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Estrogen and Progesterone Receptors in Breast Cancer: Comparison Between Enzyme Immunoassay and Computer-Assisted Image Analysis of Immunocytochemical Assay

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Evaluation of estrogen (ER) and progesterone (PR) receptor content is now an important procedure in the management of breast cancer patients. Production of monoclonal antibodies to ER and PR has permitted development of an enzyme immunoassay (EIA) and immunocytochemical assay (ICA). This study compared the results of ICA and EIA to evaluate ER and PR in 197 breast cancers using the same monoclonal antibodies. The ICA results were obtained by automated computer-assisted image analysis using CAS 200. The cut-off values adopted were 15 fmol/mg protein for EIA and 10% of the positive neoplastic area of the nuclei for ICA. For statistical analysis, Spearman's correlation coefficient and χ^2 were used. There was good correlation between ICA and EIA for both ER ($r = 0.714$; $P < 0.0001$) and PR ($r = 0.815$; $P < 0.0001$). Of 197 tumors, 136 (69.04%) were ER-ICA$^+$, and 138 (70.05%) were ER-EIA$^+$; 111 (56.35%) were PR-ICA$^+$, and 115 (58.38%) were PR-EIA$^+$. Results were concordant, positive or negative with both methods, in 175 cases for ER and in 173 cases for PR. ER and PR results were only discordant in 22 and 24 cases, respectively. Concordance of results obtained by the two methods was 88.83% ($P < 0.0001$) for ER and 87.81% ($P < 0.0001$) for PR. Correlation of results obtained by EIA and ICA to determine ER and PR was good. The data obtained suggest that ICA with automated image analysis is an effective means for evaluating ER and PR content in human breast cancer, especially when, as happens ever more frequently nowadays, the tumor is too small to perform EIA or when retrospective studies are performed.

Key terms: Estrogen receptor, progesterone receptor, breast cancer, immunocytochemical assay, enzyme immunoassay

The evaluation of estrogen (ER) and progesterone (PR) receptor content in breast cancer has become an important procedure in the management of breast cancer patients, because, together with other data, it provides information pertaining to prognosis or the therapy strategy to be employed (7,15,21). Until recently, ER and PR receptor determination was done in most instances by the dextran-coated charcoal (DCC) biochemical method (13), which has been the test most commonly used for measuring receptor content.

The production of monoclonal antibodies to ER and PR has permitted the development of an enzyme immunoassay (EIA) based on direct recognition of steroid receptor molecules; however, this method requires the homogenization of tissue samples with loss of cell integrity (4,10). For this reason, by using the same monoclonal antibodies as in EIA, the immunocytochemical assay (ICA), which allows direct recognition of positively staining nuclei with ER and PR receptors in tissue sections, was developed (8,11,16,18,19). By using ICA, pathologists can visualize the receptor status of individual tumor cells directly by light microscopy on histological sections.

A good correlation has been demonstrated between EIA or ICA and DCC for both ER and PR receptors (2,9,11,12,14,17,18,20,22). Some data are also available that assess the relationship between the results obtained

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by EIA and ICA evaluated by using semiquantitative methods (3,5,6). However, to our knowledge, no data are available so far that pertain to the relationship between the results obtained by EIA and ICA, as determined by using computer-assisted image analysis.

In this study, we compared the results of EIA with those of ICA, as evaluated by automated computer-assisted image analysis with the CAS 200, for ER and PR in 197 breast cancers using the same primary monoclonal antibodies in both methods.

Table 1
Morphologic Subtypes of Tumors

Histotype	No.	Percentage
Infiltrating ductal carcinoma	159	80.71
Infiltrating lobular carcinoma	10	5.08
Mixed ductal and lobular carcinoma	16	8.12
Medullary carcinoma	5	2.54
Mucinous carcinoma	5	2.54
Tubular carcinoma	2	1.01
Totals	197	100.00

MATERIALS AND METHODS

One hundred and ninety-seven cases of breast carcinoma, observed between January 1992 and December 1994 at the Institute of Pathological Anatomy and Histology, Perugia University, were selected for this study. These cases consisted of 195 primary breast cancers and two recurrences, all of which had sufficient neoplastic tissue for complete EIA and ICA analysis. The age of patients ranged from 26 to 85 years of age (mean 58.8 years).

