Prognostication and treatment decision-making in early breast cancer

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The prognostic value of hormone receptor detection by enzyme immuno assay and immunohistochemistry; a prospective study in patients with early breast cancer.

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ABSTRACT

Background: The main reason to determine the oestrogen (ER) and progesterone receptor (PR) in breast cancer is their predictive value for response to endocrine therapy. In addition, ER and PR receptors are often used as prognostic indicators. Enzyme immuno assay (EIA) and immunohistochemistry (ICA) are two methods for determining ER and PR receptors. These two methods have not been compared to each other on clinical endpoints.

Methods: In the present study we prospectively evaluated the prognostic value of ER and of PR, as determined both by ICA and by EIA, in 223 and 207 patients, respectively with early breast cancer.

Results: ER was positive in approximately 77% of patients, PR was positive in approximately 65% of patients. The proportion of potential agreement beyond chance between EIA and ICA was 0,58 and 0,65 for ER and PR respectively. The median follow-up period was 86 months. Both ER and PR appeared to be weak prognostic factors. No differences in prognostic value according to time-point of analysis or cut-off value chosen were found. No differences in prognostic value of hormone receptors detected by ICA or EIA were found.

Conclusions: Both methods appear to be equivalent with respect to qualification and with respect to prognostic value.

INTRODUCTION

Oestrogen- (ER) and progesterone-receptors (PR) are routinely used in the clinical management of breast cancer. The main reason to determine ER and PR is their predictive value for response to hormonal therapy.[1,2] It has been noted that oestrogen- and progesterone-receptors are also weak prognostic factors. However, long-term disease free and overall survival are not significantly influenced by the hormone receptor status.[3]

There are three commonly used techniques for hormone receptor determination. Until recently the ligand binding assay (LBA) has been the most commonly used method. With this method the rates of binding affinity and capacity of a radioactively labelled steroid hormone with its receptors in cytosol are measured. Nowadays most hospitals in the Netherlands use immunocytochemical assays (ICA) for determination of the presence of hormone receptors in tumour cells. With this qualitative technique highly specific monoclonal antibodies directed against the partially purified receptor are used. ICA has advantages over LBA: it is more sensitive and specific in the identification of low concentrations of hormone receptor positive tumour cells or in identifying hormone receptors in benign epithelium under direct microscopic visualization.[4,5] Several efforts have been made to (semi-)quantify ICA results. Good intra- and inter-observer reproducibility have been reported.[6,7] McClelland et al., however, compared the quantitative analyses of eight experienced, independent pathologists in the interpretation of ER and PR immunocytochemically stained breast tumour sections and observed a high interobserver variability.[8] The method of enzyme immunoassay (EIA) also uses specific monoclonal antibodies for hormone receptor determination, but in a quantitative way. It therefore shares many of the advantages of LBA and ICA. However, it lacks the control of presence or absence of receptor proteins in tumour cells. Concordance rates of 75% - 85% and

correlation coefficients of 0.70 – 0.97 between EIA, ICA and LBA have been reported and are found to be acceptable.[5-7,9-17]

The predictive and prognostic values both of EIA and of ICA appear of the same magnitude compared with that of LBA.[11,18,19] The prognostic value of ICA and EIA have not been compared with each other. To our knowledge there has been only one study comparing the predictive value of EIA and ICA.[15] In the present study we prospectively evaluated the prognostic value detected both by ICA and by EIA of ER in 223 and of PR in 207 breast cancer patients after a median follow-up of 86 months.

PATIENTS AND METHODS

Patients and primary treatment

In 5 hospitals affiliated with the Comprehensive Cancer Centre Middle Netherlands (IKMN) patients with operable breast cancer, diagnosed between October 1989 and March 1993, were asked to participate in a registration study on prognostic factors. 463 patients with stage I-III breast cancer gave their written informed consent. Follow-up information from all patients was collected until August 1999. ER-ICA, ER-EIA, PR-ICA and PR-EIA were determined in this multicentre study in 328, 337, 318 and 321 patients respectively. ER-ICA as well as ER-EIA was determined in 223 patients. Both ER-ICA and ER-EIA were not determined in 21 patients. PR-ICA as well as PR-EIA was determined in 207 cases. Both PR-ICA and PR-EIA were not determined in 30 patients. Survival analyses for ER and PR were performed on these 223 and 207 patients, respectively. Analyses were also performed on those patients in whom hormone receptors were not measured in order to exclude significant selection bias.

