Quantitative Immunohistochemistry of Estrogen Receptor in Breast Cancer

"Much Ado About Nothing!"

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Abstract: "The value of a clinical test should be assessed in the overall context of disease management." The ultimate goal of an assay for detection of estrogen receptor (ER) content in breast cancer tissue is to identify patients who will or will not benefit from endocrine therapy. In the past 2 decades, scenarios for ER testing of patient samples have shifted from tissue homogenate-based, biochemical ligand-binding assays to the more practical and clinically relevant slide-based immunohistochemical methods. Although the superiority of the predictive value of ER-immunohistochemistry (ER-IHC) over ligand-binding techniques has been established to everyone's satisfaction, there remains the controversial issue of quantitation of immunohistochemical results. The assumption that ER-IHC should be quantitative stems largely from the fact that the old biochemical assay results were numerical. Seasoned immunohistochemists, nevertheless, know that IHC of routinely fixed and processed tissue does not yield itself to accurate quantitation of results, even when performed by well-qualified laboratories. Furthermore, in the case of ER, immunohistochemical methods only identify a segment or epitope of ER protein that is immunologically reactive with the used antibody. Hence, as it is, an immunohistochemical technique gives no information about the functional status of ER molecule, and/or that of the complex downstream ER pathways. This may be one of the reasons why one-third of patients with ER-positive breast cancers initially, and another one-third eventually, do not respond to endocrine treatment modalities. In this review, I attempt to present an argument that is based on our current information; quantitation of ER-IHC is neither technically reliable nor clinically relevant.

Key Words: breast cancer, estrogen receptor, immunohistochemistry, quantitative immunohistochemistry

(*Appl Immunohistochem Mol Morphol* 2008;16:105–107)

Received for publication October 26, 2007; accepted October 29, 2007.
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WHY QUANTITATION OF IMMUNOHISTOCHEMISTRY IS NOT RELIABLE?

Immunohistochemical localization of a protein in routinely fixed and processed clinical specimens is affected by numerous preanalytical factors; the standardization of which is inordinately difficult, if not outright impossible. The multitude of factors that in one way or another impact the immunohistochemical results are related to the nature of tissue itself and the way it is handled from the time of surgical excision to immunohistochemical staining. Some of these variables include the type and consistency of the tissue, the prefixation periods of warm and cold ischemia, type of fixative, duration of fixation, thickness of tissue block, type and temperature of processing method, and the time lapse between microtomy and use of slide for immunohistochemistry (IHC).

Add to that, the many analytical factors inherent to the immunohistochemical technique itself: the type, affinity, and specificity of the primary antibody, the nature, duration, and temperature of antigen retrieval step, the sensitivity of the detection technique, and the overall reproducibility of results. Not surprisingly, pathologists have always been aware of the limitation of IHC in providing quantitatively reproducible signals even in duplicate runs of the same tissue block.

IS THERE A NEED FOR QUANTITATION OF ESTROGEN RECEPTOR-IHC?

Since the switch to IHC from the ligand-binding estrogen receptor (ER) assays, there has always been the assumption that ER-IHC results should also be quantitative.[1–7] This has lead to the introduction and utilization of various scoring systems to semiquantitate ER-IHC signals. The presupposition, however, that there is a direct linear relationship between the quantity of ER in tumor cells and immunohistochemically detectable antigen has proved to be unrealistic, as it very much is dependent on preanalytical factors and the overall sensitivity of the system.

I suggest that the scoring of ER-IHC is not necessary for 2 main reasons; first, the technical variability of ER detection, and second, the natural biology of ER expression in breast cancer. With regard to the preanalytical and analytical factors, it has been clearly demonstrated that the ER-IHC scores vary considerably

as a result of fixation,[8–10] antigen retrieval,[11] and the overall sensitivity of the system.[12–14] In other words, the ER-IHC scores are not reproducible because of the effects of a host of technical and analytical factors. For the same reason, it will be naive to assume that the ill effects of technical variables could be corrected by the use of computer-assisted quantitative imaging of the final staining results.

Second, recent observations have documented that when preanalytical factors are well controlled, the expression of ER in most breast cancers is bimodal; tumor cells are either uniformly positive, or clearly negative.[10,15,16] Variability in ER staining, either in intensity or in proportion of positive cells is infrequent, and when it happens, it is usually due to poor preservation of antigen; for example, inadequate fixation.[10] The observation of any-or-none ER-IHC results is further supported by the concordance of ER expression in fine needle or core biopsies with subsequent excision specimens.[16–18] This implies that no matter how small the sample, its ER status is representative of the whole tumor.

DOES QUANTITATION OF ER-IHC HAVE AN IMPACT ON MANAGEMENT OF PATIENTS WITH BREAST CANCER?

