Neoadjuvant therapy-specimen handling and reporting

25 mins
Pathology Issues

- Predictors of likelihood of response in order to select patients – e.g. on core biopsy
- Markers during response in research studies/sequential core biopsies
- Specimen handling of excision specimens
- Reporting of degree of response
Markers of prediction of response

• Pre-treatment biopsies from 517 patients with locally advanced cancer treated with preoperative epirubicine/cyclophosphamide [EC]
• pCR 11.9% (38/319)
• Patients with high grade, lower ER, PgR, Bcl-2 or higher proliferation had greater benefit
• Younger patients, with negative PgR & high proliferation index had highest benefit (56% pCR)

Assessment of pathological response – What’s the problem?
Assessment of pathological response – why?

- Complete clinical response to neoadjuvant therapy does not imply pathological complete regression
- 30-50% of patients with complete clinical regression have residual tumour pathologically

- Neoadjuvant chemotherapy in 932 patients
- Median F-U 5 years (range 0.4 - 9.4)
- 5-year disease-specific survival 96% for patients who experienced pCR (n = 130 (14%)) c.f. 87% for patients who did not (n = 802)


- i.e. pCR provides prognostic information
Scattered/Scanty Residual Carcinoma
Variation in pCR?

“rates of pathological complete response (pCR) to neoadjuvant systemic therapy vary according to the regimen used, ranging from 6% to 15% with anthracycline-based regimens to almost 30% with the addition of a noncross-resistant agent such as a taxane”

Detailed clinical information on request form -> site of tumour(s), especially if good response
Post-therapy Specimen Handling

• Marker such as a wire coil at time of initial biopsy or during therapy
• As with all specimens, sent immediately to pathology laboratory, ideally fresh -> fixation and fresh tissue for biological & genetic assays
Microscopic changes

- Fibrosis
- Mucinous or myxomatous change
- Lymphocytic & histiocytic infiltrate
- Haemosiderin
- Reaction to coil
- Cancer cells may mimic histiocytes and vice versa
- LCIS may mimic DCIS
- Adjacent breast epithelial changes may mimic DCIS
• 36 year old, 41mm mobile hypoechoic mobile mass
• B5, invasive, provisional grade 3 NST
• ER = 6/8; PGR = 0/8; HER2 = 0
Changes post-chemotherapy

Changes in lobules & normal epithelium

• Periductal & perilobular fibrosis
• Atrophy of lobular acini
• “Atypia” in epithelium
What to record if not pCR?
TNM stage after neoadjuvant chemotherapy

- 132 patients with non-metastatic breast cancer after neoadjuvant chemotherapy
- Median follow-up 5 yrs, pathological stage after neoadjuvant chemotherapy strongly associated with both DDFS & OS. DDFS:
- stage 0: 95%
- stage I: 84%
- stage II: 72%
- stage III: 47%
- “Classification .....after neoadjuvant chemotherapy using the revised AJCC TNM system is useful for predicting distant relapse and survival”
- ypT? ypN?
Residual Carcinoma Size

Measurement simple if single residual focus
Residual Carcinoma Size

When multiple scattered foci, overall maximum dimensions of area occupied by scattered foci has to be given.
Residual Carcinoma Size
Axillary Lymph Nodes

• Lymph nodes harder to find post-chemotherapy, fewer numbers

• Tumour response/regression seen as:
  – fibrosis (may be wedge-shaped)
  – mucin pools
  – aggregates of foamy macrophages

• These patients have intermediate prognosis between those with residual lymph node metastasis and those with negative nodes - Newman LA et al. Ann Surg Oncol 2003; 10: 734-739
Cytokeratin IHC - AE1/AE3

From Sami Shousha
Reporting of LNs post-chemotherapy

• Number involved

• 5-year DFS post-treatment by no. involved nodes:
  \( n = 0 \), 86%; \( n = 1–3 \), 64%; \( n = 4–9 \), 44%; \( n > 10 \) positive: 25% \((p<0.01)\) [Shien T et al. Breast Cancer Res Treat. 2009; 113; 307-313]