Enzyme immunoassay

EIA for specimens from all institutions was performed at the department of Endocrinology of the University Medical Centre Utrecht. Cytosols were prepared according to the EORTC procedure.[20] EIA was performed according to the instructions of the manufacturer (Abbott Laboratories, Chicago, IL, USA). Briefly, cytosol was incubated with beads coated with an anti-receptor monoclonal antibody (H222 for ER and KD68 for PR). Unbound material present in the cytosol was removed by aspirating the fluid and washing the beads. A second monoclonal anti-receptor antibody conjugated with horseradish peroxidase detected the presence of immune reactions in standards, controls, and cytosol samples. The chromogenic substrate was represented by orthophenylendiamine, developing a colour that was analysed by a spectrophotometer at 492 nm. and allowed a measurement of bound receptor conjugate, expressed as fmol/mg protein. Specimens with receptor values > 15 fmol/mg protein were considered positive according to the instructions of the manufacturer.

Immunocytochemical assay

ER- and PR-determination by ICA were performed at the local pathology department on fresh frozen tumour-tissue. ER-ICA and PR-ICA were performed according to the instructions of the manufacturer (Abbott Laboratories, Chicago, IL, USA) using monoclonal rat antibodies to respectively human ER and PR. Tumours were considered hormone receptor positive if more than 10% of tumour cells showed positive staining.[11,12,16] In this study ICA data were obtained from routine pathology reports and are therefore reported as positive or negative.

Table 4.1. Treatment modalities and tumour characteristics.

	Oestrogen receptor			Progesterone receptor		
	Control Group	**Study group**		**Control group**	**Study group**	
Number of patients	240	223		256	207	
Primary surgical treatment						
Modified radical mastectomy	38%	43%		39%	43%	
Breast conserving therapy	60%	55%		59%	55%	
Local excision only	2%	2%		2%	2%	
Radiation therapy	67%	64%		67%	64%	
Adjuvant chemotherapy	15%	16%		15%	16%	
Adjuvant hormonal therapy	27%	35%	†	28%	35%	
Tumour diameter						
0 – 10 mm.	22%	11%	*ƒ*	22%	10%	*ƒ*
11 – 20 mm.	35%	48%		35%	49%	
> 20 mm.	38%	40%		38%	41%	
Unknown	5%	1%		5%	0%	
Axillary lymph node status						
Tumour negative	61%	55%		60%	56%	
Tumour positive	38%	43%		38%	43%	
Unknown	2%	2%		2%	2%	
Age						
0 – 45 years	18%	19%		18%	20%	
46 – 55 years	31%	26%		29%	27%	
56 – 70 years	33%	32%		33%	31%	
> 70 years	19%	23%		19%	22%	

ƒ p<0.001; † p<0.05.

Statistics

Statistical analysis was carried out using the statistical package SPSS for Windows, release 9.0 (SPSS Inc.). Kappa statistics were used to measure the degree of agreement as determined by the two methods. Univariate associations between hormone receptor-status by ICA or EIA and control groups, treatment modalities and other categorized prognostic variables were assessed by the Pearson chi-square test. Endpoints of the study were disease free survival (DFI) and overall survival (OS). For DFI time to failure was computed from the date of surgery until recurrence (loco regional recurrence or distant metastasis) or until the last date patient was known to be free of disease. Patients who developed contralateral breast cancer were censored at the date of diagnosis. Patients who died from a cause not related to breast cancer were censored at the date of decease. Overall survival was calculated from the date of surgery until death or until the date the patient was last known to be alive. Univariate analyses were performed with life tables and with the time-fixed Cox regression procedure. For survival analyses follow-up was truncated at 84 months. Events that took place after more than 84 months of follow-up were not included in the analyses.

