A new look at the prognostic value of the estrogen, progesterone and epidermal growth factor receptors in breast cancer tissue

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The aim of the study was to evaluate the influence of the receptor status of primary breast cancer and of a number of selected clinical and morphological patient characteristics on survival. The receptors were determined by biochemical radiocompetitive methods. Disease free survival (DFS) and overall survival (OS) were determined by Cox proportional hazard model. The influence of ER, PR, and EGFR on patient survival was analyzed in two ways: 1) as a separate parameter of each receptor and 2) as a common parameter consisting of 8 variables of concomitant presence or absence of the receptors.

The first set of analyses had shown that EGFR as an independent parameter had no prognostic value either for DFS or OS because of a lack of statistical significance. Higher ER concentrations were positive and lower concentrations were negative prognostic factors, but only for DFS. PR was always a positive prognostic factor for DFS and OS and its prognostic value increased with concentration increase.

In the second analysis it was found that patients with receptor status ER+PR+EGFR+; ER–PR+EGFR–; ER+-PR+EGFR–; and ER–PR–EGFR– were having better parameters of DFS and OS (relative risks for DFS or OS were between 0.22–1.16). The patients with receptor status: ER–PR+EGFR+; ER+PR–EGFR–, ER–PR–EGFR+ and ER+PR–EGFR+ exhibited a more aggressive disease course (relative risks for DFS and OS were between 1.46–3.95). Moreover, it was found that tumor size, nodal status and patient age were independent prognostic factors for DFS and OS of patients.

Key words: estrogen, epidermal growth factor, progesterone, receptors, survival

Breast cancers may either maintain or lose reactivity to hormones and growth factors, which is usually manifested by either the presence or the lack of hormone and growth factor receptors in cancer tissues. Four types of receptors are most common in breast cancer tissue - estrogen receptors (ER), progesterone receptors (PR), insulin-like growth factor receptors (IGF1R) and epidermal growth factor receptors (EGFR). They all present a number of mutual dependencies, which may essentially affect the process of cancer cell proliferation and differentiation. It is a common belief that the interactions of these two processes determine the biology of the tumor, thus affecting the course of the disease and patient survival.

For many years ER and EGFR have been recognized as the basic predictive factors in the treatment of breast cancer. ER is considered to be predictive for hormontherapy efficacy and EGFR – for therapy based on anti-EGFR antibodies [2, 8, 14, 18].

Literary data concerning the influence of EGFR on survival of women with breast cancer are inconsistent. FERRERO et al [5], KOENDERS et al [10] and KLIJN et al [9] report no influence of EGFR on survival of patients. TORREGROSA et al [23] maintain that EGFR has prognostic value only in women with lymph node metastases, while FOX et al [6] claim, that EGFR is of prognostic value for OS in women with metastases-free lymph nodes only. SAINSBURY et al [20, 21], NICHOLSON et al [16], AZIZ et al [1] and TSUTSUI et al [25] confirm the role of EGFR as an independent prognostic factor.

Our previous reports [13, 18] and data yet unpublished suggest that EGFR assessed as separate factor is of no prognostic value both for disease free survival (DFS) and overall survival (OS) of women with breast cancer. However, in selected groups, for example in patients with ER and PR negative tumors the presence of EGFR is a negative prognostic factor, but in ER and PR positive tumors the

presence of EGFR is a positive prognostic factor [13, 18].

Presented paper evaluates the influence of the common presence or absence of ER, PR and EGFR in breast cancer tissue on DFS and OS of women with breast cancer. We believe that such a complex approach will contribute to a better understanding of the course of the disease.

Patients 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 Center and Institute of Oncology in Warsaw. Primary tumor sizes varied between 0.5 and 5 cm. Clinically, lymph node status was N0 in 85 cases, N1 in 68 cases and N2-N3 in 31 cases.

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 was set at a minimum of 5 years.

Histopathologically we found 134 cases of ductal carcinoma, 30 cases of lobular carcinoma and 20 cases of other carcinomas. Histopathological lymph node status was pN0 in 101 patients and pN1–pN3 in 83 patients.

