Estrogen and progesterone receptor testing of primary breast cancer: clinical importance and technical validation

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ER Testing

ASCO guidelines:
- Clinical validation
- Technical validation
- Influence therapeutic decision making

ASCO expert panel, J Clin Oncol, 1998

Clinical validation:
Test identifies subsets of patients with significantly different risks of recurrence/survival

Clinical Validation

Prognostic factor
• Factor that provides information on clinical outcome in the absence of therapy

Predictive factor
• Factor that provides information on likelihood of response to therapy

ER Testing (historical)

• Biochemical method
  – A portion (1g) of fresh tumour taken
  – Frozen in liquid nitrogen
  – ER content evaluated by DCC method
  – Positive result: 10 fmol/mg (Ontario)
ER Testing (IHC)

- Assessed by Immunohistochemistry for > 20 years
- **Clinical validation:**
  - *WEAK* Prognostic indicator
  - approx 25 studies, > 5000 cumulative pts
  - few studies involved untreated pts
    - 10-15% recurrence/survival benefit

ER Testing

- **Clinical validation:**
  - *STRONG* Predictive factor
  - advanced stage disease; approx 25 studies, ~1500 cumulative pts,
    - 70% ER+ pts showed significant clinical response
    - 85% ER- pts showed no response

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ER Testing

- **Clinical validation:**
  - *STRONG* Predictive factor
  - adjuvant setting; few studies
  - 25-30% recurrence/survival benefit in ER+ pts

Ferno et al, Act Oncol, 1996
Harvey et al, J Clin Oncol, 1999

ER and PR Testing

**ASCO guidelines:**
- Clinical validation
- Technical validation
- Influence therapeutic decision making

ASCO expert panel, J Clin Oncol, 1998
ER and PR Testing

Technical validation:
- Sensitive
- Specific
- Reproducible
- Interpreted in uniform manner from lab to lab

ASCO expert panel, J Clin Oncol, 1998

- **Sensitivity** – the percentage of positive test results obtained when evaluating only specimens that are truly positive
- **Specificity** – The percentage of negative test results reported when only truly negative specimens are evaluated

Technical Validation

- **Pre-analytic**
  - tissue handling and fixation:
- **Analytic**
  - assay validation/equipment calibration
  - type of antigen retrieval
  - controls
  - automation
- **Post-analytic**
  - interpretation
  - mandatory reporting elements
  - QA

- **Sensitivity** – the percentage of positive test results obtained when evaluating only specimens that are truly positive
- **Specificity** – The percentage of negative test results reported when only truly negative specimens are evaluated
Tissue Handling and Fixation

• Time from specimen excision to placement in fixative should be minimized
• Samples sliced at 5 -10 mm intervals after appropriate gross inspection
• Sufficient volume of 10% neutral buffered formalin

Time of fixation:
• Optimally 6-48 hours in 10% neutral buffered formalin
  – 6 hours for core biopsies
  – 24-48 hours more appropriate for larger specimens

Technical Validation

• Pre-analytic
  – tissue handling and fixation:
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  – assay validation/equipment calibration
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ER Testing

- IHC scoring
  - Most labs - > 10%
  - Some labs - > 20%
  - Harvey et al, (1999): adjuvant setting
    1-10% weakly ER+ cells

Harvey et al (1999):
- ER evaluated in 1,982 primary BC pts
- Antibody 6F11
- Allred score 0-8
- Results compared to Ligand Binding Assay (biochemical method) and clinical outcome

IHC Semiquantitative Scoring System (Allred et al JNCI, 85;1993; Modern Path, 11; 1998)

Proportion Score (PS)
0 1/100 1/10 1/3 2/3 1

Intensity Score (IS)
0 = negative 1 = weak 2 = intermed 3 = strong
IHC Semiquantitative Scoring System (Allred et al, *JNCI, 85;1993; Modern Path, 11; 1998*)

Proportion Score (PS) | Intensity Score (IS)
--- | ---
0 | 0 = negative
1/100 | 1 = weak
1/10 | 2 = intermed
1/3 | 3 = strong
2/3 | 5
1 | 5

Harvey et al (1999)

ER status by IHC better at predicting DFS and equivalent at predicting OS compared with ER status by LBA (biochem).
ER and PR Testing

- Elledge et al, 2000
  - ER/PR by Biochem (LBA) and IHC
  - Metastatic breast cancer (SWOG 8228)
  - Treatment with Tamoxifen, 9 years median follow up

ER and PR Testing

- Quality control
- Quality assurance

ER Testing

- Interlab variability:
  - NEQAS-ICC: 200 labs in 26 countries
  - Circulated tumors with high, medium, low levels of ER
    - > 80% labs detected ER in tumors with high and medium ER levels
    - 37% labs detected ER in tumors with low ER levels

Rhodes et al, J Clin Pathol, 2000
ER Testing

• Interlab variability: cut-offs
  – NEQAS-ICC: 200 labs in 26 countries
  – Low ER cases circulated:
    • For labs using 10% cut off, false negative rate = 66%
    • For labs using 1% cut off, false negative rate = 30%

Rhodes et al, J Clin Pathol, 2000

ER and PR Testing

• Canadian QC in IHC/CAP National Standards Committee
  – 18 labs across Canada (37 cases)
  – ER: 98.5% sensitivity: 98.3% specificity
    • Concordance: 98.5%
  – PR: 93.5% sensitivity: 95.4% specificity
    • Concordance: 94.4%

Terry et al, submitted

ER and PR Testing

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• Fix in 10% neutral buffered formalin for 8-24 hours, following slicing to allow adequate fixation
• Baylor abs and method:
  – ER, 6F11: PgR, 1294
• Allred scoring system

CAP consensus, 2000; Goldhirsch et al, 2001; NIH consensus document, 2000

ER and PR Testing

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Reporting

% positive tumor nuclei Classification
0 Negative
1-9% Low positive
10-100% Positive

Goldhirsch et al, 2001; NIH consensus document, 2000
**Synoptic Reports:**

**Estrogen Receptor Protein:** POSITIVE
- % positive cells: > 90%
- Antibody used: 6F11, LSAB procedure

**Progesterone Receptor Protein:** POSITIVE
- % positive cells: Approx 60%
- Antibody used: PGR 1294, LSAB procedure

Positive and negative laboratory controls stained appropriately

**Threshold for Positive ER/PR Result:** > 1% nuclear positivity of tumour cells (Harvey et al, JCO 17:1474-1481, 1999)

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**Information for Medical Oncologists**

- Don't accept “positive” or “negative” result
- Insist on reporting of % positivity, antibodies used and methodology (CAP requirements)
- Know lab’s cut-off point and studies that this is based on