The simple answer to the above question is no! Current observations support the notion that although the presence or absence of ER in a breast cancer is a major factor in clinical decision making, the amount of it is not.[19] Studies have shown that even when less than 1% of tumor cells are ER-positive, patients benefit from endocrine therapy.[4,20] Therefore, the simple "positive" or "negative" reporting of ER-IHC is not only practical and reproducible, but is also clinically relevant in predicting the response to endocrine therapy.[20]

A legitimate concern could be raised that when the assay's sensitivity is incorrectly low, a tumor with few minimally reactive cells will be reported as negative, hence depriving the patient from appropriate therapy. This highlights the importance of strict adherence to quality assurance measures, including interlaboratory validation of results, to ensure optimal sensitivity and specificity of ER-IHC. In addition to good laboratory practices, the pathologists could also use other measures to gauge the sensitivity of their ER-IHC at the microscopic level. For example, the best indicator of an adequate technical sensitivity is the heterogeneous expression of ER by normal or non-neoplastic mammary epithelium in the same tissue block.[10] When these benign elements are absent, the histologic type and nuclear grade of the tumor may in turn be used as important indicators of accurate results. This is because the immunohistochemical expression of ER is reliably predictable in certain histologic types of breast cancer; in the absence of such correlation technical problems should be suspected.[10] Although most typical examples of tubular, lobular, colloid, and papillary carcinomas of breast are ER-positive, the medullary, apocrine, and metaplastic carcinomas are ER-negative. Furthermore, among ductal carcinomas of no special type, those with low nuclear grades are usually positive, whereas high nuclear grade tumors are less likely to contain ER.[10] Finally, because the presence of progesterone receptor (PR) in mammary carcinomas is believed to be a surrogate marker for an intact and functional ER molecule, all PR-positive tumors should be ER-positive.[10] Therefore, an ER-negative, PR-positive result in breast cancer—but not in gynecologic cancer—must be regarded erroneous. The ER-negative, PR-positive IHC reporting is not an infrequent laboratory error; in most instances it is due to inadequate tissue fixation, which may lead to the loss of "fixation-sensitive" ER epitopes.

IS THE ER-IHC THE LAST WORD IN PREDICTION OF ENDOCRINE RESPONSE IN BREAST CANCER?

Although at the present time detection of ER expression by IHC is considered to be the best available test, it is by no means the most clinically relevant one. Over two-thirds of patients with ER-positive tumors either, initially or eventually, do not respond to endocrine therapy. Factors responsible for ER-positive, endocrine-refractory tumors could be summarized into 2 general categories: tumor-determinants, and germline-determinants or patient-determinants.

Tumor factors are all cellular biomolecules that play major roles in the kinetics of complex ER signal transduction.[21] They include the integrity of the receptor molecule, its sensitivity to the ligand, ER splice variants that may be constitutively active, ER efficiency in mobilizing the estrogen response elements, and in recruiting coactivators and corepressors leading to transcription of genes involved in cell proliferation.[21] In some ER-positive breast cancers, the lack of response to endocrine therapy may also be the result of ER activation through nonligand pathways. To that end, several growth factors and receptors, including epidermal growth factor and human epidermal growth factor receptor 2 have been shown to stimulate ER activity through phosphorylation and in the absence of estrogen.[21]

The germline-determinants or patient-determinants of response to endocrine therapy are generally those related to the individual's genomic variations, such as single nucleotide polymorphisms (SNPs) in genes encoding drug metabolizing enzymes. The drugs currently used for endocrine therapy in breast cancer are selective ER modulators and antiestrogens such as aromatase inhibitors. Members of these drug groups are metabolized by the cytochrome family of enzymes. For example, tamoxifen—the prototype selective ER modulators—is oxidized by cytochrome CYP2D6 to its active metabolite, 4-hydroxy-tamoxifen that is functionally about 100-fold more potent ER antagonist than tamoxifen.[22] Studies have shown that up to 10% of women carry SNPs in CYP2D6 that renders the enzyme inactive. Naturally, these patients will not benefit from treatments that include tamoxifen.[23] Similarly, aromatase inhibitors exert their effect by blocking aromatase enzyme—CYP19—that

normally converts androgens to estrogens. There is evidence that a significant proportion of patients carry CYP19-SNP variations that limit aromatase activity and hence, these patients derive no benefit from aromatase inhibitors.[24]

CONCLUSIONS

Today, we use IHC—a technique that does not yield itself to reliable quantitation—to measure an analyte, ER, which may or may not be biologically active in breast cancer. We then semiquantitate the staining results by setting arbitrary thresholds and assume that our numerical report will have an impact on patient's response to endocrine therapy. As it turns out, although the presence or absence of ER in breast cancer is of certain predictive value, the amount of it is not.

We have to remind ourselves, therefore, that at the present time the ER-IHC is only the *best available* predictive test for breast cancer. The search is on, nevertheless, to identify and validate clinically relevant biomolecular profiles that could guide clinicians to a more personal and tailor-made approach to the management of each patient with breast cancer.[25] Until that time we have to accept ER-IHC for what it is and not for what we would like it to be.

ACKNOWLEDGMENTS

The author thanks the constructive comments by Drs Christian Wunsch and Clive Taylor.

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