Determination of estrogen and progesterone receptors in cytosol fraction. The estrogen and progesterone receptors were determined in cytosol fraction of tumorous tissues by the radiocompetitive charcoal-dextran method [19]. Briefly: triplicate samples of cytosol (0.1 ml) from tumor tissue were incubated overnight at 4 °C with increasing concentrations of ^{3}H-estradiol (0.0625–10 nM) or with ^{3}H ORG2058 (0.0625–10 nM) in the presence or absence of 100-fold excess of non-labeled competitor – diethylstilbestrol or ORG2058 respectively. The specific binding of estradiol or progestagen (ORG2058) in the cytosol was obtained from the difference between the total binding and the non-specific binding. The concentrations of specific binding calculated by 5 point assay of Scatchard analysis were expressed as fmols/mg protein of cytosol and were assumed to be as appropriate cytosol receptors – ER or PR. Some of the cases were determined as one point assay at maximal saturation conditions (usually 10 nM) of ^{3}H-estradiol or ^{3}H ORG2058 as triplicate samples in absence or presence of 100 fold excess of unlabelled competitor.

Determination of epidermal growth factor receptor. The concentrations of epidermal growth factor receptor (EGFR) in plasma membranes of cancerous tissues were determined by the radiocompetitive method according to SKASKO et al [22]. Briefly: assay was performed using multipoint analysis with triplicate samples. Increasing concentrations of ^{125}I-EGF (0.2–10.0 nM) as triplicate samples were incubated at room temperature for one hour with 100 μg of membrane protein in the absence or presence of 100 times higher concentration of non-labeled EGF. Specific binding of ^{125}I-EGF to cell membranes was obtained from the difference between total and non-specific bonds. The concentration of specific binding of ^{125}I-EGF in plasma membranes was calculated with Scatchard plot and was expressed in fmols/mg of membrane protein. Some of the cases were determined as one point assay at maximal saturation conditions (4 nM) of ^{125}I-EGF as triplicate samples in absence or presence of 100 fold excess of unlabelled EGF. The details of EGF-R determinations were described earlier [15, 22].

In some instances tumor receptor status was expressed as receptor positive (+) or negative (–). EGF-R, ER and PR were assumed to be positive when the concentrations of the respective receptors were equal to or greater than 10 fmol/mg of cytosol or cell membrane protein. Lowry's method was used for protein determination in cytosol and membrane fraction [11].

Statistical analysis. The influence of the biochemical, clinical and morphological parameters of patients on DFS and OS was analyzed according to Cox's multivariate proportional hazard analysis (backward LR version of SPSS software). In backward LR method all selected variables are first entered into the model in a single step. Then the variables are examined for removal. The probability for the removal of variable from equation equals or exceeds 0.1.

Results

Cox's proportional hazard method was used to analyze the relations between the presence or absence of ER, PR and EGFR in breast cancer tissue and patient survival. The analyzed biochemical data included two or three concentration ranges of estrogen, progesterone and epidermal growth factor receptors. To achieve a more objective approach several clinical and morphological parameters were analyzed jointly – i.e. patient age, tumor size and histopathologic type of tumor, clinical and histopathological evaluation of the lymph node status and adjuvant therapy (hormontherapy or chemotherapy).

The first series of analyses were designed to evaluate the separate influence of each of the receptors – ER, PR and EGFR – on DFS and OS of patients (Tab. 1).

ER concentration entered into Cox's equation with significance level (s.l.) 0.0207 only for DFS analysis, while for OS analysis it was removed from the equation see Table 1 and Figure 1. The probability of variable removal was set at 0.1. PR concentration entered Cox's equation both for DFS and OS with a statistical significance level of 0.0001 and 0.0180, respectively (Tab. 1, Fig. 2 and 3). EGFR concentration did not enter the equation both for DFS and OS due to lack of statistical significance, as was also the case for

