancer and 5 Reference Genes From 3 Studies

**PROLIFERATION**
- Ki-67
- STK15
- Survivin
- Cyclin B1
- MYBL2

**ESTROGEN**
- ER
- PR
- Bcl2
- SCUBE2

**GSTM1**

**BAG1**

**INVASION**
- Stromelysin 3
- Cathepsin L2

**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

<table>
<thead>
<tr>
<th>Category</th>
<th>RS (0 - 100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low risk</td>
<td>RS &lt;18</td>
</tr>
<tr>
<td>Int risk</td>
<td>RS 18 - 30</td>
</tr>
<tr>
<td>High risk</td>
<td>RS ≥ 31</td>
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</tbody>
</table>

Tam vs placebo n= 2644, studies on 668 HR+, LN- patients
**Oncotype DX® Clinical Validation: B-14 Results – Distant Recurrence**

<table>
<thead>
<tr>
<th>Risk Group</th>
<th>% of Patients</th>
<th>10-yr Rate of Recurrence</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low (RS &lt;18)</td>
<td>51%</td>
<td>6.8%</td>
<td>4.0%, 9.6%</td>
</tr>
<tr>
<td>Intermediate (RS 18-30)</td>
<td>22%</td>
<td>14.3%</td>
<td>8.3%, 20.3%</td>
</tr>
<tr>
<td>High (RS ≥31)</td>
<td>27%</td>
<td>30.5%</td>
<td>23.6%, 37.4%</td>
</tr>
</tbody>
</table>

Test for the 10-year Distant Recurrence comparison between the low-and high-risk groups: $P < 0.001$

B-20 Results: Tam vs Tam + Chemo

Low RS  
Low Risk Patients (RS<18)  
N  |  Events  
TAM + Chemo  | 218  | 8  
TAM  | 135  | 4  

p = 0.61

Int RS  
Int Risk Patients (RS 18-30)  
N  |  Events  
TAM + Chemo  | 89  | 9  
TAM  | 45  | 4  

p = 0.39

High RS  
High Risk Patients (RS≥31)  
N  |  Events  
TAM + Chemo  | 117  | 13  
TAM  | 47  | 18  

p < 0.001

28% absolute benefit from tam + chemo

Standardized Quantitative Oncotype DX Assay

Recurrence Score in N-, ER+ patients

-**Lower RS’s**
  - Lower likelihood of recurrence
  - Greater magnitude of TAM benefit
  - Minimal, if any, chemotherapy benefit

-**Higher RS’s**
  - Greater likelihood of recurrence
  - Lower magnitude of TAM benefit
  - Clear chemotherapy benefit

3) Paik et al JCO 2006, 4) Gianni et al JCO 2005
**MO Treatment Recommendations Changed 31.5% of the Time**

<table>
<thead>
<tr>
<th>MO Pre to Post-RS Assay Treatment Recommendation</th>
<th>Number of Cases(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHT to HT</td>
<td>20 (22.5)</td>
</tr>
<tr>
<td>HT to CHT</td>
<td>3 (3.4)</td>
</tr>
<tr>
<td>CHT or HT to Equipoise</td>
<td>5 (5.6)</td>
</tr>
<tr>
<td>Treatment plan did not change</td>
<td>61 (68.5)</td>
</tr>
<tr>
<td>Total</td>
<td>89 (100)</td>
</tr>
</tbody>
</table>

- Treatment recommendation changed for 28 (31.5%) cases after results of RS Assay known.
- The most common change was from a recommendation of CHT to HT in 22.5% of cases

ASCO 2007, Abstract #577
843 patients from 3 US labs
99% concordance for HER2 negative cases
36 HER2 amplified by ISH but only of these 45% were positive by Oncotype DX
Unacceptably high false negative rate
Oncotype Dx in Australia

- Oncotype Dx is only multigene assay recommended by ASCO and NCCN in USA.
- Paraffin block or 15 x 5\(\mu\)m sections sent to Genomic Health in USA (via Healthscope).
- RNA extracted, PCR based assay.
- No rebate, approx cost $4000 with 2 week TAT.
- Is it worth it?
• OncotypeDX™ still returns 40-66% of cases as intermediate risk with no clear data to suggest a benefit of chemotherapy
• may not outperform routinely performed relatively cheap standard assays
Value of Oncotype Dx?

