Quantification of oestrogen receptors in breast cancer: radiochemical assay on cytosols and cryostat sections compared with semiquantitative immunocytochemical analysis

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SUMMARY A radiochemical oestrogen receptor assay on cytosol was correlated with a radiochemical and an immunohistochemical oestrogen receptor assay using cryostat sections from 50 breast cancer specimens. Oestrogen receptors were reliably quantitated in 6 μm cryostat sections with Scatchard analysis using radiolabelled oestradiol, and a good quantitative and qualitative relation with cytosol oestrogen receptor assay was found. Parallel sections were used for routine histological tissue verification and for direct comparison with immunohistochemistry for oestrogen receptor. Specific immunoperoxidase staining with a rat monoclonal antibody was scored by semiquantitative evaluation of the staining intensity of cancer cell nuclei. Oestrogen receptor scoring was highly reproducible when performed by the same observer. The semiquantitative immunohistochemical oestrogen receptor score correlated significantly better with the radiochemical assay on sections than with cytosol assay.

Oestrogen receptor in breast cancer can be reliably assayed by semiquantitative evaluation of cryostat sections immunostained for oestrogen receptor, but only if the procedure is adequately standardised. The results underline the importance of cellular heterogeneity as a cause of variation in oestrogen receptor assay evaluation in breast cancer.

Oestrogen receptor assays on cytosol preparations from malignant tissue are valuable for prognosis and for selecting patients with breast cancer for endocrine treatment.[1-3] Steroid receptors are routinely measured with radiolabelled ligands through their specific binding function. Enzyme immunoassays using monoclonal antibodies that recognise determinants on the receptor protein have also recently been applied to assess oestrogen receptor concentrations in cytosol or nuclear fractions of breast cancer biopsy specimens.[4-6] Oestrogen receptor assays on cytosols, however, do not have an optimal predictive value. A positive cytosol oestrogen receptor assay does not guarantee a positive response to oestrogen therapy: only 60% of the oestrogen receptor positive patients benefit from endocrine treatment. On the other hand, prediction of response to hormone treatment is more reliable for patients with oestrogen receptor negative tumours of whom only about 10% react favourably.[1 2] The heterogeneous nature of most breast cancer specimens

is presumably one of the main factors responsible for the limited predictive value of receptor assays on homogenised tissue, and highly variable oestrogen receptor concentrations within a biopsy specimen have been found.[7-12] In many cases of oestrogen receptor analysis the tissue composition—that is, the relative amount of connective tissue, normal tissue, or necrosis of malignant tissue—is unknown. As the receptor content is expressed in fmol/mg of protein, this may be an important source of errors.[7 10 11 13 14]

Assays of tissue sections could overcome some of the limitations of oestrogen receptor assays in homogenised neoplastic tissues.[15-18] We developed a radiochemical oestrogen receptor assay on cryostat sections of target tissues, which permits Scatchard analysis of both soluble and section bound oestrogen receptor on relatively small samples (50–80 mg of tissue).[19] With this technique, parallel sections can be used to verify tissue composition or to perform histochemical and immunohistochemical correlative studies.

The development of specific anti-oestrogen receptor

monoclonal antibodies has permitted the visualisation of oestrogen receptor with immunohistochemical techniques,[20][21] and several published reports describe the semi-quantification of the immunohistochemical reaction.[22-24] A correlative study was recently reported in which oestrogen receptor immunohistochemical analysis and a radioligand binding assay on adjacent breast cancer sections were applied,[25] In a similar study we compared an oestrogen receptor assay with radiolabelled oestradiol and semi-quantitative histochemical detection with a monoclonal antibody using parallel cryostat sections of breast cancer tissue.

Material and methods

TISSUE SAMPLES
Tissue samples from 50 surgically removed primary breast carcinomas were selected and histologically typed in the surgical pathology division of our department. Immature bovine uterus was used as standard tissue for the radiochemical oestrogen receptor section assay. The tissue samples were rapidly frozen in isopentane cooled with dry ice ($-70°$ C) and stored at $-80°$ C.

Each tumour block was divided into five adjacent parts. The first two parts were used for diagnostic purposes. The third and fifth part were homogenised for the preparation of cytosol; the fourth part, adjacent to the blocks for the cytosol assay, was used for the preparation of cryostat sections.

Serial 6 μm frozen sections were used for radiochemical detection of oestrogen receptor and for the immunocytochemical localisation of the oestrogen receptor protein.