Table 1. Multivariate analysis for DFS and OS in 184 breast cancer patients

Variable	DFS		OS	
	Significance	Relative risk Exp (B) 95% CI**	Significance	Relative risk Exp (B) 95% CI**
Age	0.0415		0.0721	
41–60 vs 19–40*	0.0147	0.46 (0.25–0.86)	0.0250	0.62 (0.41–0.94)
61–83 vs 19–40*	0.0300	0.42 (0.20–0.92)	0.1815	1.38 (0.86–2.22)
Nodal status (clin.)	0.0242		0.0232	
N1 vs N0*	0.0399	2.01 (1.03–3.91)	0.0137	3.12 (1.26–7.71)
N2–N3 vs N0*	0.0097	2.73 (1.28–5.86)	0.0259	2.99 (1.14–7.86)
Nodal status (histpat.)				
pN1–pN3 vs pN0*	0.0000	4.17 (2.29–7.59)	0.0039	2.89 (1.41–5.96)
Adjuvant therapy (chem. or horm.)				
+ vs –*	0.0319	0.50 (0.27–0.94)	0.0048	0.28 (0.12–0.68)
ER fmol/mg prot.	0.0207			
10–90 vs 0–9*	0.0375	2.15 (1.04–4.43)		Variable out of
91–400 vs 0–9*	0.0591	0.28 (0.07–1.05)		the equation
PR fmol/mg prot.	0.0001		0.0180	
21–60 vs 0–20*	0.2672	0.68 (0.34–1.35)	0.2919	0.66 (0.31–1.42)
61–1200 vs 0–20*	0.0002	0.23 (0.11–0.49)	0.0099	0.33 (0.14–0.77)
Variables not in the equation***	EGFR Tumor size Histology type		EGFR ER Tumor size Histology type	

* – reference variable; CI** – confidence interval; *** – the probability for removal of variable from equation equals or exceed 0.1.

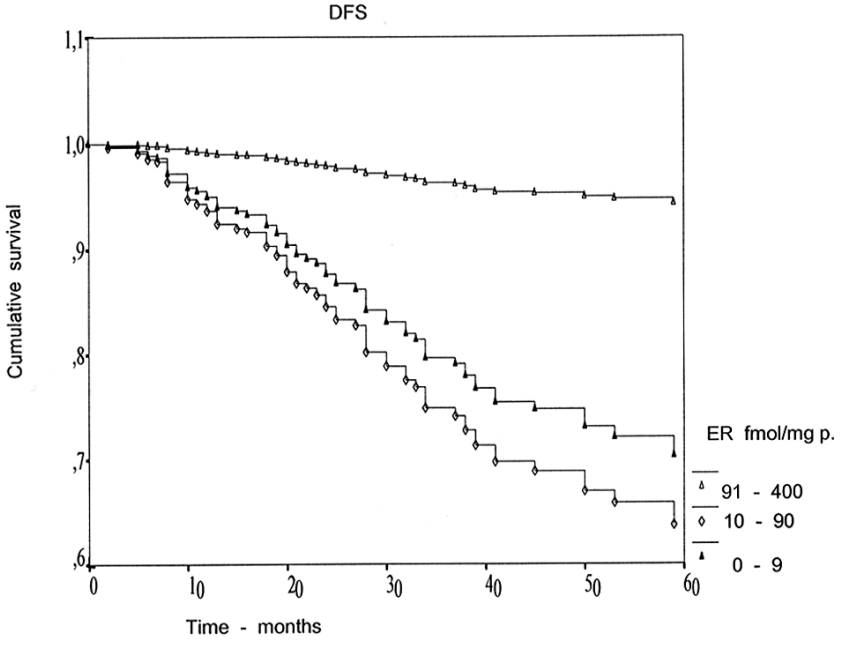

Figure 1. Disease free survival curves of breast cancer patient with different concentrations of estrogen receptor in their tumors; for details see Table 1.

tumor size and histopathological type of breast cancer (Tab. 1).

In the second series of multivariate analyses we applied a different approach to receptor parameters of breast cancer. Receptor parameters of the tumors – ER, PR and EGFR were entered into Cox's equation as one common parameter – tumors receptor status consisting of 8 possible variables of coexistence or absence of appropriate receptor. This overall receptor status entered the equation as a common parameter with a significance level of 0.0074 for DFS and of 0.0394 for OS (see Tab. 2, Fig. 4 and 5). This second series of Cox's analyses included not only the receptor status of tumors, but also clinical and morphological parameters of patients (Tab. 2).

