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FROM THE DEPARTMENT OF ONCOLOGY, UNIVERSITY HOSPITAL, LUND, SWEDEN.

ESTROGEN AND PROGESTERONE RECEPTOR ANALYSES IN MORE THAN 4000 HUMAN BREAST CANCER SAMPLES

A study with special reference to age at diagnosis and stability of analyses

M. FERNÖ, Å. BORG, U. JOHANSSON, A. NORGREN, H. OLSSON, S. RYDÉN and G. SELLBERG
SOUTHERN SWEDISH BREAST CANCER STUDY GROUP

Abstract

Estrogen (ER) and progesterone receptors (PgR) were measured in the same laboratory in more than 4000 breast cancer biopsy samples obtained from 15 different hospitals during ten years. ER was measured with isoelectric focusing and PgR with the dextran-coated charcoal method and Scatchard analysis. The distribution pattern for both ER and PgR was during this time period and for the different hospitals rather similar indicating a good stability of the analytical methods. ER concentration was positively correlated with patient age, with a higher percentage of positive samples and higher concentrations in patients ≥50 years of age compared with patients <50 years. PgR concentration increased with age for patients under 50 years, but a considerable reduction of PgR concentration and of the proportion of positive samples was seen in patients between 50 and 59 years of age. Above this age the PgR concentration again increased with increasing age. The PgR/ER ratio and the proportion of ER− PgR+ samples were higher in patients under 50 years compared to older patients. ER and PgR values decreased during tamoxifen treatment, during pregnancy and after preoperative radiotherapy. Wet weight, DNA and protein were compared as reference parameters for the expression of ER and PgR concentrations. Strong correlations were obtained suggesting that similar information can be obtained with either of these reference parameters.

Key words: Breast cancer, estrogen receptor, progesterone receptor, age, stability of analyses.

The value of estrogen (ER) and progesterone receptor (PgR) analyses for predicting the response to endocrine therapy in metastatic breast cancer is well documented (1). Several investigations have also shown that steroid receptor status is of importance for the prognosis of the disease (recurrence-free survival) and for the effect of adjuvant therapy (2–4). Some studies, however, have not confirmed these results (5–7). These discrepancies may partly be explained by differences in the performance of the receptor analyses. A further explanation can be the fact that the hospitals in a multicenter study may have a varying quality in handling and transportation of samples for receptor assays. It is therefore of importance to compare the receptor content in samples from the different hospitals and from different time periods.

In the present work, we present data from more than 4000 ER and PgR analyses (1977–1987), performed in the same laboratory. The relation between receptor content and age was studied. Certain factors of importance for the measurement were considered as temperature of the sample on arrival, protein concentration in the supernatant used for analysis, tamoxifen therapy, pregnancy at the time of operation and preoperative radiotherapy.

Material and Methods

Sampling of tumor material. The receptor laboratory at the Department of Oncology, University Hospital in Lund, performs ER and PgR determinations in breast cancer samples from the Southern Swedish Health Care Region (1.5 million inhabitants) in cooperation with the Southern Swedish Breast Cancer Study Group. The samples consist of biopsy material from primary tumors, local recurrences or distant metastasis. It has previously been shown that the median age of menopause in Scandinavia corresponds to 50 years of age (8). Fifty years of age was therefore chosen in the present study for subdivision of the patient population. For more detailed investigations on the relation between ER/PgR and age, a 'perimenopausal' group was defined consisting of patients between 45 and 54 years of age. The steroid receptor measurements

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started in 1976 and became routine in 1978. Since then, the proportion of primary breast cancers in this region examined by receptor analyses has risen from 29 to about 70% of the about 800 new breast cancers detected every year. The present study includes results from 1977, when the analytical methods were well established. The laboratory receives samples from 15 different hospitals, located at a distance of 0–200 km, mainly by transporting them in solid CO_2. After arrival, the tumor pieces are kept in liquid nitrogen for not more than two weeks until analysis. Samples reaching the laboratory at an improper temperature, samples too diluted during cytosol preparation (less than 0.5 mg protein/ml dextran-coated charcoal-treated supernatant), samples from patients on endocrine treatment or being pregnant at the time of operation, or samples taken after preoperative radiotherapy, were considered unsatisfactory and therefore evaluated separately. After these exclusions ER analysis was performed in 4323 out of 4948 breast cancer biopsy samples (87%). The corresponding figures for PgR were 3640 out of 4152 samples (88%).

