Eur J Cancer Clin Oncol, Vol. 20, No. 8, pp. 1011–1018, 1984.
Printed in Great Britain.

0277-5379/84$03.00+0.00

Concordance and Discordance of Estrogen and Progesterone Receptor Content in Sequential Biopsies of Patients with Advanced Breast Cancer: Relation to Survival

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Abstract—*In 75 patients with advanced breast cancer, sequential biopsies were analyzed for estrogen receptor (ER). In 50 of these patients progesterone receptor (PgR) was also measured. All pairs of biopsies met the following criteria: (i) interval between the two biopsies: at least* 6 weeks; *(ii) biopsies performed at least* 6 weeks *after stopping endocrine therapy; and (iii) concordant histology. Discordance in ER was found in* 14 *of* 75 *patients* (18.7%); *PgR was discordant in* 14 *of* 50 *patients* (28.0%). *No significant differences were found between concordant and discordant groups of patients in age at first diagnosis, menopausal state, diameter of the primary tumor, time interval between the two biopsies and intervening therapy. The initial ER level in patients whose ER changed from positive to negative was significantly lower than in patients whose ER remained positive. PgR levels exhibited a rise only when ER rose at the same time. Sequential assays have increased the prognostic significance of ER and as a consequence the estimated survival time for patients whose tumors were ER-negative in both biopsies was significantly shorter than for patients whose tumors were ER-negative in only one of the two biopsies. We found no prognostic significance for PgR in either single measurements or repeated biopsies.*

INTRODUCTION

MEASUREMENT of estrogen receptor activity (ER) in tumor tissue of patients with breast cancer proves to be useful in selecting patients likely to respond to endocrine treatment. However, a positive ER assay does not guarantee a response to endocrine treatment since about 40% of these patients fail to respond [1, 2].

At present it is still controversial whether data about progesterone receptor activity (PgR) will increase the predictability of the response to endocrine treatment [2–5]. The considerable number of failures in predicting the response to endocrine therapy could be due to inconsistency in the results of sequential measurements of receptor activity for estradiol and progesterone. From the scarce data in the literature it becomes clear that changes in receptor status in the course of time have occurred in about 20% of subjects [6].

We measured ER and PgR sequentially in patients with advanced breast cancer and related these data to clinical characteristics and survival rates.

MATERIALS AND METHODS

Sequential ER assays were performed in 75 patients with locally relapsed or advanced breast cancer, in 50 of whom PgR was also measured. All sequential biopsies met the following criteria: (i) interval between the two biopsies: at least 6 weeks; (ii) biopsies performed at least 6 weeks after cessation of endocrine treatment; and (iii) concordance in histology in the two biopsies. The majority of the patients ($n = 64$) were under treatment in the breast clinic of our hospital. The case records of all patients were reviewed and classified to (i) age at first diagnosis; (ii)

Accepted 6 February 1984.

menopausal state; (iii) diameter of the primary tumor; (iv) site of biopsies; (v) histology; (vi) intervening therapy; (vii) time interval between the two biopsies; (viii) disease-free interval; and (ix) survival times. Patients were considered to be postmenopausal if menses had ceased for at least 1 yr or after ovariectomy was performed. In almost all patients the elevated levels of gonadotropins in blood samples supported this classification. In 10 of the patients surgery was the only therapy between the two biopsies; 65 patients received either radiotherapy alone ($n = 13$), radiotherapy combined with systemic treatment ($n = 37$) or systemic treatment alone ($n = 15$) between the two biopsies. Initial systemic treatment after metastases were detected included endocrine modalities (ovariectomy, tamoxifen or estrogens), endocrine treatment combined with chemotherapy (5-fluorouracil and tamoxifen, or the latter in combination with 5-fluorouracil, methotrexate and cyclophosphamide, CMF) or chemotherapy alone (CMF), following treatment protocols. Later treatment modalities included aminoglutethimide, medroxyprogesterone acetate or the combination of adriamycin and cyclophosphamide. Survival times were reckoned from the date of first metastasis. One patient with an inoperable primary tumor but no distant metastases, whose sequential biopsies were taken from the primary tumor, was not included in this analysis. The estimated survival functions were calculated by the method of Kaplan and Meier [7], the differences between such functions being tested by Gehan and Mantel's non-parametric test [8, 9] (P denoted by p^*). Further statistical analyses were performed using Fishers's chi square test (P denoted by p) and Wilcoxon's two-sample test (P denoted by p^{**}). Correlation coefficients were calculated according to Spearman's rank non-parametric analysis of variance (P denoted by p^{***}).

