With main focus on the Estrogen Receptor

Guidelines for hormone receptor analysis in breast carcinoma

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Commentary

“The best way to achieve optimal treatment of today’s patients is to ensure the availability of reliable and timely pathological assessment in routine practice including treatment target identification”

Levels of evidence for biomarker analyses

<table>
<thead>
<tr>
<th>Question</th>
<th>Feature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Is it true?</td>
<td>Analytical Validity</td>
</tr>
<tr>
<td>Is it meaningful?</td>
<td>Clinical Validity</td>
</tr>
<tr>
<td>Is it useful?</td>
<td>Clinical Utility</td>
</tr>
</tbody>
</table>

Analytic Validity
- Refers to its accuracy in measuring what it is supposed to measure
  - Sensitivity and specificity of the assay
  - Assay robustness

Clinical Validity
- Refers to the predictive value of a biomarker for a given clinical outcome
  - Ie ER

Clinical Utility
- Is the biomarker helpful in improving or maintaining the health of patients
  - Treatment strategy

Intrinsic subtypes in breast cancer

- Luminal A: ER-positive, HER2-negative, Ki67- low and
- Luminal B: ER-positive, HER2-negative, Ki67> 14% or
- Luminal B: ER-positive, HER2-overexpressed or amplified, any Ki67
- HER2-positive (non luminal): HER2 overexpressed or amplified and ER negative
- Triple-negative: ER-negative, (PR-negative), HER2-negative
**Risk of recurrence pr. year**

N = 3,562 patients

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**Hormone Receptors (HR)**

- Positive in app. 80% (ER) of breast carcinomas
- cut off ≥ 1%

<table>
<thead>
<tr>
<th>ER-pos./PR-pos.</th>
<th>ER-pos./PR-neg.</th>
<th>ER-neg./PR-pos.</th>
<th>ER-neg./PR-neg.</th>
<th>Serving as control for false negative ER?</th>
</tr>
</thead>
</table>

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**Determination ER and PR 2007-2008 DBCG (Total: 7623)**

<table>
<thead>
<tr>
<th>ER</th>
<th>Unknown (%)</th>
<th>Negative (%)</th>
<th>Positive (%)</th>
<th>TOTAL (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unknown</td>
<td>54 (0.71)</td>
<td>1 (0.01)</td>
<td>2 (0.03)</td>
<td>57 (0.75)</td>
</tr>
<tr>
<td>Negative</td>
<td>55 (0.72)</td>
<td>1269 (16.65)</td>
<td>4518 (59.27)</td>
<td>6219 (81.58)</td>
</tr>
<tr>
<td>Positive</td>
<td>547 (7.18)</td>
<td>1154 (15.14)</td>
<td>4543 (59.60)</td>
<td>7623 (100)</td>
</tr>
</tbody>
</table>

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**Risk Factors and Risk Groups**

Danish Breast Cancer Cooperative Group (DBCG) 2013

www.dbcg.dk

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**Distribution of ER and PR levels (cut-off ≥ 10%) 2007-2008 DBCG (Total: 7623)**

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**Relevance of measured ER and PR status on the effects of about 5 years of tamoxifen in the 30% probability of recurrence (EIC) TCG**

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Treatment allocation according to Risk Group

www.dbcg.dk

<table>
<thead>
<tr>
<th>Risk Group</th>
<th>ER (%)</th>
<th>Age</th>
<th>Risk factor</th>
<th>Treatment allocation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low Risk</td>
<td>0%</td>
<td>No</td>
<td>No adjuvant treatment</td>
<td>C (± T)</td>
</tr>
<tr>
<td></td>
<td>1-9%</td>
<td></td>
<td></td>
<td>C + E (± T)</td>
</tr>
<tr>
<td>High Risk</td>
<td>&lt; 40 years</td>
<td></td>
<td></td>
<td>C + E (± T)</td>
</tr>
<tr>
<td></td>
<td>≥ 40 years</td>
<td></td>
<td></td>
<td>No E</td>
</tr>
<tr>
<td></td>
<td>≥ 60 years</td>
<td></td>
<td>HER2 negative</td>
<td>E</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>HER2 positive</td>
<td>C + E + T</td>
</tr>
</tbody>
</table>

C: Chemotherapy; E: Endocrine treatment; T: Trastuzumab

Preanalytical standardization
- Fixation
  - 10% NBF within 1 hour
  - Fixation time: 6 (at least) – 72 (no more than) hours.
- Analytical standardization
  - Antibody/Antigen Retrieval/Detection Systems
  - Control samples
- Postanalytical standardization
  - Interpretation
  - Cut-off level
  - Internal quality control
  - Tissue / Material
  - Image Analysis?
- Participation in quality assurance programs

Preanalytical standardization
- Antibody
  - ERα Dilution
    - SP1 monoclonal rabbit (12) (Thermocycler or Ventana)
    - Ready To Use (majority)
    - Optiview-DAB
      - Ultraview-DAB
        - Envision
          - EnVision Flex+ Rabbit
    - Clone 6F11
      - Monoclonal mouse (1) (Novocastra)
      - 1:100
      - Ultraw-DAB
        - EnVision
          - EnVision Flex+ Rabbit

The majority of the pathology laboratories in Denmark apply the Benchmark Ultra (Ventana Medical Systems) for ER staining. A few laboratories apply the Dako autostainer (DAKO /Denmark).