Analytical procedures. The assays of ER and PgR have been described in detail elsewhere (9). ER was measured in the 20000×g supernatant with isoelectric focusing (IF) in polyacrylamide gel. Presence of ER was indicated by a radioactive peak at pH 6.3–6.5. The specificity and saturability of this peak was sometimes controlled by the addition of a 100-fold excess of non-radioactive estradiol. The concentration of PgR was assessed with the multiple-point dextran-coated charcoal (DCC) technique and Scatchard analysis. During the time of study, methodological improvements resulted in increased sensitivity of receptor measurements and an increased recovery of ER and PgR. To include receptor values obtained before and after the methodological modifications, control experiments were performed using the two variants of methods in each sample. These experiments, as well as the validity of the conversion, are described in more detail elsewhere (10). As IF is not an internationally common routine method for ER analysis, it has (in agreement with the recommendations from a consensus meeting at the National Institute of Health in 1979 (11)) been compared with DCC, which resulted in a good concordance (r_s=0.92; 12). Our steroid binding assays for both ER and PgR have recently also showed very good correlations with enzyme immunoassay (13, 14).

ER and PgR were expressed as fmol specifically bound ^{3}H-estradiol and ^{3}H-R5020 respectively, per mg protein, which was determined according to Lowry et al. (15). As a comparison also DNA, determined according to Burton (16), and wet weight were used as reference parameters. The sensitivity of the analyses was approximately 1.0 fmol/ml for ER and 10 fmol/ml for PgR (9). When dividing the samples according to receptor positivity (+) and negativity (−), 10 fmol ER/mg protein and 30 fmol PgR/mg protein were chosen as cut-off points.

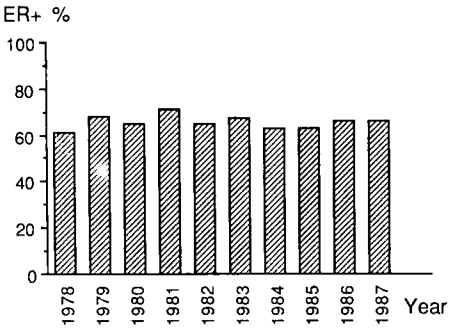

Fig. 1. Percentage ER+ (≥10 fmol/mg protein) samples for different years.

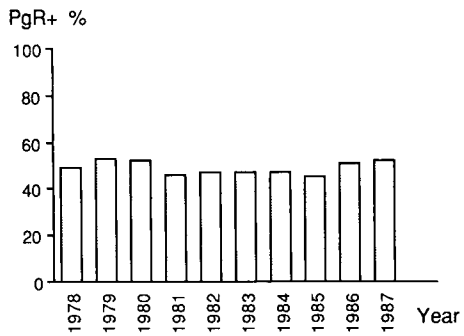

Fig. 2. Percentage PgR+ (≥30 fmol/mg protein) samples for different years.

Statistics. The possible linear relation between age and steroid receptor concentration was studied by regression analysis. Receptor concentrations were square root transformed in order to obtain a better approximation to the normal distribution. Comparisons of differences between receptor positivity were performed with the χ^2-test. Two-sided p-values were used. Correlations between different reference parameters were calculated with one-sided Spearman's rank correlation test.

Results

ER and PgR in samples from different years and hospitals. The mean percentage (±SD) of ER and PgR positivity during the 10-year period of analyses was stable: 66±2.8 and 49±2.9 respectively (Figs 1 and 2). The mean concentration (fmol/mg protein) was 190±25 for ER and 180±33 for PgR. The percentage of ER+ and PgR+ samples from the 15 different hospitals in the region was also quite stable (Figs 3 and 4). The mean percentage of positive samples was 66±8.9 for ER and 47±3.8 for PgR. The corresponding figures for the mean concentrations were 120±23 and 160±38 respectively.

ER and PgR in relation to age. The proportion of ER+ samples was higher for samples from patients ≥50 years (70%) compared to those from patients younger than 50 years (56%; p≤0.0001; Table 1). No corresponding difference was found regarding PgR (Table 1).