• Cuzick et al – study on transATAC trial material n=1125, validated in 2\textsuperscript{nd} cohort of n=786

• Oncotype Dx RS vs algorithm for ER, PR, Her2 and Ki67 IHC

\[
\text{IHC4} = 94.7 \times \{-0.100 \text{ ER}_1 - 0.079 \text{ PgR}_1 + 0.586 \text{ HER2} + 0.240 \ln (1 + 10 \times \text{Ki67})\}.
\]

similar amounts of prognostic information are contained in 4 widely performed IHC assays as in the GHI-RS.

J Clin Oncol. 2011 Nov 10;29(32)
Trial Assigning Individualized Options for Treatment (Rx) (TAILORx)

Schema: TAILORx

Node-Neg, ER-Pos Breast Cancer

Register Specimen banking

Oncotype DX® Assay

RS ≤10 Hormone Therapy Registry

RS 11-25
Randomize Hormone Rx vs Chemotherapy + Hormone Rx

RS >25 Chemotherapy + Hormone Rx

Primary study group
Routine pathology and IHC as “molecular pathology”?
RCT of 498 patients treated with BCT and XRT +/- Boost
Could surrogate subtypes predict outcome?

<table>
<thead>
<tr>
<th>Subtype</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luminal A</td>
<td>ER+ and/or PR+ and HER2 ISH -</td>
</tr>
<tr>
<td>Luminal B</td>
<td>ER+ and/or PR+ and HER2 ISH +</td>
</tr>
<tr>
<td>HER2</td>
<td>ER-, PR- and HER2 ISH+</td>
</tr>
<tr>
<td>Basal</td>
<td>ER-, PR-, HER2 ISH-, CK5/6 + and/or EGFR+</td>
</tr>
<tr>
<td>Unclassified (5 marker negative)</td>
<td>Negative for all 5 markers</td>
</tr>
</tbody>
</table>

* surrogate signatures adapted from Cheang et al JNCI 2009 using ISH for HER2
Surrogate subtypes define prognostic groups

![Graphs showing cumulative survival over time for different subtypes.]

**Table 3. Median Time to Event, in Months, According to Molecular Subtype**

<table>
<thead>
<tr>
<th>Event</th>
<th>Whole Cohort</th>
<th>Luminal A</th>
<th>Luminal B</th>
<th>Basal</th>
<th>HER2</th>
<th>Unclassified</th>
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</thead>
<tbody>
<tr>
<td>IBTR</td>
<td>60</td>
<td>80.5</td>
<td>78</td>
<td>20</td>
<td>23</td>
<td>30</td>
</tr>
<tr>
<td>LRR</td>
<td>49</td>
<td>72</td>
<td>78</td>
<td>26</td>
<td>25</td>
<td>28.5</td>
</tr>
<tr>
<td>DDFS</td>
<td>33</td>
<td>44</td>
<td>59</td>
<td>23</td>
<td>25</td>
<td>27</td>
</tr>
<tr>
<td>Breast cancer death</td>
<td>61</td>
<td>66</td>
<td>94.5</td>
<td>23</td>
<td>33</td>
<td>18</td>
</tr>
</tbody>
</table>

Abbreviations: IBTR, ipsilateral breast tumor recurrence; LRR, locoregional recurrence; DDFS, distant disease-free survival.
IHC/ISH surrogate subtype signatures

• surrogate subtype signatures able to predict outcome
• **BUT** inferior prediction of outcome compared to standard pathology variables:
  – grade, size, LN status, LVI and hormone receptors

Routine pathology and IHC still the gold standard
IHC/ISH surrogate subtype signatures

- IBCSG Trials VIII & IX (n=1220)
- highly significant differences in survival between IHC surrogate subtypes
- BUT subtype was not significant in multivariate analysis
- p53 or cyclin D1 could not improve definition of luminal tumours
- IHC/ISH subtyping complements traditional pathology

Millar, Coates, O’Toole et al ASCO oral presentation 2012
Molecular Breast Pathology in practice

- Good morphology
- ER, PR, HER2, (Ki67)

*Easy, right?*
Routine IHC

- ER positive rates ranging from 26 to 100% of breast cancers in a multi-laboratory audit

Frequency and reliability of oestrogen receptor, progesterone receptor and HER2 in breast carcinoma determined by immunohistochemistry in Australasia: results of the RCPA Quality Assurance Program

Glenn D Francis, Margaret Dimech, Leanne Giles, Alison Hopkins

Hormone testing

Judicial inquiry probes faulty breast cancer tests

Last Updated March 18, 2008
CBC News

October 2005. Health officials in Newfoundland and Labrador reveal there had been serious errors in breast cancer tests conducted on women in St. John's — and that they were suspending them for breast cancer patients. Tests on more than 1,000 women over the previous eight years were suspect, and had been sent to a hospital in Toronto for retesting.

