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Relationship between estrogen, progesterone and epithelial growth factor receptor status of primary breast cancer and the survival of women patients

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Aim. The aim of the study was to evaluate the influence of estrogen (ER), progesterone (PR), and epidermal growth factor (EGFR) receptor concentration in primary breast cancer tissues on the survival of patients. Methods: The receptors were determined by biochemical radiocompetitive methods. Disease free survival of women patients (DFS) and overall survival (OS) were determined by the Kaplan – Meier method.

Results. It has been found that between DFS and ER or PR concentration in primary breast cancer tissue there is a significant positive relation. There also exists a statistically significant positive relation between the OS of women breast cancer patients and PR concentration in their cancer tissue. On the other hand, ER concentrations have no impact on OS of the same group of patients.

There is no relation between EGFR concentration in breast cancer tissue and both DFS and OS of patients. However, such a relation may be observed when EGFR concentration is analysed in view of the presence or absence of ER or PR within the cancer tissue. The presence of higher concentrations of EGFR in breast cancer tissue with a concomitant lack of ER or PR relates to shorter DFS and OS of patients; while the concomitant lack of both EGFR and ER or PR relates to longer DFS and OS. Patients presenting with higher concentrations of EGFR and ER or EGFR and PR in their breast cancer tissue achieve longer DFS and OS, as compared to women presenting with high EGFR concentrations and negligible concentrations of either ER or PR.

Conclusions. To summarise – we believe that the influence of EGFR status in breast cancer tissue on the survival of women patients depends upon the ER and PR status of the tumour. In the presence of high concentrations of ER or PR in breast cancer tissue a high concentration of EGFR has a positive impact on DFS and OS of patients, while with the concomitant lack of PR and ER high EGFR concentrations affect both DFS and OS of patients negatively.

Zależności między zawartością receptorów estrogenów, progesteronu i naskórkowego czynnika wzrostu w tkankach pierwotnego raka piersi a przeżyciem chorych kobiet

Cel pracy. Celem badań było określenie zależności, jakie zachodzą między przeżyciem kobiet chorych na raka piersi a zawartymi w ich pierwotnych nowotworach receptorami estrogenów (ER), progesteronu (PR) i naskórkowego czynnika wzrostu (EGF-R).

Materiał i metody. Badania prowadzono u 188 chorych kobiet przez około 5 lat. Spośród obserwacji klinicznych, do porównań wyłoniono dwa parametry: a) bezobjawowy pooperacyjny okres przeżycia chorych (DFS) oraz b) całkowite przeżycie chorych (OS). Krzywe przeżycia chorych wyliczano wg Kaplana i Meiera, a ich porównania wykonano kilkoma rangowymi testami statystycznymi: (Log rank test, test Breslowa i test Tarone - Ware). Receptory ER, PR i EGFR oznaczono biochemicznymi metodami radiokompetycyjnymi.

Wynik. Wykazano, że dłuższym okresom DFS chorych odpowiadają większe a krótszym mniejsze stężenia ER lub PR w nowotworze. Długość całkowitego przeżycia (OS) kobiet chorych na raka piersi jest powiązana przede wszystkim ze stężeniem PR w pierwotnym nowotworze. Między długością OS a stężeniami ER w pierwotnym nowotworze wyraźnej zależności nie stwierdza się. Stężenia EGFR w tkankach raków piersi całej puli zbadanych kobiet nie wykazywały znamiennej współzależności z długością DFS lub OS. Jednakże zależność taką wykazano, gdy stężenie EGF-R w nowotworze rozpatrywano łącznie z towarzyszącymi mu receptorami estrogenów lub progesteronu.

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Wnioski. Obecność w rakach piersi dużych stężeń EGF-R przy jednoczesnym braku lub znikomych stężeniach ER lub PR wiąże się z krótszym okresem DFS i OS kobiet, natomiast duże stężenia EGF-R w rakach piersi w obecności dużych stężeń ER i PR powiązane były z dłuższymi przeżyciami chorych.

Key words: estrogen receptors, epidermal growth factor receptors, progesterone receptors, patient survival
Słowa kluczowe: receptory estrogenów, progesteronu i naskórkowego czynnika wzrostu, przeżycia chorych

Introduction

Between the years 1972 and 1998 at the Maria Sklodowska-Curie Memorial Cancer Centre and Institute of Oncology we had conducted extensive studies on the presence of estrogen (ER), progesterone (PR), epithelial growth factor (EGFR) and insulin-like growth factor (IGFR) receptors in the tissues of primary breast cancers in women. We have also correlated the receptor status of breast cancers with their morphology [1, 2]. These studies essentially set up our understanding of the quantitative and qualitative receptor status of primary breast cancers and allowed us to identify patients most likely to profit from hormonotherapy. We reported our results in a number of papers and communications [1-4].

All the receptors listed above may be found in breast cancer tissue, however their quantitative ratios may vary between tumours. Some tumours lose the expression of one of the receptors, while the expression of other receptors may be preserved or even increased. This mechanism lies behind the lack of, or presence, of hormonoreactivity of cancer cells. The relations between the receptors may significantly influence the processes of cancer cell division and differentiation. It is a common belief that these two processes define the biology of the tumour, thus bearing on the course of the disease and on patient survival.

The ER and EGFR receptors arouse more interest, as they are considered to be predictive in the treatment of breast cancer. ER is believed to be a predictive factor for hormonotherapy, and EGFR for specialist treatment based on anti-EGFR antibodies [5, 6], while little attention is paid to PR and IGFR.

As a predictive and prognostic factor EGFR has been widely described in literature, however the reports tend to be contradictory. Some authors present EGFR as a significantly negative prognostic factor [7-10], others believe that the prognostic impact of EGFR may be related to other factors, while a group of authors rule out the prognostic value of EGFR [11-13].

Our initial reports [14, 15], and data yet unpublished, suggest that EGFR has no prognostic value for disease free survival (DFS) and overall survival (OS) of women with breast cancer when analysed as an independent parameter. Only in specially selected patient groups – for instance ER+PR+ or ER–PR–, EGFR appears to be a predictive factor of patient survival – in the first case a positive and in the second case a negative factor. [14, 15]. We have also stressed the fact that the presence of PR in breast cancer tissue is an important, constant and underestimated prognostic factor [9].

