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Recommended Citation

Qureshi, A., Pervez, S. (2010). Allred scoring for ER reporting and it's impact in clearly distinguishing ER negative from ER positive breast cancers. *Journal Pakistan Medical Association*, 60(5), 350-353.

Available at: http://ecommons.aku.edu/pakistan_fhs_mc_pathol_microbiol/212

Original Article

Allred scoring for ER reporting and its impact in clearly distinguishing ER negative from ER positive breast cancers

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Abstract

Objective: To determine the scoring of Estrogen Receptor (ER) status in carcinoma breast by Allred method that is essentially bimodal and to compare the results with a conventional scoring system.

Materials and Methods: A retrospective, comparative study carried out at Aga Khan University Hospital Section of Histopathology over a period of 18 months i.e. Jan 2005 to June 2006. Anti ER antibody (clone D07) was used for all IHC stains using envision detection system. ER stains of 860 consecutive breast cancer cases were reviewed and rescored by both conventional and Allred method of ER scoring.

Results: Comparison of results showed that there was a substantial decrease in weak positive cases from 18% to 5% by rescoring using Allred scoring system compared to conventional scoring. The data was analyzed using chi square test.

Conclusion: The sensitivity and specificity of Allred method were calculated; Sensitivity of Allred method was 99.4% & Specificity of Allred method was 99.5% whereas sensitivity and specificity of conventional method was 88.0 % and 84 % respectively (JPMA 60:350; 2010).

Introduction

Estrogen receptor is a regulator of mammary epithelial growth, proliferation and differentiation whose complex cellular interactions are mediated by a magnitude of ligands, cofactors and other stimuli.¹

The use of immunohistochemistry (IHC) to assess the estrogen receptor (ER) status of breast cancer in formalin fixed and paraffin embedded sections is now a routine practice worldwide.² Although ER status as determined by IHC analysis has been shown to be a prognostic factor for patients with breast cancer, major aim of determining the ER receptor status is to assess predictive response to hormonal therapy.^{3,4} Carcinoma of breast is the most common malignancy in

women in Karachi, Pakistan and various scoring systems for ER and PR are being used at different centres.⁵ However in spite of its widespread use, lack of standardized scoring and standardization of threshold for ER positivity has raised concerns that a subset of patients is being misclassified with regard to their ER status. There has been particular concern that weakly ER positive tumours may erroneously be categorized as ER negative resulting in turn being denied potentially beneficial anti estrogen therapy⁶ or vice versa.

It is because of the weak positive group, Allred scoring system was introduced in various university hospitals in North America to minimize the borderline cases and to put them into either positive or negative groups. Allred scoring reduces the

borderline or weak positive groups remarkably.

It is clear that most important factors in ER IHC are pre analytical which include fixation time, processing quality, antigen retrieval, clone of antibody used and detection system. In the post staining scenario, interpretation of staining in terms of number of cells stained and intensity of staining become important to conclude whether a particular slide is classified as positive or negative. In the conventional scoring system described by Mc Carthy and also adopted by us, slides were scored negative, weak positive, intermediate positive and strong positive. Current literature however suggests that scoring of ER into weak, intermediate and strong positive is at times misleading. This is based on experience that if pre analytical factors are controlled, ER is either un- equivocally positive or negative.

Materials and Methods

Study Population:

ER stains of 860 consecutive breast cancer cases over an 18 month period (Jan 2005-june 2006) reported in the section of histopathology, Aga Khan University hospital were reviewed.

ER Immunohistochemical Analysis:

IHC for ER was performed on formalin fixed paraffin embedded tissue sections as part of the routine clinical evaluation of these cases using anti-ER antibody (clone D07, DAKO) by using Envision system for detection. A positive control sample consisting of invasive breast cancer known to express ER was included with each staining batch. Inbuilt control i.e. normal breast tissue was evaluated for ER staining wherever included with tumour. For negative control primary antibody was replaced by normal buffer. Those cases where no normal breast tissue was present an additional control on the top end of the slide was applied.

