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The epidermal growth factor receptor as a prognostic marker: Results of 370 patients and review of 3009 patients

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Summary

Epidermal growth factor receptor (EGFR) and estrogen receptor (ER) were assayed by ligand binding in tumors from 370 patients with primary breast carcinoma with a median follow up of 18 months. Forty seven percent (175/370) and 57% (210/370) of tumors had >20 fmol/mg and >10 fmol/mg of EGFR and ER respectively. There was a highly significant inverse relationship between EGFR and ER (p=0.0032). There was also a significant association between EGFR and patient age (p=0.0006) but no correlation between EGFR and lymph node status, tumor grade, or tumor size (p=0.104, p=0.198, and p=0.085 respectively). In a univariate analysis of all patients, EGFR expression was not associated with a significant reduction in overall survival (OS). However, there was a significant decrease in relapse-free survival (RFS) and OS in node negative EGFR positive patients (p=0.03 and p=0.05 respectively). In a multivariate analysis (Cox proportional hazard model) of all patients, lymph node status was an independent prognostic indicator for OS and RFS (p<0.00005 and p=0.00005 respectively), ER status for RFS (p=0.0006), and EGFR (in the node negative model) for RFS (p=0.03). When all patients were stratified for EGFR and ER, there was a significant difference in RFS and OS such that EGFR positive and ER negative had the worst prognosis (p=0.0034 and p=0.005 respectively). A similar relationship was observed for OS in node negative patients (p=0.004) and for RFS in node positive patients (p=0.009). In a review of 3009 patients with follow-up, 11/16 series showed high EGFR was associated with shorter RFS or OS in univariate analysis, and 4 showed this in multivariate analysis. However, most series had inadequate follow-up time and most did not include multivariate analysis. This highlights the need for uniform criteria of reporting trials of prognostic factors.

Introduction

Overexpression of the epidermal growth factor receptor (EGFR) is considered an important autocrine stimulatory pathway for breast carcinoma cell growth, and its expression is associated with

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Table 1. Clinicopathological characteristics of patients and tumors

Patient characteristic	All patients (370)	Node positive (169)	Node negative (201)
Age (median, range)	58 (28-92)	59 (32-92)	55 (28-86)
<50 years	106	41	67
≥50 years	264	128	134
Surgical treatment			
Partial mastectomy	263	101	162
Simple mastectomy	107	68	39
Adjuvant treatment			
Chemotherapy	103	72	30
Tamoxifen	200	119	81
Tumor size (median, range)	2.3 (0.5-15)	2.5 (0.5-15)	2.0 (0.5-14.5)
<2 cm	117	36	81
≥2 cm	253	133	120
Histology			
Ductal	277	125	152
Lobular	36	18	18
Mixed	33	16	17
Others	24	10	14
Grade			
I	47	12	35
II	117	56	61
III	113	57	56
ER[a] (median, range)	14 (0-742)	13 (0-742)	15 (0-695)
<10	160	73	87
≥10	210	96	114
EGFR[a] (median, range)	20 (0-733)	19 (0-733)	20 (0-710)
<20	195	90	105
≥20	175	79	96
Survival follow-up			
Median, range (months)	21 (0.1-49)	18 (0.1-49)	32 (0.3-48)
Deaths	41	32	9
Recurrences	70	49	21

[a] fmol/mg protein

an enhanced metastatic potential in model systems. There is a consistent inverse relationship between presence of high affinity EGFRs and estrogen receptors in primary human breast carcinomas, and tumors which express high levels of EGFR are associated with a shorter relapse-free survival (RFS) and overall survival (OS) [1,2]. However, at present there is no agreement as to the value of EGFR as a prognostic factor in human breast carcinoma [3,4]. This is primarily a function of different study designs, which have used a variety of assays and cut-off points for EGFR positivity, relatively small patient numbers, and statistical analysis which may not take into account the effect of ER status and established prognostic indicators [1,2,5-22].