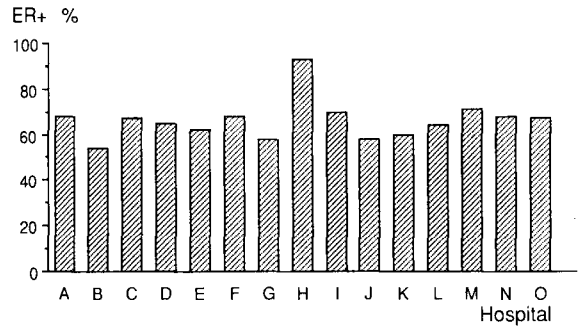

Fig. 3. Percentage ER+ samples from 15 different hospitals. (From hospital H only 14 samples was obtained.)

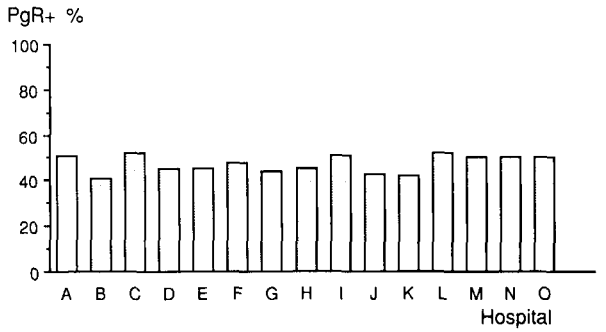

Fig. 4. Percentage PgR+ samples from 15 different hospitals.

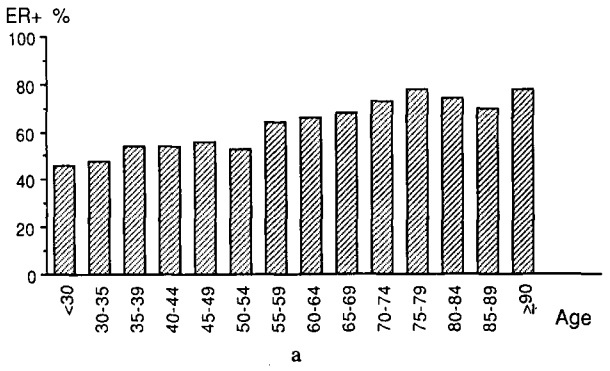

Fig. 5a. Proportions of ER positive samples for different 5-year age categories.

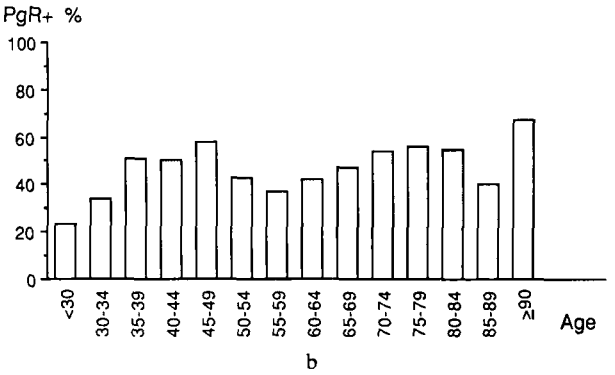

Fig. 5b. Proportions of PgR positive samples for different 5-year age categories.

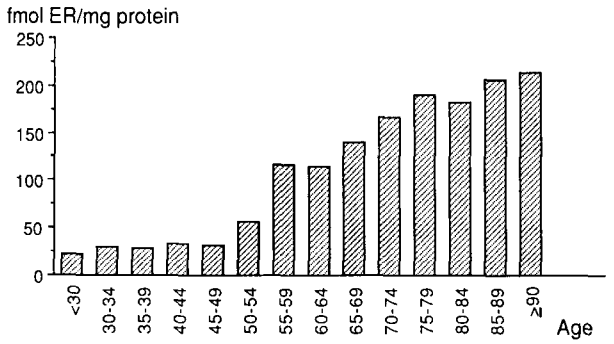

Fig. 6. Mean ER concentrations for different 5-year age categories.

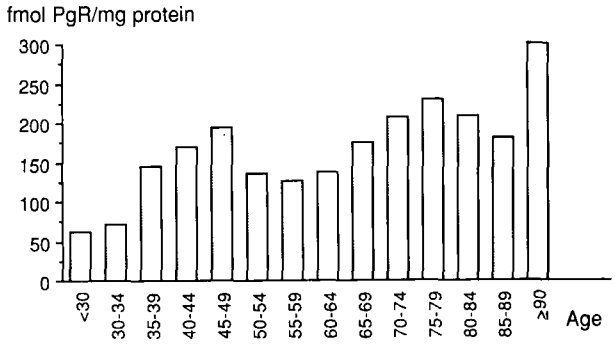

Fig. 7. Mean PgR concentrations for different 5-year age categories.

