Assessment of Tissue Estrogen and Progesterone Receptor Levels: A Survey of Current Practice, Techniques, and Quantitation Methods

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Abstract: The assessment of steroid hormone receptors in resected breast carcinoma tissue is currently the standard of practice. The traditional method for assessment of receptor status is the ligand binding assay. More recently, immunohistochemistry (IHC) has become a popular method for such testing. Despite the widespread use of IHC and the availability of many antibodies, standardization of quantitative IHC for assessment of estrogen and progesterone receptors has not been achieved. While the College of American Pathologists (CAP) offers a Quality Assurance (QA) program for IHC quantitation of estrogen receptor (ER) and progesterone receptor (PgR), no universal standard is currently recognized in assessment of ER and PgR by IHC. We surveyed 300 laboratories within the United States for their current practices regarding the assessment of ER and PgR status in breast cancer tissue specimens. Eighty usable responses were received. Forty-nine (61%) laboratories performed the assay in-house, while the remainder sent the material out for assay. All responding laboratories performing their steroid receptor analysis in-house used the IHC technique. Forty-three (80%) laboratories answering the question on material accepted for analysis performed the assay only on paraffin-embedded material, three (6%) used either paraffin block or frozen material, and two (4%) used only frozen material. Eighty-eight percent of laboratories performing steroid receptor analysis in-house used a manual quantitation technique. Four (8%) used computer-assisted image analysis, and a single laboratory used laser scanning cytometry. Eight different antibodies were used among the 44 laboratories documenting the antibody supplier, and for any given commercially prepared antibody a wide variety of dilutions were used, with the exception of the standard solution used with the Ventana antibody. Of the laboratories using manual estimation techniques, 61% simply estimated the percentage of positive cells, 29% evaluated both the intensity of staining and percentage of nuclei staining, 6% used formal H-score analysis, 2% evaluated only intensity of nuclear staining, and 2% mainly counted the percentage of nuclei staining for ER but used a formal H score in the assessment of PgR. Cutoff points for the separation of positive and negative results varied widely, with some laboratories assessing any demonstrable positivity as a positive result, while others required as many as 19% of the nuclei to stain before a specimen was declared positive. Standardization techniques differed considerably among laboratories. Eighty-six percent used the CAP program for QA. While all laboratories utilized some form of intralaboratory control for assessment of ER and PgR, the nature of that control varied from laboratory to laboratory. Our survey indicates that a majority of laboratories perform their steroid hormone receptor

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analysis in-house using IHC. There is considerable variability in the antibodies utilized, the dilutions applied, and the quantitation method and level of expression used to dichotomize specimens into positive and negative groups. Finally, no universal control for interlaboratory standardization appears to exist. ■

Key Words: breast carcinoma, estrogen receptor, progesterone receptor, steroid hormone assay

Estrogen receptor (ER) and progesterone receptor (PgR), by their interaction with their respective steroid hormones play important roles in regulating the proliferation and differentiation of normal breast epithelium (1). The level of steroid hormone receptor expression in breast carcinoma cells is believed to be associated with the responsiveness of the neoplastic cells to circulating estrogen and progesterone. During the past quarter century, many studies have measured tissue levels of ER and PgR by biochemical methods and correlated them with both prognosis and response to hormone therapy (2–5). Within the past decade, a variety of antibodies against both the ERs and PgRs have become available on a commercial basis. Many studies have compared the results of immunohistochemically determined steroid receptor values with those obtained by ligand binding analysis. In general, the correlation has been good (6–11). Despite agreement of results derived from individual antibodies used in immunohistochemistry (IHC) determinations and the ligand binding technique, significant variability has been documented in the results obtained by IHC using different commercially available antibodies (12,13). While variability between the results achieved by different antibodies may exist, the overall value of IHC-determined ER and PgR levels for the prediction of response to hormonal therapy and overall prognosis appears high (9,14–16). Some studies have documented IHC determination of ER to be superior to the ligand binding assay for the prediction of response to adjuvant endocrine therapy in breast cancer (17).

