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Estrogen Receptor Analyses in Breast Cancer: Comparison of Monoclonal Immunohistochemical and Biochemical Methods

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Abstract—*The estrogen receptor content in 100 female breast carcinomas was determined using the biochemical dextran-coated charcoal (DCC) method and the monoclonal estrogen receptor immunocytochemical assay (ER-ICA). Great care was taken to make procedures optimal in order to minimize preanalytical errors. A statistically highly significant correlation was found between the results of the two methods, which were qualitatively in accordance in 91 cases, while nine gave a negative ER-ICA but a weak positive DCC result. The quantitative correlations were also statistically significant but varied considerably in individual cases. Under optimal conditions, ER-ICA provides results similar to the DCC method, but it is still uncertain if it can stand alone in routine diagnosis.*

INTRODUCTION

THE VALUE of estrogen receptor determination in predicting a positive effect of endocrine therapy for breast cancer is well established [1–3]. The conventional biochemical steroid binding assay using dextran-coated charcoal (DCC) has several limitations, some of which can be overcome using an estrogen receptor immunocytochemical assay (EA-ICA) with direct visualization of receptor localization and content. Comparisons between the biochemical and histochemical fluorescence methods for estrogen and progesterone receptors have given promising but not fully satisfactory agreement [4]. In recent years several reports comparing the results of DCC and monoclonal histochemical estrogen immunoperoxidase methods have shown better results, although mainly qualitatively [5–14].

This study was performed to establish the correlation between the results of DCC and ER-ICA in a practical setting as close to the ideal as possible in order to minimize the known preanalytical sources of errors, i.e. all material came from one surgical department to one institute of pathology in the same hospital with the shortest possible delay from resection of the tumor to freezing down tissue for immunohistochemical and biological analyses. All procedures were done by specially instructed personnel to ensure optimal selection of tumor tissue and processing. All biochemical results were evaluated and reported by one of us (K.O.P.) without prior knowledge of the corresponding immunohistochemical results and vice versa.

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MATERIALS AND METHODS

Materials

From 26 August 1986 to 1 September 1987, tumor tissue samples for biochemical and immunohistochemical analyses were collected at the Institute of Pathology from 123 consecutive cases of female breast cancer operated at the surgical department of Aalborg Hospital. For various reasons 23 cases were excluded (Table 1). The reasons for the exclusion of the last 12 cases in Table 1 were as follows: five cases used for other scientific purposes, two cases with bad quality of frozen sections, and five cases with insufficient tumor tissue. The latter originated from small tumors (mean diameter 12 mm, range 10–18 mm). Tissue for diagnostic histopathology and biochemical assay had preference. The remaining 100 breast carcinomas (81%) form the material for this investigation.

Methods

The extirpated tumors were treated as previously described [4]. At least 0.5 cm^3 of tumor freed from surrounding tissues was at once frozen in liquid

Table 1. Tumor material. Reason for exclusion

Total number of breast cancer cases	123
Excluded:	
Two primary tumors in same breast	2
Bilateral breast cancer	1
Too long transport time	5
No material for DCC	3
No material or unsuitable for ER-ICA	12
Final number of breast cancers	100

nitrogen for biochemical analysis. Control histology of the tissue slices used for the biochemical method was not done. The biochemical method used for estrogen and progesterone receptor determination was the dextran-coated charcoal method recommended by the EORTC [15, 16] involving seven-point binding and Scatchard analysis using tritiated estradiol and progesterone (TRK.322 and ORG.2058 from Amersham) covering final labelled hormone concentration ranges during cytosol incubations of 0.08–5.0 and 0.31–18.0 nmol/l, respectively. Protein determination was performed with the Bio-Rad method with Kabi Diagnostica human serum albumin as standard. Receptor concentrations were expressed relative to the protein concentration determined in the DCC-treated cytosol and considered positive when the following three criteria were met: reasonably straight Scatchard plot, dissociation constant $<10^{-9}$ mol/l and binding capacity $\geq$10 fmol/mg cytosol protein. A pooled freeze-dried calf uterus cytosol was included in each run and the monthly between-run precision of this control was 10–20%. Participation twice yearly since 1981 in the EORTC Quality Assessment of steroid receptor assays consistently showed results within one standard deviation above the mean participating laboratories.

For immunohistochemical analysis, a slice of tumor tissue, at least 0.5 cm^2 in size, was placed in tissue tape and frozen down. The immunohistochemical staining procedure for estrogen receptors was performed according to the instructions with the ER-ICA kit from Abbott on the frozen tumor tissue. A hematoxylin and eosin section was made for estimation of tumor cellularity. The histological sections were evaluated independently by two of us (S.M.P. and G.S.), and whenever disagreement on any aspect was found, the sections were reviewed in a double microscope for a final decision.