Being in the possession of data on the receptor status of primary breast cancers in women we have launched a study aimed at assessing whether the receptor status of the primary breast cancer lesion may allow for predicting the course of the disease. Follow-up was designed to be 5 years. The results were analysed in view of a couple of different methods of evaluating the receptor status and several approaches to calculating survival. This paper presents an analysis of the impact of ER, PR and EGFR concentration in primary breast cancer tissue on postoperative disease-free survival (DFS) and overall survival (OS) performed with the Kaplan-Meier method (K-M).

Material and methods

Patient characteristics

The study included 184 (83 premenopausal and 101 postmenopausal) women with breast cancer aged between 27 and 83 years (mean age – 54.5±12.8 SD), treated at the Maria Sklodowska-Curie Memorial Cancer Centre and Institute of Oncology in Warsaw.

All patients were treated surgically; adjuvant hormontherapy or chemotherapy was applied to 88 patients, while the remaining 96 patients received no adjuvant therapy.

Follow-up of patients was set at a minimum of 5 years. In the material there were 134 cases of ductal carcinoma, 30 cases of lobular carcinoma and 20 cases of other carcinomas. The morphological studies included determination of the histological type of the tumours according to the present WHO classification [16].

Determination of estrogens and progesterone receptors in the cytosol fraction.

The receptors of estrogens and progesterone were determined in the cytosol fraction by the radiocompetitive charcoal-dextran method of multipoint assay or in one point assay analysis at maximal saturating concentrations of labelled hormones. The results of receptor determinations were expressed in fmoles/mg of cytosol protein (fm/mg c.p.). The details of determination have been described elsewhere [1]. The concentration of protein in the cytosol and membrane fractions was determined with the Lowry method [17].

Determination of epidermal growth factor receptors.

For EGF receptor determination the radiocompetitive method was applied. EGF receptor was determined in membrane fraction using one-point assay analysis at maximal saturating concentration (4nM ^{125}I-EGF) with the use of triplicate samples for total and unspecific bindings. In some cases the multipoint assay of EGFR was used. The details of determination have been described elsewhere [2,3]. The results of receptor determinations were expressed in fmols/mg of membrane protein (fm/mg m.p.).

Statistic methods

DFS and OS analysis was performed with the Kaplan-Meier method; (SPSS software).

Table I. The relationships between DFS and OS of patients with breast cancer and the concentration of estrogen receptors in their tumours

N_0 of group		Ranges of compared ER concentrations (fm/mg c.p.) (Number of cases) in group	DFS – months Mean survival ± SE Confidence limit* Difference between groups		Significance level three methods of calculation**	OS – months Mean survival ± SE Confidence limit* Difference between groups		Significance level three methods of calculation**
	a	0 – 9 (53)	48 ± 3 (42–54)		0.5566	55 ± 2 (52–59)		0.6910
1.		vs		2	0.5871		7	0.5635
	b	10 – 400 (135)	50 ± 2 (47–54)		0.5841	62 ± 2 (59–66)		0.6061
	a	0 – 20 (99)	49 ± 2 (44–53)		0.4233	55 ± 2 (51–59)		0.2328
2.		vs		1	0.4796		9	0.2029
	b	21 – 400 (89)	50 ± 3 (46–55)		0.4603	64 ± 2 (60–69)		0.2241
	a	0 – 30 (127)	48 ± 2 (44–52)		0.0476	60 ± 2 (56–64)		0.2007
3.		vs		6	0.0587		1	0.1344
	b	31 – 400 (61)	54 ± 3 (49–59)		0.0534	59 ± 2 (55–62)		0.1643
	a	0 – 40 (140)	48 ± 2 (44–51)		0.0250	61 ± 2 (57–64)		0.1087
4.		vs		7	0.0226		2	0.0923
	b	41 – 400 (48)	55 ± 3 (50–61)		0.0223	59 ± 2 (56–63)		0.0992
	a	0 – 60 (140)	47 ± 2 (43–51)		0.0242	61 ± 2 (58–65)		0.2808
5.		vs		9	0.0140		1	0.1751
	b	61 – 400 (48)	56 ± 2 (52–61)		0.0182	60 ± 2 (56–65)		0.2182
	a	0 – 90 (173)	48 ± 2 (45–52)		0.0168	61 ± 2 (58–65)		0.0674
6.		vs		16	0.0120		3	0.0719
	b	91 – 400 (15)	64 ± 1 (52–61)		0.0132	64 ± 1 (62–66)		0.0803

* Confidence limits set at 95%

** Parameters of DFS and OS calculated by the Kaplan – Meier method; significance level between groups "a" and "b" calculated by the Log rank test, the Breslow test and the Tarone – Ware test; results expressed as three consecutive values

Results

1. Relations between ER concentration in breast cancer tissue of women patients and their DFS or OS.

Results are presented in Table 1 (some also illustrated on figures). Data shows that in women with lesser ranges of ER concentrations (0-9; 0-20; 0-30; 0-40; 0-60 and 0-90 fm/mg c.p., Table I, pos. 1a-6a) arithmetic mean values of DFS were between 47-49 months. In women with greater ranges of ER concentrations (10-400; 21-400; 31-400; 41-400; 61-400 and 91-400 fm/mg c.p.; Table I; pos. 1b-6b) mean DFS values were between 50-64 months. Generally, the higher the ER concentration – the longer DFS. However, differences between mean DFS at ER concentrations of 0-9 fm/mg and 10-400 fm/mg c.p. and, respectively, 0-20 fm/mg c.p. and 21-400 fm/mg c.p. fail to reach statistical significance (see Table I; groups 1 and 2). The lowest ER concentrations above which the length of DFS increases significantly lies between 30 fm/mg c.p. and 40 fm/mg c.p. (for example Figure 1a).