We have therefore analysed a new series of 370 breast carcinomas using both univariate analysis and a Cox proportional hazard model to assess the relationship of EGFR to other prognostic variables, and have assessed the importance of EGFR expression on both RFS and OS. In

Table 2. Results of a univariate Cox proportional hazard analysis of all patients

Prognostic indicator	Survival			Relapse		
	Hazard ratio	95% CI	p-value	Hazard ratio	95% CI	p-value
Age	1.16	0.6,2.3	0.65	1.1	0.6,1.7	0.9
Tumour size	2.1	1.0,4.6	0.05	1.9	1.1,3.3	0.02
Histology	0.9	0.5,1.8	0.74	1.1	0.6,1.8	0.05
Grade	0.3	0.1,1.4	0.09	0.5	0.2,1.1	0.85
ER	0.4	0.3,0.7	0.001	0.3	0.3,0.6	0.001
EGFR	1.35	0.7,2.5	0.35	1.3	0.8,2.1	0.29
Lymph nodes						
1-3	1.9	0.8,4.4	0.12	1.2	0.7,2.1	0.61
≥4	12.2	1.8,85	0.004	3.6	1,12.3	0.04

CI = confidence interval

contrast to our previous studies [1,2], patients in this series received adjuvant chemotherapy and hormone therapy.

Patients and methods

370 patients with operable breast carcinomas were treated by simple mastectomy or lumpectomy and postoperative radiotherapy. Axillary nodes were sampled in all patients at the time of surgery. In all patients, adjuvant radiotherapy was administered to the ipsilateral axilla if lymph nodes had histological evidence of metastasis. Table 1 shows the patient characteristics of the different groups where survival data is recorded. Samples were analysed for ER and EGFR, and tumors with concentrations greater than 10 fmol ER/mg cytosolic protein and 20 fmol EGFR/mg membrane protein respectively were considered positive. (The cut-off for EGFR has been altered from the previous published 10 fmol/mg membrane protein to 20 fmol/mg membrane protein due to changes in the assay.) Grading was performed according to the modified Bloom and Richardson method [23]. Follow-up was conducted every three months for the first 18 months, and 6 monthly until 3 years. Patients with confirmed recurrent disease were treated by endocrine manipulation for soft tissue or skeletal disease or by chemotherapy for visceral disease or failed endocrine therapy. Patients with isolated soft tissue relapse additionally received radiotherapy. Adjuvant treatment details are summarised in Table 1. Median follow-up was 21 months (range 0.1-49 months).

Statistics

Analysis of patient and tumor characteristics was performed within the various subgroups using contingency tables and the Chi squared test for categorical variables and Spearmans Rank test for continuous variables. Curves were plotted using the method of Kaplan and Meier, and significance for RFS and OS was calculated using either the log rank test, or univariate or multivariate Cox proportional hazard models.

Results

Relationship of EGFR to other prognostic indicators

EGFR levels ranged from 0-733 fmol/mg protein (median 20 fmol/mg protein) (Table 1). There

Table 3. Results of a univariate Cox proportional hazard analysis of node positive patients

Prognostic indicator	Survival			Relapse		
	Hazard ratio	95% CI	p-value	Hazard ratio	95% CI	p-value
Age	1.37	0.3,3.1	0.4	0.9	0.5,1.7	0.8
Tumour size	2.0	0.8,6.9	0.07	1.8	0.8,3.8	0.12
Histology	0.95	0.5,2.0	0.89	0.9	0.5,1.7	0.8
Grade	0.9	0.2,3.7	0.8	0.5	0.2,1.4	0.3
ER	0.4	0.2,0.9	0.02	0.4	0.2,0.6	0.001
EGFR	0.9	0.5,2.0	0.98	0.9	0.6,1.7	0.96
Lymph nodes 1-3 *vs* ≥4	3.5	1.7,7.3	0.0006	2.6	1.5,4.6	0.009

CI = confidence interval

was a significant correlation between EGFR expression and patient age (cut off <50 years) (p=0.0006) but no association with tumor grade, size (cut off ≥2 cm), or lymph node status (p=0.085, 0.198, and p=0.11 respectively). ER expression ranged from 0-742 fmol/mg protein (median 14 fmol/mg protein) and showed a significant correlation with tumor grade (p<0.0001) and patient age (p<0.0001) but not tumor size (p=0.56) or lymph node status (p=0.08). There was a significant inverse correlation between EGFR and ER expression when assessed as either categorical (p=0.002) or ranked (p<0.0001) variables.

