STANDARDIZATION OF HORMONE RECEPTORS IN BREAST CANCER

A History of Hormone Receptors in Breast Cancer: Lessons for the Future

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• Schinzinger (1889) suggested “endocrine abalation” in treatment of breast cancer.
• Beatson (1896) performed the first operation to remove ovaries in a patient with inoperable breast cancer. “8 months after the operation the disease had disappeared”.
• Boyd (1900) 54 patients, 35% complete remission of disease.
...next several decades

• Should removal of ovaries in patients with breast cancer be prophylactic, or therapeutic, based on advanced stage?
Radioisotopic ("radioactive") estrogen accumulates in target tissues—pituitary gland, vagina, uterus.

Radioisotopes were found in the cytoplasm and nucleus of target cells.

Suggest that ablation of the pituitary or adrenal gland may be a treatment to eliminate sources of estrogen.
“Prelude to ER Testing”


Dextran-Coated Charcoal/Ligand Binding Method

• Principle: measurement of available cytoplasmic estrogen receptor binding proteins (ERBP), measured as a fraction of the total sample protein content.
DCC/LB..the steps

- Homeogenate of tissue-centrifuge and isolate “cytosol”
- Cytosol total protein measured
- Sucrose density gradient fractionates the cytosol
- exposed to tritiated (radioisotopic) estrogen, binds to ER.
- DCC removes unbound estrogen
- Scintillation counting.
- Exposure to estrogen to determine “nonspecific binding”
- Final result expressed in “femtomoles/mg cytosol protein”
- Femto= ten to the minus 15. (.000000000000001)
Dextran-Coated Charcoal Method/Ligand Binding

• Requires large amount of fresh tissue.
• Immediate freezing of fresh tissue when removed from patient.
• Radioactive reagents.
• Carcinogenic reagents
• Expensive laboratory equipment not usually found in hospitals.
• “Blind sampling”. Samples for assay are largely independent of what is examined histologically.
• Tumor-poor cellularity may lead to false negative assay result.
• Non-tumor areas sampled, necrotic areas yield false negative results.
• No direct visualization of assay sample*.
Immunofluorescent Detection of Estrogen Receptors in Breast Cancer

- Using estrogen polymer, labeled with fluorescein.
- Principle: the polymer binds to the estrogen receptor and is localized with a fluorescence microscope.
- Receptors were found in the cytoplasm and nucleus.
- 90% correlation with DCC/LB method.
“The technique can be performed by the average surgical pathology laboratory”

“in general, tumors with less than 10% positive cells were negative by DCC/LB, and those with 11-20% positive were borderline by DCC/LB”.

A Two Stage Method for Estrogen Receptor Analysis:
Correlation with Morphologic Parameters of Breast Carcinoma

- Enhanced sensitivity over direct methods.
- Nuclear expression dominates.
- Correlates well with morphology; better-differentiated tumors are “estrogen rich”.

Eusebi et al Tumori 1981;67:315-23
Devitalized tissue may yield false negative results:
- Comparison of fresh frozen tissue and the subsequent mastectomy specimen.
- Markedly lower DCC/LB results in mastectomy sample.
- A low expressor may become falsely negative.

- Immunocytochemical staining of estrogen receptor in paraffin sections of breast cancer by use of monoclonal antibodies: Comparison with frozen sections.
- Frozen, paraffin (IP and ABC methods)
- All correlate well with DCC/LB
• Use of the “H Score” (Histochemical).
• The sum of proportion of cells with nuclear staining times the intensity of staining (graded 0-4).
• Frozen tissue with antibody H22 (Abbot).
• Quantitative comparison with biochemical method, sensitivity 93%, specificity 89% based on clinical outcome.
Quality Issues with DCC/Ligand Binding Method: Thorpe SM
Breast Cancer Res Treat 1987;9: 175-89

• Biopsy Composition/ inability to distinguish normal from tumor
• Homegenization
• Incubation time
• Adsorption of ligand-free surfaces
• Adsorption of free steroid by DCC
• Adsorption of cytosol protein by DCC
• Scintillation counting