RADIOCHEMICAL ASSAY ON CYTOSOL PREPARATIONS
Cytosol oestrogen receptor was measured with a multiple point dextran-coated charcoal assay according to the recommendations of the EORTC group[26] (with ^{125}I-estradiol). These assays were performed in the laboratory of the De Wever Hospital, Heerlen (Dr A Gijzen). Both laboratories participate in a steroid receptor assay quality control programme.[27]

RADIOCHEMICAL ASSAY ON FROZEN SECTIONS
Frozen sections were cut at 6 μm and mounted on gelatin chrome alum-coated coverslips (40 × 20 mm) as reported previously by De Goeij *et al.*[19] Several non-mounted sections were used to determine protein concentration according to the method of Bradford.[28] Briefly, the mounted and dried unfixed sections were defatted by immersion in petroleum ether (40°–60° C) for five minutes, three times, and dried in air. Subsequently the sections were overlayed with 150 μl of buffer containing 0·01 M K_2HPO_4/KH_2PO_4 (pH 7·5),

0·0015 M K_2 edetic acid, 0·003 M NaN_3, 0·01 M monothioglycerol, and 10% glycerol (EORTC buffer).[25] Incubation in triplicate with 4, 2, 1, 0·5 and 0·25 nM [^{3}H]-estradiol (Amersham International, UK) in the presence and absence of a 100-fold excess of diethylstilboestrol was performed in a humid chamber overnight at 4° C. During incubation, part of the tissue receptor content diffused into the overlaying buffer. The specific protein-bound radioactivity which eluted from the sections was determined with a dextran-coated charcoal assay and designated as "soluble" receptor. After washing the sections with EORTC buffer the binding of radiolabel to the sections was measured by direct liquid scintillation counting of the coverglass-bound sections. This determination yielded the section-bound receptor. The total tissue oestrogen receptor content in the section assay was defined as the sum of "soluble" and section-bound oestrogen receptor and expressed as fmol/mg of total protein.

PROTEIN DETERMINATIONS
Non-mounted sections were solubilised with 150 μl 1 N NaOH at 100° C for 10 minutes in stoppered glass tubes. Protein content of 100 μl aliquots of solubilised sections, homogenates, and cytosol samples was determined according to the method of Bradford[28] using bovine serum albumin as a standard.

IMMUNOSTAINING PROCEDURE ON SECTIONS
The immunostaining was performed using the oestrogen receptor-immunocytochemical assay kit according to the manufacturer's instructions (Abbot Laboratories, Diagnostic Division, The Netherlands), with one modification: the primary antibody was used in a dilution of 1/4. Briefly, cryostat sections of 6 μm were fixed in picric acid-paraformaldehyde[29] for 10 minutes at 4° C and stored at $-20°$ C in specimen storage medium (42·8 g sucrose, 0·33 g $MgCl_2$ in 500 ml phosphate buffered saline (PBS) and glycerol 1/1) for a maximum of two weeks before staining. The sections were incubated for 15 minutes with normal goat serum (1/5) to reduce non-specific binding of the antibodies. Subsequently the sections were incubated overnight at room temperature with the rat-anti-oestrogen receptor antibody developed by Greene *et al*[30] (H222Sp γ, diluted 1/4 in 1% bovine serum albumin-phosphate buffered saline) rinsed twice for five minutes in PBS, and then incubated with goat anti-rat IgG for 30 minutes, rinsed twice for five minutes in PBS and incubated 30 minutes with the peroxidase-antiperoxidase complex, rinsed in PBS and finally incubated for seven minutes in chromogen (diaminobenzidine with 0·03% hydrogen peroxide in PBS), counterstained with Harris's hematoxylin, and mounted in Entellan. As a negative control, an adjacent

section was stained according to the same procedure but with normal rat IgG. For every staining procedure, oestrogen receptor positive cells, supplied with the Abbott kit or frozen sections of oestrogen receptor positive mammary carcinomas served as positive controls.

The intensity and distribution of the specific staining was visually evaluated using a modification of the semiquantitative analysis of McCarty *et al.*[22] The immunohistochemical oestrogen receptor score was expressed as follows:

$$\text{Oestrogen receptor score} = \sum_{i=0}^{i=4} P(i) \times i, \text{ where}$$

i = intensity of staining (0–4) and P(i) = percentage of stained tumour cells in category i (0–100%).

The classification of the staining intensity was assessed on at least three separate cohorts of 100 tumour cells from each tumour of different high power (× 400) fields, which allowed heterogeneity in tumour cell receptor content to be assessed. Scoring was performed by two pairs of independent observers working without prior knowledge of the cytosol oestrogen receptor content. Before scoring of the breast cancer sample the observer pairs reached a consensus on classification by using the reference preparation included in the oestrogen receptor-ICA kit. The final oestrogen receptor score of the sample was obtained by calculating the mean of all scores.