EGFR, ER, and survival

In a univariate analysis of all patients, there was no significant difference in either RFS or OS times between patients with EGFR negative vs. positive tumors (p=0.29 and p=0.35 respectively) (Table 2). Analysis of the node positive subgroup by EGFR status showed no significant differences in RFS or OS (p=0.98 and p=0.96) (Table 3, Figure 1). Analysis of node negative patients alone by EGFR status showed that there was a significant reduction in RFS (p=0.03) and OS (p=0.05) with elevated EGFR (Table 4) (Figure 1).

A significant difference in both RFS and OS was observed when stratifying all patients (p= 0.001 and p=0.001 respectively) and node positive patients (p=0.02 and p=0.001 respectively) for ER (Tables 2-4). There was no significant difference in RFS in the node negative subgroup of patients stratified by ER (p=0.15). Too few events occurred for analysis of OS.

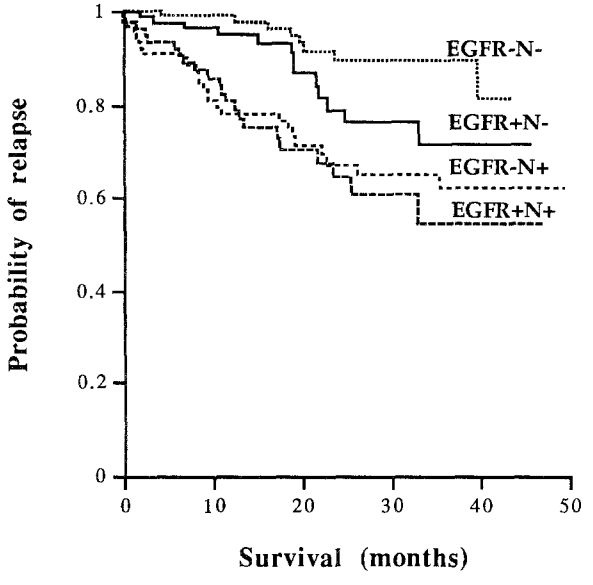
There was a significant interaction between the four EGFR/ER subgroups for all patients for both RFS (p=0.0034) and OS (p=0.005). Analysis of

Table 4. Results of a univariate Cox proportional hazard analysis of node negative patients

Prognostic indicator	Survival			Relapse		
	Hazard ratio	95% CI	p-value	Hazard ratio	95% CI	p-value
Age	0.6	0.2,2.5	0.5	0.9	0.4,2.4	0.98
Tumour size	0.7	0.2,3.0	0.7	1.2	0.5,3.1	0.65
Histology	1.1	0.2,5.4	0.9	1.6	0.5,4.8	0.4
ER	**	**	**	0.5	0.2,1.3	0.15
EGFR	4.4	0.9,21.7	0.05	2.3	1.0,5.6	0.03

CI = confidence interval.

** Too few events occurred for analysis. Likewise, grade was omitted from this table because too few events occurred in some groups to allow analysis.



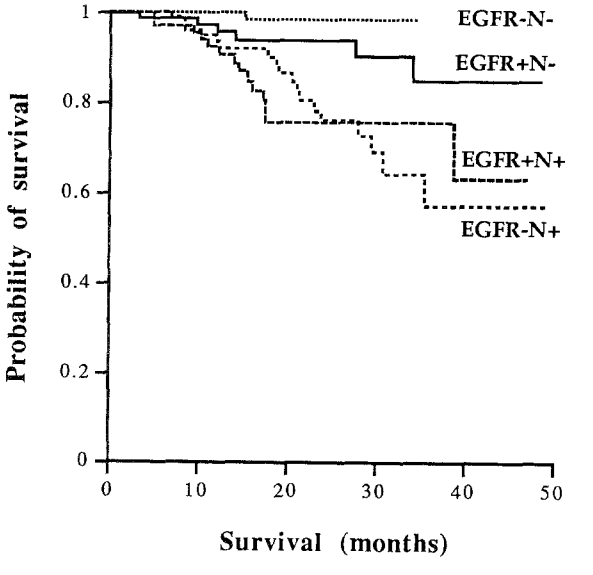
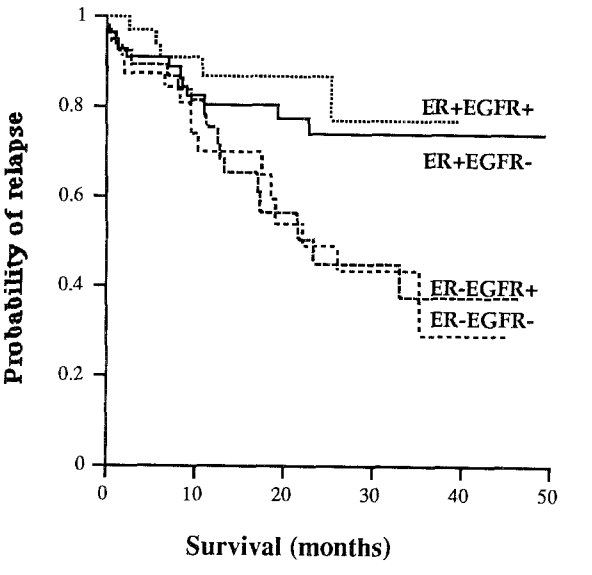