• No. of previously involved? – selection of patients for supraclavicular RT

• “In the setting of presurgical or neoadjuvant therapy, small nodal metastases are indicative of an incomplete response to systemic therapy and have the same significance as larger metastases” WHO 2012
Assessment of Pathological Response
<table>
<thead>
<tr>
<th>Name of system</th>
<th>Reference</th>
<th>Factors evaluated in the breast</th>
<th>pCR in the breast</th>
<th>Lymph nodes included</th>
<th>No. of categories of partial response</th>
</tr>
</thead>
<tbody>
<tr>
<td>B-18</td>
<td>{449}</td>
<td>Any treatment effect on invasive carcinoma</td>
<td>No invasive carcinoma</td>
<td>Yes, size of largest metastasis</td>
<td>1</td>
</tr>
<tr>
<td>Chevallier</td>
<td>{262}</td>
<td>Presence of invasive carcinoma with sclerosis or fibrosis</td>
<td>No invasive or in situ carcinoma</td>
<td>Yes</td>
<td>1</td>
</tr>
<tr>
<td>Sataloff</td>
<td>{1266}</td>
<td>Presence of invasive carcinoma</td>
<td>Total or near total therapeutic effect</td>
<td>Yes, ± treatment effect</td>
<td>2</td>
</tr>
<tr>
<td>Miller-Payne</td>
<td>{1023}</td>
<td>Presence of invasive carcinoma Cellularity</td>
<td>No invasive carcinoma</td>
<td>No</td>
<td>3</td>
</tr>
<tr>
<td>RCB (residual cancer burden)</td>
<td>{1390}</td>
<td>Size of tumour bed in two dimensions Cellularity of residual invasive carcinoma</td>
<td>No invasive carcinoma</td>
<td>Yes, number and size of largest deposit (with individual scores calculated for each case)</td>
<td>2</td>
</tr>
<tr>
<td>AJCC (y)</td>
<td>{221}</td>
<td>Size of invasive carcinoma</td>
<td>No invasive carcinoma</td>
<td>Yes, number</td>
<td>Up to 4 (dependent on the initial AJCC T and N categories)</td>
</tr>
<tr>
<td>MNPI (Modified Nottingham Prognostic Index)</td>
<td>{11}</td>
<td>Size of invasive carcinoma Cellularity of residual invasive carcinoma</td>
<td>No invasive carcinoma</td>
<td>Yes, number</td>
<td>3</td>
</tr>
<tr>
<td>Pinder</td>
<td>{1109}</td>
<td>% of tumour remaining in breast</td>
<td>No invasive carcinoma</td>
<td>Yes, presence of evidence of response Breast: 3</td>
<td>Lymph nodes: 1</td>
</tr>
</tbody>
</table>

AJCC, American Joint Committee on Cancer; ER, estrogen receptor; N, node; pCR, pathological complete response; T, tumour.

* Survival according to lymph-node status was analysed separately from response in the breast.
# Degree of Tumour Response

<table>
<thead>
<tr>
<th>Grade 1</th>
<th>Bonadonna, 1990 Smith, 2002</th>
<th>Chevallier, 1993</th>
<th>Sataloff, 1995</th>
</tr>
</thead>
<tbody>
<tr>
<td>Some alteration to cells, no reduction in numbers of core biopsy</td>
<td>Disappearance of all tumour</td>
<td>T-A Total or near total effect</td>
<td></td>
</tr>
<tr>
<td>Grade 2</td>
<td>Mild loss of invasive cells</td>
<td>No invasive carcinoma or metastasis in LNs, in situ present</td>
<td>T-B &gt;50% effect but &lt; total</td>
</tr>
<tr>
<td>Grade 3</td>
<td>Up to 90% loss of tumour cells</td>
<td>Invasive carcinoma present with stromal changes</td>
<td>T-C &lt;50% effect, but evident</td>
</tr>
<tr>
<td>Grade 4</td>
<td>Only small clusters, widely dispersed remain</td>
<td>Few changes to tumour</td>
<td>T-D No therapeutic effect</td>
</tr>
<tr>
<td>Grade 5</td>
<td>No invasive carcinoma, in situ may be present</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Comparison of systems