RESULTS

In the present registration study 463 patients were suitable for survival analysis. Both ER-EIA and ER-ICA were determined in 223 patients. The remaining 240 patients were used as control group in order to exclude selection bias. Both PR-EIA and PR-ICA were determined in 207 patients; the other 256 patients were used as a control group. Treatment modalities and tumour characteristics in the study groups were compared with those of the control groups (Table 4.1). Breast conserving therapy was performed in 55% - 60%, mastectomy in 38% - 43% of patients. Local excision only was done in 2% of patients. Radiation therapy was

Table 4.2. Percentages hormone-receptor positive tumours according to tumour characteristics and adjuvant treatment modalities.

	Oestrogen receptor			Progesterone receptor		
	ER-ICA	**ER-EIA**		**PR-ICA**	**PR-EIA**	
Total	77%	78%		67%	63%	
Adjuvant chemotherapy						
Yes	75%	72%		71%	76%	
No	77%	79%		66%	60%	
Adjuvant hormonal therapy						
Yes	80%	85%		63%	57%	
No	75%	74%		70%	66%	
Tumour diameter						
0 – 10 mm.	58%	58%	†	38%	§ 38%	‡
11 – 20 mm.	81%	83%		75%	70%	
> 20 mm.	75%	79%		64%	60%	
Axillary lymph node status						
Tumour negative	72%	73%		63%	60%	
Tumour positive	81%	83%		72%	65%	
Age						
0 – 45 years	67%	67%		68%	68%	
46 – 55 years	74%	76%		73%	71%	
56 – 70 years	77%	77%		56%	55%	
> 70 years	86%	90%		74%	59%	

§ p<0.01; ‡ p<0.025; † p<0.05

administered in 64% - 67% of patients, and adjuvant chemotherapy in 15% - 16% of patients. The percentage of patients that received adjuvant hormonal therapy was higher in the groups in whom both ER-EIA and ER-ICA were determined

compared to the control group, 35% vs. 27%. Of 21 patients in whom ER was not determined by ICA or EIA, 7 (33%) received adjuvant hormonal therapy. In the study group hormonal therapy was not given significantly more in ER-positive tumours compared to ER-negative tumours (table 4.2). The control groups contained significantly more small tumours with a diameter < 11 mm compared to the study groups (22% vs. 11%). In all groups almost 60% of tumours were less than 2 cm in diameter, 55% - 61% of tumours were axillary lymph node negative.