Figure 1. Relapse-free (top) and overall (bottom) survival curves plotted for EGFR and node status.



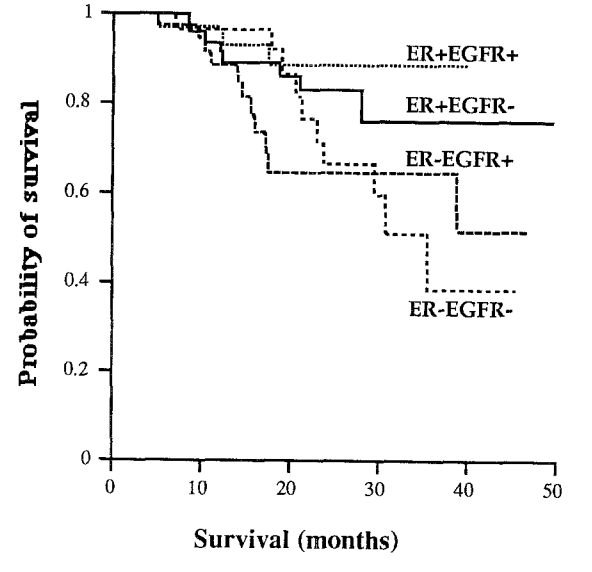


Figure 2. Relapse-free (top) and overall (bottom) survival curves plotted for ER and EGFR in node-positive patients.

the interaction between EGFR/ER groups in the node positive subgroup demonstrated a significant difference in RFS (p=0.009) but not OS (p=0.09) (Figure 2). When examining the interaction between the EGFR/ER groups in the node negative subgroup, a significant difference only in OS (p=0.004) but not in RFS (p=0.19) was observed (Figure 3).