The receptor assays were performed using the dextran-coated charcoal method as described before [10]. In our laboratory the lowest receptor values actually measured in human breast tumor cytosols were 5 and 7 fmol/mg protein for ER and PgR respectively.

Discordance in receptor status was defined as a qualitative change from positive to negative or the reverse.

RESULTS

Clinical characteristics (*Table* 1)

Table 1 shows that 14 out of 75 pairs of biopsies gave discordant results for ER and 14 out of 50 pairs for PgR. There was no statistically significant difference between patients with ER or PgR concordant and discordant biopsies in age at first diagnosis, menopausal state and diameter of the primary tumor. As might be expected, we found a statistically significant relation between the disease-free interval of patients whose first biopsy was taken from the primary tumor ($n = 46$) and the interval between the two biopsies ($r = 0,6$, $p^{***} < 0.05$). However, the time interval between the two biopsies did not differ significantly between the groups of patients with concordant and discordant results of ER or PgR. It has to be noted that in the ER concordant group of patients significantly more second biopsies were taken from lymph nodes than in the ER discordant group of patients ($p < 0.05$).

Variability in receptor status (*Tables* 2–4)

In this group of patients, 57.3% ER-positive (ER+) tumors and 46.0% PgR-positive (PgR+) tumors were found at first biopsy (Table 2). The table also shows the distribution of the various receptor phenotypes: ER+ PgR+, 38.0%; ER+ PgR−, 28.0%; ER− PgR−, 26.0%; and ER− PgR+, 8.0%. Table 3 shows the variability of the receptor status during the course of the disease. As mentioned above, discordance in ER status was present in 14 out of 75 patients (18.7%). In eight of them ER changed from positive to negative and in the remaining six patients the reverse was observed. With regard to the PgR status, the discordance rate was even higher: 14 of 50 patients (28.0%). In ten of these 14 patients PgR changed from positive to negative and in four from negative to positive. Table 4 outlines the consistency of the receptor phenotypes in sequential biopsies. Of the 23 initially PgR-positive tumors ten were negative at the second biopsy. Nine of these ten tumors were ER-positive at first biopsy and seven of these nine remained so, despite the change in PgR. Thus in the group of 19 patients with an ER+ PgR+ tumor at first biopsy, PgR became negative in nine tumors whereas ER changed in only two ($p < 0.05$). Furthermore, it appeared that the receptor phenotype ER− PgR− was the most stable one: concordance 84.6%. All ER−PgR+ tumors at first biopsy ($n = 4$) changed their phenotype at second biopsy. Three of these tumors changed to ER+ PgR+.

Quantitative changes in ER and PgR (*Figs* 1–3)

In Fig. 1 the absolute values of ER at first biopsy are depicted. The median ER value of the 43 ER-positive tumors at first biopsy was 60 fmol/mg protein. At second biopsy 35 of these tumors remained ER-positive. The median ER value at first biopsy of this group of patients was about equal, 71 fmol/mg protein. The mean ER value at first biopsy of the remaining eight

Table 1. Clinical characteristics in relation to the qualitative results of sequential ER and PgR assays

	ER		PgR	
	Concordant ($n=61$)	Discordant ($n=14$)	Concordant ($n=36$)	Discordant ($n=14$)
Age (yr):				
median	51	51	50	50
range	29–77	31–72	31–76	38–77
Menopausal state:				
premenopausal	10	2	7	3
postmenopausal	35	7	21	8
change pre/post	16	4	7	3
unknown		1	1	
Diameter of primary tumor:*				
≤5 cm	18	3	10	4
>5 cm	21	4	9	5
Site of 1st and 2nd biopsies respectively:				
primary tumor	39 3	7 0	19 1	9 0
skin	15 35	5 12	11 19	3 9
lymph node	6 19	2 0†	6 12	1 3
others	1 4	0 2	0 4	1 2
Intervening therapy:				
radiotherapy	40	10	21	9
systemic treatment	41	11	23	8
Interval between biopsies (months):				
median	19	24	17	20
range	2–66	7–76	2–54	2–66
Disease-free survival (months):				
median	22	30	23	28
range	4–168	7–74	5–80	8–168

