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VARIATIONS IN THE CONTENT OF STEROID RECEPTORS IN BREAST CANCER

Comparison between primary tumors and metastatic lesions

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Steroid receptors were determined by the dextran-coated charcoal method in 193 breast cancer patients in different clinical stages of disease. Quantitative estrogen and progesterone values in primary tumors (n = 69) were compared with receptors in regional lymph node metastases (n = 28) and in distant malignant deposits (n = 65). The groups including receptor values from primaries and regional lymph node metastases (n = 15) and from primaries and distant metastatic lesions (n = 16) in the same patients were also analyzed. The obtained results indicated relative stability of both receptors in loco-regional disease, but with a tendency towards lower receptor values in lesions from advanced disease. This tendency is probably caused by the disease progression itself, but the influence of radio- or chemotherapy cannot be excluded.

Estrogen and progesterone receptor levels of primary tumors are often used as a guide for systemic therapy at breast cancer relapse. However, the stability of steroid receptor values during disease progression and/or after chemo- or radiotherapy or endocrine therapy is still a matter of discussion. Various data concerning steroid receptor levels in primaries and regional lymph node metastases have been reported (1-3). However, remarkably fewer papers on steroid receptor status in distant malignant lesions have been presented (4). In papers discussing receptor status in simultaneous or subsequent biopsies of breast cancer mostly qualitative receptor values have been reported. In the present study we compared qualitative and quantitative steroid receptor values from primaries with values from regional metastatic lymph nodes and different other secondary lesions (distant metastases and skin invaded by inflammatory carcinoma) in patients with advanced breast cancer.

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Material and Methods

Patients. Estrogen and progesterone receptors were determined in 193 malignant lesions from breast cancer patients:

- primary tumors (n = 69),
- regional lymph node metastases (n = 28),
- primary tumor and corresponding regional lymph node metastases from the same patients (n = 15) obtained at the initial surgery,
- different malignant lesions (n = 65) including distant (contralateral and supraclavicular) lymph node metastases (n = 10), skin metastases (n = 17), metastatic pleural effusions (n = 23), and skin invaded by inflammatory carcinoma (n = 15).
- primary tumors and distant metastatic lesions (5 skin metastases, 8 metastatic pleural effusions, 3 distant metastatic lymph nodes) from the same patients (n = 16) after different disease-free intervals.

Malignancy was histologically verified in all deposits. All specimens from primary tumors, regional lymph node

Table 1

Quantitative ER and PR levels (range and median) in different secondary deposits (n = 65)

Site	n	Previous therapy (n)				Receptor value fmol/mg protein range (median)	
		Radio	Chemo	Combined radio/chemo	None	ER	PR
Distant lymph node metastases	10	2	3	0	5	0–246(0)	0–2 (0)
Skin metastases	17	2	1	6	8	0–697(6)	0–92(0)
Metastatic pleural effusions	23	1	7	3	12	0–57(0)	0–30(0)
Skin invaded with inflammatory carcinoma	15	0	0	0	15	0–149(0)	0–56(0)

metastases and skin invaded by inflammatory carcinoma derived from patients who had not received any therapy prior to the receptor determination. The group in which the receptor status of primary tumor and regional lymph node metastases was determined simultaneously was composed of patients with localized, operable carcinoma with axillary lymph node involvement. In the group of different malignant lesions, 25/65 patients had received adjuvant radio- and/or chemotherapy (Table 1). In the group composed of primary tumors and distant metastatic lesions from the same patients, 8/16 patients had received radio- and/or chemotherapy after the receptor determination in primary tumor but before the receptor analysis of the secondary deposits (Table 2). However, only patients who had been without any therapy during at least four months before the receptor determination were included. No patients who had received endocrine therapy were included, due to the fact that these patients seldom had discontinued tamoxifen before appearance of the secondary lesions.

