Bimodal Frequency Distribution of Estrogen Receptor Immunohistochemical Staining Results in Breast Cancer

An Analysis of 825 Cases

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Abstract

Immunohistochemical analysis is used routinely to determine the estrogen receptor (ER) status of breast cancers in paraffin sections. However, lack of standardization has raised concerns that weakly ER+ tumors often are classified erroneously as ER–. To determine the frequency of weakly ER+ tumors, we reviewed ER immunostains of 825 breast cancers. For each case, we estimated the proportion of ER+ tumor cells and also determined an Allred score (which results in scores of 0 or 2 through 8, based on staining intensity and proportion of positive cells). In 817 cases (99.0%), tumor cells showed complete absence of staining or staining in 70% or more of the cells. Similarly, 818 cases (99.2%) exhibited Allred scores of 0 or of 7 or 8. Thus, with the immunohistochemical method used in our laboratory, ER staining is essentially bimodal. The overwhelming majority of breast cancers are either completely ER– or unambiguously ER+, and cases with weak ER immunostaining are rare.

The use of immunohistochemical analysis to assess the estrogen receptor (ER) status of breast cancers in paraffin sections is now a routine part of pathology practice worldwide.[1] Although ER status as determined by immunohistochemical analysis has been shown to be a prognostic factor for patients with breast cancer, the major goal of determining ER status in current clinical practice is to assess the likelihood of response to hormonal therapy.[2] In this regard, several studies have indicated that ER by immunohistochemical analysis is not only predictive of response to endocrine therapy but also that its ability to predict such responses is superior to that of ER status as determined by ligand-binding assays.[3-5]

Despite the widespread use of this procedure, the lack of standardization of methods, scoring, and threshold for ER positivity has raised concerns that a substantial minority of patients is being misclassified with regard to the ER status of their tumors when immunohistochemical analysis performed on paraffin sections is used for this purpose. There has been particular concern that weakly ER+ tumors are erroneously being categorized as ER– and that this in turn results in such patients being denied potentially beneficial antiestrogen therapy.[6,7] It has been our experience that weakly ER+ tumors with the ER immunohistochemical method used in our laboratory are distinctly uncommon. To address this issue in a formal manner, we reviewed all ER immunohistochemical stains performed in our laboratory during a 2-year period. The results of this analysis indicated that in more than 99% of the cases we studied, the ER staining results were completely negative or unequivocally positive and that weakly positive cases are encountered only infrequently.

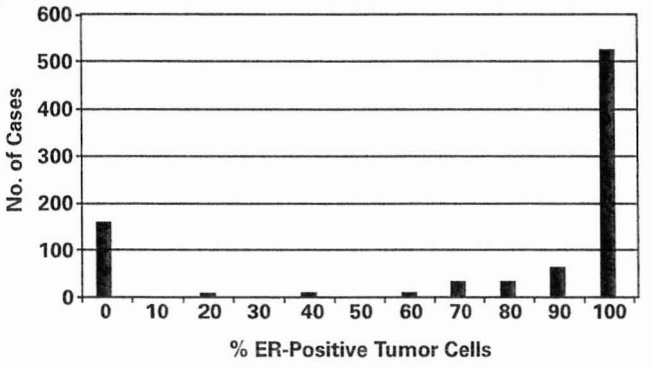

Figure 1 Frequency distribution of the percentage of cells showing nuclear staining for estrogen receptor among 825 primary breast cancers.

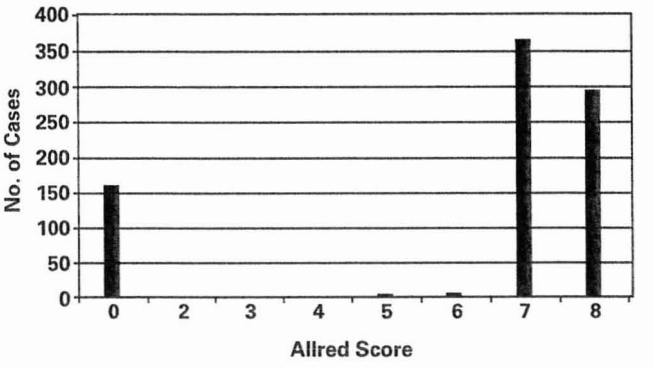

Figure 2 Frequency distribution of Allred scores among 825 primary breast cancers.

cases, the Allred score was 5 in 3 cases and 6 in 4. All 7 of these cases were in-house core needle biopsy specimens.

Discussion

The results of this analysis of more than 800 ER immunostains performed on primary invasive breast cancers from a 2-year period indicate that with the anti-ER antibody and the method used for ER immunohistochemical analysis in our laboratory, the distribution of ER staining results was bimodal and that cases with weak ER staining are encountered only rarely. In particular, in more than 99% of cases we studied, the tumors were completely ER– or unequivocally ER+. There were only a few cases in this series, accounting for approximately 1% of the study population, in which the proportion of ER+ cells was other than 0 or more than 70% or in which the Allred score was something other than 0 or 7 or 8. The results of the present study are similar to those reported by Nadji et al.[8] In that study, almost 6,000 breast cancers were evaluated for ER expression by immunohistochemical analysis with the same anti-ER antibody used in the present study (clone 1D5). These authors found, as we did, that most tumors were uniformly ER+ or completely ER–. In particular, 92.0% of the ER+ tumors showed diffuse, intense immunoreactivity. ER staining was more variable in 8.0% of their ER+ cases, but in most of those cases, this was attributable to inadequate fixation or tumor necrosis.

Until fairly recently, ligand-binding assays, such as the dextran-coated charcoal assay, were the standard methods used to determine the ER status of breast cancers, and the results of such assays were reported in a quantitative manner (as fmol/mg of protein). Studies of ER status using these assays revealed a broad range of values for ER content among ER+ breast cancers. As a consequence of this experience with biochemical assays, there had been (and still remains) the expectation that immunohistochemical assays for ER should result in a similar distribution of results, with a similarly broad range of values among ER+ cases. In fact, numerous studies have advocated the use of computer-assisted image analysis to quantify the ER content of breast cancers so that results of immunohistochemical assays could be reported in a manner analogous to that of the ligand-binding assays.[9-11] However, this approach presupposes that there is a direct, linear relationship between the amount of ER present in the tumor cells and the amount of ER antigen detected by immunohistochemical analysis. While some studies certainly have suggested such a direct relationship, the association between the actual quantity of ER protein in the tumor cell nuclei and the apparent amount of ER antigen demonstrated by immunohistochemical assays is highly complex and may be as much a function of preanalytic factors (such as details of tissue fixation and processing) and assay sensitivity as of the actual amount of antigen present in the tumor cells.[12-18]

A number of recent studies that support this contention are particularly noteworthy. Rhodes et al,[15] based on data obtained from 66 laboratories participating in a United Kingdom external quality assurance program, found that ER immunohistochemical staining results were highly affected by the efficiency of the antigen-retrieval step and that this was, in fact, the single most important factor contributing to interlaboratory reproducibility.

Goldstein et al[16] noted that immunohistochemical staining results for ER were highly dependent on the time of tissue fixation. With the assay used in their laboratory, the minimum fixation time for optimal ER immunohistochemical staining results was 6 to 8 hours, regardless of specimen type or size. Of note in that study, underfixation had a more detrimental effect on immunohistochemical staining results than did overfixation.[16]

Vassallo et al[18] performed immunohistochemical assays for ER on 20 invasive ductal carcinomas using 2 anti-ER antibodies

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of Harvey et al[5] and Barnes et al.[3] The only cases we report as ER– are those in which there is complete absence of tumor cell staining for ER.

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