Discussion

The study has been designed to evaluate, with the aid of Cox's proportional regression model, the influence of the ER, PR and EGFR status of tumors on the survival of breast cancer patients. The analysis involved also clinical and morphological parameters (Tab. 1 and 2).

The first analysis has shown that PR concentration in tumors is an independent prognostic factor for both DFS and OS (Tab. 1, s.l. 0.0001 and 0.0180, respectively). It may be noticed that the positive effect of PR concentration on patient survival increased with an increase of its concentrations in the tumor tissue. The level of relative risk (RR) observed for these phenomena shifted from 0.68 to 0.23 for DFS and 0.66 to 0.33 for OS. The significance level also shifted from 0.2672 to 0.0002 for DFS and from 0.2919 to 0.0099 for OS (Tab. 1 and Fig. 2 and 3).

Literature reports on PR status as a prognostic factor in breast cancer present contradictive data. Results similar to ours have been reported by TORREGROSA et al [23] who have shown, in a unilateral analysis, that the PR status is a prognostic factor for DFS and OS in women with breast cancer. However, they do not refer to the role of PR status in their multivariate analysis. CASTAGNETTA et al

[3] have also reported a statistically significant role of PR status on the survival of women with breast cancer. For instance PR (–) patients presents earlier recurrence than PR(+) patients. On the other hand, FERRERO et al [5] maintain that PR status plays no part in the survival prognoses of breast cancer patients.

Our study has shown, that the ER status is a prognostic factor of DFS only (s.l. 0.0207) but for OS is insignificant. The prognostic value of ER for DFS depends upon the receptor concentration within the tumor. When comparing the reference values of ER concentration (0–9 fmol/mg p.) to the higher ER concentrations (91–400 fmol/mg p.) ER have a positive influence on DFS (RR=0.28) but lower ER concentrations (10–90 fmol/mg p.) have a negative influence (RR=2.15) on patient DFS (Tab. 1 and Fig. 1).

According to TSUTSUI et al [25] ER (–) is a negative factor for DFS (RR=1.92) and for OS (RR=2.23) when compared with ER (+) tumors. In the course of a unilateral analysis NICHOLSON et al [16] have shown, that ER is a significant prognostic factor for DFS and OS, while its influence has been shown to be insignificant in the course of multivariate analysis.

Since SAINSBURY et al [20, 21] had reported that EGFR negatively influences the course of breast cancer in women the matter has been widely investigated [23, 25]. However, due to the heterogeneity of the material, varying methods of EGFR values evaluation (radioligand or immunohistochemical staining) and the application of different statistical methods the presented conclusions vary.

Table 2. Multivariate analysis for DFS and OS in 184 breast cancer patients; tumor receptor status was expressed as 8 subvariables

Variable	DFS		OS	
	Significance	Relative risk Exp (B) 95% CI**	Significance	Relative risk Exp (B) 95% CI**
Age	0.0158		0.0224	
41–60 vs 19–40*	0.0208	0.47 (0.25–0.89)	0.0474	0.46 (0.21–0.99)
61–83 vs 19–40*	0.0061	0.37 (0.18–0.75)	0.0727	2.03 (0.94–4.41)
Tumor size 0.5–5 cm	0.0035	1.71 (1.19–2.46)	0.0975	1.50 (0.93–2.44)
Nodal status (clin.)	0.0295		0.0250	
N1 vs N0*	0.0194	2.22 (1.14–4.34)	0.0078	3.56 (1.40–9.05)
N2–N3 vs N0*	0.0172	2.53 (1.17–5.44)	0.0318	3.05 (1.10–8.45)
Nodal status (histpat.)				
pN1–pN3 vs pN0*	<0.0001	3.91 (2.17–7.07)	0.0005	3.84(1.81–8.15)
Adjuvant therapy (chem. or horm.)				
+ vs –*			0.0124	0.30 (0.12–0.77)
Tumor receptor status of subvariables 1–8	0.0074		0.0394	
ER PR EGFR n#				
1. + + + 28	0.0855	0.45 (0.18–1.11)	0.0248	0.22 (0.06–0.83)
2. – + – 9	0.4783	0.63 (0.18–2.23)	0.1895	0.26 (0.03–1.96)
3. + + –* 64				
4. – – – 11	0.9297	1.07 (0.23–5.03)	0.8914	1.16 (0.14–9.47)
5. – + + 11	0.5020	1.46 (0.49–4.39)	0.0516	3.16 (0.99–10.07)
6. + – – 33	0.0350	2.14 (1.06–4.35)	0.0454	2.56 (1.02–6.42)
7. – – + 21	0.0337	2.23 (1.06–4.70)	0.0712	2.32 (0.93–5.80)
8. + – + 7	0.0332	3.31 (1.10–9.98)	0.0383	3.95 (1.07–14.48)
Total 184				
Variables not in the equation***	Adjuvant therapy Histology type		Histology type	