Receptor assay. Estrogen receptor (ER) and progesterone receptor (PR) were determined by a five-point dextran-coated charcoal (DCC) assay in cytosolic fractions of frozen tumor tissue (5). Pleural effusions required a special procedure. The effusions were collected in heparine-containing bottles. The cells were sedimented and the erythrocytes lysed. Smears were then made for cytological verification. The cells were counted and their viability determined by trypan-blue exclusion. Only effusions having more than 5×10^6 cells were processed for receptor determination. Tumor cells were washed with buffer for homogenization, stored in liquid nitrogen and processed in the same way as solid tumor specimens. Protein content of

Table 2

ER and PR in primary tumor and secondary distant deposit in patients without (Nos. 1–8) and with (Nos. 9–16) radio- and/or chemotherapy after the primary surgery

Case No.	Primary tumor		Therapy after primary surgery	Distant lesion		
	ER fmol/mg	PR protein		ER fmol/mg	PR protein	
1	21	25	None	7	0	(lymph node)
2	35	35	None	27	15	(skin)
3	254	20	None	69	22	(skin)
4	103	141	None	0	0	(pleura)
5	6	48	None	98	25	(pleura)
6	46	243	None	0	0	(pleura)
7	220	13	None	0	0	(pleura)
8	66	35	None	0	0	(pleura)
9	3	0	Chemother.	0	0	(lymph node)
10	10	25	Chemother.	0	0	(skin)
11	42	0	Chemother.	42	0	(pleura)
12	25	46	Radio/chemother.	0	0	(skin)
13	109	0	Radio/chemother.	697	22	(skin)
14	4	0	Radio/chemother.	0	0	(pleura)
15	7	42	Radiother.	48	3	(lymph node)
16	0	0	Radiother.	0	0	(skin)

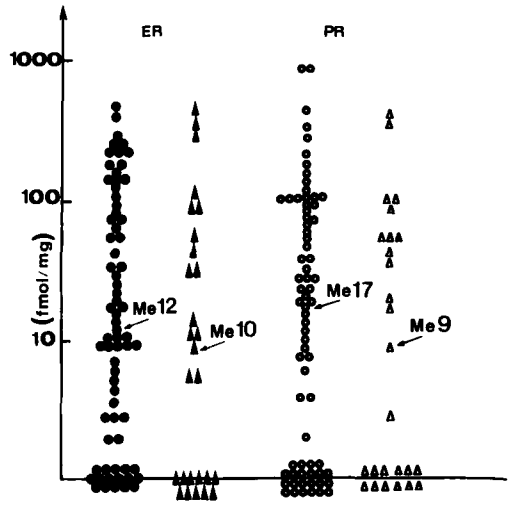

Fig. 1. Distribution of quantitative values of ER and PR in primary tumors (● ER, ○ PR, n = 69) and regional lymph node metastases (▲ ER, △ PR, n = 28). Arrows mark the median values.

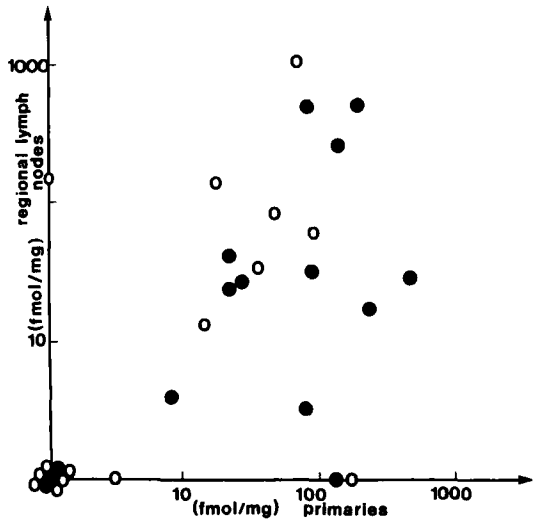

Fig. 2. Correlation between quantitative receptor values in primary tumors and axillary lymph node metastases from the same patients (● ER, ○ PR, n = 15) ($p < 0.05$).

the cytosol was measured by the method of Lowry et al. (6) and the results were analyzed by Scatchard plot (7). All results were expressed in fmol/mg cytosol protein. Cut-off point for the qualitative classification of positive receptor content was 10 fmol/mg protein for estrogen and 20 fmol/mg protein for progesterone receptors.

Statistical methods. For statistical analysis χ^2, Spearman's and Mann-Whitney's non-parametric tests were used.