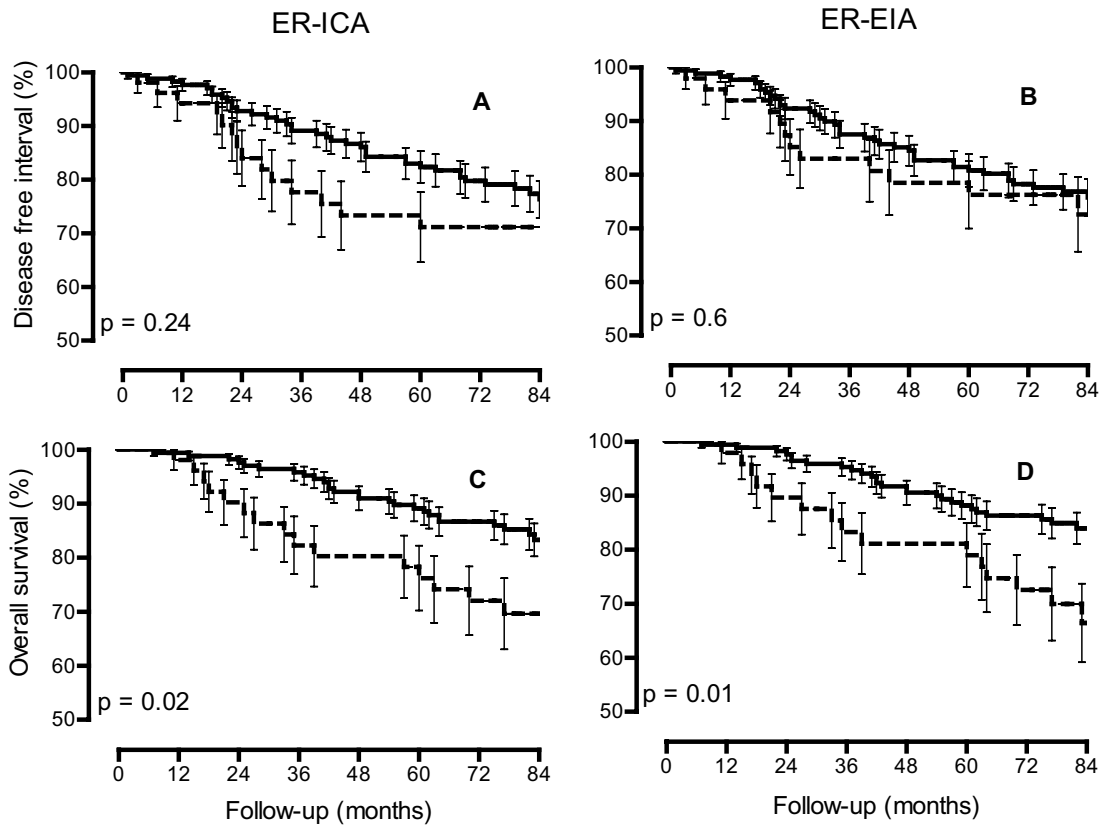
Table 4.3. 2 x 2 tables ICA and EIA.

		ER-ICA		
		Negative	**Positive**	**Total**
ER-EIA	**Negative**	34	15	49
	Positive	18	156	174
	Total	52	171	223

		PR-ICA		
		Negative	**Positive**	**Total**
PR-EIA	**Negative**	56	21	77
	Positive	12	118	130
	Total	68	139	207

Median ER-EIA value was 101 fmol/mg protein (range 0 – 1975); median PR-EIA value was 44 fmol/mg protein (range 0 – 1985). ER-EIA and ER-ICA were positive in 174 (78%) and 171 (77%) cases, respectively. PR-EIA and PR-ICA were positive in 130 (63%) and 139 (67%) cases, respectively. Small tumours (< 11 mm.) were significantly less often ER- or PR-positive compared to larger tumours (Table 4.2). The proportion of potential agreement beyond chance (Kappa) between EIA and ICA was moderate to substantial. Results from ER-EIA

Figure 4.1. Oestrogen receptor and disease free interval (A and B) and overall survival (C and D). Solid line: receptor positive tumours; dotted line: receptor negative tumours. ER-ICA: A and C; ER-EIA: B and D.