Despite the documented value and accuracy of ER and PgR assayed by IHC, a wide variety of antibodies and quantitation techniques are currently in use. The extent to which these variabilities in technique and material affect the predictive value and standardization of IHC determination of ER and PgR is unknown. Of significance, there is no universally accepted control for standardization of the assays of ER and PgR by IHC. Hence interlaboratory comparisons of steroid receptors as determined by IHC may not be entirely valid. The authors are aware of only a single quality assurance/quality control (QA/QC) program within the United States for IHC determination of steroid receptors, again bringing into question the overall consistency of results obtainable between laboratories. Despite such problems, the overall robustness of the method appears to compensate for the lack of precision associated with the technique. Several laboratories are working to refine protocols and standardization methods and Riera et al. (18) have recently proposed tissue cell culture lines as a universal control.

In order to clarify the current status of ER and PgR level determinations, we surveyed 300 laboratories to determine their methods and interpretative approach for the estimation of steroid hormone receptors in breast tissue and whether they take part in a regional or national QA/QC program in this field. Herein we report the results of that survey.

MATERIALS AND METHODS

Questionnaires were mailed with return addressed and stamped envelopes to 300 hospital pathology departments. The first 200 questionnaire recipients were drawn from the College of American Pathologists (CAP) directory by randomly selecting four pathologists from each state. An additional 100 pathologists with interest and expertise in the area of breast pathology were selected on the basis of a literature search for publications concerning estrogen and progesterone receptors.

The questionnaire contains questions relating to size and type of hospital practice. The questionnaire asked if they routinely ordered ER and PgR assays on newly diagnosed breast carcinomas and whether this analysis was done in-house or if it was sent out. If they performed in-house analysis, questions about the method used [IHC, dextran-coated charcoal (DCC) assay, polymerase chain reaction (PCR), or flow cytometry], type of material accepted [paraffin embedded, frozen tissue, or fine needle aspiration (FNA)], type of antibody, and dilution employed were included. The pathologists were also asked how quantitation was performed (image analysis, manually calculating the nuclei staining percentage, estimating the intensity of staining, H score), what the cutoff point was for differentiating positive and negative results, what protocol was used for standardization, and what controls were used. Lastly, the laboratories were asked whether they participated in any of the interinstitutional QA programs and, if so, which one.

In addition, 150 questionnaires were mailed with return addressed and stamped envelopes to hematology/

oncology departments in all 50 states. One hundred and ten oncologists were program directors at teaching hospitals. The remaining were randomly selected oncologists based at community hospitals. The questionnaire contains questions relating to the method used by the laboratory for the analysis of ER and PgR, the quantitation method, and the cutoff point used to separate positive and negative results. The questionnaire asked whether their treatment approach changed following a switch from DCC to IHC, whether they equated immunohistochemical expression of ER and PgR to specific femtomol values, and whether they required quantitation or merely positive and negative results. The oncologists were also asked whether they were influenced in choice of therapy by PgR status, or whether they gave tamoxifen regardless of steroid hormone receptor status to all postmenopausal patients. They were also asked if ER and PgR status had ever changed during treatment. Finally, their opinion regarding current recommendations (19,20) on chemotherapy and endocrine therapy and the duration of such treatment (21) (2 years versus 5 years) was sought.

Following mailing of the survey questionnaire, 3 months were allowed to pass before closure of the data collection period, allowing for adequate response time. The responses were entered on a spreadsheet program (Excel 7.0, Microsoft, Redmond, WA) and analyzed.

RESULTS

Responses were received from 80 of the 300 questionnaire recipients, geographically representing 35 states. Thirty (38%) were obtained from institutions describing themselves as community/general hospitals, seven from nonacademic tertiary care centers, 41 from academic tertiary care centers, 1 from a reference laboratory, and 1 did not indicate the type of institution. Respondents were almost equally distributed between hospitals with fewer than 250 beds (22), hospitals between 250 and 500 beds (26), and hospitals with more than 500 beds (29). Three respondents did not state the size of their institution.

