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Comparison of Immunohistochemical and Biochemical Assay of Steroid Receptors in Primary Breast Cancer

Clinical Associations and Reasons for Discrepancies

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Estrogen (ER) and progesterone receptor (PgR) status was analysed in paraffin-embedded breast cancer material with immunohistochemical (IHC) technique and compared with corresponding analyses in cytosols (CYT). ER showed the same status (positive/negative) with both methods in 88% of the samples (352/402). The concordance was also high for PgR status (81% [321/394]). Besides values near cut-off, heterogeneity in the distribution of receptor positive and negative nuclei within a tumour sample was the main reason for discordances. Histological type, presence of sclerosis, necrosis and non-invasive cells, and technical artefacts seem to be of only limited importance for explaining discordances. All patients have been treated with adjuvant tamoxifen for two years. The two subgroups, which were $ER_{CYT}+/ER_{IHC}+$ or $ER_{CYT}-/ER_{IHC}+$, both had a significantly better progression-free survival (PFS; median follow-up: almost 6 years) than the $ER_{CYT}-/ER_{IHC}-$ group ($p < 0.001$ and $p = 0.007$, respectively). The remaining group, $ER_{CYT}+/ER_{IHC}-$, had an intermediate PFS. For PgR, the associations with PFS were weaker, with significantly better PFS than the $PgR_{CYT}-/PgR_{IHC}-$ group being found only for the $PgR_{CYT}+/PgR_{IHC}-$ group ($p = 0.03$).

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Estrogen receptor (ER) is a well-accepted predictor for the effect of endocrine therapy in both metastatic and primary breast cancer (1, 2). Although the clinical importance of progesterone receptor (PgR) is not so well established, according to the meta-analysis (2) several groups, including our own, have demonstrated its value as a complement to ER for predicting sensitivity to adjuvant endocrine therapy (3–6). Initially, ER and PgR were measured in cytosols (CYT) from frozen tissue with ligand binding assays and later with enzyme immuno assay. During the last decade antibodies against ER and PgR have been developed, working on formalin-fixed, paraffin-embedded tissue. The major advantages of the immunohistochemical (IHC) techniques in paraffin-embedded material are that, in principle, all cases can be analysed, and that the evaluation of staining can be restricted to morphologically identified cancer cell nuclei. The major disadvantage with the IHC is that quality assurance procedures remain to be established for both the staining technique and the assessment of staining. Several studies have demonstrated a good concordance (79–94%) in ER status between IHC and CYT (7–13). A similar good concordance (83–87%) has also been demonstrated in some studies for PgR (8, 13). A lower degree of concordance (59–69%) for ER has, however, been reported (14, 15). In addition to a standardized assay, it is also necessary to demonstrate that IHC yields a similar association with the response of endocrine treatment compared with CYT. For ER this has been demonstrated in some studies for both metastatic (10, 14) and primary breast cancer (11, 12). So far, less attention has been paid to evaluation of PgR assays in paraffin-embedded material from breast cancer patients treated with tamoxifen (10).

The aim of the present study was therefore to perform an extensive comparison between IHC in paraffin-embedded breast cancer tissue and CYT for the analyses of ER and PgR, in a relatively large ($n = 425$) cohort of breast cancer patients treated with adjuvant tamoxifen. Our specific aims were to evaluate: (a) the concordance in receptor status between the two methods, (b) reasons for discordances, and (c) the association between progression-free survival (PFS) and overall survival, and receptor status, when measured with IHC or with CYT.

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MATERIAL AND METHODS

Study design

The series comprised 425 patients (79 pre- and 346 postmenopausal), with stage II tumours, diagnosed in the South Sweden Health Care Region between 1985 and 1994. ER and PgR have previously been analysed in cytosols from frozen tumour tissue. The patients were participating in two prospective randomized clinical trials (one for pre- and one for postmenopausal patients), launched by the South Sweden Breast Cancer Group in order to investigate the effect of adjuvant tamoxifen at 20 mg daily for 2 or 5 years. Primary surgery consisted of either modified radical mastectomy or breast-conserving surgery, both in combination with axillary lymph node dissection. Radiotherapy (50 Gy) to the breast was offered to all patients treated with breast-conserving surgery, and loco-regional radiotherapy was delivered to all patients with lymph node positive breast cancer. In the present study only patients treated with tamoxifen for 2 years were included. Two hundred and ninety cases (68%) had lymph node positive disease and for 299 cases (70%) the tumour size was larger than 20 mm.