Breast cancer specimens were received from the operating room 5–60 min after surgical removal from patients. When the time interval exceeded 10 min, tissue was placed in dry ice for transportation. The tissue was divided into two parts. In the first 113 cases, the surgeon divided the tumor and sent one part to the pathologist for intraoperative diagnosis and ICA and sent the other part to the Division of Medical Oncology for EIA. In the remaining 84 cases, the tumors were sent to the pathologist, who divided each tumor into at least two parts, one part for intraoperative diagnosis and ICA and the other part for EIA. ER and PR immunostaining was performed in accordance with the manufacturer's instructions using monoclonal antibodies H222 (ER-ICA kit; Abbott) for ER and KD68 (PR-ICA kit; Abbott) for PR. Briefly, 5-μm-thick frozen sections were fixed in 3.7% formaldehyde phosphate-buffered saline (PBS) solution, pH 7.4, followed by immersion in cold methanol and acetone and then incubated with normal goat serum to prevent nonspecific binding of subsequent reagents. The samples were then incubated with primary rat monoclonal antibodies to human ER and PR followed by goat antirat immunoglobulins and the peroxidase-antiperoxidase (PAP) complex of rat origin. The reaction product was made visible with the chromogen diaminobenzidine tetrahydrochloride (DAB) and hydrogen peroxide in PBS. The sections were then counterstained with ethyl green for CAS measurement, dehydrated in graded alcohols, cleared in xylene, and permanently mounted with Pertex. Appropriate positive and negative controls were done.

Computer-assisted image analysis was performed with Cell Analysis System's 200 machine (CAS 200). At least 30 random fields of the tumor at ×40 were evaluated. The results were expressed as the percentage of the positive area of neoplastic nuclei compared with the total nuclear area of the examined neoplastic cells. The cut-off value adopted was 10%. The enzyme immunoassay for ER and PR was performed using the same monoclonal antibodies as in ICA, according to the manufacturer's instructions (ER-EIA and PR-EIA; Abbott). The cut-off value adopted was 15 fmol/mg protein.

The EIA and ICA results were obtained independently. Correlation between the percentage of positive nuclear neoplastic area by ICA and receptor concentrations by EIA was analyzed by using Spearman's correlation coefficient. χ^2 Cross correlation was used to analyze the significance of concordant and discordant cases.

RESULTS

Table 1 gives the morphologic subtypes of the 197 tumors. Microscopic evaluation of the tumors showed that most positive cases were heterogeneous with regard to steroid receptor stains, because they contained both positive and negative cells. The positive cells contained nuclei in which the degree or intensity of staining was variable, resulting perhaps from the varying concentration of receptors. A good correlation was demonstrated between the percentage of positive nuclear neoplastic area by ER-ICA and the concentration of ER by EIA ($r = 0.714$; $P < 0.0001$; Fig. 1).

Table 2 shows that 136 tumors were positive by ICA (ER-ICA$^+$), and 138 were positive by EIA (ER-EIA$^+$); 126 tumors were positive by both methods, and 49 were negative by both methods. In 22 cases, the results obtained were discordant. In fact, 10 cases were ER-ICA$^+$/ER-EIA$^-$, and 12 cases were ER-ICA$^-$/ER-EIA$^+$. Concordance between the results obtained with the two methods was 88.83% ($P < 0.0001$; sensitivity = 91.30%; specificity = 83.05%). The positive predictive value and the negative predictive value were 92.64 and 80.30%, respectively.

A good correlation was also demonstrated between the percentage of the positive nuclear neoplastic area of PR by ICA and the concentration of PR by EIA ($r = 0,815$; $P < 0.0001$; Fig. 2). Table 3 shows that 111 tumors were PR-ICA$^+$, and 115 tumors were PR-EIA$^+$; 101 tumors were PR-ICA$^+$ and PR-EIA$^+$, and 72 tumors were PR-ICA$^-$ and PR-EIA$^-$. In 24 cases, the results obtained were discordant. In fact, 10 cases were ICA$^+$/EIA$^-$, and 14 cases were ICA$^-$/EIA$^+$. Concordance between the results obtained with the two methods was 87.81% ($P < 0.0001$; sensitivity = 87.82%; specificity = 87.80%). The positive and negative predictive values were 90.99 and

