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

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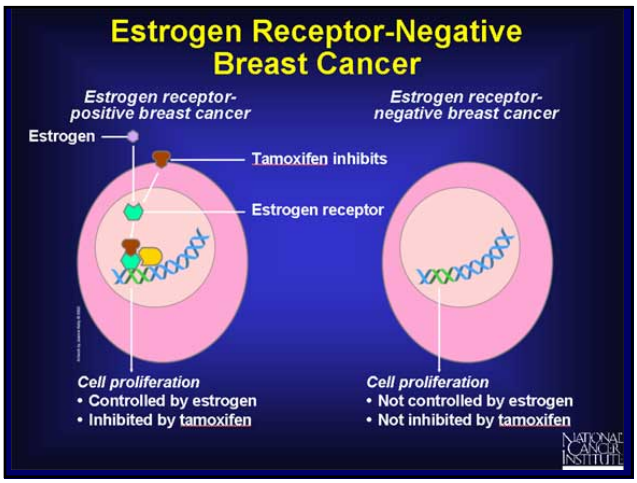

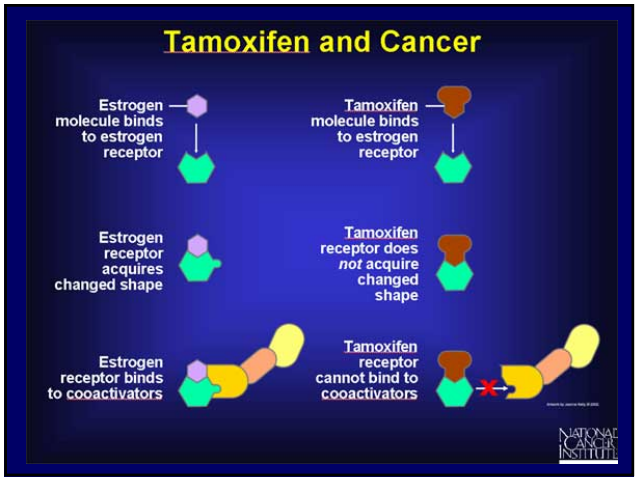

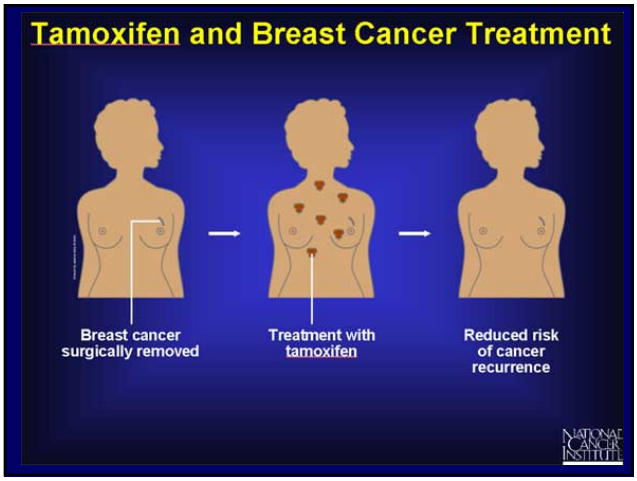

ER Testing

ASCO guidelines:

- Clinical validation
- Technical validation
- Influence therapeutic decision making

ASCO expert panel, J Clin Oncol, 1998

ER Testing

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

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

ER and PR Testing

Technical validation:

- Sensitive – *several antibodies*
- Specific – *several antibodies*
- Reproducible – *different IHC methods*
- Interpreted in uniform manner from lab to lab – *arbitrary cut-offs and methods of scoring*

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

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

Tissue Handling and Fixation

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
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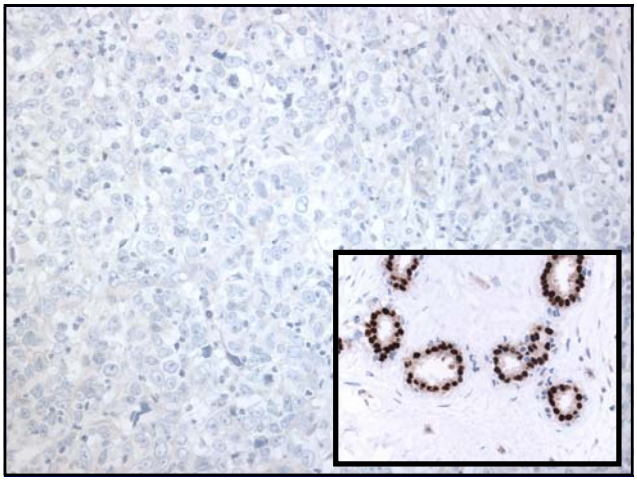