Results

COMPARISON OF RADIOCHEMICAL DETECTION OF OESTROGEN RECEPTOR IN CYTOSOL AND FROZEN SECTIONS

The Scatchard plots of the binding of radiolabelled oestradiol to 6 μm unfixed frozen sections of two oestrogen receptor positive breast cancer specimens are shown in fig 1. This radiochemical section assay
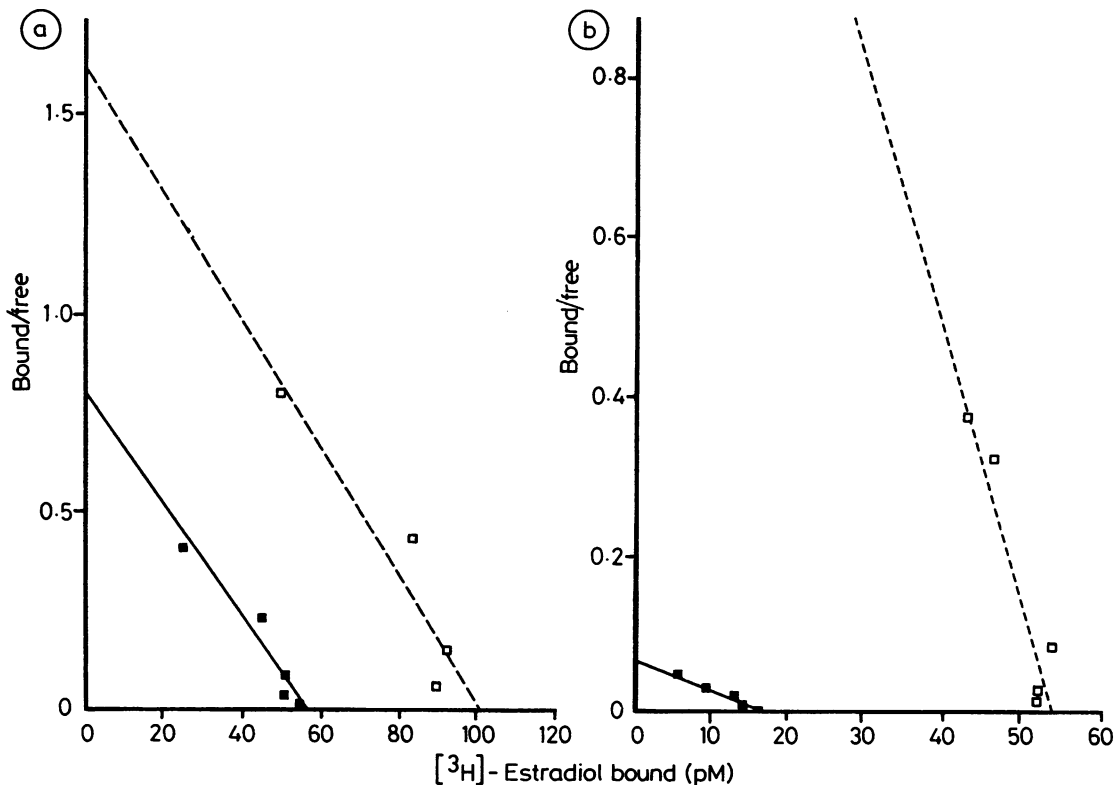

Fig 1 *Scatchard analysis of radiochemical oestrogen receptor assay on cryostat sections of two human breast cancer samples* □ - - - □: *soluble oestrogen receptor;* ■————■: *section-bound oestrogen receptor. (a) Section-bound: Bmax = 373 fmol/mg tissue protein, Kd = 0·07 nM; soluble: Bmax = 663 fmol/mg tissue protein, Kd = 0·06 nM. (b) Section-bound: Bmax = 40 fmol/mg tissue protein, Kd = 0·28 nM; soluble: Bmax = 122 fmol/mg tissue protein, Kd = 0·03 nM.*
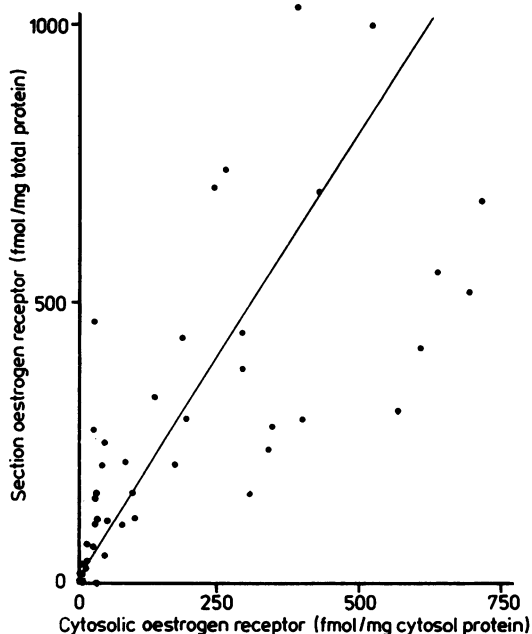
Fig 2   *Comparison of radiochemical oestrogen receptor in cytosol and cryostat sections from 50 breast cancer samples. Linear regression is characterised by: y = 17·5 + 1·55 x; r = 0·70; p < 0·00001. Note that cytosol oestrogen receptor is expressed as fmol/mg cytosol protein and section oestrogen receptor (soluble + section-bound) as fmol/mg total tissue protein.*

shows the presence of oestrogen receptors, characterised by saturable and high affinity binding of oestradiol (dissociation constant kd = 0·05—1·0 nM). The reproducibility of this assay was determined by multiple assays (n = 9) on calf uterus, and the inter-assay variation for oestrogen receptor determination