Many cancer patients don't realize a pathologist, often working in the background, has such a huge influence on their treatment.
ER & PR testing recommendations

- >1% expression any intensity is +
- (report % and intensity)
- review controls internal and external
- does the result make sense? eg a G1 tumour being ER-
- 70-80% of Br Ca are ER pos, 60-70% are PR pos
- tissue fixation critical, participate in QAP, laboratory audit of results, reliable antibody
Identifying a poor prognosis ER+ group

- Routine pathology and IHC?
- Key issue to identify which patients require more than endocrine Rx alone
- Which group more likely to show primary or secondary resistance to endocrine Rx?
- “Luminal B” population – how to define?
## Defining “Luminal B” using standard IHC

<table>
<thead>
<tr>
<th>Millar et al Boost XRT trial</th>
<th>Tamini et Nurse Health Study</th>
<th>Wang et al</th>
<th>Rakha et al</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER+ and/or PR+ or HER2 FISH+ or Ki67&gt;10% or p53&gt;10%</td>
<td>ER+ and/or PR+ and HER2+ or Grade 3+</td>
<td>ER+ and Ki67&gt;14%</td>
<td>ER+ &amp; PR-</td>
</tr>
<tr>
<td>Intrinsic Subtype</td>
<td>Clinico-pathologic definition</td>
<td>Notes</td>
<td></td>
</tr>
<tr>
<td>------------------</td>
<td>-------------------------------</td>
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<td></td>
</tr>
<tr>
<td>Luminal A</td>
<td>‘Luminal A’</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ER and/or PgR positive(76)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>HER2 negative (77)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ki-67 low (&lt;14%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Luminal B&quot;</td>
<td>‘Luminal B (HER2 negative)’</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ER and/or PgR positive</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>HER2 negative</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Ki-67 high</td>
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<tr>
<td></td>
<td>‘Luminal B (HER2 positive)’</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>ER and/or PgR positive</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Any Ki-67</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>HER2 over-expressed or amplified</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

This cut-point for Ki-67 labelling index was established by comparison with PAM50 intrinsic subtyping (7). Local quality control of Ki-67 staining is important.

Genes indicative of higher proliferation are markers of poor prognosis in multiple genetic assays (78). If reliable Ki-67 measurement is not available, some alternative assessment of tumor proliferation such as grade may be used to distinguish between ‘Luminal A’ and ‘Luminal B (HER2 negative)’.

Both endocrine and anti-HER2 therapy may be indicated.
“Luminal B” by IHC

• All these definitions have been shown to be prognostic in sizeable cohorts
• which to choose?
• proliferation is a continuum in ER+ patients
• no obvious cut point, reflected in difficulties separating these populations
• Pathologist should be able to convey to oncologist that this patient has higher risk of poor outcome
Ki67

- Expressed in all cells not in G0 (resting)
- measure of tumour cell proliferation
- shown to be a prognostic factor in breast cancer in numerous studies
- variable cut points – 10%, 13.25%, 14%, 19% 20%?
- variable results in trial and retrospective cohorts

Viale et al JNCI 2008, Cheang et al JNCI 2009
Ki67 – predictive adjuvant setting

• predictive for benefit of adding docetaxel to FU and epirubicin (Hugh JCO 2009)
• IBCSG Trials VIII&IX no benefit for adding chemo seen even if Ki67 high (Viale et al JNCI 2008)
Ki67 – predictive neoadjuvant setting

- endpoint of neoadjuvant endocrine therapy (IMPACT study) – helps identify a group of patients with such a low risk of recurrence on endocrine therapy alone that they can be spared chemoRx (Ellis et al JNCI 2008)
- P024 study found Ki67 independently associated with RFS and OS (Ellis et al Can Res 2003)
- Ki67 may be used to triage ER+ patients away from chemo in this setting
Ki67 – working group recommendations

pre-analytical factors

• Ki67 is fairly robust, not as sensitive to fixation as ER
• 10% NBF
• deterioration with cut sections though (up to 2 weeks OK)

Dowsett et al JNCI Nov 2011
Ki67 – working group recommendations

analytical factors

• MIB1 clone is the most widely used (SP6 promising and may be more sensitive but not as much data)