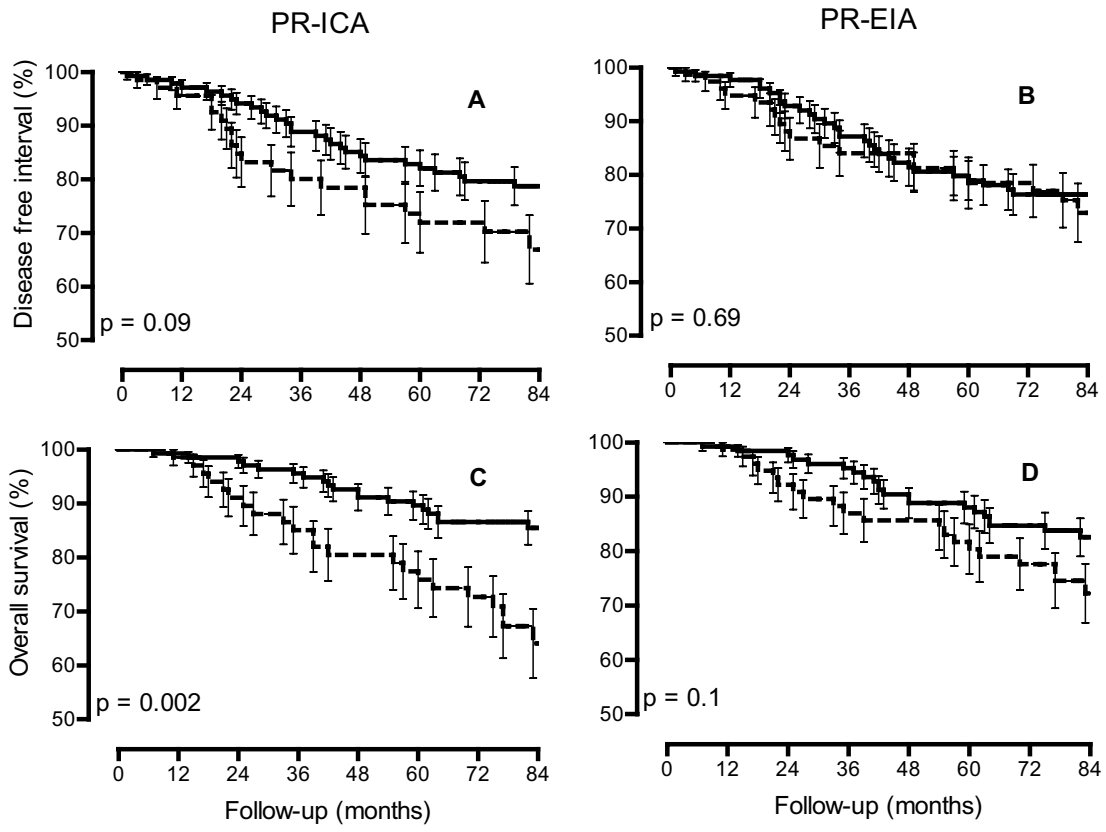


and ER-ICA agreed in 85% of cases (Kappa 0.58). Results from PR-EIA and PR-ICA agreed in 84% of cases (Kappa 0.65). Two by two tables are depicted in table 4.3. Immunohistochemistry of discordant specimens from one of the three pathology departments was re-examined. None of 7 ER-ICA negative and 6 PR-ICA negative marked specimens were converted to positive, 1 of 4 ER-ICA positive and 1 of 4 PR-ICA positive marked specimens were converted to negative (the cells that were stained positive were interpretated as carcinoma in situ). Unfortunately we were not able to re-evaluate EIA measurements.

The median follow-up was 86 months (range 44 – 110). For survival analyses follow-up was truncated at 84 months. During 84 months of follow-up 17% - 20% of patients died, 12% - 14% died related to breast cancer. Contra-lateral breast cancer was diagnosed in 3% - 5% of patients. In 23% of patients breast cancer relapsed. Distant metastases were diagnosed in 19% - 20% of patients, loco-regional relapses in 7% - 10% of patients. The rate of events did not differ significantly between study- and control-groups. DFI and OS did not differ significantly between study- and control-populations. After 84 months of follow-up ER-ICA, ER-EIA and PR-ICA were significant prognosticators of OS. Significance remained after stratification for adjuvant hormonal therapy. No significance was found for DFI after 7 years (Figure 4.1 and 4.2). EIA measurements were quantitative. The prognostic significance of ER-EIA and PR-EIA as continuous variables was determined. No significance was found for DFI or OS. Three, 5 and 7 year DFI- and OS-rates were determined and compared (Table 4.4). No differences were found between study- and control groups. Three, 5 and 7 year DFI was 86%, 81% and 75% respectively. DFI-rates in hormone receptor positive patients were slightly higher compared to hormone receptor negative patients. These differences were not statistically significant. Three, 5 and 7 year OS was 93%, 87% and 80% respectively. Differences between OS-rates in hormone receptor positive and negative patients were greater and frequently statistical significant (Table 4.4).