Seventy-seven of the 80 (96%) responding practices routinely performed ER and PgR analysis on both in situ and invasive carcinoma of the breast. Two performed it only on invasive carcinoma, and a single respondent did not indicate his practice pattern. Both institutions performing ER and PgR analysis only on invasive carcinomas were community hospitals. Forty-nine of the 80 (61%) respondents performed steroid receptor hormone analysis in their own laboratories, 30 routinely sent tissue out for such analysis, and 1 laboratory performed in-house IHC but sent out tissue to have DCC ligand binding assay performed on some specimens. Table 1 shows the distribution of institutions performing steroid receptor analysis by hospital type. Academic tertiary care hospitals were most likely to perform the assays within their institution. Similarly, hospitals with more than 500 beds were more likely to perform steroid receptor analysis in their own laboratories. Community/general hospitals were most likely to send out ER and PgR analyses (23; 77%). A wide variety of academic tertiary care medical centers and specialty commercial laboratories were used for referral of tissue for steroid hormone analysis.

All institutions performing ER and PgR analysis in-house used immunohistochemistry. A single institution performed in-house IHC but sent out material for ligand binding assay in selected cases. Forty-three (80%) of the institutions answering the question on tissue acceptable for analysis performed ER/PgR analysis only on paraffin tissue, 6 (11%) performed the analysis on paraffin-embedded and FNA material, 2 (4%) performed the assay on paraffin-embedded, frozen, and FNA material, while 2 (4%) performed the assay only on frozen material. One (2%) respondent performed the analysis on paraffin-embedded and frozen material.

Methods of quantitation varied among the laboratories responding to our survey. Forty-two (88%) used a manual counting method for quantitation, 4 (8%) used computer-aided image analysis, and 1 (2%) used laser scanning cytometry. One additional laboratory (2%) used computer-assisted image analysis between 1988 and 1998 but recently changed to a manual technique. Of the laboratories utilizing a manual counting method for quantitation of ER and PgR levels, 30 (61%) manually counted the number of tumor cell nuclei staining positively and calculated a staining percentage. Fourteen respondents (29%) used both the percentage of positive nuclei and the intensity of staining. Three laboratories (6%) used formal H-score analysis. One laboratory (2%) measured only the intensity of staining. A single

Table 1. Hospital Type

Type	Number	Percentage
Community/general	30	37.5
Nonacademic tertiary	7	8.75
Academic tertiary	41	51.25
Reference laboratory	1	1.25
No response	1	1.25
Total	80	100

Table 2. Suppliers of Antibodies Used for ER Analysis by Responding Laboratories

Type	Number	Percentage
Dako	17	39
Ventana	15	34
Novacastra	3	7
AMAC	2	5
Immunotech	4	9
Abbott	1	2
Biogenex	1	2
Zymed	1	2
Total	44	100

Table 3. Range of Dilutions Used

ER	
Dako	5–2,000
Ventana	Predilution
AMAC	50–500
Novacastra	40–200
Immunotech	50–400
PR	
Dako	20–160
Biogenex	25–350
Novacastra	50–100

laboratory manually counted the nuclei staining percentage for ER but employed the H-score technique for PgR analysis.

Table 2 shows the different commercial suppliers of antibodies used for IHC analysis of ER and PgR. Antibodies were supplied by eight different companies, and antibody dilutions varied considerably, as indicated in Table 3. Cutoff points for separation of positive and negative results varied widely among laboratories. Even when the H-score system was used, the cutoff point was not uniform. Table 4 shows the cutoff points reported by the respondents. Some laboratories accepted any visually detectable staining as indicative of positive ER while others required the nuclei staining percentage to be as high as 20% before a tissue specimen was considered positive. Two laboratories (4%) did not interpret the results as either positive or negative, but simply estimated the nuclei staining percentage and intensity of staining present, leaving interpretation to the clinicians.

Protocols for standardization are listed in Table 5. There was considerable variation, with reliance on manufacturer protocols, DCC validation, or CAP survey. The controls used for standardization were also inconsistent among laboratories, with the majority of institutions (42; 74%) using known positive and negative cases as their controls. Table 6 shows the types of controls used by the responding institutions. Sixty-nine of the 80 institutions responding (86%) took part in the CAP program. The remaining 11 institutions did not take part in any QA/QC program.