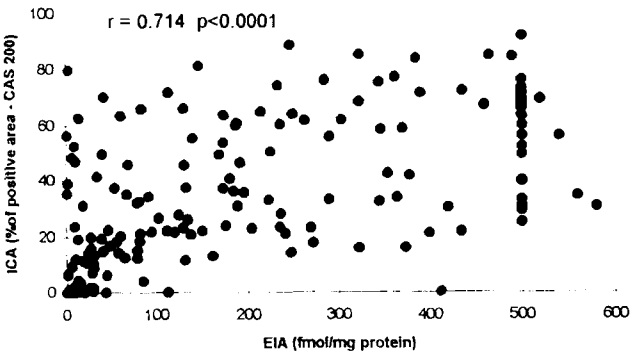

FIG. 1. Spearman's correlation coefficient: estrogen (ER). Enzyme immunoassay (EIA) vs. immunocytochemical assay (ICA).

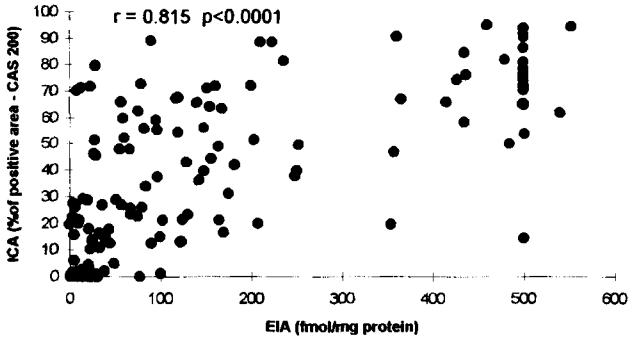

FIG. 2. Spearman's correlation coefficient: progesterone (PR). EIA vs. ICA.

Table 2
Distribution of Estrogen According to Immunocytochemical Assay (ICA) and Enzyme Immunoassay (EIA) Methods[a]

	EIA +		EIA −			
	No.	Percent	No.	Percent	No.	Percent
ICA +	126	63.96	10	5.08	136	69.04
ICA −	12	6.09	49	24.87	61	30.96
Totals	138	70.05	59	29.95	197	100

[a]Concordance, 175/197 (88.83%); $P < 0.0001$.

Table 3
Distribution of Progesterone According to Immunocytochemical Assay (ICA) and Enzyme Immunoassay (EIA) Methods[a]

	EIA +		EIA −			
	No.	Percent	No.	Percent	No.	Percent
ICA +	101	51.27	10	5.08	111	56.35
ICA −	14	7.11	72	36.54	86	43.65
Totals	115	58.38	82	41.62	197	100

[a]Concordance, 173/197 (87.81%); $P < 0.0001$.

83.72%, respectively. Table 4 gives the analysis of discrepancies between ICA and EIA results.

Estrogen Receptors

Ten tumors were ICA^+/EIA^-. Histological examination of these tumors showed that, in five cases, there was marked stromal proliferation associated with a small number of neoplastic epithelial cells. In the remaining five cases, there was no plausible explanation, suggesting the possibility of an error in sampling or improper handling of specimens. Twelve tumors were ICA^-/EIA^+. In this group, one tumor showed focal positivity of the neoplastic cells of the intraductal component using ICA methodology, and two tumors showed focal positivity of nonneoplastic ducts and lobules adjacent to or mixed with the neoplastic cells. In one case, there was nonspecific cytoplasmic staining. In the remaining eight cases, there was no plausible explanation.

Progesterone Receptors

Ten tumors were ICA^+/EIA^-. Of these, two consisted predominantly of a fibrous stromal component associated with low epithelial content. In six, there was a low percentage of the positive nuclear neoplastic area using ICA (15–27%) associated with a low staining intensity. In two cases, there was no plausible explanation. Fourteen cases were ICA^-/EIA^+. In this group, five tumors showed focal positivity of normal ducts and lobules adjacent to or mixed with the neoplastic negative cells, and, in one case, there was nonspecific cytoplasmic staining. In the remaining eight cases, there was no plausible explanation.

Table 4
Analysis of Discordant Cases

	Estrogen	Progesterone	Possible explanation
ICA^+/EIA^-			
	5	2	Low epithelial cellularity
	—	6	Low positivity at ICA
	5	2	Unexplained (inaccurate sampling?)
Totals	10	10	
ICA^-/EIA^+	1	—	Focal positivity of intraductal component at ICA
	2	5	Focal positivity of normal ducts and lobules
	1	1	Cytoplasmic staining
	8	8	Unexplained (inaccurate sampling?)
Totals	12	14	

DISCUSSION

Determining hormone receptors by using ICA in human breast cancers is now a routine practice for the identification of those patients with better prognoses who might be more likely to benefit from hormone therapy.