- First introduction of antibody to PR.
- Allowed for routine analysis of PR.
- PR was not routinely done with the DCC method because it required substantial tissue.
PR is an independent prognostic factor and ER+/PR+ patients respond better as a group (70% responders) to endocrine therapy

<table>
<thead>
<tr>
<th>ER+PR+</th>
<th>ER+PR-</th>
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</thead>
<tbody>
<tr>
<td>74%</td>
<td>28%</td>
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</table>

MCGUIRE WL
Hormone Receptors: Role in Predicting Prognosis and Response to Endocrine Therapy
*Semin Oncol* 1978 5(4) 428-33
The Call to Standardize HR by IHC

Pertschuk et al ER1D5 in paraffin predicts endocrine response better than ICA or cytosol methods. Cancer 1996;77:2514-9

- Percent of cells staining (10%), exclusive of intensity.
• The decision whether to recommend adjuvant hormonal therapy should be based on the presence of hormone receptors, as assessed by immunohistochemical staining of breast cancer tissue.
Is There a Problem?

• LBA testing was almost entirely replaced by IHC testing in the early to mid-1990s based on assumptions that the assay was accurate according to several concordance studies between IHC and LBA.
• There were few if any systematic recommendations or guidelines regarding optimization or reproducibility of IHC testing procedures, nor has there been standardized proficiency testing in the United States for ER and PgR.
In the UK, there has been an external quality assurance program (UKNEQAS) for hormonal receptors since 1994, and in 1998, a survey evaluated the frequency of hormone receptor positive cases in 30% of its participating laboratories involving over 7,000 cases.


• Proficiency testing for ER, PgR and HER2 was developed by the Royal College of Pathologists of Australasia Quality Assurance Program (RCPA QAP) in 2001 and participation is mandatory in Australia and New Zealand. Data on more than 8,000 patients from two audits was reported in 2007.49 These results also indicated significant variation across laboratories in the reporting of ER, PgR, and HER2 status.
The inquiry into ER testing practices in Newfoundland revealed that about a third of 1,023 ER tests on patients in that Canadian Province tested between 1997 and 2005 were scored falsely negative when retested in a central lab in Ontario.
• ER cases that were identified as falsely negative were found to have one or more of three characteristics: poor fixation, negative internal control (when normal duct epithelium was present, it was negative), and absent internal controls (no internal control to evaluate)

• Uncontrolled fixation.
• Different fixatives, antibodies, IHC methods/platforms, detection systems.
• Scoring methods, positive/negative cutoffs.
• Clinician variation—some refuse to adhere to established guidelines.
Richard D. Gelber and Shari Gelber for the International Breast Cancer Study Group (IBCSG) and the Breast International Group (BIG): Facilitating consensus by examining patterns of treatment effects. The Breast, 2009,

• Over 20% of tumors that tested locally as ER-negative were shown to exhibit at least some expression of ER (false-negative) upon central review.
Different procedures inter- and intra laboratory compromise standardization

3^{14} = 4.8 mil procedures (3 choices in 14 steps)
Where in the world did ASCO/CAP come from?

Guidelines for Hormone Receptor Testing by Immunohistochemistry October, 2009

• Prognostic/Predictive
• 1980’S-DCC/LIGAND BINDING METHOD-tissue quantity; sendout; biochem lab; QA centers. Grind/bind.
• MID-90’S IHC-heterogenuity; no outcomes; QA undefined. Direct visualization.
• Central vs non ~20% variance.
• Harvey et all IHC outcomes-Allred Score
• An ad-hoc group of pathologists, laboratory scientists and technical experts, representing academia, community hospitals, industry and reference laboratories, conducted a full day consensus meeting (Santa Barbara, CA; January 27, 2008) to discuss these critically important issues in an effort to develop rational evidence-based guidelines for best practices in the assessment of ER by IHC.
Consensus Recommendations on Estrogen Receptor Testing in Breast Cancer By Immunohistochemistry Appl Immunohistochem & Molec Morphol 2008