Scoring for ER Immunostains:

(a) Conventional scoring: The immunohistochemical localization of ER was scored in a semi quantitative fashion incorporating both the intensity and the distribution of specific staining as described by Mc Carthy, Jr et al.⁴ The evaluations were recorded as percentages of positively stained tumour cells in each of the five intensity categories denoted as zero (no staining), 1+ (weak but detectable), 2+ (mildly distinct), 3+ (moderately distinct) and 4+ (strong). For each tissue a value designated as HSCORE was derived by summing up the percentages of cells staining at each intensity multiplied by the weighted intensity of staining. An HSCORE of less than 50 was established as negative, between 51 to 100 as mild (weak positive), 101 to 200 as moderate (intermediate positive),

while 200 and more as strong positive.⁴

(b) Allred scoring: In addition, we determined for each case an Allred score which is semi quantitative system that takes into consideration the proportion of positive cells (scored on a scale of 0-5) and staining intensity (scored on a scale of 0-3). The proportion and intensity were then summed to produce total scores of 0 or 2 through 8. A score of 0 -2 was regarded as negative while 3 - 8 as positive (Figure-1).^{3,4} Idea conceived from original paper.⁴

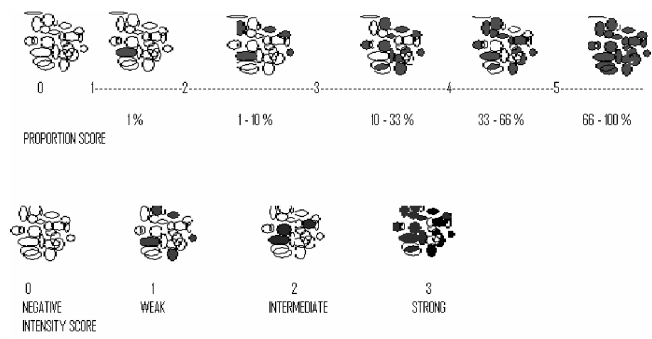


Figure-1: Diagrammatic representation of Interpretation of Allred Score.

Results

These 860 cases studied for ER immuno-stains included core needle biopsies, lumpectomies, mastectomies and wide local excision biopsy specimens. Of these, 767 (89%) cases were infiltrating ductal carcinomas, 60 (7%) were infiltrating lobular carcinomas and 33 (4%) were minor variants of breast cancer.

♦ The frequency distribution of ER immunohistochemical results based on estimated percentage of tumour cells by conventional methods showed 457 (53%) to be completely negative, 251 (29%) were intermediate to strong positive and 152 (18%) were weak positive (Figure-2).

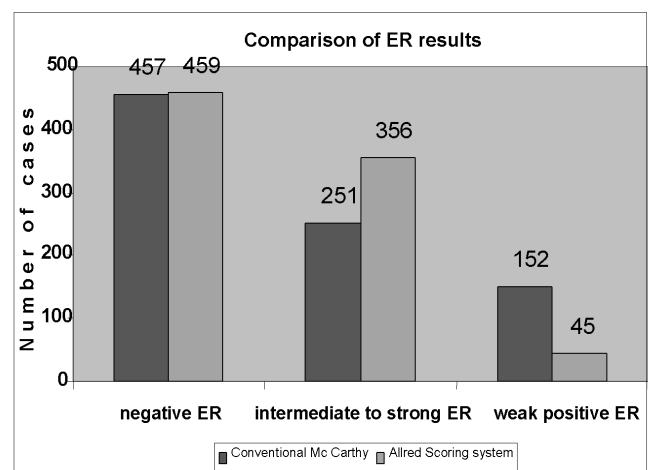


Figure-2: Comparison of ER results, conventional versus Allred.

Table: Guidelines for interpretation of ER results by Allred Method.

Proportion Score (PS)	Observation	Intensity Score (IS)	Observation
0	NONE	0	None
1	1%	1	Weak
2	1-10%	2	Intermediate
3	10-33%	3	Strong
4	33-66%		
5	66-100%		
Total Score			Interpretation
Sum of proportion score and intensity score			
0-2			Negative
3-8			Positive

◆ These cases were rescored according to Allred score which showed 459 (53%) tumors to be completely negative (SCORE 0), 356 (42%) to be intermediate to strong positive (SCORE 5-8) and only 45 (5%) to be weak positive (SCORE 3-4) a reduction of 13% when compared to conventional scoring (Figure-2).