In continuous variables the cut-off level used for survival analysis can be chosen at an arbitrary level. The cut-off level for EIA of 15 fmol/mg protein used in the present study was advised by the manufacturer of the antibodies. Other cut-off values were studied (Figure 4.3). The relative risk of disease free survival of patients with EIA negative- compared to EIA positive tumours varied between 0.4 and 1.1 for ER, and between 0.5 and 1.0 for PR. The relative risk of overall survival of patients with EIA negative- compared to EIA positive tumours varied

Figure 4.2. Progesterone receptor and disease free interval (A and B) and overall survival (C and D). Solid line: receptor positive tumours; dotted line: receptor negative tumours. PR-ICA: A and C; PR-EIA: B and D.



between 0.5 and 0.6 for ER, and between 0.3 and 0.7 for PR. The differences in hazard ratios for the different cut-off levels were not significant.

DISCUSSION

Both EIA and ICA are commonly used methods for determining hormone receptors in breast cancer. The main purpose to determine hormone receptors is

their ability to predict efficacy of endocrine therapy. But hormone receptors are also used as a prognostic indicator. We have prospectively compared the prognostic value of the oestrogen- and progesterone receptor values as determined by ICA and EIA in a routine clinical setting.

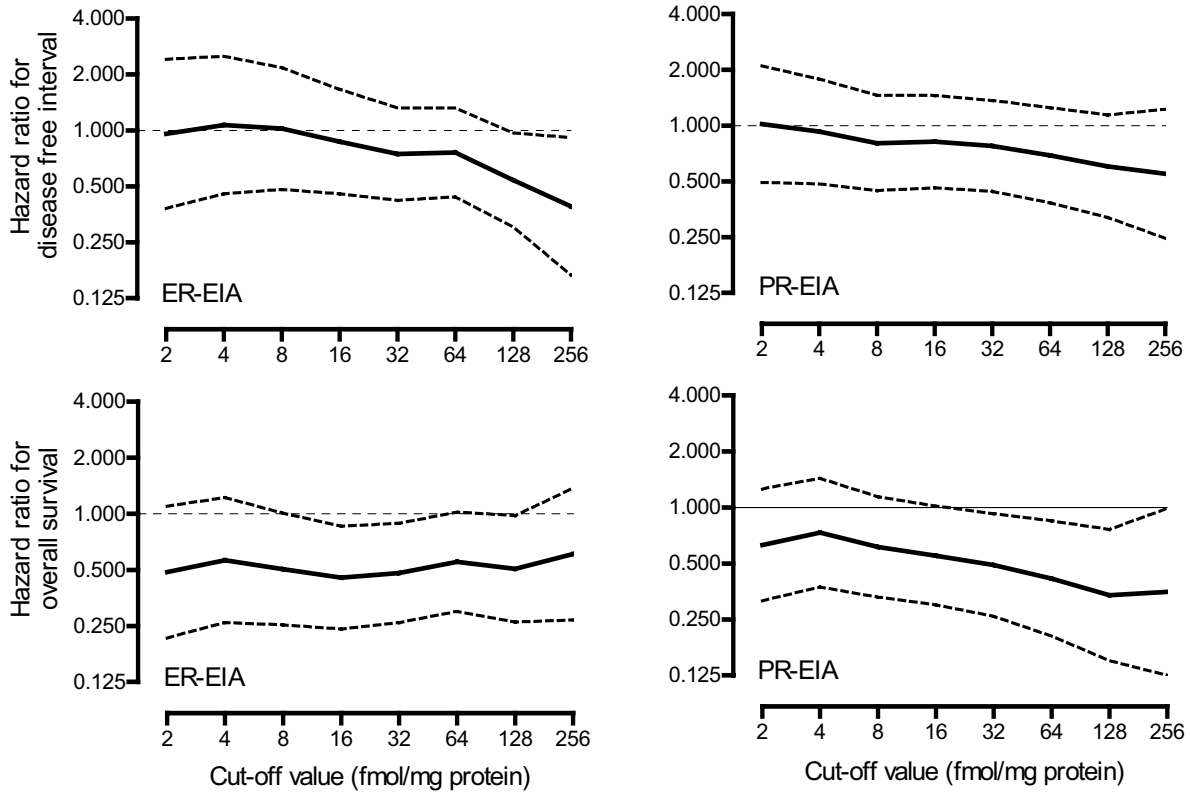
Between 1989 and 1993 in total 463 early breast cancer patients were included in a multicentre, prospective registration study on prognostic factors. ER and PR could be determined both by EIA and by ICA in less than 50% of patients (48% and 45% respectively). In order to evaluate a potential bias, the remaining patients in whom ICA and/or EIA were not determined were used as a control group. Most tumour characteristics and primary treatment modalities differed not significantly between the study and the control groups. However, the percentage of patients that received adjuvant hormonal therapy was higher in the ER-study group compared with that of the ER-control group. We could not find a suitable explanation for this phenomenon. Treatment selection based on hormone receptor values is not likely since hormonal therapy was not given significantly more in ER-positive tumours compared with that of ER-negative tumours. In tumours in which the ER was not determined at all, hormonal therapy was provided to 33% of patients. At the time of patient inclusion hormone receptors were not used as predictive factor. The rate of small tumours (< 11 mm.) was significantly higher in the control groups compared to the study groups. This was at least partly due to selection, since it is not possible to perform an adequate and reliable EIA in micro-invasive cancer. However, the consequences of this bias appear to be low. During follow-up the rate of events did not differ significantly between study- and control groups. No differences in Cox-regression analyses and in 3, 5 and 7 year survival rates were found between study- and control groups either. Therefore, we conclude that the groups of patients in whom ER and PR were determined were representative for the whole population of breast cancer patients.