Only 26 (17%) useful responses were received from the 150 questionnaires sent out to directors of hematology/oncology programs (representing 17 states). All 26 respondents routinely ordered steroid hormone receptor analysis on all newly diagnosed breast carcinomas. One of these did not routinely request such an analysis on intraductal carcinoma. Twenty-five respondents believed the technique used for the analysis was IHC, while one received information from the ligand binding (DCC)

Table 4. Criteria Used to Separate Positive and Negative Results

	Number	Percentage
10% positive cells	15	34
5% positive cells	12	28
Any positivity	4	9
Combination formula (number of cells and intensity)	3	7
<9% = negative, 9–19% = borderline, >19% = positive	2	4
Different criteria for ER and PgR	2	4
20% ER, 5% PgR		
20% ER, 10% PgR		
Number and intensity supplied for clinician interpretation	2	4
Combination of number and intensity		
10% positive with ≥2 (scale of 1–4)	1	2
H score of 50	1	2
H score of 10	1	2
San Antonio score		
1–2 = negative, 3 = borderline, 4–8 = positive	1	2
Intensity (SI 0–3), PP = percentage 0–4, IS = SI × PP		
0–1 = negative, 2 or more = positive	1	2
Total	45	100

Table 5. Protocols Used for Standardization

Protocol	Number
Positive and negative controls	3
DCC validation	3
Ventana automated stainer	2
Dako protocol	2
Do not know (DNK)	1
In-house standardized protocol (Techmate Instrumentation) and standardized commercial reagents	1
Parallel testing for new kits and new antibody lot	1
CAP survey	1
Modified Techmate	1
All slides reviewed by the director	1
Ventana/microwave antigen retrieval	1
No protocol	1
DCC and reference laboratories	1
HIER target antigen retrieval (Dako)	1
CAS 2000	1
Manufacturers' guidelines/journals/textbooks	1

analysis. Of the 25 individuals experiencing a shift in analytic technique from DCC to IHC, only one changed their treatment approach because of the modification in technique. Thirteen of 25 (52%) hematologists/oncologists equated negative or low IHC values of ER and PgR with specific femtomol values. The other 12 did not directly correlate IHC results with femtomol levels.

In agreement with the results of the survey of pathologists, there was considerable variation in the cutoff points used by hematologists and oncologists to separate positive from negative ER results. These values ranged from 1 to 30%. Four hematologists/oncologists (18%) did not know the value used for stratifying ER results into positive and negative. Table 7 shows the distribution of cutoff points used by the responding hematologists and oncologists. Eleven respondents required quantitation in their practice, while 16 required only a statement of positive or negative. Thirteen of 25 respondents agreed with the recommendations for treatment of early breast cancer as stated in the *British Journal of Cancer* (19) and in the *Review of Seminars in Oncology* (20).

Table 7. Distribution of Thresholds for Establishing ER Positivity Used by Hematologists/Oncologists

Threshold	Number	Percentage
No response	7	26
10%	5	19
Do not know	4	15
10 fmol	4	15
5%	2	7
30%	2	7
1%	1	4
20% = negative, <30% = borderline, >30% = positive	1	4
Laboratory does it	1	3
Total	27	100

Sixteen of 25 (64%) responding hematologists/oncologists would not treat an ER-negative carcinoma in a postmenopausal patient with tamoxifen. Twenty-three of 27 responding hematologists/oncologists stated that they were influenced in their treatment decisions by the presence or absence of PgR positivity in the neoplasm. Twenty-five of 26 hematologists/oncologists stated that they had modified their treatment plans based on the ER or PgR status of the patient. Finally, 20 of 27 responding hematologists/oncologists stated that they had not changed the duration of treatment after publication of the Swedish Breast Cancer Cooperative Group results in *Journal of the National Cancer Institute* (21).

DISCUSSION

Since the recognition of the relationship between ER and PgR levels in breast carcinoma and patient prognosis and response to hormonal therapy, the assessment of steroid hormone receptors has become a widely accepted component in the examination of breast carcinomas (23). Initially assessment of ER and PgR levels was performed using a ligand binding technology (DCC) (2–5). More recently, ER and PgR assessment by IHC has become popular, if not the predominant technique. Many

