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Estrogen and Progesterone Receptor Content of Primary and Secondary Breast Carcinoma: Influence of Time and Treatment

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Abstract—*ER and PgR concentrations were assayed in primary and secondary breast carcinoma specimens from patients classified into 3 groups*: (1) *both specimens excised on the same occasion* (61 *patients*); (2) *specimens obtained on separate occasions with no intervening treatment* (43 *patients*); (3) *specimens obtained on separate occasions with intervening chemotherapy and/or irradiation* (25 *patients*). *There were highly significant linear correlations* ($P < 0.001$) *between the concentrations of ER* (*expressed as* $\log_{10}$) *in primary and secondary specimens in all groups. The relationship between PgR concentrations in primary and secondary specimens in groups* 1 *and* 2 *was highly significant, although there appeared to be a greater tendency for loss of PgR in sequential, than in simultaneous secondary biopsies.*

When expressed in terms of hormone receptor status (*HRS*), *the same rate of discordance was observed in groups* 1 *and* 2 (30% *when concentrations were expressed in terms of cytosol protein*). *In group* 1 *the major cause of discordance was the occurrence of receptor* +*ve secondaries in association with receptor* −*ve primaries, possibly because of the high cellularity of many involved axillary nodes. In group* 2, *the major cause of discordance was the occurrence of receptor* −*ve secondaries derived from receptor* +*ve primaries. In both groups discordance in PgR status was more frequent than in ER status. In group* 3, *overall discordance in HRS was* 24% *and was due equally to ER and PgR; however, the high concordance rate for PgR was probably due to the fact that the tumours were initially PgR* −*ve, and the secondaries were also* −*ve.*

These results confirm that ER content tends to be stable, even after long periods of time and the administration of chemotherapy and/or irradiation. Progesterone receptor content is much less stable, and may decrease during quite short time intervals even in the absence of treatment.

INTRODUCTION

THE ROLE of the estrogen and progesterone receptor (ER and PgR) status of primary breast carcinoma as an aid in prognosis and in choice of therapy for recurrent disease is well established. However, the increasingly frequent use of systemic adjuvant therapy raises the possibility that the hormone receptor status (HRS) of recurrent carcinoma appearing during or after this period of therapy may be altered. In that event, the HRS of the primary disease would be less useful as an indication of the likely response of metastatic disease to any particular therapeutic regimen. Apart from the effect of therapy, it is also possible that the hormone receptor content may change spontaneously over time. In particular, it might be expected that the functional activity of ER (as assessed by the presence of PgR) would be lost before the loss of ER itself. In order to investigate possible changes in HR concentration brought about by time or treatment, it is necessary to compare the differences in receptor concentration in primary and secondary breast carcinoma specimens removed from the same patient on the same occasion with the differences observed when time, with and without treatment, has intervened between primary and secondary surgery. The emphasis in many published investigations of this topic has been on differences in hormone receptor content in terms of receptor status, i.e. receptor positivity or negativity, rather than in terms of quantitative differences [e.g. 1–4]. The conclusions reached are thus influenced

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by the "cut-off" points used to discriminate between positive and negative receptor status, and also by the precision of the assays at values near the "cut-off" points, which may vary between laboratories, particularly for PgR. Also since the receptor concentrations form a continuous, rather than a discontinuous, population of values it seems more appropriate to examine the data in quantitative terms. This approach has been used mainly for ER [5, 6]: fewer data are available for PgR [7, 8].

In this paper, we have examined in both quantitative and qualitative terms the relationships between receptor concentrations in primary and secondary breast carcinoma specimens from patients undergoing breast surgery at Women's College Hospital, Toronto, between January 1974 and September 1985. The majority of the patients were seen and followed-up at the Henrietta Banting Breast Centre.

PATIENTS, MATERIALS AND METHODS

Patients

Primary and secondary breast cancer specimens were obtained from 129 patients, who were classified into 3 groups:

Group 1. Both specimens obtained on the same occasion and assayed at the same time. This group included 61 patients, of whom 36 were postmenopausal, and 25 pre- or perimenopausal. Since hormonal changes after the menopause are gradual [9], perimenopausal patients are defined as those within 5 years of a natural menopause, and hysterectomized patients between 50 and 55 years old whose ovaries were left intact. Postmenopausal patients are defined as those 5 years or more beyond a natural menopause, and all those who have undergone ovarian ablation. In this group, all except one of the secondary biopsies were lymph nodes, mainly from the axilla. The exception was a skin nodule on the chest wall.