Table 1   *Correlation of radiochemical oestrogen receptor assay in cytosol and sections: classification* of receptor positive and negative breast cancer samples (n = 50)*

	Cytosol assay (fmol oestrogen receptor/ mg cytosol protein)	
	< 10	≥ 10
Section assay (fmol oestrogen receptor/mg tissue protein)		
< 33	10 (20%)	1† (2%)
≥ 33	1‡ (2%)	38 (76%)

*For cytosol assay a cut off value of 10 fmol/mg cytosol protein was used, corresponding to 33 fmol/mg tissue protein according to equation y = 17·5 + 1·55 x, experimentally determined in experiments from fig 2. Specificity = 91%; sensitivity = 97·5%.
†This sample had 10 fmol oestrogen receptor/mg of cytosol protein in the cytosol and 16 fmol oestrogen receptor/mg tissue protein in the sections.
‡This sample had no detectable oestrogen receptor/mg cytosol protein in the cytosol and 37 fmol/mg tissue protein in the sections.

resulted in a coefficient of variation of 17·5%.

The results of the radiochemical quantitation of oestrogen receptor sites in cytosol and cryostat sections in a series of 50 breast cancer specimens are illustrated in fig 2. The two oestrogen receptor assays showed a significant linear correlation (r = 0·70; p < 0·00001). The relative amount of soluble receptors varied from 20 to 80% of the total sample content and no significant relation was found between the number of oestrogen receptors in the cytosol and in the soluble fraction of the sections (results not shown). The mean (SEM) Kd for the cytosol oestrogen receptor assay was 0·31 (0·61) nM compared with 0·30 (0·43) nM for the section-bound oestrogen receptor and 0·16 (0·20) nM for the soluble oestrogen receptor. The mean Kd for the section-bound and soluble oestrogen receptor differed significantly from each other according to Students's t test (p < 0·01). These results show that the radiochemical assays on cytosol and frozen sections detect high affinity binding sites corresponding to oestrogen receptors. The Kd data also indicate that the oestrogen receptors eluted from the sections and detected as soluble receptors show a higher binding affinity than the receptors which remain bound to the sections.

Classification of the 50 samples into oestrogen receptor positive and negative breast cancers, as determined with both assays, is shown in table 1. For the cytosol assay a cut off value of 10 fmol oestrogen receptor/mg of cytosol protein was generally used. The corresponding cut off value for the section assay was 33 fmol oestrogen receptor/mg tissue protein. The agreement between both assays was excellent, with a sensitivity of 97·5% and a specificity of 91%. Two cases were discordant, but receptor contents were near the borderline values (table 1).

The receptor values were expressed in a different way for the cytosol and the section assay. For obvious reasons the cytosol oestrogen receptor content was determined in fmol/mg *cytosol* protein and section oestrogen receptor in fmol/mg *total tissue* protein. To compare the results of both assays based on the same amount of tissue the cytosol oestrogen receptor content was also expressed per mg total tissue protein. Cytosol protein represents 48 (9%) (n = 50) of the total tissue protein. Recalculation of the cytosol receptor data on the basis of the amount of total tissue protein in the sample showed that about three times more oestrogen receptor was recovered from the same amount of tissue with a section assay compared with a cytosol assay.

COMPARISON OF IMMUNOCYTOCHEMICAL AND RADIOCHEMICAL DETECTION OF OESTROGEN RECEPTOR
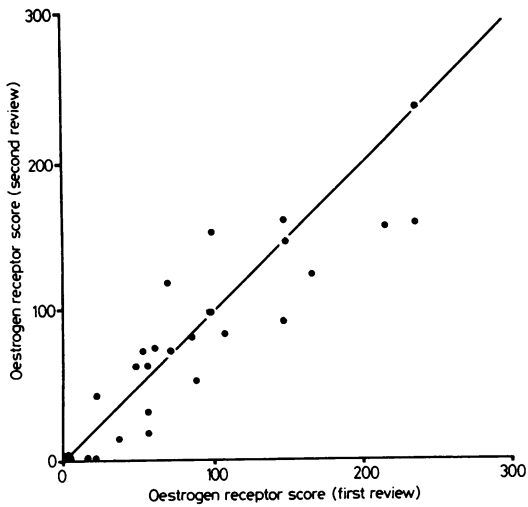Only nuclear staining could be observed in the

Fig 3 *Intraobserver variability of oestrogen receptor score in 26 breast cancer samples, obtained in two separate evaluations by one observer. (r = 0·96, p < 0·00001).*