The relations between OS and ER concentrations in primary breast tumours are also presented in Table I (OS). We have compared the OS of women with higher and lower ER concentrations. The ranges of higher and

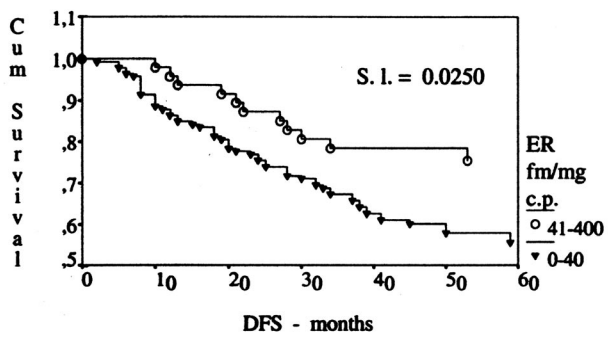

Figure 1a

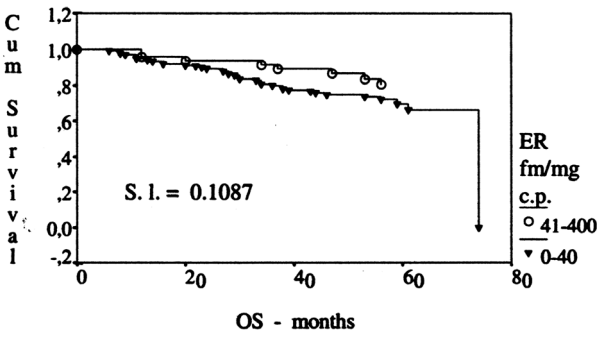

Figure 1b

Figures 1a and 1b. The survival curves of women with breast cancer with either higher or lower concentrations of estrogen receptors (ER) within tumour tissue. The DFS curves (1a) differ significantly, the differences for OS are non-significant (2b)

The significance levels on the figures present only Log Rank test. For complete of significance levels tests see Tables 1 – 4.

lower concentrations were the same to those analysed for DFS. The mean OS values of women with a lower ranges of ER concentration were 55-61 months, and with a higher ranges of ER – 59-64 months. The differences in survival between the groups with higher and lower ER concentrations only in two cases reached as much as 7 and 9 months (Table I; OS pos. 1 and 2), while in the remaining cases they varied between 1 and 3 months, however all differences failed to reach statistical significance, e.g. Figure 1b.

2. Relations between PR concentration in breast cancer tissue of women patients and their DFS or OS.

We performed comparisons between groups with lower ranges of PR concentrations (0-4; 0-9; 0-20; 0-30; 0-40; 0-60 and 0-90 fm/mg c.p., Table II; pos. 1a-7a) and higher ranges of PR concentration (5-1200; 10-1200; 21-1200; 31-1200; 41-1200; 61-1200; and 91-1200 fm/mg c.p., Table II, pos. 1b-7b). Results are presented in Table II, and some are shown on figures.

Data shows that in women with a lesser PR concentration mean values of DFS were shorter (41-47 months). In women with greater PR concentrations mean values of DFS were longer (53-58 months). In all groups of patients compared relating to the lower or higher PR concentration these differences were statistically significant, e.g. Figure 2a.

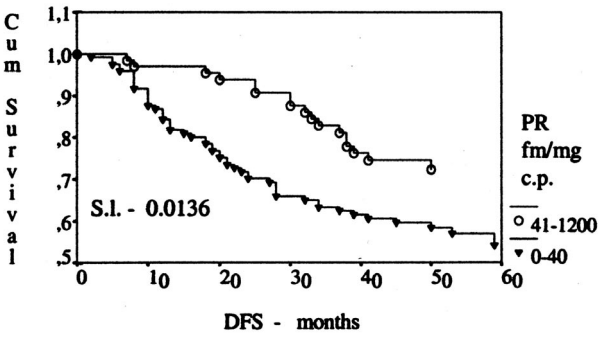

Figure 2a

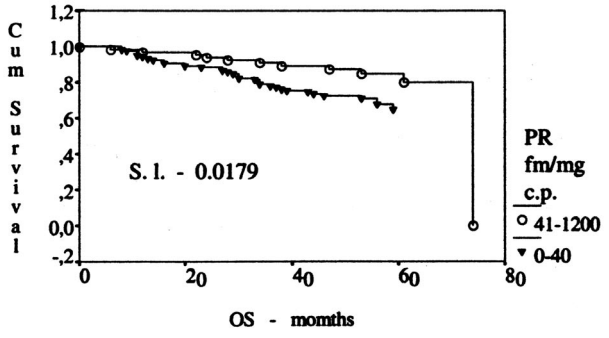

Figure 2b

Figures 2a and 2b. The survival curves of women with breast cancer with either higher or lower concentrations of progesterone receptors (PR) within tumour tissue. The differences are statistically significant both for DFS (2a) and OS (2b)

Table II. The relationships between DFS and OS of patients with breast cancer and the concentration of progesterone receptor in their tumours

N_0 of group		Ranges of compared PR concentrations (fm/mg c.p.) (Number of cases)	DFS – months Mean survival ± SE Confidence limits* Difference between groups		Significance level three methods of calculation**	OS – months Mean survival ± SE Confidence limits* Difference between groups		Significance level three methods of calculation**
1	a	0 – 4 (51)	41 ± 3 (34–48)	12	0.0320	49 ± 3 (43–55)	16	0.0017
		vs			0.0190			0.0020
	b	5 –1200 (137)	53 ± 2 (49–56)		0.0240	65 ± 2 (62–68)		0.0019
2	a	0 – 9 (72)	44 ± 3 (38–49)	9	0.0105	52 ± 2 (48–57)	13	0.0257
		vs			0.0084			0.0311
	b	10 – 1200 (116)	53 ± 3 (50–57)		0.0100	65 ± 2 (61–68)		0.0281
3	a	0 – 20 (97)	45 ± 3 (40–50)	9	0.0069	53 ± 2 (49–57)	13	0.0169
		vs			0.0031			0.0133
	b	21 – 1200 (91)	54 ± 2 (51–58)		0.0047	66 ± 2 (62–70)		0.0141
4	a	0 – 30 (110)	46 ± 2 (42–51)	8	0.0320	55 ± 2 (51–58)	10	0.1130
		vs			0.0125			0.0724
	b	31 – 1200 (78)	54 ± 2 (50–58)		0.0202	65 ± 2 (61–69)		0.0853
5	a	0 – 40 (121)	46 ± 2 (42–51)	9	0.0136	54 ± 2 (51–58)	13	0.0179
		vs			0.0064			0.0159
	b	41 – 1200 (67)	55 ± 2 (51–60)		0.0093	67 ± 2 (63–71)		0.0156
6	a	0 – 60 (140)	47 ± 2 (43–51)	9	0.0242	55 ± 2 (52–58)	12	0.0500
		vs			0.0140			0.0491
	b	61 – 1200 (48)	56 ± 2 (52–61)		0.0182	67 ± 3 (62–72)		0.0460
7	a	0– 90 (147)	47 ± 2 (44–51)	11	0.0134	55 ± 2 (57–58)	13	0.0375
		vs			0.0075			0.0323
	b	91 – 1200 (41)	58 ± 2 (53–62)		0.0098	68 ± 3 (62–73)		0.0317