Table 4.4. Three, 5 and 7 year disease free interval and overall survival.

	Cumulative disease free interval			Cumulative overall survival					
	3 year rate (SE)	5 year rate (SE)	7 year rate (SE)	3 year rate (SE)		5 year rate (SE)		7 year rate (SE)	
Oestrogen receptor									
Study	0.86 (0.02)	0.80 (0.03)	0.75 (0.03)	0.94 (0.02)		0.86 (0.02)		0.80 (0.03)	
Control	0.87 (0.02)	0.81 (0.03)	0.75 (0.03)	0.93 (0.02)		0.87 (0.02)		0.80 (0.03)	
ER-ICA									
Negative	0.78 (0.06)	0.73 (0.06)	0.71 (0.07)	0.82 (0.05)	‡	0.78 (0.06)		0.69 (0.07)	†
Positive	0.89 (0.02)	0.83 (0.03)	0.76 (0.03)	0.96 (0.02)		0.89 (0.02)		0.83 (0.03)	
ER-EIA									
Negative	0.83 (0.06)	0.78 (0.06)	0.73 (0.07)	0.83 (0.05)	†	0.81 (0.06)	†	0.67 (0.07)	†
Positive	0.88 (0.03)	0.81 (0.03)	0.76 (0.03)	0.95 (0.02)		0.88 (0.02)		0.84 (0.03)	
Progesterone receptor									
Study	0.86 (0.02)	0.81 (0.03)	0.75 (0.03)	0.94 (0.01)		0.87 (0.02)		0.82 (0.03)	
Control	0.86 (0.02)	0.80 (0.03)	0.75 (0.03)	0.92 (0.02)		0.86 (0.02)		0.79 (0.03)	
PR-ICA									
Negative	0.80 (0.05)	0.74 (0.06)	0.67 (0.06)	0.85 (0.04)	‡	0.77 (0.05)	†	0.64 (0.06)	§
Positive	0.89 (0.03)	0.83 (0.03)	0.79 (0.04)	0.96 (0.02)		0.90 (0.03)		0.86 (0.03)	
PR-EIA									
Negative	0.84 (0.04)	0.80 (0.05)	0.73 (0.05)	0.87 (0.04)	†	0.82 (0.04)		0.72 (0.05)	
Positive	0.87 (0.03)	0.80 (0.04)	0.76 (0.04)	0.95 (0.02)		0.88 (0.03)		0.82 (0.04)	

§ p<0.01; ‡ p<0.025; † p<0.05

Figure 4.3. Relative risk of disease free- and overall survival (solid line) with 95% confidence interval (dotted lines) at progressively higher cut-off values for ER-EIA and PR-EIA.