Several biochemical ligand-binding assays have been developed to measure receptor content, and DCC has become the most commonly used test. The production of monoclonal antibodies has permitted the development of EIA, which has substituted DCC. However, the values obtained with both methods give the average of receptor concentration of the entire specimen and do not allow one to specifically morphologically identify and confirm those cells that are positive as the malignant cells, the percentage of neoplastic cells that are positive, or, alternatively, the percentage of positive nuclear neoplastic area. For this reason, there has recently been a significant development in the use of ICA for evaluating breast cancer receptor content.

Several studies have shown good correlation between EIA and DCC (9,12,14,22) and ICA and DCC (2,11,17, 18,20). Recent data have also shown that there is a good correlation between results obtained with EIA and ICA for ER evaluation (5,6) and, more recently, for ER and PR as well (3). By using the same primary monoclonal antibodies with both methods, this study evaluated the correlation between ICA and EIA in the determination of hormone receptor content. The results obtained with ICA were evaluated by automated computer-assisted image analysis using the CAS 200. The results have shown good correlation between the percentage of the positive nuclear neoplastic area by using ICA and the concentration of receptors by using EIA for both ER ($r = 0.714$; $P < 0.0001$) and PR ($r = 0.815$; $P < 0.0001$).

There was good agreement between the results obtained by using the two methods for ER (88.83%) and for PR (87.81%). This data agrees with that from the literature that indicate an average concordance rate of 86% (range 77–96%) for DCC and ICA using frozen sections or 88% (range 82–96%) using paraffin sections (1) and with data indicating a concordance rate of 73.0–84.5% between EIA and ICA evaluated by semiquantitative methods (3).

The possible causes for discordance in the results we obtained by the two methods were also investigated. ICA^+/EIA^- tumors were, in some cases, tumors with an intense desmoplastic component and a concomitant, scanty epithelial neoplastic component, which might explain the negative results by EIA. The other discrepancies found between the two methods might have been due to improper handling of the specimens or to unsuitable samples of the tumor sent for EIA. Thus, it is possible that, at least in some cases, EIA was performed on fragments that were not shown histologically to consist solely of neoplastic tissue. In those cases, it is possible that pieces of tissue adjacent to the neoplasia were included, because the difference, which, in some cases, was up to "70% positivity of the nuclear neoplastic area at ICA" and "8 fmol/mg protein at EIA," is difficult to explain on the basis of neoplastic heterogeneity alone. This interpretation is supported by the fact that these discrepant results were observed only during the first part of the study, when the sample for EIA was sent directly from the operating room and was not chosen by the pathologist.

The discrepancies between the ICA^-/EIA^+ tumors involved low-positive EIA values that could be explained on the basis of residual ER^+ or PR^+ benign breast epithelium of ducts and lobules in the EIA specimens or by the presence in some neoplasias of a positive intraductal component at ICA. There were only two exceptions: in one case, ER = 412 fmol/mg protein; in the other case, PR = 180 fmol/mg protein. Discovering such apparently positive cases at EIA is possible, because direct microscopic examination of positive neoplastic cells can be done by using ICA. In two cases, an intracytoplasmic component was seen at ICA. Errors in sampling or improper handling of the specimens could explain other discrepancies.

In conclusion, there is good correlation between the results obtained by using EIA and ICA methods for the determination of ER and PR receptor content. The analysis of discordant data suggests that a significant proportion of all discordant results between EIA and ICA represent "false positive" and "false negative" EIA test findings; therefore, this suggests that ICA is a more specific and more sensitive test for the measurement of receptor content in breast cancer. Our data suggest that ICA, with automated image analysis, provides an effective means of evaluating ER and PR content in human breast cancers and has a great advantage over EIA, because it is able to histologically recognize and therefore evaluate only neoplastic cells and to identify the percentage of positive nuclear neoplastic area. The ICA method may also be employed for evaluating receptor status retrospectively on paraffin blocks of formalin-fixed tumors that were not evaluated by EIA, which requires fresh or frozen tissue. Finally, on frozen sections or paraffin blocks, ICA is the only method that clinicians and pathologists have to evaluate the receptor status in very small tumors, where there is not sufficient material for EIA analysis.

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