*Including only those patients whose first biopsy was taken from the primary tumor.
†Statistical significance ($P<0.05$) between the site of biopsy (lymph nodes) in the concordant and discordant ER groups.

Table 2. Receptor phenotypes in the first biopsy

	n	%
ER ($n=75$)		
ER-positive	43	57.3
ER-negative	32	42.7
PgR ($n=50$)		
PgR-positive	23	46.0
PgR-negative	27	54.0
ERPgR ($n=50$)		
ER+ PgR+	19	38.0
ER+ PgR-	14	28.0
ER- PgR-	13	26.0
ER- PgR+	4	8.0

Table 3. Receptor phenotypes in repeated biopsies

	Receptor analyzed			
	ER ($n=75$)		PgR ($n=50$)	
Phenotypes	n	%	n	%
Both positive	35	46.7	13	26.0
Both negative	26	34.7	23	46.0
Change pos→neg	8	10.7	10	20.0
Change neg→pos	6	8.0	4	8.0
% changed	18.7		28.0	

Table 4. Consistency of receptor phenotype in sequential biopsies

First biopsy ER PgR	n	Second biopsy +/+	+/-	-/-	-/+
+/+	19	10/19	7/19	2/19	0/19
+/-	14	2/14	10/14	2/14	0/14
-/-	13	2/13	0/13	11/13	0/13
-/+	4	3/4	0/4	1/4	0/4

patients, whose tumor became ER-negative at second biopsy, was significantly lower than the mean ER value of the tumors which remained ER-positive (44.5 ± 29.4 vs 191.9 ± 275.4 fmol/mg protein respectively, $p^{**}<0.05$). Such a difference could not be established for PgR, as shown in Fig. 2: the median PgR value of the 23 PgR-positive tumors at first biopsy ws 51 fmol/mg protein; 13 of these patients remained PgR-positive at second biopsy, with an initially median PgR value of 74 fmol/mg protein; there was no statistically significant difference between the mean PgR value at first biopsy of the ten patients whose tumor became PgR-negative at second biopsy and the mean PgR value of the 13 tumors that remained PgR-positive (86.5 ± 140

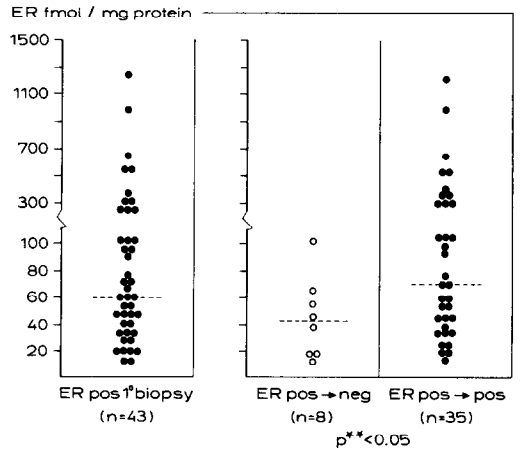

Fig. 1. Absolute ER levels of all patients with ER-positive tumors at first biopsy (n = 43)*, of those whose ER status remained positive* (n = 35) *and of those whose ER status became negative* (n = 8) *at second biopsy.*