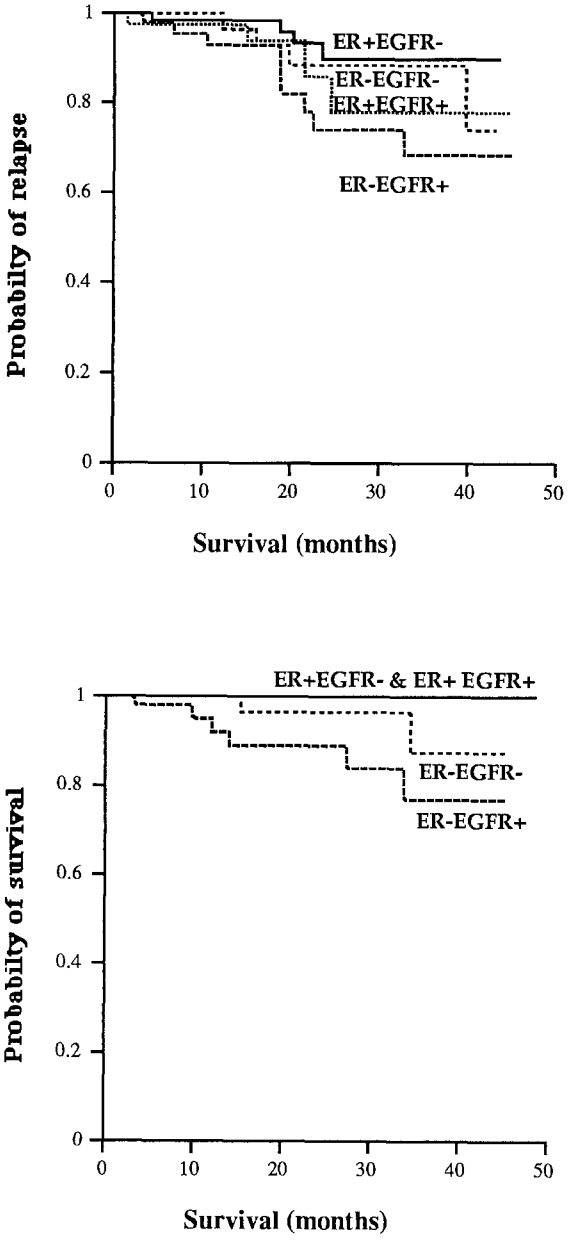


Figure 3. Relapse-free (top) and overall (bottom) survival curves plotted for ER and EGFR in node-negative patients.

Multivariate analysis of survival

In a Cox proportional hazard model of all patients there was a negligible influence on RFS and OS for EGFR (p=0.33 and p=0.5), tumor size (p=0.06 and p=0.52), and grade (p=0.95 and p=0.62) when assessed with nodal status and ER. The nodal status remains significant for RFS (p=0.06 [nodes 1-3] / p=0.00005 [nodes>4]) and OS (p=0.016 [nodes 1-3] / p=0.00005 [nodes>4]) and ER level for RFS (p=0.0006) and OS (p=0.06). In the node positive group for both RFS and OS, only tumor size (p=0.009 and p=0.04), nodal status (p=0.02 and p=0.01), and (for RFS) ER levels (p=0.001) remain significant; both EGFR (p=0.5 and p=0.9) and tumor grade (p=0.9 and p=0.9) drop from the model. When examining the node negative subgroup, we find EGFR to be a significant predictor for RFS (p=0.03) but not for OS (p=0.13), and ER levels for OS (p=0.01).

Discussion

Although several thousand patients have been reported with correlations of EGFR and other variables (see Klijn review) [3,4], there are only 16 series with follow-up data and survival analysis on 3009 patients (Table 5). In this review, in which we also include this present patient data set, where there have been several publications by one group, only the largest series with longest follow-up have been analysed [1,2,5-23].

In 12/16 studies there is a significant association of RFS or OS with EGFR expression, using univariate analysis [1,6,8,10,12,15-19,21]. In most studies this applies to all patients, but in others only to the node positive [10,16] or node negative [1,19] groups. There are several possible explanations for the variation in these results. Most of these series have follow-up data for less than three years, median or maximum. This will only allow detection of factors associated with risk of early relapse and is too short for adequate assessment of OS. The short follow-up may also explain why an effect on RFS is more frequently reported than on OS (11 compared to 5 studies). Similar variability in significance has been reported for cathepsin D, c-*erb*B-2, ER, and other biological factors, but as larger series with longer

Table 5. Summary of published series reporting the relationship between EGFR and prognosis

Author	n	Adjuvant chemotherapy		Follow-up	Method	Cut-off	% +ve	Analysis			
		Criteria	Treatment (n)					Univariate		Multivariate	
								RFS	OS	RFS	OS
Rios et al [15] 1988	179	Not stated	–	30 max	LB	1 fmol/mg	43	p<0.05			
Costa et al [6] 1988	376	N+	C±H 180	12 med	LB	10 fmol/mg	–	p<0.01			
Grimaux et al [10] 1989	55	>3N+	C±H 41	65 mean	LB	5 fmol/mg	33	NS	p=0.051**	p=0.014	p<0.015
Foekens et al [7] 1989	203	N+	C 42; H 11	42 med	LB	none	*91	NS		NS	
Lewis et al [12] 1990	90	None	–	36 max	IH	>2+	14	p<0.003		p=0.04	
Spyratos et al [19] 1990	109	'High risk'	C±H 34	60 mean	LB	10 fmol/mg	34	p=0.05		p=0.03	
Nicholson et al [1] 1991	231	No chemo	H 40	45 med	LB	10 fmol/mg	35	p<0.001	p<0.001	p=0.03	NS
Toi et al [16] 1991	135	N+	C±H 59; H 75	31 med	LB	1 fmol/mg	41	p<0.05			
Hawkins et al [11] 1991	120	Not stated	–	20 med	LB	1 fmol/mg	43	NS	NS	NS	NS
Shrestha et al [17] 1992	50	Not stated	–	60 min	IH	any	44	p<0.05	p<0.05		
Gasparini et al [8] 1992	164	N+	C 51; H 30	36 med	IH	>5% cells	56	p=0.003	NS	p=0.0049	
Osaki et al [18] 1992	115	N+; T>3cm	C±H 53; H 69	32 mean	LB	1 fmol/mg	35	p<0.01			
Bolla et al [5] 1992	272	N+/G3/ER–	C±H 190	16 med	LB	3 fmol/mg	51	NS		NS	
Murray et al [13] 1993	107	Not stated	–	60 med	mRNA	+ or ++	51	NS	NS		
Koenders et al [21] 1993	376	N+	C 52; H 96	24 med	LB	50 fmol/mg	22	p=0.03	p=0.002	NS	NS
Fox et al 1993	370	N+/G3/ER–	C 103; H 200	18 med	LB	20 fmol/mg	47	p=0.03(N-)	p=0.05(N-)	p=0.03(N-)	NS