Group 2. Primary and secondary specimens obtained on different occasions 1–75 months apart (mean interval 21 months, median 16 months), with no intervening therapy. Of the 43 patients in this group, 27 were postmenopausal, 15 pre- or perimenopausal, and one changed her menopausal status from peri- to postmenopausal during the time interval between biopsies. The sites of the secondary biopsies were more varied than in group 1: 18 from the skin or muscle of the chest wall, 17 lymph nodes, 3 from breast tissue, 2 from the mastectomy scar, and one each from the muscle of the back, the abdominal wall and the lung.

Group 3. Primary and secondary specimens obtained on different occasions 4–87 months apart (mean interval 25 months, median 17 months), with intervening chemotherapy and/or irradiation (25 patients). The sites of the secondary biopsies were similar to those in group 2: 11 from the skin or muscle of the chest wall, 9 lymph nodes, 3 from the mastectomy scar and one each from neck muscle and peritoneum. The treatments given are listed in Table 1. Patients given hormonal treatments were omitted from this study, due to the difficulty in intepreting data obtained from patients who were on Tamoxifen treatment at the time of secondary biopsy. Of the 25 patients who had non-hormonal treatment, 13 were postmenopausal, and 12 were pre- or perimenopausal at the time of primary surgery. Two of the latter (aged 40 and 51 years) stopped menstruating during the course of chemotherapy.

Table 1. Treatment of patients in group 3

Treatment	No. of patients
Irradiation alone	
To primary excision site and regional nodes	2
To non-operable primary tumour	1
To area of local recurrence	3
Chemotherapy alone	
CMF*	15
CMF + oral BCG*	1
Adriamycin and cyclophosphamide	1
Irradiation and chemotherapy	
Irradiation and CMF	1
Irradiation and FAC*, followed by CMF	1

*CMF: cyclophosphamide, methotrexate, 5-fluorouracil; BCG: B. Calmette Guerin; FAC: 5-fluorouracil, adriamycin, cyclophosphamide.

The proportion of pre- and perimenopausal patients in this study [52/129 (40%)] is somewhat higher than in the overall population of breast cancer patients from whom we have samples for receptor assay (approximately 33%). This may reflect the generally greater aggressiveness of the disease in younger women, resulting in more recurrences than in the postmenopausal patients during the time frame of this study.

Receptor assays

Reagents. [2, 4, 6, 7][^{3}H]Estradiol (specific activity 90–115 Ci per mmol), [17α-methyl-^{3}H]promegestone (R5020) (specific activity 70–87 Ci per mmol) and radioinert R5020 were obtained from New England Nuclear, Boston, MA. The radioisotopes were diluted on receipt to stock solutions containing 10 μCi per ml redistilled benzene : ethanol, 9 : 1 v/v. Radioinert estradiol, cortisol and dihydrostestosterone were obtained from Sigma Chemical Company, St. Louis, MO; dextran T 70 from Pharmacia, Montreal; and charcoal (Norit

A) from Matheson, Coleman and Bell, OH. The scintillator used was PCS (Amersham Corp., Arlington, IL): toluene 2 : 1 v/v.

Receptor assays: Tumours were chilled on ice and transferred to the laboratory where they were stored in liquid nitrogen, usually for less than 10 days. Cytosol preparation and receptor assays were carried out as described previously [10]. Dextran-coated charcoal was used to separate free and bound steroid after the incubation of replicate aliquots of cytosol with 1 nM [^{3}H]estradiol or 5 nM [^{3}H]R5020, in the absence or presence of appropriate excess cold steroids to correct for non-specific binding. Cytosol was prepared in the absence of glycerol but the latter was included in the incubations with [^{3}H]R5020 in order to stabilize PgR. It is therefore likely that PgR was dissociated from endogenous ligand prior to incubation, and that the assay quantitates total PgR [11]. Cytosol protein was assayed by the Lowry method [12].

Progesterone receptor assays were carried out only after November 1977: the number of patients for whom PgR assay data are available for both primary and secondary specimens is therefore 98, as compared with 129 for the ER assay. The assay results were expressed as fm/mg tissue wet wt for the whole series: results obtained after May 1980 were also expressed as fm/mg cytosol protein. Binding data from primary and secondary specimens from 70 patients were thus expressed in terms of both weight and cytosol protein.