Results

There was no significant difference (Mann-Whitney's non-parametric test) between primaries and regional lymph metastases concerning ER and PR levels (Fig. 1). The median ER values were 12 fmol/mg in primaries and 10 fmol/mg in metastatic lymph nodes and the corresponding PR values 17 fmol/mg and 9 fmol/mg respectively.

Qualitative analysis of the same data (χ^2-test, $p > 0.05$) showed no significant difference in the proportion of positive/negative ER and PR values between primaries and regional lymph node metastases (Table 3).

Receptor determinations were not performed twice (simultaneously or subsequently) in all patients. We therefore formed two groups in which two biopsies from the same patients were analyzed. In the first group receptor status was determined simultaneously in primaries and regional lymph node metastases (n = 15). In the second group subsequent biopsies of primaries and distant metastatic breast cancer lesions were analyzed (n = 16). A significant positive correlation ($p < 0.05$) was found both for ER and PR quantitative values estimated in primaries and regional lymph node metastases from the same patients (Spearman's test, Fig. 2).

Nevertheless, there was a significant difference ($p < 0.05$) between primaries and distant secondary lesions concerning the quantitative values of both receptors (Mann-Whitney's test, Fig. 3). The median values for ER were 12 fmol/mg protein in primaries and 0 fmol/mg protein in different secondary malignant lesions. The median values for PR were 17 fmol/mg protein in primaries and 0 fmol/mg protein in the secondary malignant lesions. There was no obvious difference in the ranges and median values between secondary deposits with different sites (Table 1). In the secondary deposits there was a higher proportion of ER and PR values of 0 fmol/mg protein and a lower proportion of values over 100 fmol/mg protein. No obvious difference in receptor values could be seen which could be clearly related to previous therapy. The quantitative ER

Table 3

Qualitative ER and PR assessment in primary tumors and axillary lymph node metastases

	ER^+PR^+	ER^+PR^-	ER^-PR^+	ER^-PR^-	Total
Primary tumors (n)	31	12	2	24	69
Regional lymph node metastases (n)	11	3	1	13	28
Total	42	15	3	37	97

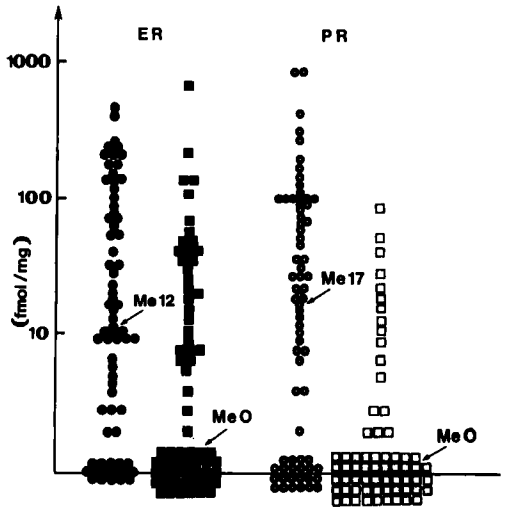

Fig. 3. Distribution of quantitative receptor values in primary tumors (n = 69) and distant malignant deposits (n = 65) ($p < 0.05$). Median values are marked by arrows. Closed symbols = ER (● primaries, ■ distant deposits); open symbols = PR (○ primaries, □ distant deposits).

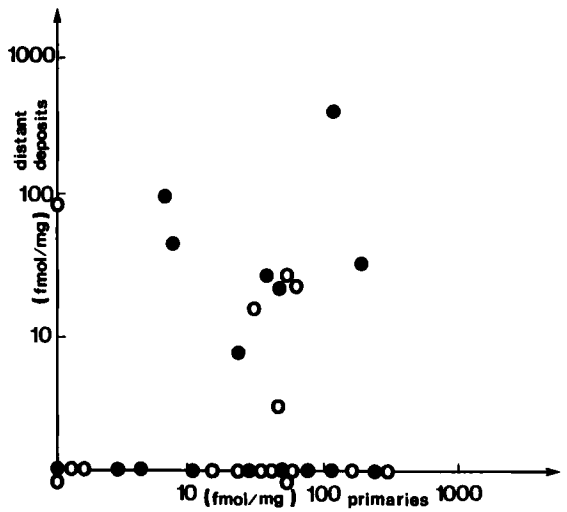

Fig. 4. Quantitative ER (●) and PR (○) values in primary tumors and in distant metastatic lesions from the same patients (n = 16). No correlation was found.