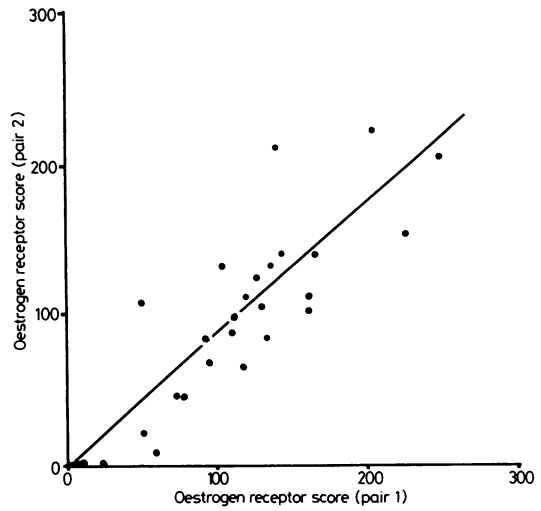Fig 4 *Interobserver variability of oestrogen receptor score in 32 breast cancer samples, obtained independently by two pairs of observers (r = 0·90; p < 0·00001; slope = 0·89; Student's t test p = 0·017).*
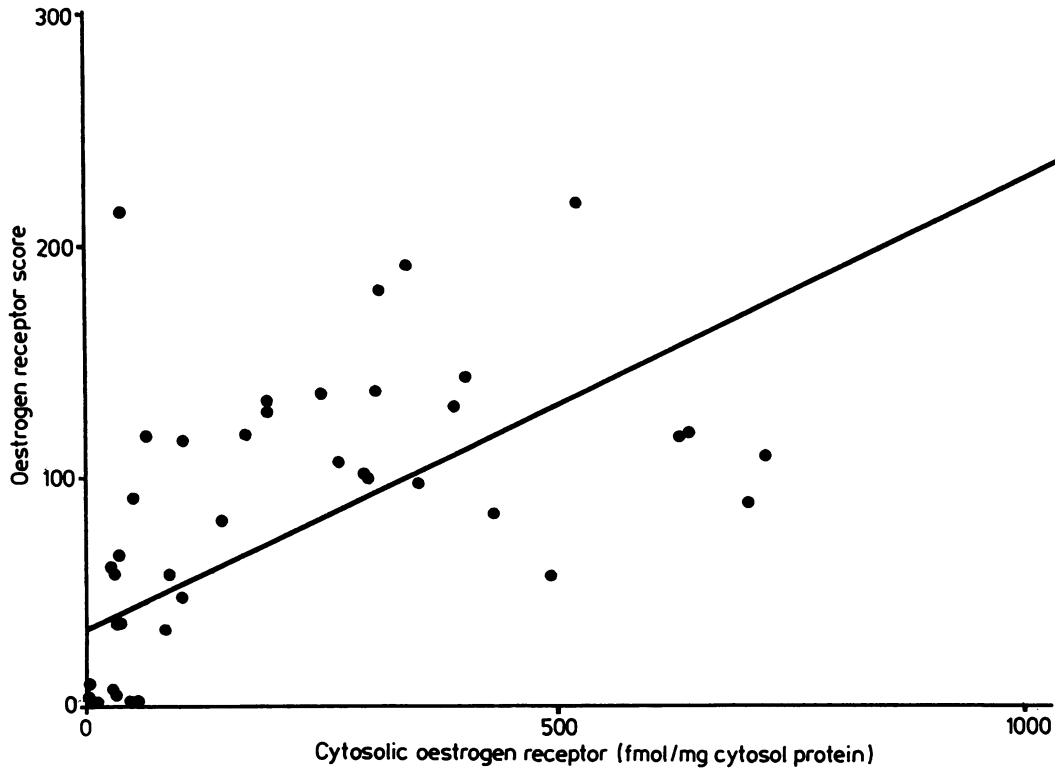

Fig 5 *Comparison of immunohistochemical oestrogen receptor score and radiochemical oestrogen receptor assay in cytosol of 50 breast cancer samples. Characteristics of correlation: r = 0·65; p < 0·00001; y = 33·8 + 0·19 x).*

immunocytochemically oestrogen receptor positive tumours. Staining was heterogeneously distributed over the malignant cells in most breast cancer samples. Adjacent epithelial cells of normal breast tissue also often showed nuclear staining. In at least three fields of each section, each field including 100 cells, the nuclear staining was classified into five categories ranging from no (0) to very intense (4) staining and an oestrogen receptor score was obtained as detailed. To facilitate comparison of immunohistochemical and radiochemical results only the total oestrogen receptor scores are presented. It is important to note, however, that significant differences often occurred between the fields.

The intraobserver variability for visual assessment of the oestrogen receptor score was studied. Fig 3 shows a significant linear correlation between both observations (r = 0·96; p < 0·00001).