The oestrogen receptor was positive in approximately 77% of patients, the progesterone receptor was positive in approximately 65% of patients. The proportion of potential agreement beyond chance between EIA and ICA was moderate to substantial (Kappa 0,58 and 0,65 respectively for ER and PR). These results are in line with that of the literature.[7,10,11,13,15-17,21-23] Concordance between EIA and ICA found in the present study was substantial (85%), but there also were a substantial number of tumours with a discordant result. Re-evaluation of 22 ICA samples, with discordant EIA/ICA results, led to only 2 conversions. Unfortunately it was not possible to re-evaluate EIA samples. Explanations for discordant EIA/ICA results are: effect of fixation and processing on the

preservation of hormone receptors,[24] intratumoural heterogeneity,[12,13] improper handling of the specimens or unsuitable samples of the tumour sent for EIA,[12] hormone receptor positive benign- or intraductal components in the EIA sample,[12] borderline EIA and ICA results.[13] The major theoretical advantage of ICA over EIA is microscopic verification of the presence of the receptor proteins in tumour cells. It has been suggested that ICA is a more specific and more sensitive test for the measurement of receptor content in breast cancer.[12] It is, however, impossible to draw conclusions concerning specificity and sensitivity and the discordant results in the present study.

After 7 years of follow-up ER-ICA, ER-EIA and PR-ICA were significant prognosticators of OS. Significance remained after stratification for adjuvant hormonal therapy. No significance was found for DFI though. The absence of prognostic significance in the present study for DFI was not unexpected. The number of patients studied was relatively small. ER and PR are considered to be weak prognostic factors.[2] The observed prognostic significance of the hormone receptors for OS was probably caused by a better response in relapsed disease to hormonal treatment of patients with initial hormone receptor positive tumours.

Although long-term DFI and OS are thought not to be significantly influenced by the hormone receptor content, hormone receptor positive tumours are thought to have a somewhat more indolent course during the first few years after primary treatment.[2] This could not be supported by the differences in DFI-rate and OS-rate between hormone receptor negative and positive tumours at 3, 5 and 7 year, as they appeared to be constant over time and independent upon time-point of analysis.

The major theoretical advantage of EIA over ICA is its objective quantification. Several efforts have been made to (semi-)quantify ICA results and good intra- and inter-observer reproducibility has been reported by several authors.[6,7] Others,

however, observed a high interobserver variability.[8] In the present study ICA-results were binominal, no efforts were made to (semi)quantify ICA using a scoring system in order to reflect the routine clinical practice. The cut-off value was arbitrarily chosen at 10% staining. Results from EIA were quantitative. The cut-off value chosen to separate receptor-negative from receptor-positive tumours was 15 fmol/mg protein, according to the instructions of the manufacturer of the antibodies. But, the prognostic value of continuous variables, such as ER and PR, may be influenced by the cut-off level chosen.[25] Therefore, other cut-off values were studied. No significant differences in prognostic value of different cut-off values were found.

To our knowledge there has been only one study comparing the predictive value of EIA and ICA .[15] No former studies have been conducted comparing the prognostic value ER and PR as determined by either EIA or ICA. In the present study we prospectively evaluated the prognostic value detected both by ICA and by EIA of ER in 223 and of PR in 207 breast cancer patients after a median follow-up of 86 months. Both ER and PR appeared to be weak prognostic factors. No differences in prognostic value according to time-point of analysis or cut-off value chosen were found. No differences in prognostic value of hormone receptors detected by ICA or EIA were found. Both methods appear to be equivalent with respect to qualification and with respect to prognostic value.

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