Analytical methods

Paraffin-embedded material. Four hundred and twenty-five blocks, one from each of the primary tumours, were collected. From 20 of the patients, material from a second synchronic ipsilateral primary tumour was also collected. Furthermore, blocks from a tumour-involved lymph node were collected in 273 of the 290 patients with lymph node positive breast cancer. The paraffin blocks were sectioned (4–5 μm sections) and stained for ER, PgR and routine histopathological evaluation (hematoxylin-eosine) at two departments. The antibodies and retrieval and staining methods are detailed in Table 1. The slides were evaluated by two pathologists (G.C., I.I.). The evaluation of staining was primarily classified into six groups: ≤ 10 stained nuclei in any high-power field (diameter of hpf = 0.5 mm), > 10 stained nuclei/hpf up to $< 1\%$, $1\%- < 10\%$, 10%–20%, 21%–50%, and $> 50\%$ stained nuclei. As receptor status is usually reported as negative or positive, we also defined two groups: $< 10\%$ and $\geq 10\%$ stained nuclei. At the consensus meeting in St Gallen in 1998, it was stated that if 10% or more of the tumour cells are stained, an unequivocal response to endocrine therapy is likely (16). The staining intensity was not taken into consideration. The concordance between the two pathologists, when evaluating the same 100 slides, was 93% for ER status and 94% for PgR status, based on the 10% cut-off. When the more detailed sub-grouping, as described above, was considered, complete agreement was obtained in 80% (ER) and 72% (PgR) of the cases. The results presented in this study are based on the two pathologists consecutively evaluating every other case according to date of birth. When the IHC staining was evaluated, some specific patterns were also recorded: (a) no invasive cancer, (b) large amount ($> 25\%$) of benign tissue and/or in situ tumour present in tumour area, (c) sclerosis (a substantial part [$> 25\%$ of the tumour area] represents stroma without tumour cells), (d) necrosis (a substantial part [$> 25\%$ of the tumour area] represents single tumour cell necrosis and/or confluent necrosis), (e) heterogeneous receptor content (irregular distribution of proportion receptor positive nuclei in the tumour section), and (f) technical artefacts (e.g. cytoplasmic and/or background staining, and artefacts due to suboptimal fixation, dehydration, or microwave treatment). Furthermore, the histological type according to WHO (see Table 3) was evaluated by one of us (G.C.). The rationale for the remarks and histology was that we suspected that they might influence the concordance in receptor status between IHC and CYT. Those cases with no invasive cancer cells present on the slides (remark [a] according to the above) were excluded from further analyses. Four hundred and two primary tumours remained for ER and 394 for PgR. In the series of lymph nodes, 251 cases remained for ER and 246 for PgR.

Cytosols from frozen tissue. These analyses have previously been performed routinely every week, within 8 days after the primary operation. ER and PgR content was measured with enzyme immuno assay according to kit instructions (Abbott Laboratories, Diagnostic Division, Chicago, IL, USA). Samples with receptor content ≥ 25 fmol/mg protein were classified as ER or PgR positive, and samples with values below these levels as ER or PgR negative. Presence of tumour cells in the samples was checked by a cytological examination of imprints from the tumour specimens.

Table 1

Description of antibodies, antigen retrieval and staining machines used in this investigation for ER and PgR analyses in paraffin-embedded breast cancer tissue

Antigen	Clone	Dilution	Obtained from	Antigen retrieval	Staining machine
ER	1D5	1:100	Dako	Oven, 75°C, 24 h	Techmate500
ER	6F11	Prediluted	Ventana	Microwave, pH 7.0	Ventana NexES
PgR	Polyclonal	1:50	Dako	Oven, 75°C, 24 h	Techmate500
PgR	1A6	Prediluted	Ventana	Microwave, pH 7.0	Ventana NexES

Statistics

Due to grossly violated proportional hazards assumptions for both ER and PgR status (both methods), we chose not to fit Cox models to the data for the entire follow-up period. Instead the log-rank test was used for comparison of progression-free and overall survival (PFS and OS) in different subgroups. However, when studying the prognostic importance for ER and PgR during different time periods, Cox models were used. Survival curves were estimated using the Kaplan–Meier method and curves were curtailed when less than five individuals remained in the risk set (17). Associations in 2×2 tables were evaluated using Fisher's exact test and presented without adjustment for multiple testing.