* Confidence limits set at 95%

** Parameters of DFS and OS calculated by the Kaplan – Meier method; significance level between groups" a" and "b" calculated by the Log rank test, the Breslow test and the Tarone – Ware test; results expressed as three consecutive values

Relations between OS and PR concentrations in primary breast tumours are also presented in Table II (OS). We have compared the OS of women with higher and lower PR concentrations. The arithmetic means of OS of women with lower ranges of PR concentrations were 49-55 months, and with higher ranges – 65-68 months. The differences in survival between the groups with higher and lower PR were between, 10-16 months. The differences reached statistical significance in all the compared groups (e.g. Figure 2b), except for one – the PR 0-30 and 31-1200 fm/mg c.p. which achieved probability levels of 0.1130; 0.0724 and 0.0853 (i.e. only approached the generally accepted significance level 0.05;- Table II; OS group 4).

3. Relations between EGFR concentration in breast cancer tissue of women patients and their DFS or OS.

In order to assess the relations between EGFR and DFS or OS we compared a number of groups of patients who presented with EGFR concentrations of 0-5; 6-15 and 16-100 fm/mg m.p. Results are presented as survival curves on figures 3a and 3b. A similar analysis of DFS and OS was performed for EGFR concentrations from 0-9 fm/mg m.p. and 10-100 fm/mg m.p. (these last results are not shown in the present paper, for details refer to the dissertation of S. Mazur MD, Ph.D.) [18].

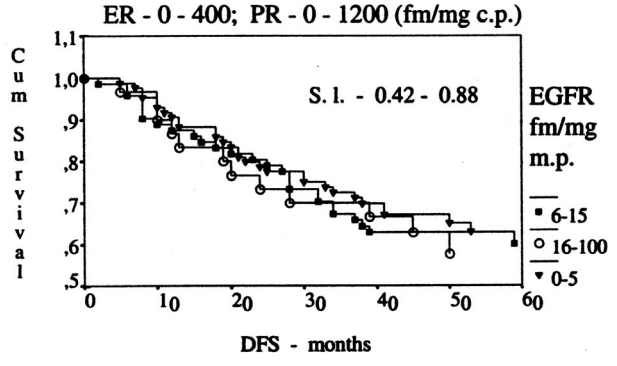

Figure 3a

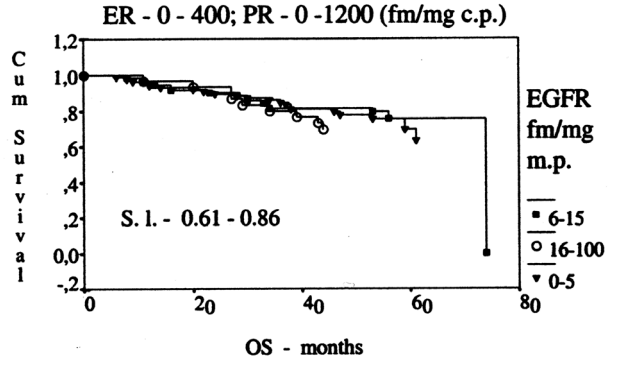

Figure 3b

Figures 3a and 3b. The survival curves of women with breast cancer with three different, increasing ranges of concentrations of EGFR. The differences between the curves are non-significant both for DFS (3a) and OS (3b)

DFS and OS were analysed with the Kaplan-Meier method, while significance levels were assessed in three tests (Log-rank, Breslow and Tarone-Ware). We have shown that the survival curves calculated according to EGFR concentrations did not differ. Significance levels performed for all possible comparisons were in the 0.42-0.88 range for DFS and in the 0.61-0.86 range for OS.

4. Relations between the concomitant presence or absence of ER and EGFR or PR and EGFR in breast cancer tissue of women patients and their DFS or OS.

In the studies cited above we have shown that there exists a significant correlation between the ER or PR concentration in breast cancer tissue and DFS and between PR concentration and OS. However we had found no such correlation between EGFR concentration and either DFS and OS. This appears inconceivable in view of the fact that in our material there exists a statically significant negative correlation between EGFR and ER concentrations and EGFR and PR concentrations. For EGFR and ERα=–0.2051; significance level 0.0000; for EGFR and PRα=–0.1279, significance level 0.014.

In our breast cancer specimens the EGFR concentrations varied greatly – from zero or indiscernible values to 9 fm/mg m.p. and from 10-100 fm/mg m.p.. The former range of concentrations is usually referred to as EGFR-negative and the latter as EGFR-positive.

In order to investigate the lack of significant relation between EGFR concentration and DFS or OS we had performed several analyses. We divided the patients into two groups – EGFR-negative and EGFR-positive. Then we analysed the relation between ER or PR concentrations and DFS or OS within these groups.

4a. Relations between the ER concentration in EGFR-negative and EGFR-positive patients and their DFS or OS.

The results of the analyses are presented in Table III. and some of them are illustrated on figures. We found that in EGFR-positive patients with a lack of or very low ER concentration (Table III, DFS; group 1-3) survival was shorter than in EGFR-negative patients with a lack of or very low ER concentration. (mean values of DFS by 10-22 months and mean values of OS by 10-11 months). These differences were statistically significant (Figure 4a and 4b) or on the verge of significance. With the increase of ER concentrations (Table III; group 4: ER range 0-15) these differences decrease to 8 and 5 months, and become non-significant.