As a numerical indication of the estrogen receptor content in ER-ICA two estrogen receptor indices (ER-1I and ER-2I) were calculated. ER-1I was formed by multiplying three values: tumor cellularity (1 = <10%, 2 = 10–50%, 3 = >50% of tumor area), intensity of ER-ICA staining (0 = negative, 1 = slight, 2 = moderate, 3 = strong), and fraction of tumor cells with a positive ER-ICA staining (0 = none, 1= <10%, 2 = 10–50%, 3 = 51–80% and 4 = >80% of tumor nuclei). The range of ER-1I will be a minimum of zero to a maximum of 36. ER-2I was formed by multiplying the values for intensity and fraction of positive tumor cells. The range of ER-2I will be a mimimum of zero and maximum of 12.

The qualitative association between ER-ICA and DCC was tested by chi-square analysis, while the quantitative agreement of the two methods was statistically tested using the Spearman test of correlations.

RESULTS

All positive staining with ER-ICA was localized to the nuclei of the carcinoma cells as well as benign epithelial cells, when such were found, which is in accordance with previous results [8, 10, 11, 17, 18]. Heterogeneity of staining intensity was often seen, in which case the most predominant intensity of staining was scored. In many cases stained and unstained nuclei were seen among each other, but we never found a clear cut regional difference with one part of the tumor ER-positive and other parts completely negative.

The evaluation of tumor cellularity and fraction of positive tumor cells was very seldom a cause of disagreement. The disagreement in the evaluation of the immunohistochemical method was almost exclusively represented by tumors with low intensity of staining reaction.

As seen from Table 2, 20% of the tumors were ER-negative with DCC and 29% with ER-ICA. The overall agreement of the two methods was 91% (chi-square 61.21, $P < 0.001$). In no case did we find a positive ER-ICA and a negative result with DCC, although nine tumors with a negative ER-ICA had a positive result in the biochemical analysis. The mean biochemical ER value for these nine tumors was 24 fmol/mg cytosol protein (range 12–43 fmol/mg). Six of the nine tumors were also progesterone receptor negative, one was slightly positive (15 fmol/mg cytosol protein) and only two were definitely progesterone receptor positive by the DCC method. The histology of these nine tumors was reviewed, but no morphological similarities or explanations of the disagreement could be found.

The relationship between the two semiquantitative ER-ICA scores (ER-1I and ER-2I) and the results of the DCC analysis are shown in Figs. 1 and 2 respectively. As is readily seen there is not an absolute correlation in every individual case as to the quantity of estrogen receptor content found with the two methods. Especially in cases with a

Table 2. Qualitative correlation between ER-ICA and DCC estrogen receptor content

DCC	Positive	Negative	Total
ER-ICA positive	71	0	71
ER-ICA negative	9	20	29
Total	80	20	100

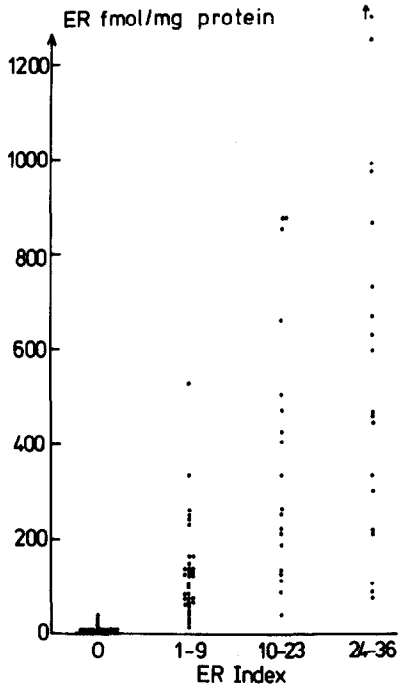

Fig. 1. The results of the semiquantitative ER-ICA score ER-1I, subdivided into a negative and three positive groups (horizontal) are compared to the results of DCC (vertical).