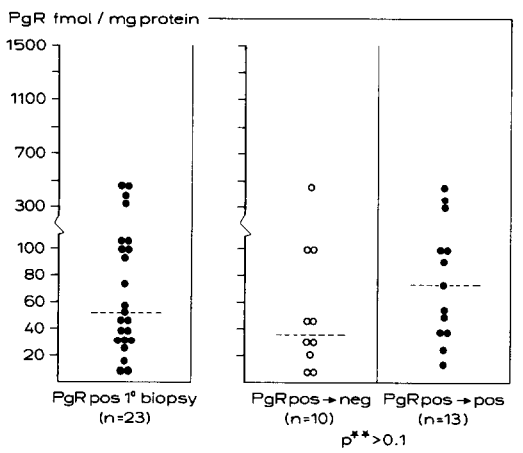

Fig. 2. Absolute PgR levels of all patients with PgR-positive tumors at first biopsy (n = 23)*, of those whose PgR status remained positive* (n = 13) *and of those whose PgR status became negative* (n = 10) *at second biopsy.*

vs 138.1 ± 151 fmol/mg protein respectively, $p^{**} > 0.1$).

With regard to those tumors whose ER or PgR status remained positive, the median values of ER or PgR at first and second biopsy were about equal: for ER 71 and 62 fmol/mg protein respectively; for PgR 74 and 45 fmol/mg protein repectively. Figure 3 shows the relation between the changes in absolute ER values (ΔER) and the changes in absolute PgR values (ΔPgR). When PgR increases, ER rises concomitantly. When ER decreases, PgR falls at the same time.

Survival and ER and PgR status (Figs 4–7)

Figure 4 shows the estimated survival time of the patients with ER-positive tumors, which is significantly longer than of those with ER-negative tumors ($p^* < 0.001$). The survival curves were related to the receptor status in the first biopsy specimen. There was no significant difference in estimated survival times between groups of patients with PgR-positive and PgR-negative tumors at first biopsy (Fig. 5). With regard to both ER and PgR at first biopsy, Fig. 6 shows that patients with ER+ PgR+ tumors survived significantly longer than patients with ER- PgR- tumors ($p^* < 0.02$). As expected, patients with ER+ PgR- tumors showed survival functions similar to those with ER+ PgR+ tumors ($p^* > 0.1$). Figure 7 shows that patients with ER-negative tumors in both biopsies had a statistically significantly shorter estimated survival time than patients with one ER-negative and one ER-positive tumor in the biopsies ($p^* < 0.02$). The 25 patients with ER-negative tumors in both biopsies had an extremely short

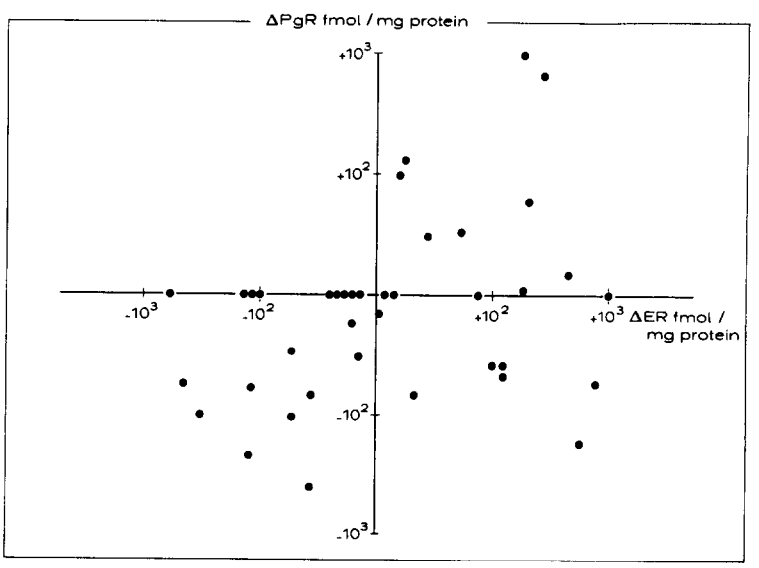

Fig. 3. Relation between the changes in absolute ER levels (ΔER) and absolute PgR levels (ΔPgR) in sequential biopsies (n = 39)*. The patients with ER- PgR- tumors at both biopsies* (n = 11) *are not included in this figure.*