- Hadi Yaziji, MD, Clive R. Taylor, MA, MD, D.Phil, Neal S. Goldstein, MD, David J. Dabbs, MD, Elizabeth H. Hammond, MD, Bryan Hewlett, ART (CSMLS), MLT (CMLTO), Alton D. Floyd, PhD, Todd S. Barry, MD, Alvin W. Martin, MD, Sunil Badve, MD, Frederick Baehner, MD, Richard W. Cartun, MD, Richard N. Eisen, MD, Paul E. Swanson, MD, Stephen M. Hewitt, MD, PhD, Mogen Vyberg, MD and David G. Hicks, MD and Members of the Standardization Ad-Hoc Consensus Committee

- M. Elizabeth H. Hammond; Daniel F. Hayes; Mitch Dowsett; D. Craig Allred; Karen L. Hagerty; Sunil Badve; Patrick L. Fitzgibbons; Glenn Francis; Neil S. Goldstein; Malcolm Hayes; David G. Hicks; Susan Lester; Richard Love; Pamela B. Mangu; Lisa McShane; Keith Miller; C. Kent Osborne; Soonmyung Paik; Jane Perlmutter; Anthony Rhodes; Hironobu Sasano; Jared N. Schwartz; Fred C. G. Sweep; Sheila Taube; Emina Emilia Torlakovic; Paul Valenstein; Giuseppe Viale; Daniel Visscher; Thomas Wheeler; R. Bruce Williams; James L. Wittliff; Antonio C. Wolff
Preanalytic—cold ischemic time, formalin exposure.

Analytic—ER & PR antibody clones, detection, FDA status, automation.

Interpretation—1% positive cells, semiquantitation of percent positive cells with intensity.

Controls—both +/−

Postanalytic: correlation with morphology, metrics for ER+/PR+, ER+/PR−, ER−/PR+, ER−/PR−.
QUANTITATION is **KEY**

Standardization of Hormone Receptors by IHC
**Table 7: IHC ER/PgR Testing Interpretation Criteria**

<table>
<thead>
<tr>
<th>Review controls (external standard and internal normal breast epithelium if present). If not as expected, the test should be repeated and not interpreted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Provide an interpretation of the assay as either Receptor Positive, Receptor Negative or Uninterpretable.</td>
</tr>
<tr>
<td>Positive interpretation requires at least 1% of tumor cells showing positive nuclear staining.</td>
</tr>
<tr>
<td>Receptor Negative is reported if less than 1% of tumor cells show staining of any intensity.</td>
</tr>
<tr>
<td>Receptor Uninterpretable is reported if the assay controls are not as expected or the preanalytic or analytic conditions do not conform to the guideline and there is no tumor staining in the absence of normally stained intrinsic epithelial elements.</td>
</tr>
<tr>
<td>Report the percentage of cells with nuclear staining using either estimation or quantitation. Quantitation may be done either by image analysis or manually.</td>
</tr>
<tr>
<td>Entire slide should be reviewed to assess the tumor containing areas. Cytology samples with limited tumor cells and little tumor staining must have at least 100 cells counted.</td>
</tr>
<tr>
<td>Report an average intensity of tumor cell nuclei recorded as strong, moderate, weak.</td>
</tr>
<tr>
<td>A score may be provided if the scoring system is specified.</td>
</tr>
<tr>
<td>Quantitative image analysis is encouraged for cases with low percentages of nuclear staining to improve quantification or in cases with multiple observers in the same institution. It is also a valuable way to quantify intensity and assure day to day consistency of control tissue activity.</td>
</tr>
</tbody>
</table>
Controls (high protein expression, low-level protein expression, negative protein expression, internal elements, ideally from included normal breast tissue included with sample)

<table>
<thead>
<tr>
<th>Adequacy of sample for evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td>*Results</td>
</tr>
<tr>
<td>- Percentage of invasive tumor cells and/or DCIS exhibiting nuclear staining</td>
</tr>
<tr>
<td>- Intensity of staining: Strong, medium, weak</td>
</tr>
<tr>
<td>- Interpretation:</td>
</tr>
<tr>
<td>- Positive (for ER or PgR receptor protein expression), Negative (for ER or PgR protein expression), Uninterpretable.</td>
</tr>
<tr>
<td>- Internal and external controls: Positive, Negative, Not present</td>
</tr>
<tr>
<td>- Standard assay conditions met/not met</td>
</tr>
<tr>
<td>- Optional score and scoring system</td>
</tr>
<tr>
<td>- Comment: Should explain reason for uninterpretable result and/or any other unusual conditions, if applicable. Should also provide correlation with histologic type of the tumor. May provide information about laboratory accreditation</td>
</tr>
</tbody>
</table>

