Detection of HER-2/*neu* Gene Amplification in Breast Cancer Using a Novel Polymerase Chain Reaction/Ligase Detection Reaction Technique

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BACKGROUND: Gene amplification is the primary mechanism of HER-2/*neu* overexpression in breast cancer and is a strong predictor of prognosis. Currently screening for HER-2/*neu* gene amplification in breast cancer is done by fluorescent in-situ hybridization (FISH), which is accurate but costly and labor intensive. We have evaluated a new PCR (polymerase chain reaction)-based assay for the detection of HER-2/*neu* gene amplification in human breast cancer.

STUDY DESIGN: A total of 15 breast cancer cell lines and 14 breast cancer specimens were evaluated. HER-2/*neu* status of the tumors was evaluated by FISH and then assessed using a quantitative polymerase chain reaction/ligase detection reaction (PCR/LRD) technique.

RESULTS: Amplification of the HER-2/*neu* gene was detected in seven cell lines previously reported to have amplification and no amplification was found in any of the six that had been reported not to have amplification. In the assessment of breast specimens the PCR/LDR and FISH assays were in complete agreement. All 10 tumors with amplification by FISH were also amplified by PCR/LDR.

CONCLUSIONS: The PCR/LDR technique successfully detects HER-2/*neu* gene amplification in clinical breast cancer specimens and shows 100% concordance with FISH. This technique is an accurate and rapid alternative to FISH with the potential for automation and high throughput analysis of HER-2/*neu* status in breast cancer. (J Am Coll Surg 2003;197:419-425. © 2003 by the American College of Surgeons)

HER-2/*neu* is a 185-kd transmembrane growth factor receptor with intrinsic tyrosine kinase activity.[1,2] Overexpression of HER-2/*neu* occurs in 25% to 30% of human breast cancers and is associated with early recurrence and mortality.[3] Recently completed phase III trials have revealed the efficacy of monoclonal anti-HER-2/*neu* antibody therapy (Herceptin, Genentech) in the treatment of patients with advanced breast cancer,[4] demonstrating the utility of HER-2/*neu* not only as a prognostic marker but also as a therapeutic target.

The pivotal role played by HER-2/*neu* in breast cancer progression and treatment underlies the need for the development of new strategies for the analysis of this oncogene in clinical specimens. The current standard for primary screening of HER-2/*neu* expression in breast cancer is immunohistochemistry.[4] The introduction of kits approved by the Food and Drug Administration has helped to standardize immunohistochemical assays; this methodology remains limited by differences in fixation and staining technique as well as by the subjectivity of its interpretation.[5-9]

Although immunohistochemical analyses attempt to quantify levels of HER-2/*neu* protein expression, evidence suggests that assays designed to detect the phenomenon of gene amplification represent a superior approach to HER-2/*neu* analysis in breast cancer. HER-2/*neu* protein overexpression has long been known to be the result of gene amplification in breast cancer and,

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