Statistical Analysis:

Conventional scoring technique by visual inspection (as explained earlier by McCarthy) was taken as gold standard and results Allred were compared with that to calculate the sensitivity and specificity with the help of 2 x 2 table.

Sensitivity of Allred method = 99.4%

Specificity of Allred method = 99.5%

Sensitivity of conventional score= 88 %

Specificity of conventional score= 84%.

Discussion

The results of ER immunostaining performed at our laboratory by conventional method, clearly indicate that the results were essentially trimodal with a very broad ER weakly positive band. However rescoring done by Allred method has markedly decreased the weakly positive band upgrading them into ER intermediate to strong positive cases. This was further strengthened by the high sensitivity and specificity values. The results of our study are comparable to Najdi et al⁹ in which 6000 breast cancer cases were evaluated by immunohistochemical analysis. These authors found that most tumours were either unequivocally ER positive or ER negative and any discrepancy to this was attributed to pre analytical factors like inadequate tissue fixation.¹⁰⁻¹²

Although the results of this study demonstrate that weakly ER positive tumours are rare using the method employed in our laboratory other studies have clearly shown that there is considerable inter laboratory variation in the identification of tumours with lower levels of ER expression.^{13,14} It could therefore be argued that it might be difficult to generalize the results of our study to other

institutions.^{15,16} Our results are a function of careful attention to technical details of the assay, use of appropriate control samples and a high index of suspicion when unexpected results are encountered.^{17,18}

Taken together the result of all this highlights the role of pre-analytical factors and assay details in determining the distribution of immunohistochemical results in any given population. In our view, justification is difficult for the routine use of quantifying the ER Immunihistochemical results in clinical practice.

In current clinical practice once a case is considered to be ER positive the degree of ER positivity has no impact on recommendations for the use of hormonal therapy. The results of this study demonstrate that weakly ER positive tumors are rare using the Allred method compared to conventional scoring methods.^{19,20} ER is viewed by clinicians as dichotomous rather than a continuous variable when assessing patient suitability for anti estrogen therapy. Use of Allred scoring gives a very clear message to clinicians with regard to ER positivity versus negativity.^{21,22} Weak positive staining report in the conventional scoring in contrast give the message of equivocality to the clinician with a potential risk of deprivation of anti estrogen therapy or otherwise.^{23,24}

Conclusion

Our data suggest that by using Allred scoring the ER staining results will be essentially bimodal i.e. completely negative or unequivocally positive. If pre analytical factors are controlled there should be very few cases which are weak ER positive and these should be considered as positive for treatment purposes. ER negative should be reserved only for those cases which show complete absence of staining.

References

- Anderson E, Clarke RB, Howell A. Estrogen responsiveness and control of normal human breast proliferation. *J Mammary Gland Biol Neoplasia* 1998; 3: 23-35.
- Elledge RM, Green S, Pugh R, Allred DC, Clark GM, Hill J, et al. Estrogen receptor (ER) and progesterone receptor (PgR), by ligand-binding assay compared with ER, PgR and pS2, by immuno-histochemistry in predicting response to tamoxifen in metastatic breast cancer: a Southwest Oncology Group Study. *Int J Cancer* 2000; 89: 111-7.
- Clark GM. Prognostic and predictive factors. In: Harris JR, Lippman ME, Morrow M, Hellman S, eds. *Diseases of the Breast*. Philadelphia: Lippincott-Raven, 1996; pp 461-85.
- Collins LC, Botero ML, Schnitt SJ. Bimodal frequency distribution of Estrogen Receptor immunohistochemical staining results in breast cancer. *Am J Clin Pathol* 2005; 123: 16-20.
- Nisa A, Bhurgri Y, Raza F, Kayani N. Comparison of ER, PR and Her/2/neu reactivity pattern with histologic grade, tumor size and lymph node status in breast cancer. *Asian J Cancer prevention* 2008; 9: 553-6.
- Allred DC, Bustamante MA, Daniel CO. Immunocytochemical analysis of estrogen receptors in human breast carcinomas. Evaluation of 130 cases and review of the literature regarding concordance with biochemical assay and clinical relevance. *Arch Surg* 1990; 125: 107-13.
- Taylor CR. Paraffin section immunocytochemistry for estrogen receptor: the time has come. *Cancer* 1996; 77: 2419-22.
- Allred DC, Harvey JM, Berardo M, Clark GM. Prognostic and predictive