* – reference variable; CI** – confidence interval; n# – number of cases in subvariables; *** – the probability for removal of variable from equation equals or exceed 0.1.

Our results show, that EGFR in breast cancer tissue does not influence neither DFS nor OS of patients as an independent parameter (Tab. 1). On the other hand, NICHOLSON et al [16] report that EGFR is a significant factor affecting DFS and OS both in a unilateral and in a multivariate analysis. TSUTSUI et al [24] have shown, that EGFR is an independent and significant prognostic factor only when the multivariate analysis does not include the ER status of the tumor, while if ER is analyzed, the EGFR status looses its prognostic independence due to a lack of statistical significance. TORREGROSA et al [23] report, that in the course of Cox's proportional hazard analysis EGFR is an independent prognostic factor of OS only, while it is insignificant for DFS. In the two latter studies ER status was included in the analyses.

Such inconsistencies in the role of hormonal receptors on patient survival have induced us to change our approach to the assessment of the prognostic value of ER, PR and EGFR. We have assumed that survival of patients depends on actual receptor status of breast cancer tissue. Therefore ER, PR and EGFR should not be analyzed separately, but rather as the current receptor status of the primary tumor.

We shall begin with discussing the results of a multivariate analysis of the receptor status of breast cancer tissue, which had consisted of 8 variables of concomitant presence or absence of ER, PR and EGFR. This common receptor status entered Cox's regression equation at a 0.0074 signifi-

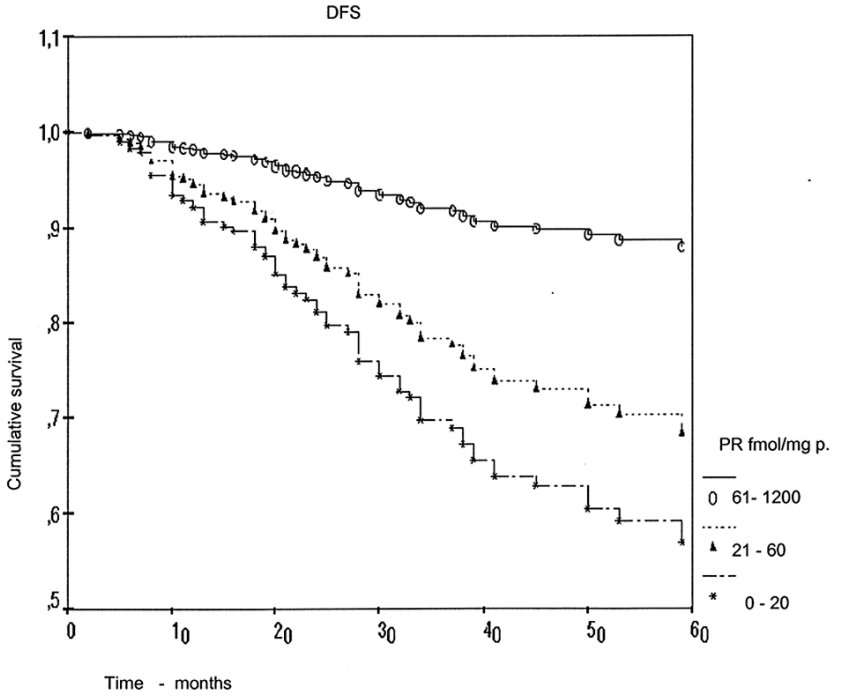

Figure 2. Disease free survival curves of breast cancer patients with different concentrations of progesterone receptor in their tumors; for details see Table 1.