With regard to the qualitative expression of the data, the "cut-off" values used to discriminate between receptor negative and receptor positive specimens in this laboratory are as follows: for ER, $<$ 10 fm/mg cytosol protein ($<$ 0.3 fm/mg wet wt) is considered negative; $\geqslant$ 10 fm/mg protein ($\geqslant$ 0.3 fm/mg wet wt) is considered positive in pre- and perimenopausal patients; 10–19 fm/mg protein (0.3–0.5 g fm/mg wet wt) is considered borderline and $\geqslant$ 20 fm/mg protein ($\geqslant$0.6 fm/mg wet wt) is considered positive for postmenopausal patients. For PgR, $<$ 45 fm/mg (1.35 fm/mg wet wt) is considered negative, 45–59 fm/mg (1.35–1.77 fm/mg wet wt) is considered borderline, and $\geqslant$ 60 fm/mg protein ($\geqslant$ 1.78 fm/mg wet wt) is considered positive for all patients. The values used for PgR are higher than those in use in most laboratories: however, use of these "cut-off" values has resulted in the following distribution of receptor status over some 2500 patients: ER + PgR + 38%, ER + PgR − 27%, ER − PgR + 2%, ER − PgR − 18%. The remaining 15% have borderline status for either ER, PgR or both. In order to simplify presentation of the data in this paper, tumours with borderline status have been included with those with positive status.

RESULTS

Group 1

Whether the binding data were expressed as fm/mg cytosol protein, or as fm/mg wet wt of tissue, there was a very strong linear correlation between the ER binding capacity (expressed as $\log_{10}$) of primary and secondary tumour specimens obtained on the same occasion (Fig. 1). Exceptions were 2 secondary specimens (involved lymph nodes) which contained very little tumour tissue. The regression line expressing the relationship was parallel to, but slightly above, the line of identity, indicating that there was a tendency for the secondary specimen to have a slightly higher binding capacity than the primaries. This may have been due to the fact that virtually all the secondaries in this patient group were involved lymph nodes, which often contained tumour less contaminated by stromal tissue than the primary tumour.

The relationship between PgR binding capacities in the primary and secondary specimens was not as close as for ER, but was still highly significant (Fig. 2). While a number of secondaries had a higher PgR content than the corresponding primaries, there were also cases in which a partial or complete loss of PgR had occurred.

With regard to the hormone receptor status (HRS) of the primary and secondary specimens, it is clear from Fig. 1 that (apart from secondaries with little malignant involvement) few discordances in ER occurred (12%), and that most of those which did occur were due to small quantitative differences. The occurrence of positive secondaries with negative primaries was as common as the converse. This also applied to PgR (Fig. 2), but with this receptor discordances were more frequent (24%) and were commonly due to larger quantitative differences. Table 2 shows the data from patients for whom both ER and PgR status was available for primary and secondary specimens. When both receptors were considered together, the discordance rate was 38% when the results were expressed in terms of tissue wet wt, and 30% when expressed in terms of cytosol protein. The largest category of discordances resulted from ER + PgR + secondaries occurring with ER + PgR − primaries. Whether the data were expressed in terms of tissue weight or cytosol protein made only a marginal difference in the receptor status distribution.

Group 2

In cases in which time had elapsed between excision of the primary and secondary specimens, there was still a highly significant positive correlation of concentration of both receptors between the paired specimens (Figs. 3 and 4). Divergence