• HIER required (avoid low pH and protease

• counterstain important to ensure all negative nuclei are recognised and counted
Ki67 – working group recommendations

Interpretation of Ki67

• any nuclear staining also mitotic figures should be counted (can get cytoplasmic staining esp if sq metaplasia, ignore)

• If homogeneous – count at least 3 x 40x randomly selected fields
Ki67 – working group recommendations

**Interpretation of Ki67**

- count all positive cells within region
- scoring requires determination of cells positive
- no interpretation of intensity
Ki67 – working group recommendations

Interpretation of Ki67 – Heterogeneous staining

1) gradient at leading edge – count 3 x 40x fields at periphery

2) Hotspots: approach varies, working grp suggest assessing the whole section and record an average score

“This issue needs clarification...and a working party ... has been established to assess which method is more robust”
Ki67 – working group recommendations

Interpretation of Ki67 – How many cells?
recommend at least 1000 cells

Cut Point?
Controversial because no current consensus
select cut point based on clinical setting
“Endpoint must be validated in separate
independent study of similar design with
same endpoints” !!!!
Ki67

- not recommended as a biomarker by ASCO guidelines on use of tumor markers\(^1\) but now recommended by St Gallen panel\(^2\)
- in practice requested by oncologists at many hospitals in Australia
- Our approach is to report score as a range and not comment on high or low, lack of consensus even in our own Dept

\(^1\) Harris et al JCO 2007, \(^2\) Goldhirsch et al Ann Oncol 2011
HER2 testing

• growth factor, amplified in around 15% of breast cancers *
• trastuzumab results in 50% reduction in mortality in early BCa and 44% in metastatic compared to non-HER2 Ca
• extremely important to correctly identify these patients

*Farshid et al, USCAP 2010
HER2 testing Australia

- ISH confirmation required for PBS funding
- BRISH done in several reference labs, FISH reimbursed only at SVH National Ref Lab
- Up to 20% of HER2 assays performed in an audit of US labs were incorrect when re-evaluated by a central testing lab
**HER2 testing algorithm**

<table>
<thead>
<tr>
<th>Result</th>
<th>Single probe (eg CISH or SISH)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>Mean HER2 copy number ≤4 signals per tumour cell nucleus</td>
</tr>
<tr>
<td>Positive</td>
<td>Mean HER2 copy number &gt;6 signals per tumour cell nucleus</td>
</tr>
<tr>
<td>Equivocal</td>
<td>Mean HER2 copy number 4-6 signals per tumour cell nucleus</td>
</tr>
</tbody>
</table>
CISH single probe

Non-amplified diploid

Amplified
Mean copy number 4.3 - Equivocal
<table>
<thead>
<tr>
<th>Result</th>
<th>Single probe (eg CISH or SISH)</th>
<th>Dual Probe (eg FISH or C/SISH with CEP17 probe)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>Mean HER2 copy number &lt;4 signals per tumour cell nucleus</td>
<td>HER2/ CEP17 ratio &lt;1.8</td>
</tr>
<tr>
<td>Positive</td>
<td>Mean HER2 copy number &gt;6 signals per tumour cell nucleus</td>
<td>HER2/ CEP17 ratio &gt;2.2</td>
</tr>
<tr>
<td>Equivocal</td>
<td>Mean HER2 copy number 4-6 signals per tumour cell nucleus</td>
<td>HER2/CEP17 ratio 1.8-2.2</td>
</tr>
</tbody>
</table>
HER2 FISH testing

Fluorescent in situ hybridisation (FISH)
Equivocal category

- problematic, 2% of cases
- count additional nuclei
- second scorer
- repeat assay on another block
- be guided by IHC
- 20 equiv cases FISH 5 large reference laboratories an overall discordance 20%
- further studies needed – response to Rx?

Dowsett et al Mod Pathol. 2007; 20: 584-91.
Issues in HER2 testing – Heterogeneity

• ASCO/CAP criteria for IHC >30% 3+ strong membranous expression
• what if smaller proportion?