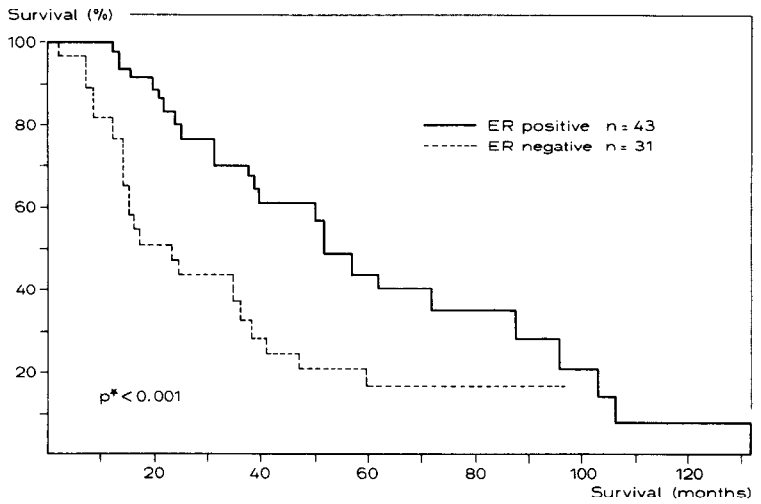

Fig. 4. Estimated survival times of the patients with ER-positive (n = 43) *or ER-negative* (n = 31) *tumors at first biopsy.*

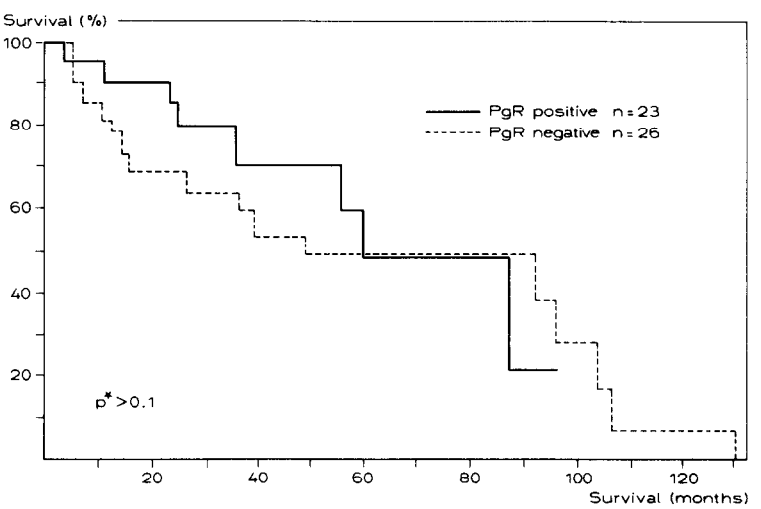

Fig. 5. Estimated survival times of the patients with PgR-positive (n = 23) *or PgR-negative* (n = 26) *tumors at first biopsy.*

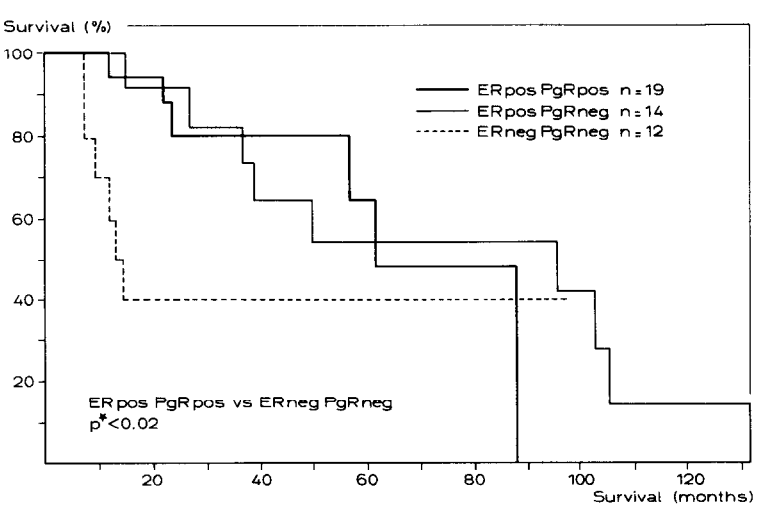

Fig. 6. Estimated survival times of the patients with ER+ PgR+ (n = 19), *ER+ PgR–*, (n = 14) *or ER– PgR–* (n = 12) *tumors at first biopsy.*