- factors in breast cancer by immunohistochemical analysis. *Mod Pathol* 1998; 11: 155-68.
9. Barnes DM, Harris WH, Smith P, Millis RR, Rubens RD. Immunohistochemical determination of oestrogen receptor: comparison of different methods of assessment of staining and correlation with clinical outcome of breast cancer patients. *Br J Cancer* 1996; 74: 1445-51.
 10. Pertschuk LP, Feldman JG, Kim YD, Braithwaite L, Schneider F, Braveman AS, et al. Estrogen receptor immunocytochemistry in paraffin embedded tissues with ER1D5 predicts breast cancer endocrine response more accurately than H222Sp gamma in frozen sections or cytosolbased ligand-binding assays. *Cancer* 1996; 77: 2514-9.
 11. Harvey JM, Clark GM, Osborne CK, Allred DC. Estrogen receptor status by immunohistochemistry is superior to the ligandbinding assay for predicting response to adjuvant endocrine therapy in breast cancer. *J Clin Oncol* 1999; 17: 1474-81.
 12. Rhodes A, Jasani B, Barnes DM, Bobrow LG, Miller KD. Reliability of immunohistochemical demonstration of oestrogen receptors in routine practice: interlaboratory variance in the sensitivity of detection and evaluation of scoring systems. *J Clin Pathol* 2000; 53: 125-30.
 13. McCann J. Better assays needed for hormone receptor status, experts say. *J Natl Cancer Inst* 2001; 93: 579-80.
 14. Esteban JM, Ahn C, Mehta P, Battifora H. Biologic significance of quantitative estrogen receptor immunohistochemical assay by image analysis in breast cancer. *Am J Clin Pathol* 1994; 102: 158-62.
 15. Sahin AA, Ordonez N. Quantitative estrogen receptor immunohistochemical analysis of breast cancer: advantages and potential problems. *Adv Anat Pathol* 1996; 3: 97-100.
 16. Lehr HA, Mankoff DA, Corwin D, Santeusanio G, Gown AM. Application of Photoshop-based image analysis to quantification of hormone receptor expression in breast cancer. *J Histochem Cytochem* 1997; 45: 1559-65.
 17. Leong AS-Y, Milios J. Comparison of antibodies to estrogen and progesterone receptors and the influence of microwaveantigen retrieval. *Appl Immunohistochem* 1993; 1: 282-8.
 18. Von Wasielewski R, Mengel M, Nolte M. Influence of fixation, antibody clones, and signal amplification on steroid receptor analysis. *Breast J* 1998; 4: 33-40.
 19. Santeusanio G, Mauriello A, Ventura L, Liberati F, Colantoni A, Lasorella R, et al. Immunohistochemical analysis of estrogen receptors in breast carcinomas using monoclonal antibodies that recognize different domains of the receptor molecule. *Appl Immunohistochem Mol Morphol* 2000; 8: 275-84.
 20. Rhodes A, Jasani B, Balaton AJ, Barnes DM, Anderson E, Bobrow LG, et al. Study of interlaboratory reliability and reproducibility of estrogen and progesterone receptor assays in Europe: documentation of poor reliability and identification of insufficient microwave antigen retrieval time as a major contributory element of unreliable assays. *Am J Clin Pathol* 2001; 115: 44-58.
 21. Layfield LJ, Goldstein N, Perkinson KR, Proia AD. Interlaboratory variation in results from immunohistochemical assessment of estrogen receptor status. *Breast J* 2003; 9: 257-9.
 22. Goldstein NS, Ferkowicz M, Odish E. Minimum formalin fixation time for consistent estrogen receptor immunohistochemical staining of invasive breast carcinoma. *Am J Clin Pathol* 2003; 120: 86-92.
 23. Eifel P, Axelson JA, Costa J, Crowley J, Curran WJ Jr, Deshler A, et al. National Institutes of Health Consensus Development Conference Statement: adjuvant therapy for breast cancer, November 1-3, 2000. *J Natl Cancer Inst* 2001; 93: 979-89.
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