Table 6. Type of Controls Used for ER and PgR Analysis

Control	Number	Percentage
Known positive and negative cases	42	71
Internal and external breast tissue control	9	17
EIA assayed breast cancers	1	2
Endometrium	1	2
ER, breast; PgR, endometrium	1	2
Abbott ER-ICA, PgR-ICA control slides for frozen and cytology	1	2
Control cases with H < 10, 10–100, and >100	1	2
Multitumor blocks	1	2
Total	57	100

studies have correlated the results of IHC with those obtained by the ligand binding method (6–12), and have confirmed the relationship of ER and PgR with patient prognosis and response to hormonal therapy (1,14–17). Despite the near uniformity in finding a high correlation between IHC and ligand binding assay results and good predictive value for IHC, these studies have used a variety of antibodies, antibody dilutions, staining and quantitation techniques, and cutoff points (23). The variation within the literature is reflected within community practice. Many of the issues relating to IHC determination of steroid hormone receptor levels reflect basic issues in quantitative IHC as discussed by a panel of experts during a recent meeting of the European Section of the International Academy of Pathology (Nice, France, October 1998).

In order to assess the current status of ER and PgR assay methods in the United States, we undertook a mail survey investigating the methodologies, controls, quantitation techniques, and cutoff points utilized by a variety of academic and nonacademic laboratories. Our study found a wide variation in the antibodies, dilutions, quantitation techniques, and cutoff points used by the respondents. While these variations do not in and of themselves negate the clinical significance of steroid hormone receptor analysis, the existence of such variations raises the potential for clinically significant discordance in reported steroid hormone receptor values between laboratories.

Commercial and large academic medical center laboratories perform approximately 38% of all ER and PgR assays, but the majority of such assays are performed in-house by local laboratories. Nearly all of these laboratories report using IHC for the assessment of ER and PgR. A number of observations can be made on the basis of this study. First, a confounding variable for interlaboratory comparisons of steroid hormone receptor results is the variety of materials accepted by various laboratories. Eighty-one percent of laboratories accept only paraffin-embedded material for analysis, but 11% used only frozen tissue or both frozen and paraffin-embedded tissue for analysis. In addition, 4% also accepted FNA specimens. Second, various antibodies were used. The laboratories in our study employed antibodies supplied by eight different manufacturers. Seventy-three percent of institutions used an antibody supplied by either Dako (Carpinteria, CA) or Ventana (Tucson, AZ). As shown in Table 3, even when using the same antibody, various institutions employed widely different antibody dilutions for their assays. These differences in antibodies and dilutions may have significant impact on the quantitative assessment of ER and PgR by IHC.

Third, methods for the quantitation of IHC results varied considerably between respondents. The majority (88%) of laboratories completing the survey questionnaire used various manual techniques for quantitation in which estimates of nuclei staining percentage were made. Multiple manual quantitation methods exist in addition to simply estimating the nuclei staining percentage. Twenty-nine percent of laboratories using a manual quantitation technique employed a technique where both the number of positive cells and the intensity with which the cell nuclei stained were estimated. Six percent used formal H-score analysis (6). We did not obtain information on the threshold of staining intensity used to accept a nucleus as positive for quantitation purposes. Neither did we obtain information on cell selection techniques. Clearly differences in counting technique can affect whether a neoplasm is designated positive or negative for ER and PgR. Recommendations for cell counting have been published (11).

Fourth, the cutoff points used for the assignment of breast cancer cell populations as positive for ER or PgR differ between laboratories. Our survey documented at least a fourfold variation in the nuclei staining percentage used by laboratories for the assignment of positivity. Twelve laboratories (27%) used a 5% cutoff point to designate a specimen as positive and four laboratories (9%) used a value of 20% as their cutoff point. Fifteen laboratories (33%) used a 10% nuclear positivity rate as an indicator of a positive ER level. Variability in cutoff point results in discordance of results even when methodologic aspects of the assay are identical. Such variability in threshold for positivity highlights the need for laboratories to include cutoff points in their reports. Thresholds used with other quantitation methods (H score) also varied. A few laboratories simply assessed the number of positively staining cells and the intensity of staining present, allowing the clinicians to interpret the data. Thus wide variability in practice exists and such variability may have a significant impact on the documentation of the presence or absence of clinically significant levels of ER and PgR.