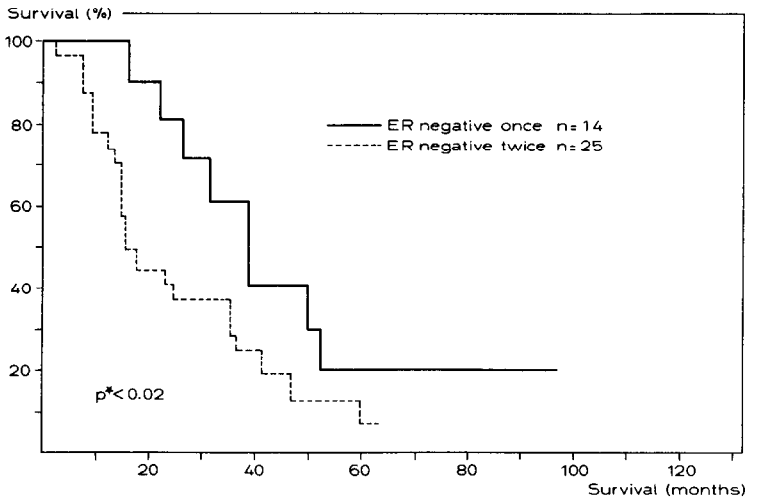

Fig. 7. Estimated survival times of the patients with ER-negative tumors at both biopsies (n = 25) *and those with only one ER-negative tumor in the two biopsies* (n = 14).

estimated survival time: half of these patients were expected to die within 18 months after first metastasis. With regard to the four PgR phenotypes at repeated biopsies (pos→pos, pos→neg, neg→neg, neg→pos), no statistically significant differences were found in the respective estimated survival functions.

DISCUSSION

The data obtained in this study indicate a discordance rate for ER in sequential biopsies of patients with advanced breast cancer of 18.7%. Hull *et al.* [11] reported a discordance rate for ER of 16.5%. As stated by Lee [6] in her review of the literature, most authors reported a more frequent change from ER-positive to ER-negative than the opposite. Several factors contribute to the variability of the ER status during the course of the disease. Rosen *et al.* [12] and Webster *et al.* [13] stated that the longer the time interval between the two biopsies, the more likely a discordant result will be obtained from the second biopsy. Our results and those of Hull *et al.* [11] and Paridaens *et al.* [14] contrast with these findings. Hull *et al.* [11] reported that a change in ER status from ER-negative to ER-positive was more frequently found in patients with primary tumours of less than 2 cm than in patients with larger primary tumors. They suggest that tissue sampling errors were made at first biopsy. In our study there was no statistically significant difference in size of the primary tumor between the ER-discordant and -concordant groups of patients. Nevertheless, one is forced to admit that the number of very small primary tumors in our study was too small to exclude the possibility that tissue sampling errors may play an important role in the variability of the receptor status. In the group of patients with ER-concordant results, significantly more second biopsies were taken from lymph nodes than in the ER-discordant group. This finding so far remains unexplained. With regard to intervening therapy between the two biopsies, it has to be noted that in five of the eight patients whose tumor ER status changed from positive to negative, instituted tamoxifen treatment was withdrawn 6 weeks before the second biopsy was performed. If we exclude these patients, the discordance rate for ER was 12.8% (9/70). Both our own and Hull's observation [11] can be explained by the findings of Fabian *et al.* [15], who reported that blood tamoxifen levels were still detectable 6 weeks after stopping this therapy.

With regard to the absolute ER levels at first biopsy, we found that ER values at first biopsy were significantly lower in the groups of patients whose ER status became negative at second biopsy than in those whose ER status remained positive. King *et al.* [3] reported similar findings, whereas Webster *et al.* [13] were unable to demonstrate such a difference in initial ER values.