The proportions of ER and PgR positivity as a function of age are shown in Fig. 5. The group between 45 and 54 years did not differ with respect to ER positivity from the group below 45 years of age, but both these groups had significantly lower ER contents than patients above or equal to 55 years of age ($p<0.0001$). For PgR no corresponding pattern was found. In contrast to the relative stable percentage of ER+ samples between different 5-year age groups for patients under 55 years, the percentage of PgR+ samples was more variable.

The mean ER and PgR concentrations for different 5-year age categories are shown in Figs 6 and 7. Regression analyses showed that ER was significantly correlated with age in patients between 45 and 54 years and in those older than 54 years. No such correlation was found in samples from patients under 45 years of age. For PgR a different pattern was obtained. PgR was negatively correlated with age in patients between 45 and 54, whereas a positive correlation was found in both younger and older age groups.

The median ratio between PgR and ER (PgR/ER), calculated for all samples with values above method sensitiv-

Table 1

ER (n=4323) and PgR (n=3640) content (percentage positive samples, mean (±SD) concentration, range) for a 10-year material of breast cancer samples

	All patients	<50 years	≥50 years
% ER+ (n)	65	56	70
% PgR+ (n)	48	51	48
ER*			
Mean	120±200	37±77	150±220
Range	0–2 200	0–1 200	0–2 200
PgR*			
Mean	180±380	170±350	190±410
Range	0–4 700	0–4 100	0–4 700

* fmol/mg protein.

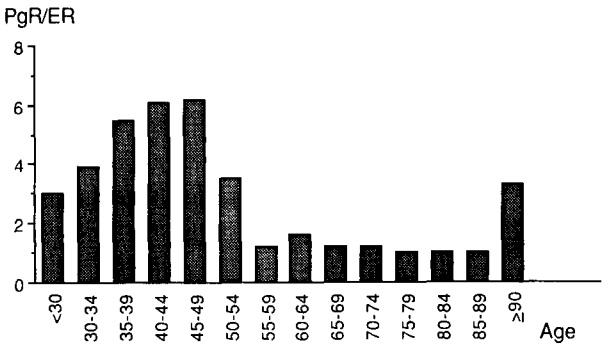

Fig. 8. The median ratio PgR/ER for all samples giving concentration values above method sensitivity for different 5-year age categories.

ity, was higher for patients <50 years compared to patients ≥50 years (median values 5.1 and 1.2 respectively; Fig. 8). Data from regression analysis for the three different age groups (<45, 45–54, ≥55 years of age) showed that, for the first age group, the ratio increased with higher age. The opposite pattern was found in the group between 45 and 54 years, whereas no correlation between the ratio PgR/ER and age was found in patients ≥55 years.

Table 2 shows the receptor distribution when both ER and PgR status were taken into consideration. One conspicuous finding is the fact that patients between 35 and 54 years of age had tumors being ER− PgR+ more often than other age groups.

Factors influencing ER and PgR content (Table 3). Inaccurate temperature during transport of the tumor samples considerably reduced both the proportion of receptor positivity and the mean concentration values. Another factor generally considered to be of importance is the protein concentration in the cytosol used for receptor determination. A concentration below 1 mg/ml has been considered to cause reduced measurable receptor levels (17). At our laboratory, a protein concentration down to 0.5 mg/ml has been found to give reliable results (10). However, receptor positivity and mean concentration values are only to a minor extent influenced by even very low protein concentrations (<0.25 mg/ml).

Tamoxifen treatment at the time for biopsy collection had a negative influence on the receptor content: the percentage of ER+ samples was reduced to one-fourth and PgR+ samples to about half. Preoperative radiotherapy caused an even more pronounced negative effect on both receptors. Among the more than 4000 samples, four derived from patients pregnant at the time of operation. All four were ER− and PgR−.

Reference parameters: protein, DNA or wet weight. For both ER and PgR, comparisons were made between the following pairs of reference parameters: protein–DNA, protein–wet weight and DNA–wet weight. For ER, Spearman's rank-correlation values (r_s) of 0.97 (n=4090), 0.98 (n=3465) and 0.97 (n=3347) respectively, were obtained. For PgR the corresponding figures were 0.99 (n=3497), 0.99 (n=2841) and 0.99 (n=2792) respectively.