O’Toole et al Pathology 2011
HER2 testing – genetic heterogeneity distinct “clones”

- A/ Prof Morey reports that this occurs in around 0.4% (33 of 9035) diagnostic HER2 ISH cases.
- IHC extremely useful to recognise his phenomenon
- report % of tumour showing amp

Heterogeneity in HER2 testing – distinct clones
Heterogeneity in HER2 testing – intermingled cells with ratio < and > 2.2
“Genetic heterogeneity in HER2”

• CAP guidelines say report if between 5-50% of cells show heterogeneity ie individual cells with ratio >2.2 admixed with non-amplified cells
• give overall report ie HER2 ISH negative but state genetic heterogeneity is present
• reported in >20% of cases.
• confusing!
• Such GH is frequently seen in cases that have equivocal ratios
Genetic Heterogeneity in HER2

- Allison et al 1329 consec FISH tested cases found 23% had “GH” if HER2/Ch17 ratio was used
- if just HER2 copy number used then only 6.5% were heterogeneous
- ratio based definition results in large numbers of non-amplified cases being classified as “heterogeneous”
- Just use ratio overall to classify a case

Allison et al Am J Clin Path 2011
Causes of Genetic heterogeneity

• gains and losses in Ch17 (rather than true Ch 17 polysomy) are common and can artificially skew the HER2/Ch17 ratio
• while 2 probes very useful can create problems that you would never know about if single probe only was used!!

• Pic of dual probe SISH
Conclusions

• New technologies may add predictive and prognostic information in some settings

• Pathologists need to stay informed about new developments but morphology and good IHC is still gold standard

• must be vigilant in maintaining highest standards of testing
Thanks

• Ewan Millar
• Rob Sutherland
• Alan Coates
• Catriona McNeil
• Tina Selinger
• Adrienne Morey
• RPA colleagues
Biopsy Outcomes of Screen Detected Microcalcifications

Audit of 2545 Cases

Gelareh Farshid, Thomas Sullivan, Peter Downey, Grantley Gill & Steve Pieterse
BreastScreen South Australia
2012

Incidence & Dx of DCIS

- 80-85% DCIS detected by mammography (96% due to bx of microcalcifications)
- BSSA: 20% malignancies
- 1,600 cases of DCIS annually in Australia
- US: 1 in 1300 MMG
- Mostly asymptomatic
- Rarely mass or nipple changes - symptomatic proportion stable over time

DCIS

- Cytologically malignant epithelial cells confined to breast duct-lobular system
- No metastatic potential
- Same risk factors as invasive ca: age, Fhx, reproductive hx, HRT, post menopausal BMI, density
- Non-obligate precursor of invasive cancer
### Invasive Cancer After DCIS Dx
**Bx Alone**

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Number</th>
<th>Cancer</th>
<th>% Inv Ca</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lewis</td>
<td>1938</td>
<td>8</td>
<td>6</td>
<td>75</td>
</tr>
<tr>
<td>Farrow</td>
<td>1970</td>
<td>25</td>
<td>5</td>
<td>20</td>
</tr>
<tr>
<td>Haagensen</td>
<td>1971</td>
<td>11</td>
<td>8</td>
<td>73</td>
</tr>
<tr>
<td>Millis</td>
<td>1975</td>
<td>8</td>
<td>2</td>
<td>25</td>
</tr>
<tr>
<td>Rosen</td>
<td>1980</td>
<td>15</td>
<td>8</td>
<td>53</td>
</tr>
<tr>
<td>Eusebi</td>
<td>1994</td>
<td>80</td>
<td>11</td>
<td>14</td>
</tr>
<tr>
<td>Page</td>
<td>1995</td>
<td>28</td>
<td>9</td>
<td>32</td>
</tr>
<tr>
<td>Collins</td>
<td>2005</td>
<td>13</td>
<td>6</td>
<td>46</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>188</td>
<td>55</td>
<td>29.2%</td>
</tr>
</tbody>
</table>

### Recurrence after Tx with BCS

<table>
<thead>
<tr>
<th>Study</th>
<th>N</th>
<th>F/U</th>
<th>Rec</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSABP B-17</td>
<td>813</td>
<td>&gt;12 y</td>
<td>32.9%</td>
</tr>
<tr>
<td>EORTC 10853</td>
<td>1010</td>
<td>10.5</td>
<td>26%</td>
</tr>
<tr>
<td>Swedish Trial</td>
<td>1046</td>
<td>8</td>
<td>32%</td>
</tr>
<tr>
<td>UK/ANZ Trial</td>
<td>1030</td>
<td>4.4</td>
<td>13.6%</td>
</tr>
</tbody>
</table>

Half local recurrences are as invasive cancers. Now, has metastatic potential.
The major goal of management of patients with DCIS is to prevent invasive cancer!