The next series of analyses were performed on EGFR-negative and EGFR-positive patients with lower ranges of ER concentrations (0-9; 0-20; 0-30; 0-40; and 0-60 fm/mg c.p., groups 5a-9a) and relatively higher ranges of ER concentrations (10-400; 21-400; 31-400; 41-400; and 61-400 fm/mg c.p., group 5b-9b). In EGFR-negative patients with low ER concentration (see Table III: DFS groups 5–9) the mean DFS values remained between 49 and 56 months, while in patients with

Table III. The relationships between DFS and OS of patients with breast cancer and the concentrations of ER in EGFR-positive or EGFR-negative tumours

N_0 of group	EGFR*	Ranges of compared ER concentrations (fm/mg c.p.) (Number of cases)	DFS – months Mean ± SE (Confidence limit)# Difference between groups		DFS – months Significance level**	OS – months Mean ± SE (Confidence limit)# Difference between groups		OS – months Significance level**
	–	0 – 4 (8)	60 ± 4 (52–68)		0.0479			
1		vs		22	0.0491	–		–
	+	0 – 4 (33)	38 ± 5 (28–48)		0.0480			
	–	0 – 9 (20)	56 ± 4 (48–63)		0.0812	62 ± 2 (57–66)		0.0406
2		vs		13	0.0480		11	0.0388
	+	0 – 9 (33)	43 ± 4 (45–51)		0.0574	51 ± 3 (44–58)		0.0395
	–	0 – 10 (23)	55 ± 3 (49–62)		0.0971	62 ± 2 (58–66)		0.0399
3		vs		10	0.0477		10	0.0392
	+	0 – 10 (37)	45 ± 4 (37–52)		0.0600	52 ± 3 (46–58)		0.0395
	–	0 – 15 (42)	53 ± 3 (46–59)		0.3828	58 ± 3 (53–63)		0.4393
4		vs		8	0.2427		5	0.2653
	+	0 – 15 (42)	45 ± 4 (37–52)		0.2879	53 ± 3 (48–59)		0.3096
		a) 0 – 9 (20)	56 ± 4 (48–63)		0.2977			
	–	vs		6	0.1967			
		b) 10 – 400 (99)	50 ± 2 (45–54)		0.2247			
5						–		–
		a) 0 – 9 (33)	43 ± 4 (35–51)		0.1364			
	+	vs		9	0.1032			
		b) 10 – 400 (36)	52 ± 3 (46–58)		0.1162			
		a) 0 – 20 (53)				55 ± 3 (49–60)		0.3668
	–	vs					2	0.3784
		b) 21 – 400 (66)				57 ± 2 (54–61)		0.3933
6			–		–			
		a) 0 – 20 (46)				54 ± 3 (49–60)		0.4662
	+	vs					11	0.3874
		b) 21 – 400 (23)				65 ± 4 (57–73)		0.4213
		a) 0 – 30 (70)	49 ± 3 (43–54)		0.2878	55 ± 2 (50–60)		0.3835
	–	vs		4	0.3036		3	0.3189
		b) 31 – 400 (49)	53 ± 3 (47–58)		0.3008	58 ± 2 (54–62)		0.3599
7								
		a) 0 – 30 (57)	46 ± 3 (40–51)		0.0824	58 ± 2 (55–67)		0.3440
	+	vs		11	0.1019		3	0.2581
		b) 31 – 400 (12)	57 ± 5 (47–66)		0.0914	61 ± 3 (55–66)		0.2919
		a) 0 – 40 (81)	49 ± 3 (44–54)		0.2317	55 ± 2 (51–60)		0.3246
	–	vs		5	0.2179		3	0.3492
		b) 41 – 400 (38)	54 ± 3 (48–60)		0.2259	58 ± 2 (53–63)		0.3459
8								
		a) 0 – 40 (59)	45 ± 3 (39–51)		0.0453	61 ± 3 (55–66)		0.1744
	+	vs		15	0.0502		2	0.1174
		b) 41 – 400 (10)	60 ± 3 (54–67)		0.0472	63 ± 1 (61–65)		0.1383
		a) 0 – 60 (98)				56 ± 2 (52–60)		0.5146
	–	vs					3	0.4294
		b) 61 – 400 (21)				59 ± 3 (53–65)		0.4761
9			–		–			
		a) 0 – 60 (62)				61 ± 3 (56–67)		0.3639
	+	vs					2	0.2449
		b) 61 – 400 (7)				63 ± 1 (61–65)		0.2908

Confidence limits set at 95%; *EGFR- 0 – 9 fm/mg m.p. *EGFR + ≥10 fm/mg m.p.

** Parameters of DFS and OS were calculated by the Kaplan – Meier method; significance level between groups “a” and “b” calculated by the Log rank test, the Breslow test and the Tarone – Ware test; results expressed as three consecutive values

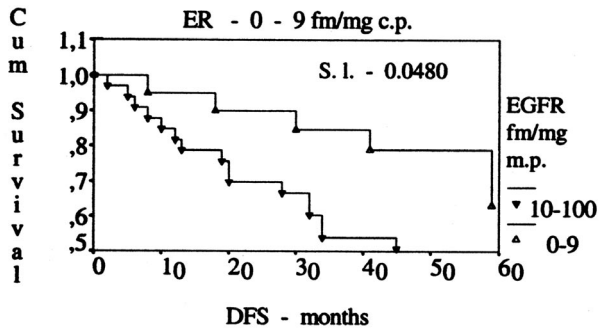

Figure 4a

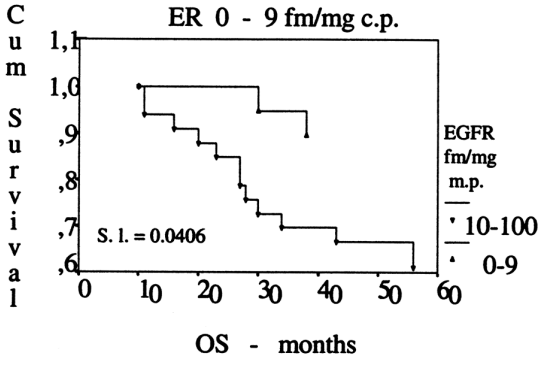

Figure 4b

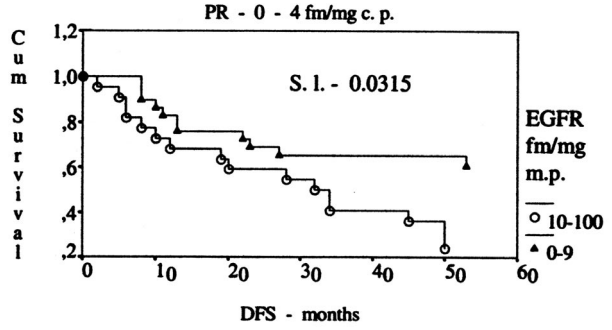

Figure 4c

Figures 4a, 4b, 4c. The DFS and OS curves of women with ER or PR negligible values and EGFR-negative vel EGFR positive breast cancer. In all cases the differences are statistically significant. The higher EGFR levels and low ER or PR levels in breast cancer is associated with poorer prognostic of women patients

relatively higher ER concentrations they varied between 50 and 54 months. The differences in the length of survival were 6, 4 and 5 months and failed to reach statistical significance.