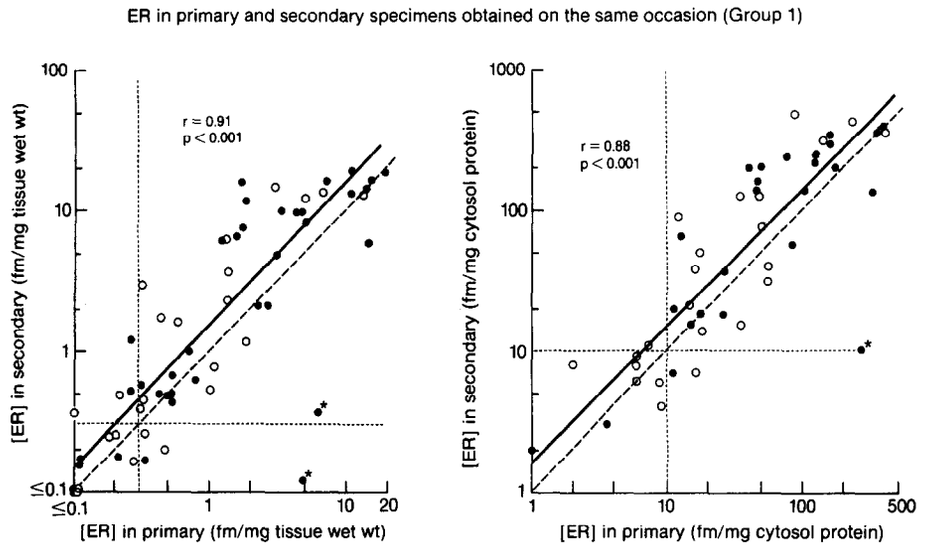

*Fig. 1. ER concentrations in primary and secondary breast carcinoma specimens removed on the same occasion (group 1). Binding data are expressed in terms of tissue wet wt (left panel) and in terms of cytosol protein (right panel). ○, Pre- and perimenopausal patients; ●, postmenopausal patients; ·····, values used as "cut-off" to discriminate between receptor +ve and −ve tumours; line of identity, ---; regression line, ——; specimens containing little tumour tissue, * :these values were omitted from the calculation of the regression.*

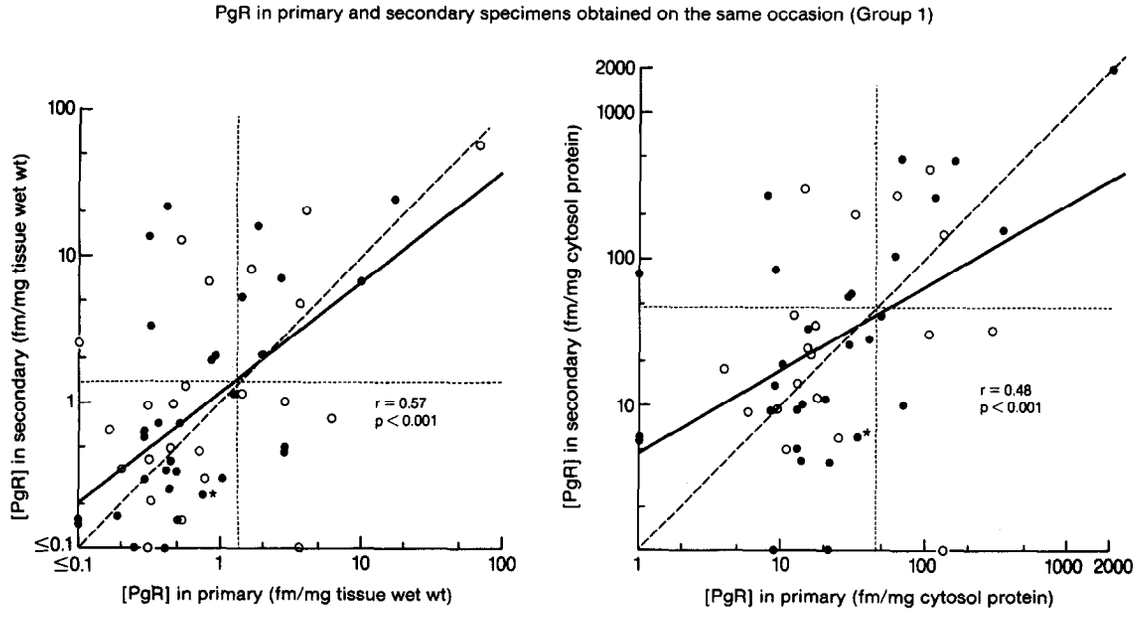

Fig. 2. PgR concentrations in primary and secondary breast carcinoma specimens removed on the same occasion (group 1). Binding data are expressed in terms of tissue wet wt (left panel) and in terms of cytosol protein (right panel). Symbols as in Fig. 1.

from the line of identity was larger for PgR than for ER, indicating a greater tendency for loss of binding capacity. Consistent with this observation, more discordances were observed in PgR, than in ER status (33% vs 19%). As in group 1, the association of positive secondaries with negative primaries as well as the converse situation was observed. When both receptors were considered together (Table 2), the overall discordance rate was 46% when the results were expressed in terms of tissue wet wt, and 30% when expressed in terms of cytosol protein. The latter value is identical for the overall discordance rate in group 1. However, whereas in group 1, the majority of discordances were due to higher receptor concentrations in the secondaries than in the primaries, the reverse was true in group 2. Neither the receptor status changes nor the changes in receptor concentrations were related to the time interval between primary and secondary surgery in this group.