Interobserver variability in oestrogen receptor scoring by two observer pairs on 26 breast cancer specimens is summarised in fig 4. The results show a significant linear correlation (r = 0·90; p< 0·00001) but a consistently lower score for one observer pair (slope 0·91, Student's *t* test p < 0·017). The interassay

Table 2 *Correlation of radiochemical oestrogen receptor assay in cytosol and immunohistochemical oestrogen receptor score for 50 breast cancer samples*

	Cytosol assay (fmol oestrogen receptor/ mg cytosol protein)	
	< 10	≥ 10
Immunohistochemical assay (oestrogen receptor score)		
< 35	11	9*
≥ 35	0	30

For cytosol assay a cut off value of 10 fmol oestrogen receptor/mg cytosol protein was used, which corresponds to an oestrogen receptor score of 35 according to equation y = 33·8 + 0·19 x, derived from data in fig 5.
*The cytosol oestrogen receptor content of these samples varied between 78 and 10 fmol/mg cytosol protein.

coefficient of variation for the whole immunohistochemical procedure including oestrogen receptor scoring was assessed on homogeneous tissue samples and was found to be 10%.

For the 50 samples the results of semiquantitative scoring of oestrogen receptor immunoreactivity were
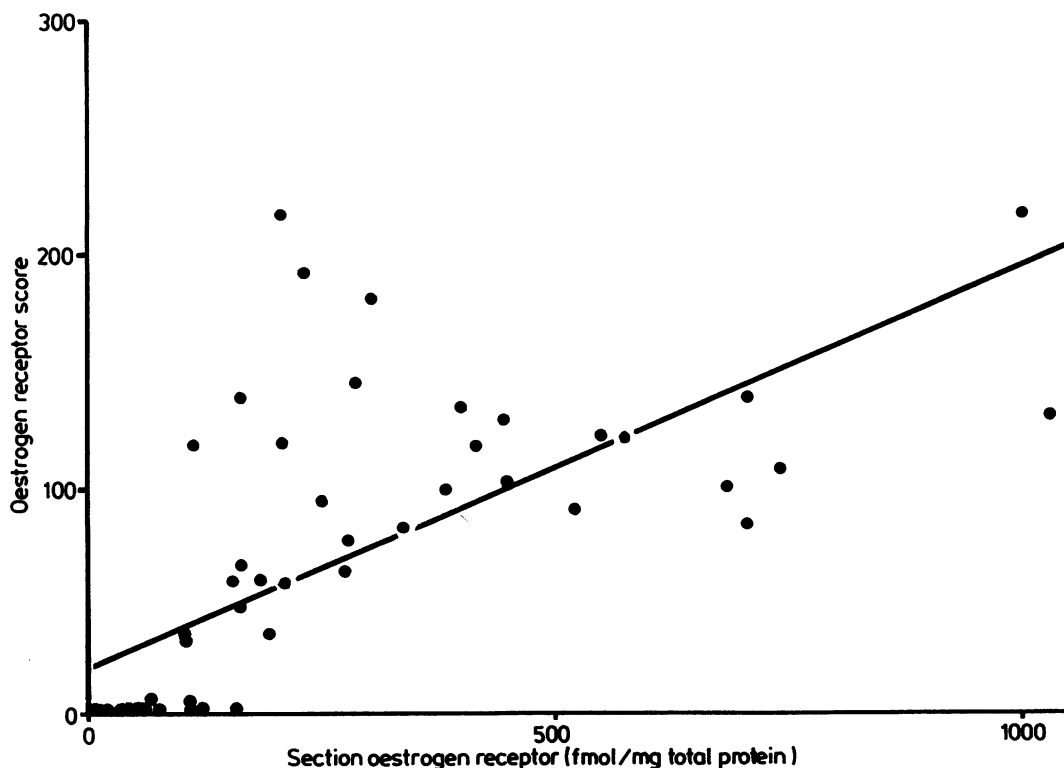

Fig 6   Comparison of immunohistochemical oestrogen receptor score and radiochemical oestrogen receptor assay in sections of 50 breast cancer samples. Characteristics of correlation: r = 0·70; p < 0·00001; y = 25·2 + 0·17 x).

compared with the radiochemical oestrogen receptor binding assays in cytosol as well as in sections. The linear correlation for oestrogen receptor assay in cytosol and the oestrogen receptor score was 0·63 and was highly significant (p < 0·00001) (fig 5). According to the data from fig 5, the cut off value of 10 fmol/mg cytosol protein corresponded to an oestrogen receptor score of 35. Application of these values resulted in the classification of the 50 breast cancers by the cytosol assay and immunohistochemical evaluation (table 2).

Fig 6 shows the linear relation for radiochemical and immunohistochemical quantitation performed on adjacent cryostat sections. The correlation coefficient of 0·70 was highly significant (p < 0·00001). The cut off value of 33 fmol/mg tissue protein corresponded to an oestrogen receptor score of 31. Classification of the 50 samples according to these assays is shown in table 3.

The results of the linear relations (figs 5 and 6) show that the radiochemical determination of oestrogen receptor content in the frozen sections was in better agreement with the semiquantitative immunohisto-chemical analysis than the oestrogen receptor measurements in the tumour cytosol.