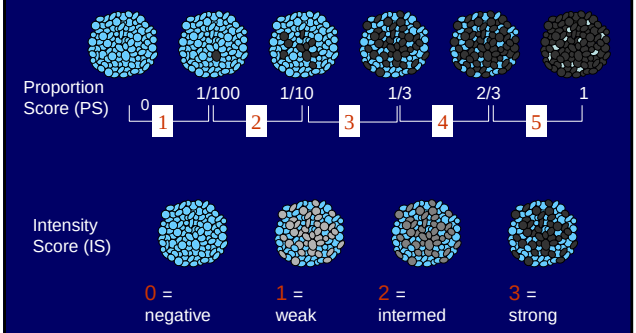
ER Testing

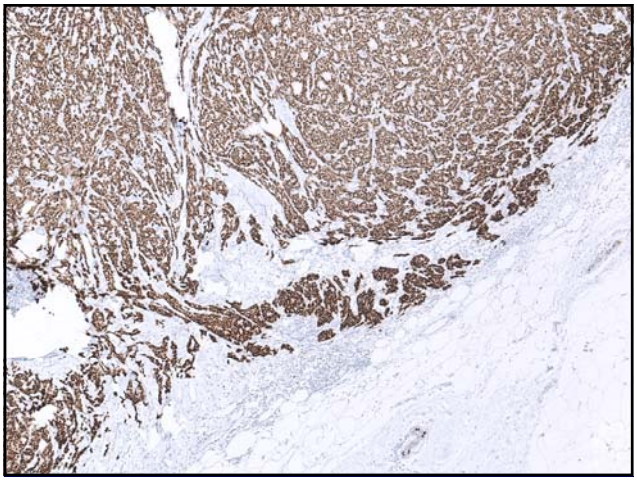
- IHC scoring
  - Most labs - > 10%
  - Some labs - > 20%
  - Harvey et al, (1999): adjuvant setting 1-10% weakly ER+ cells

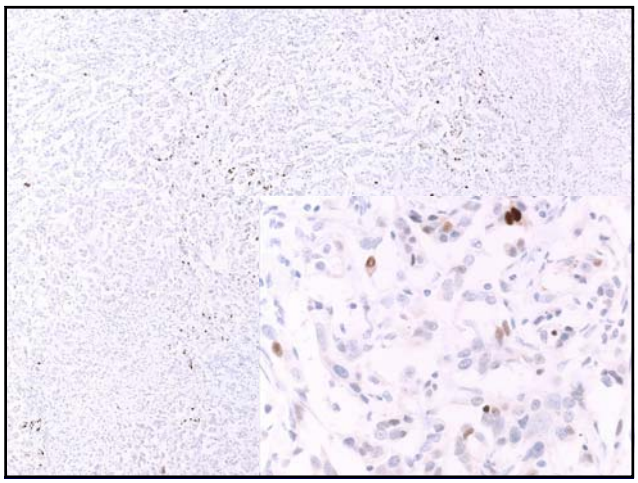
ER Testing

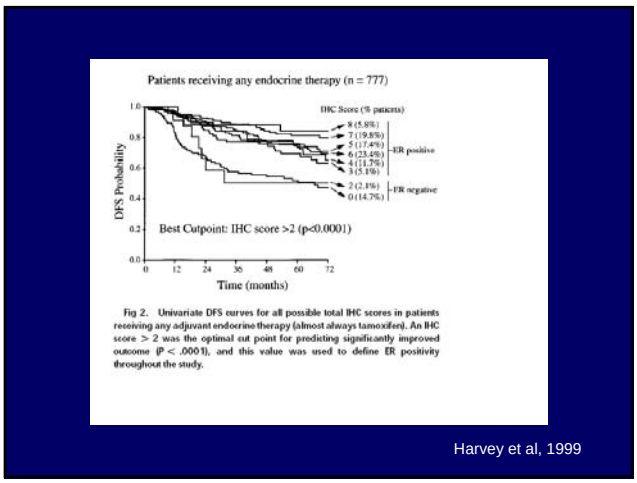
- Harvey et al (1999):
  - ER evaluated in 1,982 primary BC pts
  - Antibody 6F11
  - Allred score 0-8
  - Results compared to Ligand Binding Assay (biochemical method) and clinical outcome