Differences in receptor content between primary and secondary specimens generally did not appear to be associated with menopausal status. However, in the patient whose status changed during the

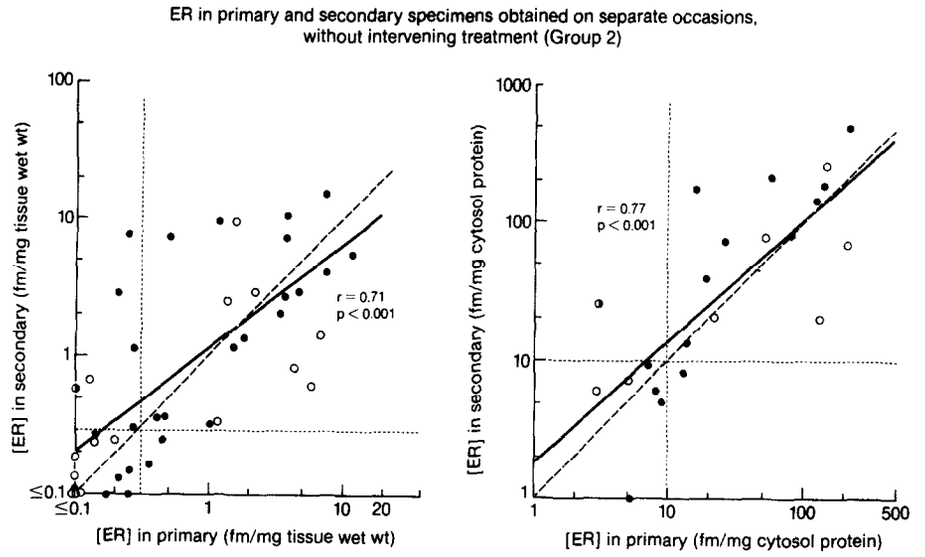

Fig. 3. ER concentrations in primary and secondary breast carcinoma specimens excised on different occasions without intervening therapy (group 2). ◑, Patient who became postmenopausal during interval between primary and secondary biopsies. Other symbols and expression of binding data as in Fig. 1.

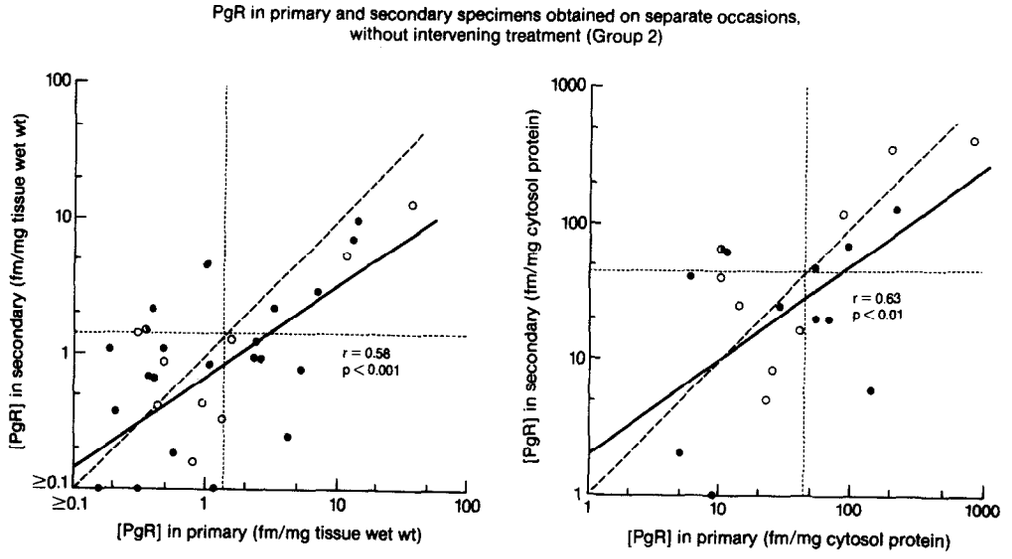

Fig. 4. PgR concentrations in primary and secondary breast carcinoma specimens excised on different occasions without intervening therapy (group 2). Symbols and expression of binding data as in Fig. 1.

interval between biopsies, both the ER and PgR concentrations in the secondary were higher than in the primary, resulting in discordance of the HRS.