Agreement with regard to receptor status was excellent (100%) for breast cancer samples, which were classified as negative with radiochemical assay on cytosol (table 2) and sections (table 3) compared with immunohistochemical oestrogen receptor score. In contrast, 23% (cytosol) and 18% (sections) of the radiochemically oestrogen receptor positive tumours had a negative score by immunohistochemistry. The heterogeneous distribution of oestrogen receptors and the strict quantitative correlation of the immunocyto-chemical and radiochemical determination of oes-trogen receptor in adjacent frozen sections taken from different parts of one tumour sample is shown in fig 7. The plot shows that for each of the four regions of the tumour sample the oestrogen receptor scores were in significant linear correlation with the radiochemical oestrogen receptor content detected in adjacent frozen tumour sections (r = 0·99, p < 0·00001).

Discussion

Oestrogen receptors can be specifically determined in frozen sections of normal target tissues with Scatchard analysis using radiolabelled oestradiol.[19] This report shows that the technique can also be applied for reliable oestrogen receptor assay in comparative studies on breast cancer sections in man.

The binding characteristics are fully compatible with specific oestrogen receptor detection and the reproducibility of the section assay is acceptable. The interassay coefficient of variation was comparable with what has been found for oestrogen receptor determinations in cytosol by several laboratories

Table 3 *Correlation of radiochemical oestrogen receptor assay in frozen sections and immunohistochemical oestrogen receptor score of 50 breast cancer samples*

	Section assay (fmol oestrogen receptor/ mg tissue protein)	
	< 33	⩾ 33
Immunohistochemical assay (oestrogen receptor score)		
< 31	11	7*
⩾ 31	0	32

For section assay a cut off value of 33 fmol oestrogen receptor/mg tissue protein was used, which corresponds to an oestrogen receptor score of 31 according to equation y = 25·2 + 0·17 x, derived from data in fig 6.
*The section oestrogen receptor content of these samples varied between 65 and 110 fmol/mg tissue protein. These samples (n = 7) are all included in the nine samples with more than 10 fmol/mg cytosol protein (table 2).

participating in steroid receptor assay quality control programmes.[27 30] A highly significant linear relation was found between the results of radiochemical assay on sections and cytosol prepared from adjacent tissue blocks.

The agreement of receptor status classification of the specimen between both radiochemical methods
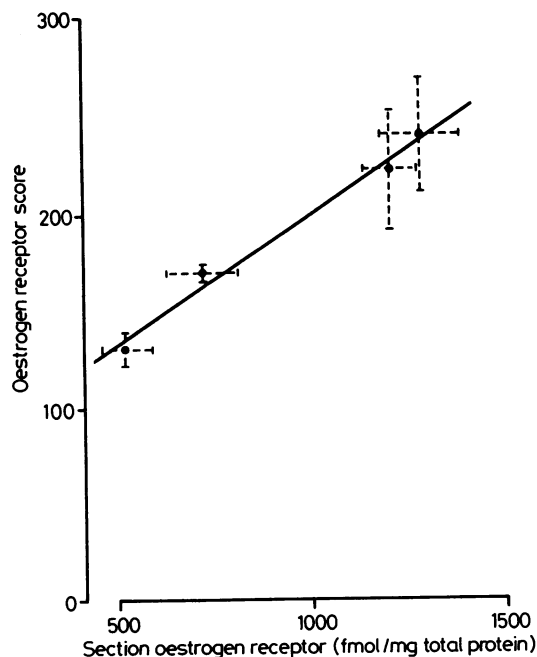

Fig 7 *Correlation between immunohistochemical oestrogen receptor score and radiochemical oestrogen receptor assay in sections for multiple samples obtained from one breast cancer biopsy specimen (r = 0·993; p < 0·00001).*

was excellent (97·5%) when a cut off value of 10 fmol oestrogen receptor/mg cytosol protein was used. Cases negative by radioligand assay but positive by immunocytochemical assay may be due to heterogeneity within the tumour samples: this has been repeatedly shown in breast cancer.[7-14] Cases positive by radioligand assay but negative by immunocytochemical assay can occur when tumour samples contain appreciable amounts of oestrogen receptor positive non-neoplastic cells, which are not taken into account in the immunocytochemical assay.

As in the previous study[19] part of the oestrogen receptor content was found to elute from the unfixed breast cancer sections into the overlaying buffer during incubation, while more than 90% of the rest remained bound to the sections, even after extensive washing. In contrast to the results on normal tissue, in this breast cancer series the relative amount of soluble receptors was highly variable.

We noticed that the mean dissociation constant for soluble receptors was lower than that for section-bound oestrogen receptor. This slight but significant difference in binding affinity suggests that there are two receptor forms or populations with different protein conformation. These conformational differences may be induced by receptor binding to cellular structures. Alternatively, the state of phosphorylation might be important. On the other hand, as all cells in a 6 μm section are opened up, part of the oestrogen receptor that is assayed in the overlay buffer might be bound to cell fragments which are lost from the unfixed section during incubation. Although the mean dissociation constants for cytosol and soluble oestrogen receptor did not differ significantly, we cannot assume that they were directly comparable; no significant relation among receptor contents was found in the 50 breast cancer samples.