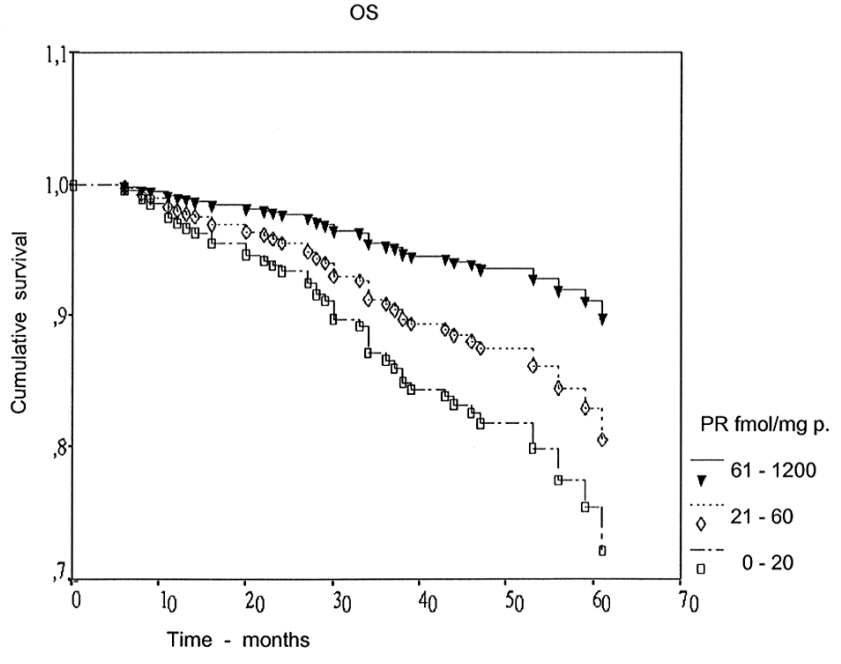

Figure 3. Overall survival curves of breast cancer patients with different concentrations of progesterone receptor in their tumors; for details see Table 1.

cance level for DFS and at a 0.0394 significance level for OS (Tab. 2). We chose the ER+ PR+ and EGFR– variable as the reference group of receptor status to which the relative risks (RR) factors were calculated. As we observed (Fig. 4 and 5 and Tab. 2) every variable had a different impact on survival, as compared to the reference group.

We found, that those patients who had presented with the ER+ PR+ EGFR+ variable had the best results for OS (Tab. 2; RR=0.22 with statistical level 0.0248) while for DFS RR is satisfactory but has not achieved full statistical significance of difference to reference parameter (RR=0.45 and s.l. 0.0855). Patients with a ER–PR+EGFR– and ER–PR–EGFR– with a lower RR for DFS and OS (0.63 and 0.26, respectively) or a slightly higher RR for DFS and OS (1.07 and 1.16, respectively), as compared to the reference group, did not achieve statistical significance (s.l. between 0.1895 and 0.9297). It may be assumed that their survival did not differ significantly from the reference group (ER+ PR+ EGFR–).

It is interesting that the ER– PR+EGFR+ group presented a slightly increased, but nevertheless statistically insignificant risk of earlier recurrence (RR=1.46, statistical significance 0.5020), while it was found to achieve much worse results in terms of overall survival (RR=3.16; statistical level approaches to significance 0.0516) – Table 2.

Three groups: the ER+PR–EGFR–, the ER–PR–EGFR+ and the ER+PR–EGFR+ achieved significantly worse results in terms of DFS and OS (RR values between 2.14 and 3.95; s.l. between 0.0712 and 0.0332; Tab. 2 and Fig. 4 and 5). It may be summarized that patients with a ER+-PR+EGFR+; ER–PR+EGFR–; ER+-PR+EGFR–; and ER–PR–EGFR– receptor status achieve better survival in terms of DFS and OS, when compared with patients having ER+PR–EGFR–, ER–PR–EGFR+ and ER+PR–EGFR+ status.