Group 3

Because of the small number of patients eligible for this group since 1980, the results have been expressed as fm/mg wet wt only (Fig. 5). However, it is clear from the results from groups 1 and 2 that expression of the binding data in this way is very similar to expression in terms of cytosol protein.

The relationship between ER concentrations in the primary and secondary specimens was similar to that in group 2: in both groups an approximately equal number of secondary specimens had ER values lower and higher than the corresponding primary, but in both groups a strong positive correlation was observed between the primary and secondary values in the tumour population as a whole. However, there was no significant correlation beteeen the primary and secondary PgR values in this group, partly due to the fact that very few of the primaries in this group were PgR +ve. Possibly this reflects selection of PgR −ve patients for non-hormonal treatment, or it may indicate that PgR −ve tumours were more likely than PgR +ve tumours to recur within the time frame of this study. Thus, few discordances in PgR status were

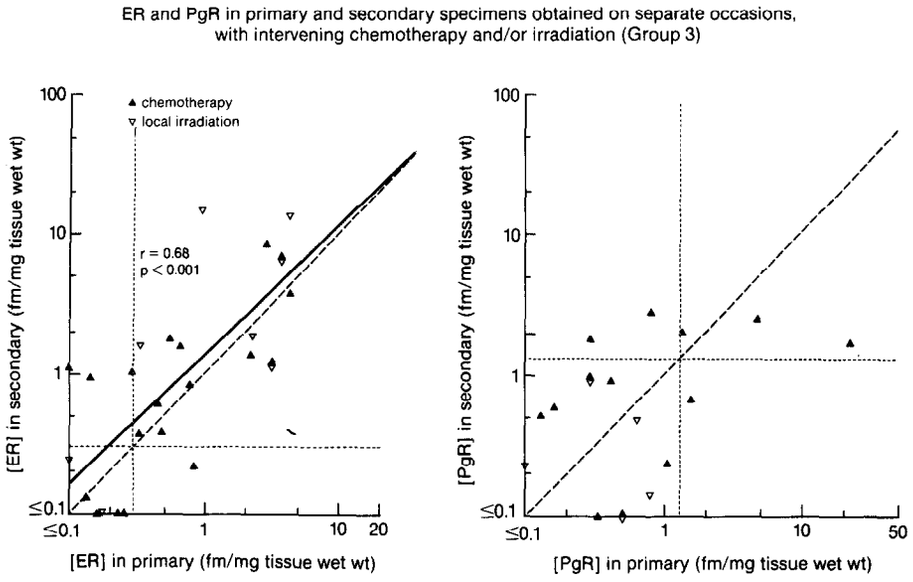

Fig. 5. ER and PgR concentrations in primary and secondary breast carcinoma specimens excised on different occasions with intervening non-hormonal treatment: ▲, chemotherapy; ▽, regional irradiation. Binding data expressed in terms of tissue wet wt.

Table 2. Hormone receptor status (HRS) in primary and secondary biopsies

HRS of secondary	Group 1 n = 53 (47)	HRS of primary ER + PgR +	ER + PgR −	ER − PgR +	ER − PgR −	Totals
	ER + PgR +	17 (19)	15 (15)	2 (2)	0 (0)	34 (36)
	ER + PgR −	8 (6)	34 (38)	0 (0)	6 (2)	48 (46)
	ER − PgR +	2 (0)	0 (0)	0 (0)	0 (0)	2 (0)
	ER − PgR−	2 (2)	2 (2)	2 (2)	11 (13)	17 (19)
	Totals	29 (27)	51 (55)	4 (4)	17 (15)	

HRS of secondary	Group 2 n = 30 (20)	HRS of primary ER + PgR +	ER + PgR −	ER − PgR +	ER − PgR −	Totals
	ER + PgR +	17 (25)	7 (5)	0 (0)	3 (5)	27 (35)
	ER + PgR −	17 (10)	27 (25)	0 (5)	7 (0)	51 (40)
	ER − PgR +	3 (0)	0 (0)	0 (5)	3 (0)	6 (5)
	ER − PgR −	0 (5)	3 (0)	3 (0)	10 (15)	16 (20)
	Totals	37 (40)	37 (30)	3 (10)	23 (20)	

n = Number of specimen pairs for which both ER and PgR values were available. HRS data are derived from receptor concentrations expressed as fm/mg wet wt and as fm/mg cytosol protein (in parentheses), and are expressed as a percentage of the total number of specimen pairs in each group.

observed in this group, the majority of both primary and secondary specimens having PgR −ve status. There were only 3 discordances in ER status (12%), and 2 of those were due to a higher ER content in the secondary than in the primary specimen: one of the patients concerned had stopped menstruating during the course of chemotherapy. Of the concordant pairs of specimens, 16 were ER +ve, and 6 ER −ve. As in group 2, there was no relationship between changes in receptor status or concentration and the time interval between biopsies.