- 370 patients, neoadjuvant chemotherapy; 26% recurrence
- 5-year DFS by Chevallier system:
  - grade 1 - 83%
  - grade 2 - 85%
  - grade 3 - 62%
  - grade 4 - 65%
- 5-year DFS by Japanese Breast Cancer Society system
  - grade 3 - 77%
  - grade 2 - 68%
  - grade 1a - 68%
  - grade 1b - 58%
  - grade 0 - 52%
- HRs not statistically significant

Measurement of Residual Breast Cancer Burden to Predict Survival After Neoadjuvant Chemotherapy

W. Fraser Symmans, Florestina Feintinger, Christos Hatzis, Radhika Rajan, Henry Kuerer, Vicente Valero, Lime Anzul, Anna Pusicha, Bryan Hennessy, Marjorie Green, Aman U. Buzykar, S. Eva Stojanovt, Gabriel N. Hortobagyi, and Lajos Pusztai

Residual Cancer Burden Calculator

*Values must be entered into all fields for the calculation results to be accurate.

1. Primary Tumor Bed
   - Primary Tumor Bed Area: 
     - Overall Cancer Cellularity (as percentage of area): 
     - Percentage of Cancer That Is in situ Disease: 

2. Lymph Nodes
   - Number of Positive Lymph Nodes: 
   - Diameter of Largest Metastasis: 

Residual Cancer Burden: 
Residual Cancer Burden Class: 

The following parameters are required from pathologic examination in order to calculate Residual Cancer Burden (RCB) after neoadjuvant treatment:

1. The largest two dimensions (mm) of the residual tumor bed in the breast (largest tumor bed if multicentric disease)
2. Submission of the entire largest cross-sectional area of the residual tumor bed for histologic mapping, with specific identification of those slides in the pathology report (e.g. "the largest cross-sectional area of primary tumor bed was submitted in cassettes A5 - A9")
   - If the residual tumor is large (i.e. largest diameter > 5 cm), then at least 5 representative cassettes from the largest cross-sectional area are sufficient, but should be identified in the original pathology report (e.g. "representative sections from the largest cross-sectional area of primary tumor bed were submitted in cassettes A5 - A9")
3. Histologic assessment of the percentage of the tumor bed area that contains carcinoma (all carcinoma, i.e. invasive and in situ), select one of the following:
   - 0% 1% 5% 10% 20% 30% 40% 50% 60% 70% 80% 90%

http://www3.mdanderson.org/app/medcalc/index.cfm?pagename=jsconvert3
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(1) Primary Tumor Bed

Primary Tumor Bed Area: 15 (mm) X 20 (mm)

Overall Cancer Cellularity (as percentage of area): 50 (%)

Percentage of Cancer That Is in situ Disease: 0 (%) 

(2) Lymph Nodes

Number of Positive Lymph Nodes: 3

Diameter of Largest Metastasis: 14 (mm)