What is Over-Diagnosis?
- Dx of disease that if left undetected and untreated would not become symptomatic
- Estimates based on statistical inference
  - range from (0-30%) of screen detected cancers
- DCIS accounts for much of the allegedly over-diagnosed malignancies
- The detection & Tx of DCIS is one of the criticisms of screening for breast cancer

Balance & Trade offs

Development of invasive breast cancer

Tx for DCIS
- Mastectomy
- BC Surgery
+/- XRT
+/- Tamoxifen
+/- SNBx

After surgery for DCIS, 10 year mortality from invasive breast cancer is <2%.

Quality of life Surveillance
Study Design

- In which microcalcifications where the only imaging abnormality
- Limited to cases with only 1 lesion
- Tabulate patient demographics, imaging features, bx findings & final histology
- Statistical analysis to determine independent predictors of malignancy.

Results

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time frame</td>
<td>Jan 1992-Dec 2007</td>
</tr>
<tr>
<td>Number of women</td>
<td>2545</td>
</tr>
<tr>
<td>Strong FHx</td>
<td>187</td>
</tr>
<tr>
<td>Side</td>
<td>L: 1118</td>
</tr>
<tr>
<td></td>
<td>R: 990</td>
</tr>
<tr>
<td>Screening Round</td>
<td>R1: 894</td>
</tr>
<tr>
<td></td>
<td>R2-15: 1651</td>
</tr>
</tbody>
</table>

Results

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imaging grade</td>
<td>3: 1215</td>
</tr>
<tr>
<td></td>
<td>4: 719</td>
</tr>
<tr>
<td></td>
<td>5: 611</td>
</tr>
<tr>
<td>Palpability</td>
<td>137</td>
</tr>
<tr>
<td>Distribution of calcs</td>
<td>Multiple clusters: 224</td>
</tr>
<tr>
<td></td>
<td>Single cluster: 1357</td>
</tr>
<tr>
<td></td>
<td>Scattered: 505</td>
</tr>
<tr>
<td>MMG extent</td>
<td>Range: 2-430mm</td>
</tr>
<tr>
<td>≤15mm</td>
<td>1229</td>
</tr>
<tr>
<td>&gt;15mm</td>
<td>859</td>
</tr>
</tbody>
</table>
Overview of Malignancies

- 47.9% malignant, 4.8% premalignant
- When malignant:
  - 2/3 (66.3%) DCIS, 1/3 (33.7%) inv ca

Invasive Cancers Presenting as Microcalcifications

- 16.1% of all calcs biopsied showed invasive cancer
- 1/3 of all malignancies
- Only 21.2% cancers were low grade
- Invasive cancer: 92.7% T1
- but few (21.2%) low grade
- and nodal metastases in 15.6% cases

<table>
<thead>
<tr>
<th>Inv ca</th>
<th># (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 1</td>
<td>87 (21.2%)</td>
</tr>
<tr>
<td>Grade 2</td>
<td>180 (43.8%)</td>
</tr>
<tr>
<td>Grade 3</td>
<td>77 (18.7%)</td>
</tr>
</tbody>
</table>

Independent Predictors

- Radiologic grade was the most important independent predictor of malignancy
- Detection after the first episode of screening, mammographic extent and association with a clinically palpable mass also significant.
Conclusions

- MMG screening and appropriate biopsy of microcalcifications are effective in diagnosing breast malignancy: overall 47.9% malignant
- Study validates RANZCR grading scheme
- Assessment of microcalcps preferentially detects biologically significant disease:
  - high grade DCIS (only 1/3 DCIS was low grade)
  - higher grade invasive cancers (1/3 of all malignancies)
  - 78.2% IG or HG & 15.6% nodal mets

Stability in Comedo DCIS Dx

![Graph showing stability in Comedo DCIS Dx](figure)


Challenges & Focus

- Much to learn about DCIS
- Stratify Tx by risk profile
  - Who needs mastectomy?
  - Who can avoid XRT?
  - Place of watchful waiting?
- Communicating risk to women, informed consent
- Methods of surveillance
41% Survival Advantage

Women participating in BSSA were significantly less likely to die from breast cancer. OR 0.59

Acknowledgements

- Past and present staff including radiologists, pathologists and surgeons
- BSSA General Manager
  Ms Lou Williamson
- Ms Jill Rogers and Ms Ada Childs for raw data extraction
- Clients of BSSA

Thank You