DISCUSSION

There are a number of factors which would be expected to contribute to quantitative differences in hormone receptor concentrations between primary and secondary specimens of the same carcinoma apart from changes in the receptor characteristics of the cell populations involved. Intratumoural heterogeneity with regard to receptor distribution has been shown to occur [13]. The portion of the primary and/or secondary carcinoma sent for assay may therefore not be representative of the whole specimen. This may be due to the distribution of epithelial and non-epithelial components and/or to the heterogeneous distribution of receptor within the epithelial component, as has been demonstrated using monoclonal antibody to ER for histochemical localization [14, 15]. Differences in cellularity would be expected to be another factor contributing

to apparent differences in hormone receptor concentration between primary tumours and involved lymph nodes or very fibrous secondary specimens such as those sometimes received from the chest wall or from muscle. The high cellularity of involved lymph nodes may account for the large proportion of secondaries in group 1 which had higher concentrations of receptors than in the corresponding primary. This was much less common in group 2, where many of the secondary specimens were from non-nodal sites. However, inspection of the data from that group showed that changes in receptor status from +ve primary to −ve secondary were in fact more common in the patients with nodal, than non-nodal, secondaries. Thus it is more likely that the difference in receptor status change patterns observed between groups 1 and 2 is due to the lapse of time between biopsies than to the different sites of the secondary specimens.

In addition to the factors inherent in the specimens themselves, the receptor assays, particularly that for PgR, contribute to the variation observed. The imprecision of the assays would be expected to contribute less to the differences between primary and secondary specimens in group 1, where specimens excised at the same time were assayed together (intra-assay variation), than those observed in groups 2 and 3, where the assays were carried out months or even years apart (inter-assay variation). In this laboratory, the coefficient of intra-assay variation in the ER and PgR assays is 13 and 22%, respectively, and inter-assay variation is 21 and 27%, respectively [16]. Poorer precision in the PgR assay than in the ER assay has been observed in a number of laboratories [16, 17]. Thus a somewhat greater dispersion about the line of identity between primary and secondary specimens in all groups would be expected for PgR than for ER concentrations, and in groups 2 and 3 than in group 1 for both receptors. However, the contribution of the assay imprecision to the observed differences in primary and secondary hormone receptor concentrations is likely to be small relative to the divergences observed.

The data reported here are in agreement with those in the majority of studies in which the hormone receptor content of primary and secondary breast carcinoma specimens have been compared. Except for hormonally manipulated patients, ER content appears to remain stable in the majority of patients over long periods of time, even after chemotherapy and/or irradiation [1, 3–6, 18, 19]. This stability is consistent with some cytological variables which have been examined [20, 21]. In common with others, we have found the length of time between sequential biopsies is not related to the degree of difference in hormone receptor content [1, 2, 7, 19]. The ER content of a primary carcinoma of the breast is therefore likely to be a good indication of the ER content of recurrent tumour, provided that no hormonal manipulation has occurred in the intervening period. Progesterone receptor concentration, and by implication, hormone sensitivity, is more likely to differ in primary and secondary biopsies, even in the absence of treatment [2, 7, 8 and these data]. Although the differences in PgR content are not always due to a decrease in receptor concentration, decreases are more likely to occur with time, even within a few weeks. This is supported by the work of Bonneterre *et al.* [22] who found that PgR assay carried out at the time of primary surgery was of no prognostic value at the time of recurrence. However PgR assay performed at the time of recurrence was of prognostic value, patients whose tumours contained > 50 fm/mg protein having a longer survival from the time of relapse than those with lower PgR concentrations. ER values > 10 fm/mg protein were of prognostic value when carried out at the time of primary surgery, or at the time of relapse. For the management of advanced breast cancer, it is therefore advisable to carry out receptor assays on tissue excised at a time as close as possible to the start of the proposed treatment.

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