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
- of a biomarker 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
- in 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 < 14%
- Luminal B: ER-positive, HER2-negative, Ki67 > 14% or 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
The oestrogen receptor as a prognostic marker
Shift from prognostic to predictive!!

Risk of recurrence pr. year
N = 3,562 patients

Hormone Receptors (HR)
- Positive in app. 80% (ER) of breast carcinomas
- cut off ≥ 1%
  - 1-9% low
  - ≥10% high

ER-pos./PR-pos.
ER-pos./PR-neg.
ER-neg./PR-pos.
ER-neg./PR-neg.

Serving as control for false negative ER?

Determination ER and PR
2007-2008 DBCG
(Total: 762)

Risk Factors and Risk Groups
Danish Breast Cancer Cooperative Group (DBCG) 2013
www.dbcg.dk

- 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
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).

### Antibody ER α

<table>
<thead>
<tr>
<th>Antibody</th>
<th>ER α</th>
<th>Dilution</th>
<th>Detection system</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPα (monoclonal rabbit) (Thermocycler or Ventana)</td>
<td>Ready To Use (majority)</td>
<td>Optiview-DAB</td>
<td>Envision Flex+ Rabbit</td>
</tr>
<tr>
<td>Clone 6F11 (monoclonal mouse) (Novocastra)</td>
<td>1:100</td>
<td>Ultraview-DAB</td>
<td>Envision Ultra-W- DAB</td>
</tr>
</tbody>
</table>

**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>Allred score</td>
<td>PS + IS</td>
<td>PS: 0: none; 1: &lt;1%; 2: 1-10%; 3: 10-33%; 4: 33-66%; 5: &gt;66%</td>
<td>0.2 - 8</td>
</tr>
<tr>
<td>H-score</td>
<td>Sum of (PS at a given IS)</td>
<td>PS: 1-50%; 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</td>
<td>Cut-off value ± 1%</td>
<td>0 - 100%</td>
</tr>
<tr>
<td>IHC subtype</td>
<td>Mathematical model</td>
<td>Creation of an IHC subtype</td>
<td>Variation of IHC</td>
</tr>
</tbody>
</table>

**Interpretation of ER**

- 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


74% measured ER positive by DCC (age<70), median value of ER 42 fmol/mg cytosol protein (0-2542)
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

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.


564 patients
500 TMA
Evaluable TMA

1D5, SP1
n=390
PharmDx
n=361

Evaluable TMA

1D5, SP1
n=321

Guideline
Positive agreement must be ≥ 90%
Negative agreement ≥ 95%
Positive results are defined as ≥ 1% immunoreactive cells compared with a clinically validated assay

Results agreement

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<th>Cut-off 1%</th>
<th></th>
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<tr>
<td>SP1</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>29</td>
<td></td>
<td>263</td>
</tr>
<tr>
<td>Overall agreement</td>
<td>(93-263)/390</td>
<td>92 (89-94)</td>
</tr>
<tr>
<td>Positive agreement</td>
<td>263/266</td>
<td>99 (97-99)</td>
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<table>
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<tr>
<th>Cut-off 10%</th>
<th></th>
<th></th>
</tr>
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<tbody>
<tr>
<td>SP1</td>
<td></td>
<td>109</td>
</tr>
<tr>
<td>24</td>
<td></td>
<td>257</td>
</tr>
<tr>
<td>Overall agreement</td>
<td>(109-257)/390</td>
<td>94 (91-96)</td>
</tr>
<tr>
<td>Positive agreement</td>
<td>257/257</td>
<td>100 (99-100)</td>
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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.

Application of control tissue

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.
Östrogen receptor

NordiQC: Positive staining reaction of the stromal cells indicates that a high sensitive protocol is being applied.

Surgical tumor specimens
Core Needle Biopsies (CNB)
Tissue Micro Array (TMA)
Small biopsies from metastatic sites i.e. bone (decalcified tissue)

ER staining sensitivity/intensity

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)

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<th>ER</th>
<th>CNB &lt; 1%</th>
<th>CNB ≥ 1%</th>
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<tr>
<td>SS ≤ 1%</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>SS &gt; 100%</td>
<td>0</td>
<td>80</td>
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No Neoadjuvant Chemotherapy
N-89, bimodal distribution
Concordance 98%
Ranked correlation 1 (rho)
Sensitivity 0.909
Specificity 2

Conclusion: With a cut-off value ≥ 1%, ER on CNB is sufficiently accurate and reliable.

ER status on Core Needle Biopsy (CNB) vs. Surgical Specimens (SS)

Histological malignancy grade, estrogen receptor and HER2 receptor status on core needle biopsy compared to surgical specimen in breast cancer: Two samples; one sample is 95% hormone receptors; Munch-Petersen H. APMIS: Special Issue: Abstracts of the Annual Meeting of the Danish Pathology Society, 14–16 March 2013, Randers, Denmark

March 2013, Volume 121, Issue Supplement s135, Page 8

In case of negative ER on Core Needle Biopsy – repeat analysis on surgical specimen
Discordance of ER, PR and HER2 after neoadjuvant chemotherapy in Breast Cancer

- Discrepancy of results
  - A change of 8-33% in Hormone Receptor status was reported
  - PR more discordant than ER
- Retesting of Hormone Receptor is recommended

Re-testing of Hormone Receptor

- Repeat staining on surgical specimens after neoadjuvant chemotherapy in any case of ER or PR negativity on primary tumor tissue
- Repeat staining on surgical specimens after neoadjuvant chemotherapy in case of HER2 discrepancy

In Conclusion

- Standardization of staining methodology
- Preanalytical
- Analytical
- Postanalytical
- Preanalytical recommendations by the American Society for Clinical Oncology (ASCO) and the College of American Pathologists (CAP) Guideline Recommendations (Arch Pathol Lab Med. 2010;134:e48-e72)

Accuracy of ER expression in TMA compared to whole section

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<tr>
<th>Study</th>
<th>Number of cases</th>
<th>Size (mm) and number of cores</th>
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Recommended publications
- On behalf of the Translational Subgroup of the NBO Breast Clinical Studies Group

Prospective studies:
- Many “pathology chart review” studies, did not re-analyse tumor samples (methodological variation)
- Biopsy had treatment consequence in 15-30%
- Benign disease/other malignancies in 4/6%
- HER2 discrepancy: 6–20%, often with gain of HER2+
- ER discrepancy: 10–20%, often with loss of receptor

In Conclusion

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