Table 2

ER and PgR status for different age categories

Age category	Percentage				n
	ER+ PgR+	ER+ PgR−	ER− PgR+	ER− PgR−	
All	45	21	4	31	3 602
≥50	46	23	2	29	2 839
<50	43	13	7	36	763
<30	23	23	0	55	22
30–34	31	17	3	48	58
35–39	41	13	10	36	135
40–44	43	11	7	39	228
45–49	47	9	11	33	320
50–54	37	16	6	42	331
55–59	35	29	2	34	373
60–64	40	26	2	32	438
65–69	45	23	2	30	449
70–74	52	21	2	26	487
75–79	55	23	1	22	376
80–84	53	21	2	25	238
85–89	40	30	0	30	110
≥90	59	19	8	14	37

In the present study the cut-off point for ER was set at 10 fmol/mg protein. Values below this level were found in 1462 (36%) tumors. Using DNA as the reference parameter, a cut-off point of 94 (fmol ER/mg DNA) was used in order to get the same number of negative samples. The number of tumors not equally classified for ER status was found to be 168 of 4090 tumors (4.1%; $p<0.0001$). For PgR a similar estimation was made when the cut-off point was set at 30 fmol PgR/mg protein, which resulted in 1866 (53%) tumors falling below this level. The corresponding value for fmol PgR/mg DNA was 260. The number of tumors not equally classified was 102 (2.9%; $p<0.0001$).

Table 3

ER and PgR contents (percentage positive samples, mean concentrations) in samples analyzed in different conditions

Condition	ER			PgR		
	n	% +	Mean value*	n	% +	Mean value*
Satisfactory	4 323	65	120	3 640	48	180
Unsatisfactory						
Thawed samples	192	31	24	138	16	42
0.25–0.5 mg protein/ml	162	69	85	126	42	220
<0.25 mg protein/ml	55	78	230	51	37	180
Antiestrogen	63	16	14	58	26	140
Preoperative radiotherapy	17	0	1.9	16	6	5.9
Pregnancy	4	0	0.35	4	0	0

* fmol/mg protein.

Discussion

This study presents data from over 4000 ER and PgR analyses in patients from the Southern Swedish Health Care Region. The proportion of positive samples and mean concentration values for both ER and PgR were quite stable during the last 10 years.

It is generally considered of importance that receptor determinations are performed in a cytosol of sufficient protein concentration (1 mg/ml (17)). The results from the present study show, however, that with our method of isoelectric focusing the ER content (percentage positive samples and mean concentrations) was not influenced by even very low protein concentrations. In agreement with others, the use of the DCC method for PgR measurement was negatively influenced by low protein concentration values.

In clinical practice, tumors are classified as either receptor positive (rich) or receptor negative (poor) on the basis of arbitrarily chosen cut-off points. The question was raised whether the choice of reference parameter in some cases would influence the classification of the patient and, ultimately, the choice of treatment. Protein is the most commonly used reference parameter for the expression of steroid receptor content, but DNA and wet weight have also been used. From the data obtained in the present study, which confirms a smaller previous study (18), it could be concluded that similar information could be obtained with either of these reference parameters.

In agreement with other studies (19), ER positivity was in the present study positively correlated with age at the time of operation. For PgR a different age distribution pattern is found. For patients below 45 years and patients ≥55 years the PgR concentration increased with age. For patients between 45 and 54 years, PgR was negatively correlated with age. Similar results have been found by Thorpe in a study of about 4000 patients (19). The increase with age in patients younger than 50 years was more pronounced for PgR than for ER. This was illustrated both by a higher proportion of ER− PgR+ samples and by the higher PgR/ER ratio. One might speculate that changes in the hormonal milieu during the reproductive period influence this pattern. Estrogens have a stimulatory effect on both ER and PgR synthesis whereas progesterone has the opposite effect (20). Some studies have shown differences between ER and PgR in breast cancer related to the phase of the menstrual cycle (21–23). A possible explanation of the lower ER concentration in premenopausal patients could be a blockade of ER by endogenous estrogens. A study, comparing enzyme immuno-assay (measuring both unoccupied and occupied ER) and IF (measuring only unoccupied ER), however, has not confirmed this theory (13). Furthermore, the different age distribution patterns for ER and PgR were also found in a smaller series of samples measured by EIA. It has also been hypothesized that ER and PgR contents in the breast cancer at time of operation reflect the endocrine milieu at the time of tumor initiation, so that the actual hormonal status of the patient might be of minor importance (24). A high frequency of ER− PgR+ samples in younger women has recently been shown to occur almost exclusively in women who have measurable ER in the nuclear fraction (after high salt extraction) which indicates methodological problems of receptor measurements in premenopausal patients (25). The difference between pre- and postmenopausal patients regarding ER and PgR status also raises the question whether it is appropriate in clinical practice to use the same cut-off points for all age groups when separating receptor positive and negative samples (10). In a recent report (26) on menopausal status and cut-off levels it was demonstrated that subgroups or subpopulations of patients could be identified on the basis of plasma FSH and estradiol concentration. Both subgroup definition and different ER/PgR profiles markedly influenced the fraction of so-called ER positive assays. In