and PR values in the skin samples from inflammatory carcinoma were not significantly different from the distant secondary lesions but they were significantly lower than the values in the primaries and axillary lymph nodes (Mann-Whitney's test). With the mentioned cut-off points the most frequent phenotypes in distant malignant lesions were ER^+PR^- and ER^-PR^-. The proportion of ER^+PR^+ (Table 4) was significantly higher (χ^2-test, $p < 0.01$) in primaries than in the distant deposits. Individual receptor values in primary tumors and distant metastatic deposits from patients without or with adjuvant chemo/radiotherapy between sequential receptor determinations are shown in Table 2. In both groups discrepancies could be observed between the first and the second determination usually with lower values in the secondary deposits. There was, however, no obvious pattern that could be related to the therapy. No correlation was found (Spearman's test) between the quantitative receptor values in primaries and distant metastatic lesions from the same patients (Fig. 4).

Discussion

In most previous studies which had shown conflicting data concerning receptor stability during breast cancer progression, analysis of receptor status was made in a qualitative manner. The cut-off points for ER and PR positivity are defined empirically, usually ranging from 3 to 20 fmol/mg protein. Some studies have pointed out the importance of the receptor quantity for prognosis and choice of therapy in breast cancer (8, 9).

Our study showed a good agreement between quantitative ER and PR levels in primary tumors and regional lymph node metastases from the same patients. Many authors have reported similar results concerning qualitative receptor estimates (1, 10, 11). According to these findings the receptor status of regional metastatic lymph nodes may be used instead of values from the primary tumor for prognostic purposes and for therapy selection. This is important particularly when receptor status of the primary tumor is missing. However, Hahnel & Twaddle (12), Holdaway et al. (13) and Jakesz et al. (14) reported various discordance between the receptor content in sequential breast cancer biopsies that could be related to the time interval between the biopsies. Simultaneous biopsies had the greatest degree of agreement while more discrepancies with change from positive to negative receptor status could be found in sequential biopsies (14).

There is an opinion that a metastatic lymph node, due to its histological pattern (high cellularity and low proportion

Table 4

Qualitative ER and PR assessment in primary tumors and distant malignant deposits

	ER^+PR^+	ER^+PR^-	ER^-PR^+	ER^-PR^-	Total
Primary tumors (n)	31	12	2	24	69
Distant malignant deposits (n)	4	18	2	41	65
Total	35	30	4	65	134

of stroma), is more suitable for DCC receptor determination than are other malignant deposits (15). However, we analyzed receptor status of distant lymph nodes we found that the receptor values deviated obviously from those in primary tumors and in regional lymph nodes metastases. It seems likely that the concordance observed between primary tumor and axillary lymph nodes has biological rather than technical reasons.

Concerning the patients with receptor determination in both primaries and distant metastases, the absence of correlation indicated that the receptor status of distant metastases could not be predicted. For the choice of therapy it may therefore be of interest to determine the receptor levels in secondary lesions, whenever possible.

As far as influence of various therapy modalities is concerned, Toma et al. (16) reported that estrogen receptors tended to regain their original levels after discontinuation of therapy. The time period sufficient for this restitution varied considerably (from 1 to 12 months). The same authors tried to analyze the effect of radiotherapy, specifically on steroid receptor content of recurrences but concluded that more precise evaluation was impossible. (The recurrences could be situated within or outside an irradiated volume, etc. (16).)

Nor is the relation between chemotherapy and variations in steroid receptor content quite clear, and it has been suggested that steroid receptors may change both from positive to negative and vice versa, and that these changes are almost unpredictable (10, 17, 18). Moreover, it has been reported that these individual changes hardly affect the receptor status of the observed group as a whole because of their opposite directions (11). Allegra et al. (19) concluded that intervening chemotherapy did not affect the ER content. In the group of patients with sequential receptor determinations, similar changes of receptor status occurred, regardless of applied chemotherapy. The number of patients with chemotherapy was small ($n = 6$) but our observation is in accordance with the data cited above. Our study suggests that, during progression of the disease, there is a tendency towards loss of steroid receptors even if it cannot completely exclude an influence of radio/chemotherapy.

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