Although both radiochemical assays could not detect the oestrogen receptor content of individual cancer cells, the assay on sections has several advantages over that of cytosol. Firstly, parallel or directly adjacent sections can be used for routine histological verification of the sample for histodiagnostic purposes and for histochemical and immunohistochemical studies. Secondly, less tissue is required for Scatchard analysis (about 50–80 mg of fresh tissue compared with at least 200 mg for cytosol assay). This could be important, in view of the tendency towards a decreasing mean size of primary breast cancer samples due to detection at an earlier stage of the disease. The difference may be due to loss of receptor binding function during cytosol preparation, such as heat denaturation during homogenisation, protease activity, and removal of the nuclear fraction.

On the other hand, better preservation of oestrogen receptor in section assay can be explained by very rapid drying of the frozen section, high stability of receptor preparations in a dry state,[19 31 32] and minimal delay in incubating the tissue with receptor ligand which stabilises the receptor binding function.

The characteristics of oestrogen receptor immunoreactivity, most notably the predominant nuclear localisation and the pronounced heterogeneous cellular distribution, agree with the findings of other studies using the same or similar monoclonal antibodies.[20-25] For the semiquantitative evaluation of immunostaining, several approaches have been described, such as determination of the percentage of positive cells,[24 25 33 34] classification in varying numbers of categories,[22 24 25 34] and various mathematical analyses.[23 24] We chose a slight modification of the method of McCarty *et al*.[22] Our intra- and interobserver variability studies showed that semiquantitative scoring of oestrogen receptor immunoreactivity was highly reproducible for one observer, but could result in significantly different oestrogen receptor scores for different observers. This effect will be more pronounced when no standard preparations for reference are used. In addition to observer and calculation variations, differences in experimental conditions may also produce significant quantitative discrepancies. Therefore, before semiquantitative immunohistochemical oestrogen receptor analysis is applied for clinical purposes, development of quality control programmes for this technique, similar to those which are currently used for radiochemical steroid receptor assays,[27 30] should be implemented.

Positive correlations between oestrogen receptor immunohistochemistry and cytosol assay have been reported.[22-25 33] Our finding that oestrogen receptor scores correlated better with radiochemical oestrogen receptor assay on parallel sections than with cytosol underlines the importance of cellular and sample heterogeneity as a cause of variation in oestrogen receptor assay evaluation. A high correlation between anti-oestrogen receptor immunohistochemistry and enzyme immunoassay and a radioligand binding assay on adjacent cryostat sections was also found by Giri *et al*,[25] although a different technical approach for the radiochemical assay was taken. Individual cases, however, may show discrepancies between radioligand assay and immunocytochemical results on adjacent cryostat sections.

Cases negative by radioligand binding assay but positive by immunohistochemical assay may be due to methodological errors in the radioligand assay or to dilution of cytosol with oestrogen receptor negative material in samples with a low oestrogen receptor positive cell content. Cases negative by immunohistochemical assay but positive by radioligand assay may be due to the methodological difference between detecting a molecule through antigen-antibody bind-

ing and receptor-ligand binding, although the monoclonal anti-oestrogen receptor antibody recognised an epitope that is distinct from the oestradiol binding site of the receptor. Furthermore, admixture of oestrogen receptor positive, non-neoplastic cells, which are not taken into account in the immunohistochemical oestrogen receptor score, may be responsible. Similar discrepancies between immunohistochemical and radioligand binding assay have been reported.[35 36] Whether breast cancers with negative oestrogen receptor scores belong to the group of non-responders to endocrine treatment in the cytosol oestrogen receptor positive group of breast cancer patients, as has been reported earlier,[36] has to be verified. Final validation of semiquantitative immunohistochemical oestrogen receptor assay for clinical use awaits assessment of its ability to predict the response to hormonal treatment and the prognosis.

Semiquantitative analysis, resulting in a single number score, does not take into account the possibility that heterogeneity may contain important biological information. We feel that the distribution pattern of the staining intensity of the tumour cells should be separately analysed in relation to clinical data. In this respect, the ability to show the presence of oestrogen receptor in paraffin sections is important. Some attempts have been reported[37-40] but as yet no validated techniques for routinely fixed malignant tissue have been described.

In conclusion, we have shown that cryostat sections of breast cancer can be used for radiochemical oestrogen receptor assay, and that the radiochemical section assay is a reliable and reproducible technique, which offers advantages over the use of cytosol. The semiquantitative immunohistochemical oestrogen receptor score correlates better with radiochemical assay on sections compared with cytosol. The results indicate that oestrogen receptors may be reliably quantitated by semiquantitative evaluation of specific immunostaining in cryostat sections of human breast cancer, but only if this technique is adequately standardised.

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