Scoring Methods used for determination of HR status

<table>
<thead>
<tr>
<th>Scoring method</th>
<th>Algorithm type</th>
<th>Definitions</th>
<th>Range of scores</th>
</tr>
</thead>
<tbody>
<tr>
<td>H-score</td>
<td>Sum of (PS at a given ISxIS)</td>
<td>PS: 0-100%; IS: 0:none; 1: weak; 2: moderate; 3:strong</td>
<td>0-300</td>
</tr>
<tr>
<td>Semi-quantitative Bright Field LM</td>
<td>Percentage positive tumor cells Cut-off value ≥ 1</td>
<td>0-100%</td>
<td></td>
</tr>
<tr>
<td>IHC4 (ER, PR, KI67, HER2)</td>
<td>Mathematical model</td>
<td>Creation of an IHC4 score</td>
<td></td>
</tr>
<tr>
<td>IHC subtype</td>
<td>Variation of IHC4</td>
<td>0-100%</td>
<td></td>
</tr>
</tbody>
</table>
Determination of ER:
Biochemical method, ex. dextran coated charcoal (DCC) and presently immunohistochemistry

74% measured ER positive by DCC (age<70), median value of ER is 4-2 fmol/mg cytosol protein (0-2542)

IHC:
• No method to determine the exact amount of protein by weight in IHC exists.
• The influence of fixation and HIER on protein loss is largely unknown, hence estimation of staining intensity can be unreliable.

Shi et al. J Histochem Cytochem 2011;59:13-32

Guideline recommendations
• Only methods with pre-analytical and analytical components conformed exactly to clinically validated assays or compared with the clinically validated assays should be used for prediction of response for endocrine treatment
  ▪ Positive agreement must be ≥90%
  ▪ Negative agreement ≥95%
  ▪ Positive results are defined as ≥1% immuno-reactive cells

Arch Pathol Lab Med 2010;134:48-72

Cheang MC et al. J Clin Oncol 2006; 24:5637-44
• The proportion of ER positive breast cancers increased from 63% to 71% when SP1 was used instead of 1D5 with superior prognostic information.
• Higher antibody affinity causes increased ER positivity in other organs i.e. lung adenocarcinomas.


Results agreement

<table>
<thead>
<tr>
<th>Cut-off 1%</th>
<th>1D5</th>
<th>-</th>
<th>+</th>
<th>Overall agreement</th>
<th>-</th>
<th>+</th>
<th>95</th>
<th>263</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative agreement</td>
<td>95/124</td>
<td>77 (68-85)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive agreement</td>
<td>263/266</td>
<td>99 (97-99)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
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<table>
<thead>
<tr>
<th>Cut-off 10%</th>
<th>1D5</th>
<th>-</th>
<th>+</th>
<th>Overall agreement</th>
<th>-</th>
<th>+</th>
<th>109</th>
<th>257</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative agreement</td>
<td>109/153</td>
<td>82 (74-88)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive agreement</td>
<td>257/257</td>
<td>100 (99-100)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Guideline
• Positive agreement must be ≥90%
• Negative agreement ≥95%
• Positive results are defined as ≥1% immunoreactive cells compared with a clinically validated assay
In conclusion:
• The proportion of ER positive breast cancer increased from 68% to 75% (cut-off 1%) when SP1 was used compared to 1D5.
• The choice of antibody and HIER influences the prevalence of ER-positivity.
• When applying changes in the staining procedure caution must be taken towards the guideline recommendations concerning positive and negative agreement with clinically validated assays.

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**Stable expression of staining intensity by repeat staining of control tissue**

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**ER in normal breast glandular tissue serving as internal controls**

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**ER staining sensitivity/ intensity**

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**Variety of material for ER analysis**

- Surgical tumor specimens
- Core Needle Biopsies (CNB)
- Tissue Micro Array (TMA)
- Small biopsies from metastatic sites i.e. bone (decalcified tissue)
Her2 and estrogen receptor status on core needle biopsy compared to surgical specimens in breast cancer – A case for treatment reaction. A study performed at the Aarhus University Hospital, Herlev, Denmark.

Methods: Histological malignancy grade, estrogen receptor and Her2 receptor status on core needle biopsy compared to surgical specimens in breast cancer. A study performed at the Aarhus University Hospital.

Results: In case of negative ER on Core Needle Biopsy – repeat analysis on surgical specimen

Conclusions: ER discrepancy: 12 – 29%, often with loss of receptor

HER2 discrepancy: 6 – 20%, often with gain of HER2+

Limitations:

• Many "pathology chart review" studies, did not re-analyse tumor samples

• Prospective studies - biopsy had treatment consequence in 15-20%

• Benign disease/other malignancies in 14%

In Conclusion

• Standardization of staining methodology

• Preanalytical

• Analytical

• Postanalytical

• Application of controls on all ER, (PR) stained slides

• Repeat staining on surgical specimen in case of ER negative result on primary Core Needle Biopsy or TMA.

• Repeat staining on surgical specimens after neoadjuvant chemotherapy in any case of ER staining.

• Attendance in external Quality Assurance Programs

"Routine ER, PR, HER2 and Ki67 used in combination as binary categories are not the same as a (centrally determined) IHC score (based on continuous variables) or multivariate genomic predictors." Dr. Lajos Pusztai, St. Gallen 2013.
ER receptor status in Core Needle Biopsy. How accurate is it?

The prevalence of ER positivity

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