Literature data concerning the variability of PgR are scarce. The discordance rate of PgR status in this series was even higher than in the ER status: 28.0%. Matsumoto *et al.* [16] reported a discordance rate for PgR of 22.2% and King *et al.* [3] found a discordance of 28.0%. In the first report from our laboratory Koenders *et al.* [17] reported a change in PgR status in 30.0% of the patients. Based upon our own findings and the literature data, we draw the conclusion that ER activity of tumor tissue is a more stable feature than PgR activity. This considerable variability of PgR in the course of time undermines the value of PgR for the prediction of responses to endocrine therapy.

The reproducibility of ER and PgR analyses in our laboratory is high. An intralaboratory controlled study, recently updated by Koenders and Benraad [18], showed a within-run variation coefficient of 7.0% for ER and 7.8% for PgR. That is, if ER and PgR analysis in a given tumor specimen are repeated on the same day, the variation coefficient for absolute ER and PgR values in the tumor cytosol is 7.0 and 7.8% for ER and PgR respectively. Koenders *et al.* [17] reported earlier that simultaneously taken biopsy specimens from various tumor sites showed a concordance rate of 92.0% for ER (11 out of 12 cases) and of 100% for PgR (7 out of 7 cases).

According to the hypothesis of Horwitz *et al.* [19], the presence of PgR in tumor tissue reflects an intact ER pathway. In general the correlation between ER and PgR is not very close [2]. Our analysis of quantitative receptor levels in sequential biopsies supports this theory: initially PgR-positive tumors exhibited a rise in PgR value in subsequent biopsies only when ER rose at the same time. Furthermore, it was shown that whenever ER levels declined in multiple biopsies, PgR fell concomitantly, and that the receptor phenotype ER− PgR+ showed discordance in all four cases studied.

We found no data in literature dealing with the prognostic significance of repeated receptor measurements in patients with advanced breast cancer. Estrogen receptor activity is generally considered an independent prognostic factor in breast cancer [1, 2], whereas the data in literature concerning PgR in this respect are at least controversial [2, 20–25]. Our results strongly support the significance of ER as a prognostic factor in patients with advanced breast cancer. Even those patients whose tumor tissue only once contained ER had a significantly longer estimated survival time than patients with two ER-negative tumors at sequential biopsies. The superiority of PgR compared with ER in predicting survival times could not be established either as a single factor or in combination with ER. We therefore conclude that sequential ER assays will increase the prognostic significance of this receptor. The determination of PgR activity, once or twice, does not add to the prognostic significance of ER.

Acknowledgements—The authors wish to thank Dr Th. Wobbes, Department of Surgery, University Hospital, Nijmegen; Dr F. E. van Dam, Department of Medicine, St. Joseph Hospital, Eindhoven; and Dr J. M. van Turnhout and Dr E. D. H. Bruggink, Department of Medicine and Surgery, Canisius-Wilhelmina Hospital, Nijmegen, for their co-operation in this study; and Dr R. Holland, Department of Pathology, University Hospital Nijmegen, for reviewing the biopsies.

REFERENCES

1. Cowan K, Lippman M. Steroid receptors in breast cancer. *Arch Intern Med* 1982, **142**, 363–366.
2. Clark GM, McGuire WL. Progesterone receptors and human breast cancer. *Breast Cancer Res Treat* 1983, **3**, 157–163
3. King RJB, Stewart JF, Millis RR, Rubens RD, Hayward JL. Quantitative comparison of estradiol and progesterone receptor content of primary and metastatic human breast tumors in relation to response to endocrine treatment. *Breast Cancer Res Treat* 1982, **2**, 339–346.
4. Beex L, Koenders A, Raemaekers J *et al.* The clinical significance of progesterone receptors (PgR) in estradiol receptor positive (ER+) tumors of patients with advanced breast cancer. 3rd EORTC Breast Cancer Working Conference, 1983, Abstract I—37.
5. Clavel B, Pichon MF, Pallud C, Milgrom E. Estradiol and progesterone receptor content and response to norethisterone treatment in advanced breast cancer. *Eur J Cancer Clin Oncol* 1982, **18**, 821–826.
6. Lee Y-TN. Variability of steroid receptors in multiple biopsies of breast cancer: effect of systemic therapy. *Breast Cancer Res Treat* 1982, **2**, 185–193.
7. Kaplan EL, Meier P. Non parametric estimation from incomplete observations. *J Am Statist Assoc* 1958, **53**, 457–481.
8. Gehan E. A generalized Wilcoxon tests for comparing arbitrarily single censored samples. *Biometrika* 1965, **52**, 203–224.
9. Mantel N. Ranking procedures for arbitrarily restricted observations. *Biometrics* 1967, **23**, 65–78.
10. Koenders AJ, Geurts-Moespot J, Kho KH, Benraad ThJ. Estradiol and progesterone receptor activities in stored lyophilized target tissue. *J Steroid Biochem 1978*, **9**, 947–950.
11. Hull DF, Clark GM, Kent Osborne C, Chamness GC, Knight WA, McGuire WL. Multiple estrogen receptor assays in human breast cancer. *Cancer Res* 1983, **43**, 413–416.
12. Rosen PP, Menendez-Botet CJ, Urban JA, Fracchia A, Schwartz MK. Estrogen receptor protein (ERP) in multiple tumor specimens from individual patients with breast cancer. *Cancer* 1977, **39**, 2194–2200.