Report should contain those elements with * as minimum.
Table 8b: Reporting elements for ER and PgR IHC Assays

<p>| *Patient identification information |  |
| *Physician identification |  |
| *Date of service |  |
| *Specimen site and type |  |
| *Specimen identification (case and block number) |  |
| Fixative |  |
| Time to fixation |  |
| Duration of fixation |  |
| Staining Method Utilized: |  |
| Primary antibody and vendor |  |
| Assay details and other reagents/vendors. |  |
| References supporting validation of assay (note: most commonly, these will be published studies performed by others which the testing laboratory is emulating). |  |
| Status of FDA approval. |  |
| Controls (high protein expression, low-level protein expression, negative protein expression, internal elements, ideally from included normal breast tissue included with sample) |  |</p>
<table>
<thead>
<tr>
<th>Year</th>
<th>ER+</th>
<th>ER-</th>
<th>TOTAL</th>
<th>% ER+</th>
</tr>
</thead>
<tbody>
<tr>
<td>2008</td>
<td>699</td>
<td>163</td>
<td>862</td>
<td>81.1%</td>
</tr>
<tr>
<td>2009</td>
<td>729</td>
<td>158</td>
<td>887</td>
<td>82.2%</td>
</tr>
<tr>
<td>2010</td>
<td>731</td>
<td>158</td>
<td>889</td>
<td>82.2%</td>
</tr>
<tr>
<td>2011</td>
<td>755</td>
<td>144</td>
<td>899</td>
<td>84.0%</td>
</tr>
</tbody>
</table>
Scoring Immunostained Slides

Proportion Score (PS)

0 = 0
1 = 1/100
2 = 1/10
3 = 1/3
4 = 2/3
5 = 1

Intensity Score (IS)

0 = negative
1 = weak
2 = intermed
3 = strong

Total Score (TS) = PS + IS (range 0-8)
**Example of Scoring**

PS = 4 (1/3rd to 2/3rd positive cells)

IS = 2 (average intensity “intermediate”)

TS = 6/8
ESTROGEN/PROGESTERONE IMMUNOHISTOCHEMICAL REPORT

Using tissue fixed 8-96 hours in 10% neutral buffered formalin with appropriate positive and negative controls, the test for the presence of these hormone receptor proteins is semi-quantitated as indicated below.

ER: ___ Percent cells staining as: (0 ___%; 1+ ___%; 2+ ___%; 3+ ___%)

PR: _____ Percent cells staining as: (0 ___%; 1+ ___%; 2+ ___%; 3+ ___%)

Estrogen receptor antibody ___, an IVD, is performed using the ___ detection on the ____.

Progesterone receptor antibody _____, an IVD, is performed using the _____ detection on the ____.
ER and PR IHC Score

• ER and PR results can be made objective using an H-score like method
  – Percentage cellular staining for intensity levels 0, 1+, 2+, 3+
  – Example
    • 0: 10%; 1+: 20%; 2+: 20%; 3+: 50%

\[ 0 + 20 + 40 + 150 = 210 \]
ER IHC vs RTPCR

- **Concordance between semiquantitative immunohistochemical assay and oncotype DX RT-PCR assay for estrogen and progesterone receptors.** (80 cases)
- **97% ER percent positive agreement with IHC; 93% PR.**
- **Semi-quantitative immunohistochemical assay versus oncotype DX(®) qRT-PCR assay for estrogen and progesterone receptors: an independent quality assurance study.** (464 cases)
- **98 % ER percent positive agreement with IHC; 94% PR.**
- **ASCO-CAP fixation guidelines, SP1 clone, whole sections, fully automated Benchmark platform, 1% cutoff, semiquantitative H Score method.**
Prediction of the Oncotype DX recurrence score: use of pathology-generated equations derived by linear regression analysis
Molly E Klein, David J Dabbs, Yongli Shuai, Adam M Brufsky, Rachel Jankowitz, Shannon L Puhalla, and Rohit Bhargava