Follow-up times may be median (med), mean, maximal (max), or minimal (min); NS=not significant; Cut-off levels are per mg protein unless stated; * any positivity; ** at 40 months not 90 months; LB=ligand binding; IH=immunohistochemistry; mRNA=dot blot hybridization; C=chemotherapy; H=hormonal therapy; G=grade; numbers of patients treated are estimates derived from published data.

follow-up have become available, some resolution of their significance has been obtained.

Many series were often small, which did not allow adequate subgroup analysis (only 6 studies had more than 200 cases and 3 more than 300). The great heterogeneity in breast cancer biology and patient populations is as apparent for EGFR as for any other prognostic marker, and therefore large series must be studied. Nevertheless, when subgroups have been examined EGFR had an effect in 3/5, 2/4, and 2/3 of studies of ER negative [1,6,7,10,12], node negative [1,10,16,19], and node positive [1,10,16] subgroups, respectively. Thus, the role of EGFR may be most useful in node negative patients, or in selecting patients for tamoxifen therapy.

In only a proportion of reports was multivariate analysis carried out, thereby taking into account the influence of other prognostic variables. When this was performed, an effect was detectable on RFS and OS in 6/10 and in 1/5 of the studies respectively. Indeed, when analysing the effects on survival it is critical which other variables are included in the multivariate analysis. Thus, in one series EGFR was no longer significant when c-*erb*B-2 was available in the statistical model [8,9].

Adjuvant therapy is also likely to affect outcome, particularly as EGFR expression is inversely related to ER and may be associated with tamoxifen resistance. This has been difficult to assess, since in 4 studies it is not stated whether adjuvant therapy was given, and in 10 studies it was given to "high risk" patients. However, in the 2 studies where adjuvant chemotherapy was not administered there was a significant effect of EGFR expression on RFS [1,12]. Furthermore, when treated and untreated groups were directly compared, EGFR status was a significant independent prognostic marker only in the untreated group [19]. In addition, assessment is further complicated because EGFR may also be a predictive factor as well as a prognostic factor for response to adjuvant therapy [24].

As displayed in the table, a variety of assay techniques including ligand binding, immunohistochemistry, and mRNA analysis have been used. Ligand binding has used different methods

of purifying membranes, preparing the ligand, and labelling the ligand, as well as different cut-points. ER assays are standardised by a quality control scheme and a similar approach should be taken for EGFR. Nevertheless, the variability in assays has still allowed definition of high and low EGFR groups such that all but 2 studies fall into the range of 35-60% positivity.

In conclusion, the majority of studies have shown some effect of EGFR expression on RFS, but this review highlights the need for journal editors to have a minimal set of criteria for accepting papers on prognostic factors. Without this, optimum use cannot be made of the multiple publications in the area. Even after a review of 3009 patients with follow-up (Table 5), firm conclusions still cannot be drawn. In this special issue of Breast Cancer Research and Treatment, many authors have updated and extended their series, so a better assessment can be made. As in our previous studies, in this series we only observed an EGFR effect (albeit less significant than ER) on RFS in node negative breast cancer patients. The use of adjuvant tamoxifen for a wider group of patients since its utility in both node positive and node negative cases was demonstrated may partly explain this discrepancy, since ER positive patients will have greater benefit from tamoxifen than ER negative patients, with the result that there is a wider separation in survival curves [25].

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