

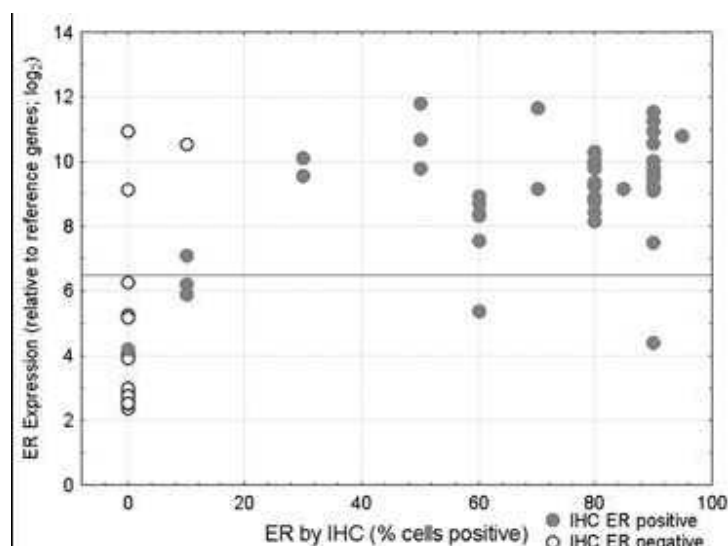
Comparison of ER, PR, HER2, and Ki-67 Quantitative Expression in Formalin Fixed, Paraffin-Embedded Breast Carcinomas by RT-PCR with Protein Expression by Immunohistochemistry

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Background: A new multi-gene quantitative assay of mRNA in fixed paraffin embedded tissues (OncoType DX) can accelerate the practical application of gene expression analysis in clinical oncology. We recently completed a study to identify candidate genes that predict recurrence in 78 patients with invasive breast carcinoma and 10 or more positive lymph nodes (Cobleigh et al, ASCO 2003). Here, we report the correlation between RT-PCR analysis of gene expression and immunohistochemistry.

Design: Patients with invasive breast cancer and 10 or more positive nodes diagnosed from 1979 to 1999 were identified. RNA was extracted from three 10 μ sections. Expression was quantified for 185 cancer-related genes using RT-PCR, and normalized relative to the expression of 5 reference genes. Primers and probes were specifically optimized to detect small RNA fragments present in fixed paraffin-embedded tissues.

Results: The relationship between ER expression by RT-PCR and ER expression by immunohistochemistry is shown (1 unit = 2-fold change in expression). The concordance is high; Kappa = 0.75 (95% CI, 0.60, 0.90) with cut-point of 6.5 units.



A similar high concordance between RNA analysis and immunohistochemistry was shown for HER2 (Kappa = 0.67; 95% CI, 0.46, 0.88; cut-point 11.5 units) and for PR (Kappa= 0.40; 95% CI, 0.20, 0.61; cut-point 5.5 units). There was no significant concordance between RT-PCR analysis of Ki-67 and the MIB-1 immunohistochemistry (Kappa = 0.15; 95% CI, -0.06, 0.37). Nor did MIB-1 immunohistochemistry correlate with expression by RT-PCR of other proliferation genes, such as PCNA and topoisomerase 2. Additional larger studies are ongoing to evaluate the utility of quantitative RNA analysis by RT-PCR for ER, PR, HER2, and proliferation genes in standard clinical assays.

Conclusions: Quantitative RT-PCR analysis of RNA expression of biomarkers in formalin fixed, paraffin-embedded tissue is feasible, even in samples more than 20 years old.