In EGFR-positive patients with lower ER concentration the mean DFS values differed from those achieved by patients with higher ER concentrations. The differences increased with the increase in ER concentration (9, 11 and 15 months), while their probability level progressed from non-significance towards the point of significance i.e. 0.05 (see: Table III: groups 5, 7 and 8).

Similar analyses were performed for OS (See: Table III; column OS). In EGFR-negative patients with low ER concentration the mean OS values remained between 55 and 56 months, while in patients with relatively higher ER concentrations they varied between 57 and 59 months. The differences in the length of survival were 2-3 months and failed to reach statistical significance.

In EGFR-positive patients with lower ER concentrations the mean OS values differed from those achieved by patients with higher ER concentrations. In patients with low ranges of ER concentrations (see Table III, OS, groups 6b-9b) the mean OS values remained between 54 and 61 months, while in patients with relatively higher ranges of ER concentration they varied between 61 and 65 months. The difference in the length of survival was the greatest in group 6 and reached 11 months, but all the differences between the groups failed to reach statistical significance.

4b. Relations between the PR concentration in EGFR-negative and EGFR-positive patients and their DFS or OS.

The results are presented in Table IV and some of them are illustrated on figures. In EGFR-negative (0-9 fm/mg m.p.) patients who also presented as PR–negative (0-4 fm/mg c.p.) DFS was longer by 16 months than in EGFR-positive but PR–negative patients. This difference achieved statistical significance (Table IV: DFS group 1; Fig. 4c). This difference rapidly fell to 9 months with a minimal increase of the PR concentration range (0-9 fm/mg c.p.; Table IV: DFS group 2:) and immediately failed to reach statistical significance.

In another series of analyses we compared the survival of EGFR negative and EGFR positive patients correlating with lower or higher ranges of PR concentration. Lower ranges of PR concentrations remained within the 0-4; 0-9; 0-20; 0-30 and 0-40 fm/mg c.p. (groups 3a-8a) and higher ranges of PR concentrations within the 5-1200; 10-1200; 21-1200; 31-1200; and 41-1200 fm/mg c.p. (groups 3b-8b).

In EGFR-negative patients with lower PR concentrations the mean DFS values remained between 47 and 48 months, while in patients with relatively higher PR concentrations they varied between 48 and 54 months. The differences in the length of survival were 1-6 months and were statistically non-significant (for example Figure 5a).

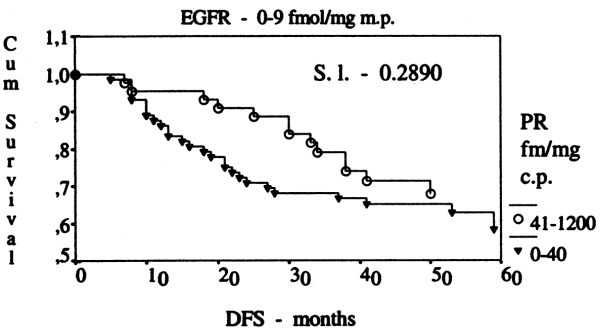

Figure 5a. The DFS curves of women with EGFR-negative breast cancer and relatively higher vel lower PR concentrations. The differences are statistically non-significant

Table IV. The relationships between DFS and OS of patients with breast cancer and the concentrations of PR in their EGFR-positive or EGFR-negative tumours

N_0 of group	EGFR*	Ranges of compared PR concentrations (fm/mg c.p.) (Number of cases) groups	DFS – months Mean survival ± SE (Confidence limit)# Difference between groups	DFS – months Significance level**	OS – months Mean ± SE (Confidence limit)# Difference between groups	OS – months Significance level**
	–	0 – 4 (29)	47 ± 5 (38–56)	0.0315		
1		vs	16	0.0596	–	–
	+	0 – 4 (22)	31 ± 4 (23–40)	0.0448		
	–	0 – 9 (44)	47 ± 4 (40–54)	0.1372		
2		vs	9	0.1763	–	–
	+	0 – 9 (28)	38 ± 4 (30–47)	0.1519		
		a) 0 – 4 (29)			52 ± 4 (44–59)	0.1065
	–	vs			6	0.1571
		b) 5 – 1200 (90)			58 ± 2 (55–61)	0.1336
3			–	–		
		a) 0 – 4 (22)			45 ± 5 (36–54)	0.0033
	+	vs			23	0.0021
		b) 5 – 1200 (47)			68 ± 2 (63–72)	0.0026
		a) 0 – 9 (44)	47 ± 4 (40–54)	0.2317	54 ± 3 (49–60)	0.3277
	–	vs	6	0.2179	4	0.4402
		b) 10 – 1200 (75)	53 ± 2 (48–58)	0.2259	58 ± 2 (54–61)	0.3882
4						
		a) 0 – 9 (28)	38 ± 4 (30–47)	0.0453	48 ± 4 (41–55)	0.0172
	+	vs	15	0.0502	19	0.0123
		b) 10 – 1200 (41)	53 ± 3 (53–67)	0.0472	67 ± 2 (63–72)	0.0144
		a) 0 – 20 (59)			55 ± 3 (50–60)	0.4766
	–	vs			2	0.4209
		b) 21 – 1200 (29)			57 ± 2 (53–61)	0.4433
5			–	–		
		a) 0 – 20 (38)			49 ± 3 (43–55)	0.0040
	+	vs			21	0.0034
		b) 21 – 1200 (21)			70 ± 2 (65–75)	0.0035
		a) 0 – 20 (59)	47 ± 3 (41–53)	0.9235		
	–	vs	1	0.7905		
		b) 21 – 90 (29)	48 ± 4 (41–56)	0.8679		
6					–	–
		a) 0 – 20 (38)	40 ± 4 (33–48)	0.0330		
	+	vs	16	0.0293		
		b) 21 – 90 (21)	56 ± 4 (48–63)	0.0308		
		a) 0 – 30 (68)	48 ± 3 (43–54)	0.3345	56 ± 2 (51–60)	0.8933
	–	vs	5	0.1975	1	0.7120
		b) 31 – 1200 (51)	53 ± 3 (48–58)	0.2604	57 ± 2 (52–61)	0.7868
7						
		a) 0 – 30 (42)	42 ± 4 (35–50)	0.0329	52 ± 3 (46–58)	0.0210
	+	vs	14	0.0220	18	0.0167
		b) 31 – 1200 (27)	56 ± 3 (50–62)	0.0266	70 ± 3 (64–75)	0.0181
		a) 0 – 40 (73)	48 ± 3 (43–54)	0.2890	56 ± 2 (51–60)	0.6316
	–	vs	6	0.1859	1	0.5159
		b) 41 – 1200 (46)	54 ± 3 (48–59)	0.2336	57 ± 2 (53–62)	0.5549
8						
		a) 0 – 40 (48)	43 ± 3 (36–49)	0.0123	52 ± 3 (46–57)	0.0016
	+	vs	16	0.0091	22	0.0022
		b) 41 – 1200 (21)	59 ± 3 (54–64)	0.0104	74 ± 0 (74–74)	0.0018