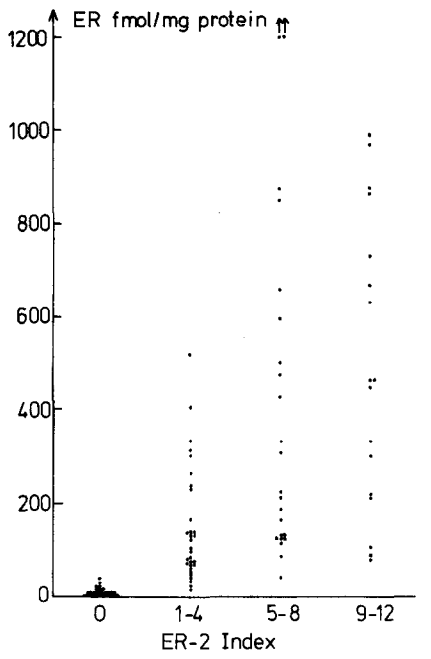

Fig. 2. The results of the semiquantitative ER-ICA score ER-2I, subdivided into a negative and three positive groups (horizontal) are compared to the results of DCC (vertical).

moderate receptor content considerable divergences between the results of the two methods are sometimes seen. The points in Figs. 1 and 2 are almost superimposable and the statistical agreement between either of the two indices and the DCC method is good ($P < 0.001$). The estimation of tumor cellularity is therefore non-contributory.

DISCUSSION

The value of estrogen receptor determination in breast carcinomas for prediction of disease-free survival, and especially for response to endocrine manipulation, is well documented for the biochemical method, and several investigations found the correlations between DCC and the response to treatment best when taking the receptor content per unit cytosol protein into consideration [1–3, 19].

As discussed in an earlier study [4], histo- and biochemical receptor determinations can hardly be expected to give identical results, as they probably measure different quantities. Nevertheless, many investigators have found a good qualitative correlation [5–10]. With ER-ICA and DCC methods the agreement has varied from 79.9% [9] to 91.5% [6]. The present work confirms the latter with an agreement of 91%. In contrast to other investigations [5–10] we had no cases with a positive ER-ICA and negative DCC. This is especially in contrast to the work of Reiner *et al.* [9], where 19% gave this combination, and might be a result of our optimal procedural conditions to avoid sampling and preanalytical errors and thereby false negative DCC results.

The rather high percentage of ER-positive tumors by the DCC method is partly due to a Danish convention of expressing results per mg cytosol protein measured after DCC treatment which induces some protein loss due to adsorption. The nine tumors with discrepant ER status all had low ER values by the DCC method and the disagreement is thus explicable by the different sensitivity of the two methods and the arbitrary low, but conventional, cut-off value of the DCC method.

The quantitative correlation between ER-ICA and DCC is more controversial [8–11, 13, 14].

Statistically we found a high degree of correlation between DCC and the results of the two semiquantative scoring systems of ER-ICA, while the results for individual cases are more difficult to compare and evaluate, but are certainly not always identical. There are several factors which are possibly involved in making a part of the individual results different. As stated above, there are methodological and probably sensitivity differences between the two methods. Receptor proteins may be lost in the processing of the tissue for immunohistochemical analysis. Common to both methods is degradative loss of receptor protein by a prolonged time of warm anoxia. In favor of the histochemical methods is the direct possibility for control, as it is possible visually to judge the intensity and distinctness of the staining of the tumor nuclei and of any background staining, and thereby appreciate the degree of tissue preservation. At the same time the visual control makes the histochemical methods overcome a serious drawback of the biochemical method: it can be directly assured that it is in fact tumor tissue that is investigated, which is not possible in biochemistry, and this fact precludes false negative results from incorrect tissue sampling.

Investigations comparing the correlations of survival and response to hormonal therapy with on one side the estrogen receptor content as determined with DCC and on the other with the results of ER-ICA are very sparse. Pertschuk *et al.* [11], in a group of 43 stage IV patients, found a significantly higher predictive value of a positive assay with ER-ICA than with DCC as concerns response to endocrine therapy (80% vs. 58%). DeSombre *et al.* [13] in their Fig. 7 found that ER-ICA and DCC had an identical power to differentiate two groups of patients with significantly different times before recurrence.

Immunohistochemical methods are now used in many departments of pathology, and the laboratory procedures and microscopical evaluation of estrogen receptor assays are not different from other routinely used immunohistochemical investigations, so their introduction in laboratories of pathology should cause only few problems. Additional advantages of immunohistochemistry are a lower cost than DCC and a rather quick service, where information of the estrogen receptor content can be given with the definitive answer of the tumor microscopy. An even better evaluation may become possible in the future, if immunohistochemical assays for progesterone receptor content and methods for immunohistochemical analysis of routinely fixed and processed tissue become available. Investigations in these fields are being done [20–25]. The latter will also make it possible to examine all tumors including those not sent for frozen sections.

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