order to investigate more carefully the relationship between ER and PgR for patients between 45–54 years of age, the PgR/ER ratio should be calculated for each patient and then viewed in relation to the time for menopause. It should furthermore be interesting to measure ER content in the nuclear fraction from samples with different PgR/ER ratios. Such a study has been started at our department.

The influence of the hormonal milieu on the measurable amount of ER and PgR is also illustrated in samples from patients on tamoxifen treatment at the time of operation. The proportion of both ER+ and PgR+ decreased after this treatment. The negative effect of pregnancy and preoperative radiotherapy on the measurable amount of ER and PgR is also in agreement with other investigations (27, 28). Regarding other influential factors, we have found that young premenopausal breast cancer patients, who at an early age have used oral contraceptives, have lower receptor concentrations than other young women (29). Other investigations have also shown that ER concentrations are lower among ever users of oral contraceptives (30–33). Preliminary findings also suggest that age at first full-term pregnancy affects receptor concentrations (34). We have therefore started studies on breast cancer risk factors and different reproductive factors in relation to receptor concentrations. The influence of menopausal estrogen administration on ER and PgR concentrations will also be considered.

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Request for reprints: Dr Mårten Fernö, Department of Oncology, University Hospital, S-221 85 Lund, Sweden.

REFERENCES

1. McGuire WL, Osborne CK, Clark GM, Knight WA. Steroid hormone receptors and carcinoma of the breast. Am J Physiol 1982; 243: 99–102.
2. McGuire WL, Clark GM, Dressler LG, Owens MA. Role of steroid hormone receptors as prognostic factors in primary breast cancer. NCI Monographs, 1986; 1: 19–23.
3. Rose C, Thorpe SM, Andersen KW, et al. Beneficial effect of adjuvant tamoxifen therapy in primary breast cancer patients with high oestrogen receptor values. Lancet 1985; 1: 16–9
4. Stewart HJ, Prescott R. Adjuvant tamoxifen therapy and receptor levels. Lancet 1985; 1: 573.
5. Baum M, Brinkley DM, Dossett JA, et al. Controlled trial of tamoxifen as single adjuvant agent in management of early breast cancer. Lancet 1985; 1: 836–40.
6. Howat JMT, Barnes DM, Harris M, Swindell R. The association of cytosol oestrogen and progesterone receptors with histological features of breast cancer and early recurrence of disease. Br J Cancer 1983; 47: 629–40.
7. Shapiro CM, Schifeling D, Bitran JD, et al. Prognostic value of the estrogen receptor level in pathologic stage I and II adenocarcinoma of the breast. J Surg Oncol 1982; 19: 119–21.
8. Villadsen S, Jeune B, Kongshavn T, Höy Pedersen S. The age of the menopause. Methodological problems in historic and geographic comparisons. Ugeskr Laeger 1985; 147: 3637–41.
9. Norgren A, Borg Å, Fernö M, Johansson U, Lindahl B, Tsiobanelis K. Improved method for assay of estradiol and progesterone receptors with special reference to breast cancer. Anticancer Res 1982; 2: 315–20.
10. Fernö M. Thesis. Malmö, Sweden: Infotryck AB, 1985.
11. DeSombre ER, Carbone PP, Jensen EV, et al. Steroid receptors in breast cancer. N Eng J Med 1979; 301: 1011–2.
12. Fernö M, Borg Å, Norgren A. A comparison of two steroid receptor assays in breast cancer: dextran coated charcoal and isoelectric focusing. Anticancer Res 1983; 3: 243–6.
13. Fernö M, Borg Å, Sellberg G. Enzyme immunoassay of the estrogen receptor in breast cancer biopsy samples. A comparison with isoelectric focusing. Acta Radiol Oncol 1986; 25: 171–5.