Confidence limits set at 95%

*EGFR- 0 – 9 fm/mg m.p.;*EGFR+ ≥10 fmol/mg m.p.

** Parameters of DFS and OS calculated by the Kaplan – Meier method; significance level between groups "a" and "b" calculated by the Log rank test, the Breslow test and the Tarone – Ware test, results expressed as three consecutive values

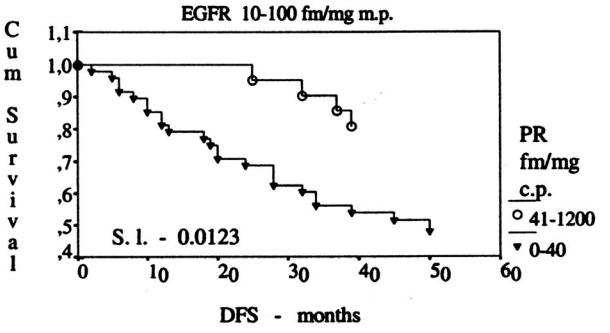

Figure 5b. The DFS curves of women with EGFR positive breast cancer and relatively higher vel lower PR concentrations. The differences are statistically significant

In EGFR-positive patients with lower PR concentrations the mean DFS values remained between 38 and 43 months, while in patients with relatively higher PR concentrations they varied between 53 and 59 months. The differences in the length of survival were 14-16 months and were statistically significant (for example Figure 5b).

In order to assess the impact of PR and EGFR concentrations on OS of women with breast cancer we used a similar approach as during DFS analysis. In EGFR-negative patients presenting with lower PR concentrations OS was 52-56 months, while in those with higher PR concentrations – 57-58 months. The differences between the groups varied between 1-6 months and were statistically non-significant (for example Figure 6a).

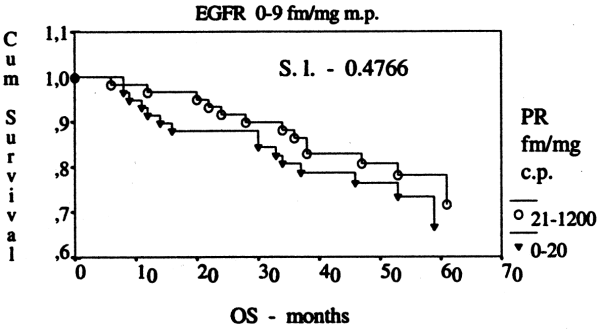

Figure 6a. The OS curves of women with EGFR negative breast cancer and relatively higher vel lower PR concentrations. The differences are statistically non-significant

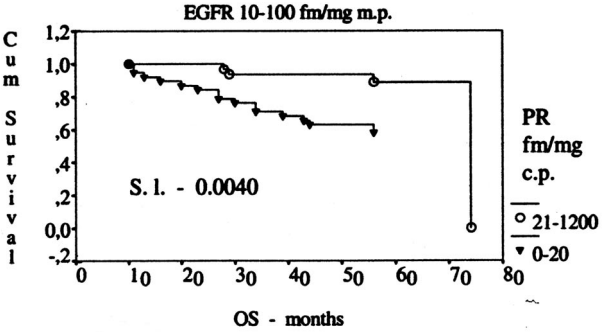

Figure 6b. The OS curves of women with EGFR positive breast cancer and relatively higher vel lower PR concentrations. The differences are statistically significant

In EGFR-positive patients presenting lower PR concentrations OS was between 45 and 52 months, while in those with higher PR concentrations – 67-74 months. The differences between the groups varied between 18 and 23 months and were highly significant statistically (e.g. Figure 6b).

Discussion

In the course of conducted studies we have shown that there exists a statistically significant relation between the concentration of ER in breast cancer tissue and DFS of women patients. In patients with higher ER concentration DFS is longer than in patients with lower ER concentrations. However, this relation becomes obvious only at ER concentration of 30 fm/mg c.p. and is maintained at all higher ER concentration. On the other hand ER concentrations have no impact in OS of the same group of patients. (Table I and Figure 1a and 1b).

The reports of other authors regarding this subject tend to vary. Tsutsui et al. [19] have shown that ER negative breast cancer patients have poorer survival parameters (DFS – RR – 1.92 and OS – RR – 2.23) than ER positive breast cancer patients. On the contrary, Nicholson et al. [20] have proven, in the course of a unilateral analysis, that ER is a significant prognostic factor of DFS and OS, while in multivariate analysis the influence of ER is non-significant.

In our other study we have applied Cox's multivariate analysis to the same data and we have concluded that ER presence is a positive prognostic factor of DFS, but not of OS [9] However, the prognostic value of ER for DFS depends on its concentration within the tumour. When compared to reference concentrations of ER (0-9 fm/mg c.p.), higher ER concentrations (91-400 fm/mg c.p.), have a positive impact but lower ER (10-90 fm/mg c.p.) – a negative impact on DFS of patients. The relative risks (RR) were 0.28 and 2.25, respectively [9].

The present study has also shown that patients with a higher PR concentration in their tumours achieve longer DFS and OS. Contrary to the situation observed with ER in case of PR concentration, the value from which differences in survival were observed was very low or on the verge of discernibility. It may be assumed that even minimal PR concentrations increase the chance of longer DFS and OS (Table II, Figure 2a and 2b).