Residual Cancer Burden: 3.827

Residual Cancer Burden Class: RCB-III
# Examples of Cellularity

<table>
<thead>
<tr>
<th>Percentage</th>
<th>Description</th>
<th>Images</th>
</tr>
</thead>
<tbody>
<tr>
<td>1%</td>
<td>Rare scattered cells or glands</td>
<td><img src="image1.png" alt="Image" />  <img src="image2.png" alt="Image" /></td>
</tr>
<tr>
<td>5%</td>
<td>Less than half, less than 1/4, around 1/20 area</td>
<td><img src="image3.png" alt="Image" />  <img src="image4.png" alt="Image" /></td>
</tr>
<tr>
<td>10%</td>
<td>Less than half, less than 1/4, around 1/10 area</td>
<td><img src="image5.png" alt="Image" />  <img src="image6.png" alt="Image" /></td>
</tr>
<tr>
<td>20%</td>
<td>Less than half, slightly less than 1/4</td>
<td><img src="image7.png" alt="Image" />  <img src="image8.png" alt="Image" /></td>
</tr>
<tr>
<td>30%</td>
<td>Less than half, more than 1/4</td>
<td><img src="image9.png" alt="Image" />  <img src="image10.png" alt="Image" /></td>
</tr>
<tr>
<td>40%</td>
<td>Slightly less than half</td>
<td><img src="image11.png" alt="Image" />  <img src="image12.png" alt="Image" /></td>
</tr>
<tr>
<td>50%</td>
<td>About half</td>
<td><img src="image13.png" alt="Image" />  <img src="image14.png" alt="Image" /></td>
</tr>
</tbody>
</table>
• 50 patients treated with 3 months of chemotherapy Vs 53 patients with 3 months of neoadjuvant letrozole
• Excised tumours compared with preoperative core bx
• Chemotherapy produced more cPR & scattered cell pattern more frequently
• Letrozole produced more central scars (31 Vs 2 cases)
  Thomas JS et al. Histopathol. 2007;51:219-26

? Implications for surgery and for pathological specimen handling in neoadjuvant endocrine Vs chemotherapy
### Table 2. Patterns of response in cores and excisions

<table>
<thead>
<tr>
<th>Pattern</th>
<th>Chemotherapy % (N)</th>
<th>Letrozole % (N)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Core biopsy patterns*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diffuse</td>
<td>53.3 (24)</td>
<td>66 (35)</td>
<td></td>
</tr>
<tr>
<td>Nodular</td>
<td>46.7 (21)</td>
<td>34 (18)</td>
<td></td>
</tr>
<tr>
<td>Excision biopsy patterns</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diffuse</td>
<td>18.0 (9)</td>
<td>5.7 (3)</td>
<td></td>
</tr>
<tr>
<td>Nodular</td>
<td>20.0 (10)</td>
<td>13.2 (7)</td>
<td></td>
</tr>
<tr>
<td>Scattered</td>
<td>40.0 (20)</td>
<td>20.8 (11)</td>
<td>0.052</td>
</tr>
<tr>
<td>Central scar</td>
<td>4.0 (2)</td>
<td>58.5 (31)</td>
<td>0.0005</td>
</tr>
<tr>
<td>Complete response</td>
<td>18.0 (9)</td>
<td>1.9 (1)</td>
<td>0.007</td>
</tr>
</tbody>
</table>

Figure 2. Excision, central scar pattern.

Thomas JS et al. Histopathol. 2007;51:219-26
Change in markers post-chemotherapy

87 cases:
- 23 (26%) changed HR status (7 ER, 16 PR)
- HER2 status; 11+ve pre-chemo were ‘down-regulated’

25 cases:
- ER same in 80%, up in 12%, down in 8%
- PgR: same in 80%, up in 8%, down in 12%
- HER2: same in 80%, up in 12%, down in 8%

<table>
<thead>
<tr>
<th>Marker (n)</th>
<th>Number of Positive Pre- (%)</th>
<th>Number of Positive Post- (%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER (38)</td>
<td>17 (45)</td>
<td>17 (45)</td>
<td>1</td>
</tr>
<tr>
<td>PR (38)</td>
<td>17 (37)</td>
<td>8 (21)</td>
<td>0.03</td>
</tr>
<tr>
<td>HER2 (37)</td>
<td>12 (32)</td>
<td>8 (22)</td>
<td>0.2</td>
</tr>
<tr>
<td>HER2FISH (7)</td>
<td>5 (71)</td>
<td>4 (57)</td>
<td>0.32</td>
</tr>
</tbody>
</table>

Conclusions

- Specimen handling of post-neoadjuvant chemotherapy specimens can be difficult

Issues for “routine” pathology:
- Tumour size assessment & margins
- Recommendation - low threshold for epithelial marker immunohistochemistry
- Methods of assessing degree of response – no global agreement
- Predictive markers (ER, PgR and HER2) may change – no good data on significance