14. Fernö M, Borg Å, Johansson U. Enzyme immunoassay of progesterone receptor in breast cancer biopsy samples. A comparison with the dextran coated charcoal method. Acta Oncol 1989; 28: 19–22.
15. Lowry OH, Roseborough N, Farr L, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951; 193: 265–75.
16. Burton K. A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. Biochem J 1956; 62: 315–23.
17. EORTC Breast Cooperative Group. Revision of the standards for the assessment of hormone receptors in human breast cancer: Report of the Second EORTC Workshop, Held on 16–17 March, 1979, in the Netherlands Cancer Institute. Eur J Cancer 1980; 16: 1513–5.
18. Norgren A, Fernö M, Borg Å. Observations on wet weight protein and DNA as reference for steroid receptors in malignant mammary tumours. Anticancer Res 1986; 6: 59–64.
19. Thorpe SM. Estrogen and progesterone receptor determinations in breast cancer. Technology, biology and clinical significance. Acta Oncol. 1988; 27: 1–19.
20. Williams RH. Textbook of Endocrinology. Washington: Saunders WB Co, 1981: 60.
21. Heise E, Görlich M. Estradiol receptor in human breast cancers throughout the menstrual cycle. Oncology 1982; 39: 340–4.
22. Saez S, Chouvet C. Influence of endogenous hormone levels on tumor estradiol and progesterone receptors. Recent Results Cancer Res 1984; 91: 150–6.
23. Weimer DA, Weimer WL. Changes in estrogen and progesterone receptor content of primary breast carcinoma during the menstrual cycle. Breast Cancer Res Treat 1987; 10: 273–8.
24. Olsson H. Reproductive events, occurring in adolescence at the time of development of reproductive organs and at the time of tumour initiation, have a bearing on growth characteristics and reproductive hormone regulation in normal and tumour tissue investigated decades later—a hypothesis. Med Hypotheses 1989; 28: 93–7.
25. Thorpe SM. Immunological quantitation of nuclear receptors in human breast cancer. Relation to cytosolic estrogen and progesterone receptors. Cancer Res 1987; 47: 1830–5.
26. Norgren A, Forsberg AH, Lindgren A, Sällström J. Meno-

pausal status and cut-off levels in steroid receptor ligand binding assays in breast cancer. Anticancer Res 1989; 9: 173–6.
27. Holdaway IM, Mason BH, Kay RG. Steroid hormone receptors in breast tumours presenting during pregnancy or lactation. J Surg Oncol 1984; 25: 38–41.
28. Janssens JPH, Bonte J, Drochmans A, et al. Effect of presurgical radiotherapy on the steroid receptor concentrations in primary breast carcinoma. Eur J Cancer 1981; 17: 659–64.
29. Olsson H, Borg Å, Ewers SB, Fernö M, Möller T, Ranstam J. A biological marker, strongly associated with early oral contraceptive use, for the selection of a high risk group for premenopausal breast cancer. Med Oncol Tumor Pharmacother 1986; 3: 77–81.
30. Hulka BS, Chambless LE, Wilkinson WE, et al. Hormonal and personal effects on estrogen receptors in breast cancer. Am J Epidemiol 1984; 119: 692–704.
31. Lesser ML, Rosen PP, Senie RT, et al. Estrogen and progesterone receptors in breast carcinoma: correlations with epidemiology and pathology. Cancer 1981; 48: 299–309.
32. Osborne MP, Rosen PP, Lesser ML, et al. The relationship between family history, exposure to exogenous hormones, and estrogen receptor protein in breast cancer. Cancer 1983; 51: 2134–8.
33. Stanford JL, Szklo M, Boring CC, et al. A case-control study of breast cancer stratified by estrogen receptor status. Am J Epidemiol 1987; 125: 184–94.
34. Thomas D. Hormones and hormone receptors in the etiology of breast cancer. Breast Cancer Res Treat 1986; 7 (Suppl): 11–22.