Literature reports regarding the value of PR in the prognosis of breast cancer are also contradictory. Results resembling ours have been reported by Torregrosa et al. [7]. In the course of a unilateral analysis they had concluded that the PR status is a factor prognostic for DFS and OS in breast cancer women patients, while in a multivariate analysis they do not mention the impact of PR status on patient survival. Castagnetta et al. [21] also report PR status to have significant prognostic value in breast cancer women patients. PR negative patients present with earlier recurrences than PR positive patients. Contradictory to our results, Ferrero et al. [11] report that PR status has no impact on the survival of breast

cancer women patients. Our other studies, in which Cox's multivariate analysis has been applied, have brought us to similar conclusions – i.e. that PR concentration in breast cancer tissue of women patients is an independent prognostic factor of both DFS and OS. Higher PR concentrations in the tumour tissue significantly decrease the risk of recurrence and death [9, 10].

Contrary to PR status and, to a certain extent, to ER status the present study has given us no evidence of any statistically significant relations between the concentration of EGFR in breast cancer tissue and DFS and OS (Figure 3a and 3b). This is in direct opposition to all our other observations, namely that there exists a statistically significant negative correlation between the concentrations of EGFR and ER or PR [2-4, 15]. In order to elucidate this issue we performed a number of additional tests – we analysed how the different ER and PR concentrations affect DFS and OS of (i) EGFR-negative patients (i.e. EGFR concentration of 0-9 fm/mg m.p.) and (ii) EGFR-positive patients (i.e. concentrations of 10-100 fm/mg m.p.).

In the course of these tests we have concluded that EGFR concentration may differ in its impact on DFS and OS, depending on the lack of or presence of ER or PR in the cancer tissue. In EGFR-positive patients, in whom ER or PR concentrations in cancer tissue were negligible, DFS and OS were shorter by 10-22 months, as compared to EGFR-negative patients with higher ER or PR concentrations (see Table III, group 1-3 and Table IV group 1). In women patients in whom cancer tissue shows a lack of ER or PR, or their very low concentrations, the presence of EGFR negatively affects DFS and OS (Figures 4a, 4b and 4c).

In EGFR-positive patients with positive ER and PR status DFS and OS were longer by 11-23 months than in EGFR-positive patients with lower ER or PR concentrations (Table III, groups 7-8 DFS and Table IV group 3-7 DFS and OS). It is therefore clear that there exists a synergistic effect in the impact of positive ER, PR and EGFR status on DFS and OS. We have not found such a communication in literature as to date.

In the aforementioned study based on Cox's multivariate analysis we have confirmed this observation [9]. It is obvious that, taken separately, EGFR status does not influence neither DFS nor OS, but in the presence of positive ER and PR status it becomes a positive prognostic factor; while when accompanied by the lack of ER and PR it is a significantly negative prognostic factor [9].

EGFR as a prognostic factor for breast cancer patient survival first appeared in a paper by Sainsbury et al. [8], where it was pronounced to be a negative prognostic factor for the survival of women with breast cancer. Since then many papers were published regarding this issue [7, 19]. Nicholson et al. [20] report in the course of both single-arm and multivariate analysis that EGFR is a significant prognostic of DFS and OS. Tsutsui et al. [22] report it to be an independent and significant prognostic factor, but only if the ER status of the tumour is omitted in the course of a multivariate analysis. On the other hand, if ER is not omitted, EGFR loses its independence due to the lack of statistical significance. Torregrosa et al. [7] have shown that in Cox's multivariate analysis EGFR is an independent significant prognostic factor only for OS, while for DFS it is non-significant – in both these analyses the ER status was included. It is quite likely that these discrepancies arise from the possibility of difference of the studied material, from varied methods applied for the assessment of EGFR expression (radioligands or immunohistochemical staining) and from different statistical approaches (Kaplan-Meier, various forms of Cox's multivariate analysis). Besides one must consider the fact that a majority of authors refer to receptor status as positive (+) or negative (-) [8, 23]. This may introduce disarray into the assessment analyses, especially in the case of positive receptor status. The assessment of the expression of EGFR by immunohistochemical staining may be charged with subjective errors. Our studies were performed as quantitative assays, therefore the concentrations of ER, PR and EGFR were presented as defined numeric values. Concentration of EGFR in few analyses was also expressed as EGFR-negative (0-9 fm/mg m.p.) or EGFR-positive (≥10 – 100 fm/mg m.p.) ranges, but in these cases negativity or positivity are presented as a defined range of values of the receptor concentration.

To summarise, it may be stated from our results that EGFR is a very important prognostic factor indicating the course of disease in cases of breast cancer, but its impact depends upon the ER and PR status of the breast cancer tissue.

Conclusions

1. There exists an evident relationship between the length of postoperative disease free survival (DFS) of women with breast cancer and both ER and PR concentration within the tissues of the primary tumour. Women with greater ER or PR concentrations present with longer DFS and women with lower PR concentrations – with shorter DFS.
2. There exists a statistically significant positive relation between overall survival (OS) of women with primary breast cancer and the PR concentration in their cancer tissue. However, there exists no such relation of OS with ER concentration.
3. There is no relation between EGFR concentration in breast cancer tissue and both postoperative disease-free survival (DFS) and overall survival (OS) of patients. However, such a relation may be observed when EGFR concentration is analysed in view of ER and PR concentrations within the cancer tissue. A detailed analysis of this phenomenon has allowed us to conclude as follows:
   a. The presence of higher concentrations of EGFR in breast cancer tissue with a concomitant lack of ER relates to shorter DFS and OS of patients; while

the concomitant lack of both EGFR and ER relates to longer DFS and OS.

b. Patients presenting with higher concentrations of EGFR and ER or EGFR and PR in their breast cancer tissue achieve longer DFS and OS, as compared to women presenting with high EGFR concentrations and negligible concentrations of ER or PR.

To summarise – we believe that the influence of EGFR status in breast cancer tissue on the survival of women patients depends upon the ER and PR status of the tumour. In the presence of high concentrations of ER or PR in breast cancer tissue high concentration of EGFR have a positive impact on DFS and OS of patients, while with the concomitant lack of PR and ER high EGFR concentrations affect both DFS and OS of patients negatively.

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