13. Webster DJT, Bronn DG, Minton JP. Estrogen receptor levels in multiple biopsies from patients with breast cancer. *Am J Surg* 1978, **136**, 337–338.
14. Paridaens R, Sylvester RJ, Ferrazzi E, Legros N, Leclercq G, Heuson JC. Clinical significance of the quantitative assessment of estrogen receptors in advanced breast cancer. *Cancer* 1980, **46**, 2889–2895.
15. Fabian C, Steruson L, El-Sarafi M, Cain L, Hearne E. Clinical pharmacology of tamoxifen in patients with breast cancer: correlation with clinical data. *Cancer* 1981, **48**, 876–882.
16. Matsumoto K, Ochi H, Nomura Y, Takatani O, Sugano H. Progesterone and estrogen receptors in Japanese breast cancer. In: McGuire WL, ed. *Hormones, Receptors and Breast Cancer. Progress in Cancer Research and Therapy*. New York, Raven Press, 1978, Vol. 10, 43–58.
17. Koenders A, Beex L, Smals A, Kloppenborg P, Benraad Th. Oestradiol and progesterone receptors in multiple tumour specimens from patients with breast cancer. *Neth J Med* 1980, **23**, 62–67.
18. Koenders A, Benraad Th. Inter-laboratory variability in estrogen and progestin receptor assays. *Second International Congress on Hormones and Cancer, Monte Carlo, 18–23 September 1983*. New York, Raven Press.
19. Horwitz KB, McGuire WL, Pearson OH, Segaloff A. Predicting response to endocrine therapy in human breast cancer: a hypothesis. *Science* 1975, **189**, 726–727.
20. Allegra JC, Lippman ME, Simon R *et al.* Association between steroid hormone receptor status and disease free interval in breast cancer. *Cancer Treat Rep* 1979, **63**, 1271–1277.
21. Pichon MF, Pallud C, Brunet M, Milgrom E. Relationship of presence of progesterone receptors to prognosis in early breast cancer. *Cancer Res* 1980, **40**, 3357–3360.
22. Stewart J, King R, Hayward J, Rubens R. Estrogen and progesterone receptors: correlation of response rates, site and timing of receptor analysis. *Breast Cancer Res Treat* 1982, **2**, 243–250.
23. Mason BH, Holdaway IM, Mullins PR, Yee LH, Kay RG. Progesterone and estrogen receptors as prognostic variables in breast cancer. *Cancer Res* 1983, **43**, 2985–2990.
24. Campbel FC, Blamey RW, Elston CW, Griffiths K, Nicholson RI. Quantitative oestrogen receptor (ER) measurements obviate the need for progesterone receptor (PgR) analysis in primary breast cancer. 3rd EORTC Breast Cancer Working Conference, 1983, Abstract I—40.
25. Harland RNL, Barnes DM, Swindell R, Redford J, Howell A, Sellwood RA. Oestrogen and progesterone receptors and the survival after mastectomy. 3rd EORTC Breast Cancer Working Conference, 1983, Abstract VI—27.