The presented data shows that both DFS and OS of breast cancer patients depend on a definite receptor status of the primary tumor. The less aggressive disease course of patients appears to depend on the presence of PR in the tumor. Namely, the presence of ER and EGFR in the tumor, combined with the presence of PR, favors the best survival of patients (Tab. 2, Fig. 4 and 5).

Worse survival is associated with the absence of PR in

tumors. The sole presence of ER or the sole presence of EGFR in tumor accompanies worse survivals (RR between 2.14 and 2.56; s.l. 0.0337–0.0712), while the combined presence of ER and EGFR appear to further decrease survival (DFS: RR=3.31, statistical significance 0.0332; OS: RR=3.95, significance level 0.0383).

We have found no literary reports of such an approach to the assessment of ER, PR and EGFR status on the survival of patients. Only MAURI et al [12] have reported, that the combined evaluation of ER and PR enhances the prognostic value of ER. Most reports are concerned with the influence of the different receptors evaluated separately or with the combined impact of ER and EGFR. These latter studies bear some resemblance to our report. For instance, TORREGROSA et al [23] stress, that patients with ER+ and EGFR+ status present with worse DFS parameters than patients with ER+EGFR– status. Our analysis has shown that ER+PR–EGFR+ patients have the worst DFS and OS parameters (RR=3.31 and 3.95, respectively). TORREGROSA et al [23] also report, that ER–EGFR– patients present with better DFS than ER–EGFR+ patients. In our study ER–PR–EGFR– patients present better DFS and OS than ER–PR–EGFR+ patients (RR=1.07 and 1.16, as compared to 2.23 and 2.32; see Tab. 2). According to HARRIS et al [7] ER–EGFR– and ER+ patients have comparably good prognosis. This is also comparable with our variables in which ER–PR–EGFR– patients are almost identical with the reference group (i.e. ER+PR+EGFR– patients) in terms of DFS and OS (DFS: RR=1.07 and OS: RR=1.16 as compared to RR of the reference group, which is, of course, 1.0).

Summarized, our results show that the different ER, PR and EGFR status of tumors may have different influence on survival of patients. The final impact depends upon the different relations between the presence and absence of the receptors. These differences in survival may arise from the existence of two different mechanisms of receptor action [4]. One of these mechanisms relies on increasing gene expression within the cells, while another involves a protein-protein interaction (e.g. bonding between ER or PR and the AP1 complex) [17]. Moreover, the differences may also arise from the presence of two kinds of estrogen receptors – α and β. Altogether, these different mechanisms of receptor action may affect both cell proliferation and differentiation in different way.

We assume that all the inconsistent opinions on the prognostic impact of ER, PR and EGFR may arise from the fact, that they are usually analyzed separately. Our complex ap-

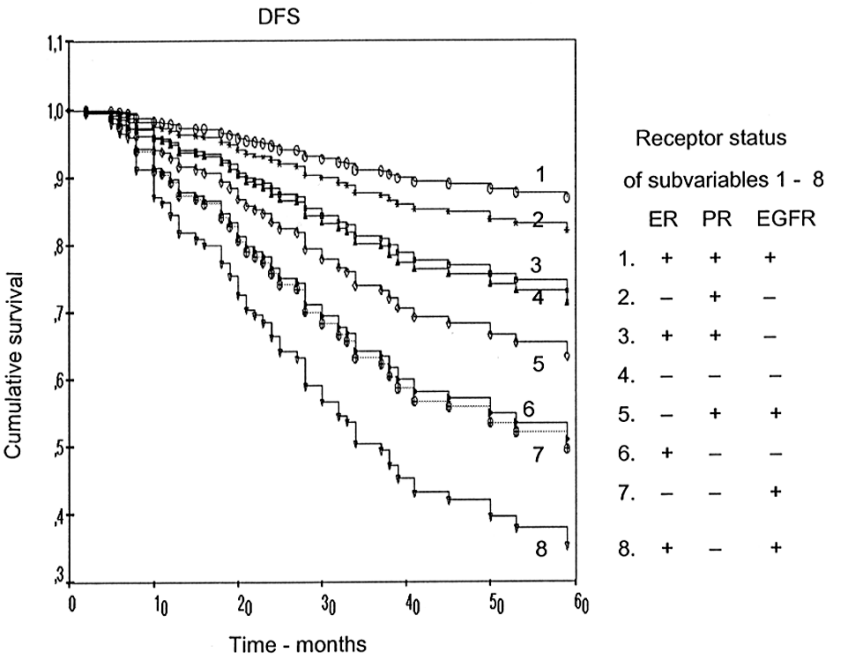

Figure 4. Disease free survival curves of groups of patients with definite receptor status (subvariables 1–8) in breast cancer tissue; for details see Table 2.