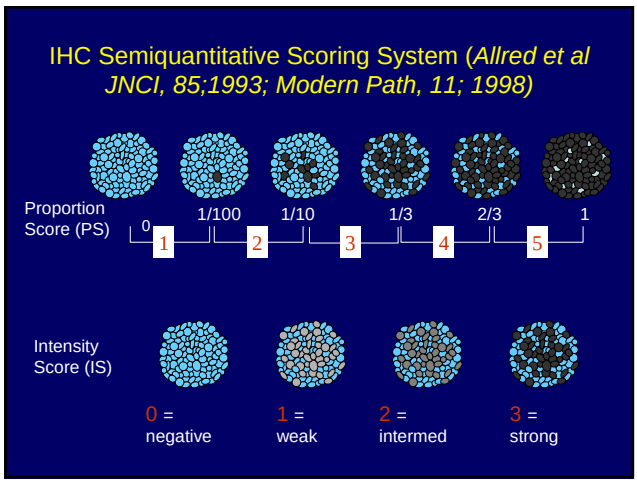
IHC Semiquantitative Scoring System (*Allred et al JNCI, 85;1993; Modern Path, 11; 1998)*

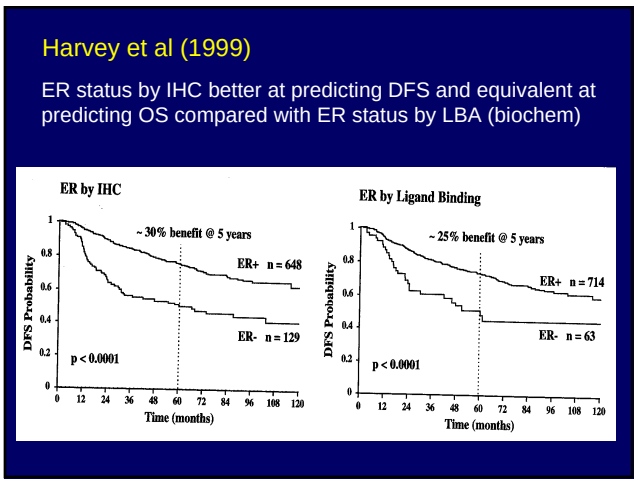







Patients receiving any endocrine therapy (n = 777)
IHC Score (% patients)
8 (5.8%)
7 (19.8%)
5 (17.4%)
6 (23.4%)
4 (11.7%)
3 (5.1%)
2 (2.1%)
0 (14.7%)
ER positive
ER negative
DFS Probability
Best Cutpoint: IHC score >2 (p<0.0001)
Time (months)
Fig 2. Univariate DFS curves for all possible total IHC scores in patients receiving any adjuvant endocrine therapy (almost always tamoxifen). An IHC score > 2 was the optimal cut point for predicting significantly improved outcome (P < .0001), and this value was used to define ER positivity throughout the study.
Harvey et al, 1999

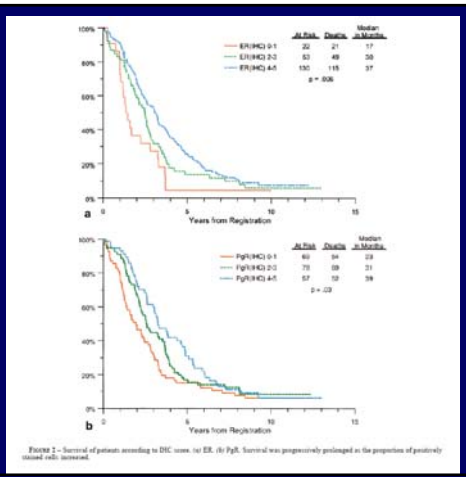

IHC Semiquantitative Scoring System (Allred et al JNCI, 85;1993; Modern Path, 11; 1998)
Proportion Score (PS)
0
1/100
1/10
1/3
2/3
1
1
2
3
4
5
Intensity Score (IS)
0 = negative
1 = weak
2 = intermed
3 = strong


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 by IHC
~ 30% benefit @ 5 years
ER+ n = 648
ER- n = 129
p < 0.0001
DFS Probability
Time (months)
ER by Ligand Binding
~ 25% benefit @ 5 years
ER+ n = 714
ER- n = 63
p < 0.0001
DFS Probability
Time (months)

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

Elledge et al, 2000



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

Mount Sinai Hospital

- 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

ER and PR Testing

Mount Sinai Hospital

Reporting

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

CAP consensus, 2000: 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)

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