- [http://path.upmc.edu/onlineTools/mageeEquations.html](http://path.upmc.edu/onlineTools/mageeEquations.html)
- **New Magee equation 1**: Recurrence score = 15.31385 + Nottingham score*1.4055 + ERIHC*(-0.01924) + PRIHC*(-0.02925) + (0 for HER2 negative, 0.77681 for equivocal, 11.58134 for HER2 positive) + tumor size*0.78677 + Ki-67 index*0.13269.
- **New Magee equation 2**: Recurrence score = 18.8042 + Nottingham score*2.34123 + ERIHC*(-0.03749) + PRIHC*(-0.03065) + (0 for HER2 negative, 1.82921 for equivocal, 11.51378 for HER2 positive) + tumor size*0.04267.
- **New Magee equation 3**: Recurrence score = 24.30812 + ERIHC*(-0.02177) + PRIHC*(-0.02884) + (0 for HER2 negative, 1.46495 for equivocal, 12.75525 for HER2 positive) + Ki-67*0.18649.
Digital Image Platforms

The Key to Quantitation of IHC?
<table>
<thead>
<tr>
<th>ER Analysis</th>
<th>Region Score</th>
<th>Slide Score (1 ROI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Positivity:</td>
<td>94</td>
<td>94</td>
</tr>
<tr>
<td>Number Nuclei:</td>
<td>48</td>
<td>48</td>
</tr>
<tr>
<td><strong>Staining Intensity</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0:</td>
<td>6%</td>
<td>6%</td>
</tr>
<tr>
<td>1:</td>
<td>13%</td>
<td>13%</td>
</tr>
<tr>
<td>2:</td>
<td>48%</td>
<td>48%</td>
</tr>
<tr>
<td>3:</td>
<td>33%</td>
<td>33%</td>
</tr>
<tr>
<td><strong>Scoring</strong></td>
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<td></td>
</tr>
<tr>
<td>H Score:</td>
<td>205</td>
<td>205</td>
</tr>
<tr>
<td>Proportion Score (PS):</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Intensity Score (IS):</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Allred Score (TS):</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td><strong>RESULT:</strong></td>
<td>POSITIVE</td>
<td></td>
</tr>
</tbody>
</table>
Quantitative determination of estrogen receptor, progesterone receptor, and HER2 mRNA in formalin-fixed paraffin-embedded tissue—a new option for predictive biomarker assessment in breast cancer.


- Comparing RT-kPCR results with standard IHC, we found a good concordance for ESR1 (agreement: 98.4%), PGR (84.4%), and HER2 (89.8%).
A New Molecular Predictor of Distant Recurrence in ER-Positive, HER2-Negative Breast Cancer Adds Independent Information to Conventional Clinical Risk Factors
Martin Filipits, Margaretha Rudas, Raimund Jakesz, et al.
Clin Cancer Res 2011;17:6012-6020. Published OnlineFirst August 1, 2011

- EndoPredict (EP), RTPCR for 8 genes: BIRC5, UBE2C, DHCR7, RBBP8, IL6ST, AZGP1, MGP, and STC2 used to generate an EP risk score (s) (1-15).
- Development: 964 pts (training set); Validation set: 2 prospective trial trials of ABCSCG (1764 pts) (Rx adjuvant endocrine therapy only).

EP clin = .35*t + 0.64*n + .28 s

EPclin had c-indices of 0.788 and 0.732 and resulted in 10-year distant recurrence rates of 4% and 4% in EPclin low-risk and 28% and 22% in EPclin high-risk patients in ABCSG-6 (P < 0.001) and ABCSG-8 (P < 0.001), respectively.

- we reach level I evidence according to Simon and colleagues because our study consisted of 2 category B studies using archived samples from 2 similar but separate prospective clinical trials.
Digital Image Platforms

The Key to Quantitation of IHC? CO$T
Problematic Issues with Technology in the United States

• Corporate “science”
• Pollution of clinical trials groups-NSABP, ECOG, etc
• Competing interests of physicians
• Independently verified?
• Cost
THANK YOU!