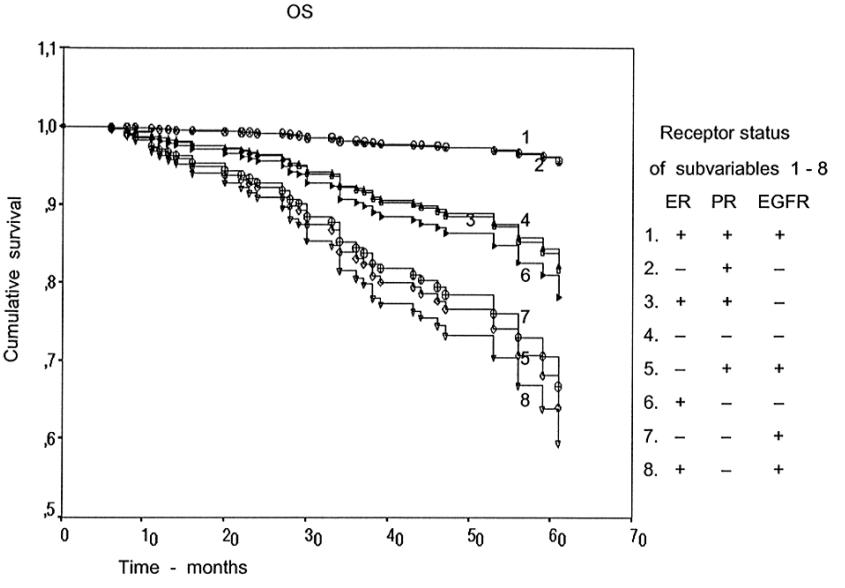

Figure 5. Overall survival curves of groups of patients with definite receptor status (subvariables 1–8) in breast cancer tissue; for details see Table 2.

proach explains these inconsistencies and allows a more exact prediction of the course of the disease – more or less aggressive.

In our data both the clinical and the histopathological nodal status, were the most significant and independent prognostic factors of patient survival (Tab. 1 and 2). RR was between 2.01 and 4.17 for DFS and OS, significance level range: 0.0000–0.0399. In a first multivariate analysis (Tab. 1) tumor size did not enter Cox's regression equation (both for DFS and OS) due to a lack of statistical significance. However, in a second multivariate analysis tumor size turned out to be a slightly negative predictive factor both for DFS and OS (Tab. 2; RR 1.71 and 1.50, respectively). FERRERO et al [5] have reported tumor size and metastatic lymph nodes to be independent prognostic factors of survival. On the other hand, TSUTSUI et al [25] have reported tumor size and lymph node metastases to be a negative prognostic factor for DFS and patient age and nodal status to be independent prognostic factors of OS in women with breast cancer. Also TORREGROSA et al [23] report tumor size and metastatic lymph nodes as negative prognostic factors of DFS and OS.

Our study shows that age in both multivariate analyses enters Cox's equation for DFS and OS (s.l. between 0.0158 and 0.0727), although its impact varies. Women aged between 41–60 years present better results in terms of DFS and OS than younger patients (19–40 yrs). The eldest group of patients (61–83 yrs) present significantly better results in terms of DFS, and worse results of OS (Tab. 1 and 2).

In the first multivariate analysis adjuvant treatment (hormontherapy and chemotherapy counted together, Tab. 1) clearly influenced both DFS and OS (RR=0.5 and 0.28 respectively, significance level 0.0319 and 0.0048, respectively). In the second analysis (Tab. 2) adjuvant treatment influenced only OS (RR=0.30, significance level 0.0124). TSUTSUI et al [25] report no impact of adjuvant treatment on DFS and OS of breast cancer patients.

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