

A comparison of estrogen receptor (ER) measurement by three methods in node-negative, estrogen receptor (ER)-positive breast cancer: ligand binding (LB), immunohistochemistry (IHC), and quantitative RT-PCR

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Background: A number of methodologies have been developed to measure ER expression. To determine both the correlations among methods and the relationship of each to distant recurrence, we compared quantitative measurement of ER gene expression by RT-PCR and measurement of ER protein by LB and IHC.

Material and Methods: Patients with node-negative, ER-positive breast cancer who were treated with tamoxifen in NSABP B-14 were studied. LB (fmol/mg) was performed by the study sites at enrollment. Quantitative expression of ER was measured by the pre-specified 21-gene *Oncotype DX*TM assay on a scale from 0 to 15 (relative to the reference genes), where a one-unit increment is associated with a 2-fold change in expression. IHC was performed by NSABP using the DakoCytomation ER/PR pharmDxTM and quantitative image analysis, and reported as % cells, intensity, and % cells x intensity. Cox models were used to evaluate the association between levels of ER and distant recurrence-free interval (DRFI).

Results: There were 297 patients treated with tamoxifen who had analysis by all three methods. Within this ER-positive cohort, the Pearson correlation of LB with either IHC or RT-PCR was fair (R = 0.3 and 0.4, respectively). The Pearson correlation of IHC with quantitative PCR was moderate (R = 0.5). The table below summarizes the associations of ER with DRFI.

ER Measures and Hazard Ratios for DRFI			
ER Measures*	Hazard Ratio	95% CI for HR	p-value
Ligand Binding (fmol/mg/100)	0.86	0.70, 1.08	0.191
IHC % cells (%/50)	0.63	0.43, 0.94	0.022
IHC intensity (score/500)	0.32	0.15, 0.71	0.005
IHC % cells x intensity (value/50000)	0.44	0.21, 0.89	0.023
Quantitative RT-PCR (expression/6)	0.14	0.07, 0.29	<0.0001

*All ER measures were rescaled so that they have comparable dynamic range

Discussion: The hazard ratios for the association of ER measured by all three methods and DRFI were less than 1.0, indicating, as expected, that higher ER expression was associated with longer DRFI in node-negative patients with ER-positive breast cancer. LB was not significantly associated with DRFI. IHC was significantly associated with DRFI. The quantitative RT-PCR measure of ER expression, as measured by the *Oncotype DX* assay, had the strongest association with DRFI.