Standardization protocols varied widely among the respondents to our survey. Only 22 respondents (28%) answered our query concerning their standardization protocol. Of these, three used DCC validation as their standardization technique, while a majority relied on manufacturer's guidelines, previously tested positive and negative intralaboratory controls, or simply review

of all slides by the laboratory director. Controls for the standardization of ER and PgR assays varied among the laboratories. The majority (52%) used previously assayed tissue blocks known to be positive and negative. This offered a degree of intralaboratory consistency but did not allow external verification of laboratory assay levels. Nine laboratories utilized internal breast tissue controls for the assessment of steroid hormone receptors in associated neoplastic tissue. Occasionally laboratories utilized endometrium as a positive control. As stated by Riera et al. (18), no universal control exists for external validation of steroid hormone receptor assays by IHC. The lack of such a control complicates comparisons between laboratories using different antibodies, dilutions of antibodies, and modifications of the IHC technique. Recently, cultured cells have been suggested as a control for quantitative immunocytochemical analysis of ER levels (18). Widespread utilization of such a standard control should increase the comparability of ER results performed at different laboratories.

Only 26 responses (17%) from our survey of 150 directors of hematology/oncology programs were received, meaning conclusions based on this small dataset should be made with caution. However, certain trends were noted. First, the respondents routinely ordered ER and PgR analysis on all newly diagnosed breast carcinomas, implying general acceptance by oncologists for measurement of ER and PgR. The majority of responding oncologists did not alter their treatment approach when their laboratory switched from the DCC to the IHC methodology. Variability existed among clinicians in how they equated IHC expression to femtomol values. Approximately half of the respondents did not equate negative or low IHC values with specific femtomol values.

In agreement with our laboratory survey findings, the threshold for calling a result positive varied widely among oncologists. Values associated with a positive result by IHC varied from 1 to 30%. Of equal importance, 59% of the responding hematologists/oncologists treating breast cancer patients did not require quantitative data but merely desired a statement by the laboratory as to whether the assay was positive or negative. This finding is of particular interest in light of a recent study showing that very high levels of ER are associated with an unfavorable prognosis (22). Simply dividing ER values into positive and negative may yield incomplete and misleading information. The reporting of femtomol equivalents, nuclei staining percentage, or the stratification of results into negative, borderline, intermediate, and high levels may be more clinically useful.

There does not appear to be uniformity in approach to the interpretation and utilization of ER and PgR data by oncologists. Only 13 respondents (48%) agreed with recently published recommendations concerning the use of endocrinology and chemotherapy in patients with breast cancer (19,20). The majority of oncologists responding to our questionnaire acknowledged that ER or PgR status had modified their treatment of patient's with breast cancer (92%). Despite this reliance on steroid hormone receptor assay results, fully one-third of responding oncologists would treat a postmenopausal patient whose carcinoma was ER negative with tamoxifen. The results of the Swedish BCCG study (21) appeared to change the treatment approach of only a minority of oncologists responding to our survey (26%).

The oncologists varied significantly in the threshold they used to classify specimens as positive or negative. Reported cutoff points for positivity varied from any staining to a cutoff point of at least 30% of nuclei staining. When specific percentages were given, there was a sixfold variation in the cutoff point (5–30%). Such variability in interpretative thresholds renders interlaboratory comparisons of ER and PgR results difficult if only positive and negative assessments are reported. Interlaboratory comparisons are more easily achieved if laboratories record the nuclei staining percentage, cutoff point used, and interpretation of the results rather than simply reporting the specimen as positive or negative.

The CAP QA program is commonly used by laboratories assessing ER and PgR in breast tissue, but its level of success in ensuring interlaboratory uniformity was not assessed by this survey. Further studies into the effectiveness of this program would be of value both to pathology laboratories performing steroid hormone receptor analysis as well as to oncologists interpreting the results.

Steroid hormone receptor assay by IHC appears robust enough to maintain the correlation with prognosis established by DCC, despite the many variations discussed. This interpretation is supported by the observation that the majority of studies in the literature using variable techniques, antibodies, titers, and cutoff points still report good correlation of the IHC results with DCC assays and demonstrate good predictive and prognostic value for the test (1,6–17). As in many areas of surgical pathology, reliability may exceed reproducibility (24). This may lead some clinicians and pathologists to adopt a nihilistic approach to standardization. However, it may be that important prognostic information associated with steroid hormone receptor levels is being

concealed by the imprecision of current IHC methods. The development of a universal control and improved standardization methods should improve the validity of interlaboratory comparison of the results of ER and PgR measurement by the IHC technique.

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