The present study has three aims: to evaluate (a) the concordance in receptor status between the two methods, (b) explanations for discordances, and (c) the association of each method with PFS and OS after adjuvant tamoxifen. It would have been desirable also to evaluate whether one method showed a stronger association with clinical outcome than the other. However, as described below, our sample was far from the size necessary to have a reasonable chance of finding significant differences in prognosis between the two groups.

It has been reported that IHC and CYT lead to concordant ER status in about 85% of all samples analysed in both ways (7–13). Hence, most patients will be non-informative when evaluating the relative prognostic value of receptor determinations using the two methods. When planning the size of the present study, using a nomogram for the log-rank test, we assumed that 15% of the samples would show discordant receptor status and that differences in either direction would be equally likely. The five-year PFS in the population from which we were planning to collect samples was estimated at 75%. To detect a hazard ratio of 2.65, corresponding to a 5-year PFS of 65% and 85% in the two groups respectively, assuming exponentially distributed progression times, with 80% power using a two-sided test at the 5% significance level, would require 40 progressions. This number corresponds to $40/(1-0.75) = 160$ discordant cases and hence to a total of 1 067 samples (907 concordant and 160 discordant). The actual number of samples included in this study was, however, limited mainly by practical considerations, leading to a sample size of 425. The computer package Stata 7.0 (18) was used for the statistical analysis.

RESULTS

Comparison between IHC and CYT

ER showed the same status (10% cut-off for IHC) with both methods in 88% of the cases (352/402; 85 negative and 267 positive). Twenty-eight cases were positive with IHC but negative with CYT, whereas the opposite pattern was found for 22 cases. As far as PgR was concerned, 81% showed concordant status (321/394; 153 negative and 168 positive). Thirty-three cases were positive with IHC and negative with CYT, whereas 40 showed the opposite pattern. For ER, the agreement was better for postmenopausal patients than for premenopausal patients (90% vs. 77%; $p = 0.003$), whereas for PgR similar figures were obtained for both menopausal groups (81% vs. 82%).

A more detailed comparison of the two methods is presented in Fig. 1, where receptor class (IHC) has been plotted against cytosol value for ER and PgR. The highest concordance rates between the two methods were found for the extreme IHC groups (≤ 10 stained nuclei and $> 50\%$ stained nuclei). For these two groups the concordance was 93% (314/337) for ER and 86% (234/271) for PgR. The concordance in the four intermediate groups was much lower for both ER (58%; 38/65) and PgR (71%; 87/123).

To investigate the importance of values near cut-off for discordant results, we defined a borderline group for each of the methods: 18–35 fmol/mg protein (CYT; symmetry on a log-scale around the cut-off of 25 with an upper limit of 35) and 1%–20% stained nuclei (IHC). For ER, 18 of the 50 discordant cases (36%) were found in at least one of these borderline groups. Similar figures were also found for PgR (33/73; 45%). These fractions were much higher than those for the groups showing concordance in receptor status (ER: 26/352 [7%]; PgR: 51/321 [16%]).

For both ER and PgR, the percentage of samples with remarks (see Table 2) was higher in the discordant than in the concordant subgroups (ER: 48% [24/50] vs. 32% [112/352], $p = 0.03$; PgR: 49% [36/73] vs. 37% [119/321], $p = 0.06$). The most obvious difference, and the only difference that remains statistically significant after Bonferroni adjustment for multiple testing, was the high proportion of cases with a heterogeneous receptor content in the discordant compared with the concordant subgroups (ER: 28% vs. 5%, $p < 0.001$; PgR: 29% vs. 6%, $p < 0.001$). This finding was most pronounced in the $ER_{CYT}-/ER_{IHC}+$ or $PgR_{CYT}-/PgR_{IHC}+$ subgroups (Table 2). For ER, sclerosis was more frequent in the concordant than in the discordant subgroups (12% vs. 2%, $p = 0.03$). No corresponding difference was found for PgR (Table 2). For all the other remarks no significant differences were found between the discordant and concordant subgroups. A striking observation was that no $ER_{CYT}+/ER_{IHC}+$ ($n = 267$) or $PgR_{CYT}+/PgR_{IHC}+$ ($n = 168$) case showed presence of necrosis. This finding should be compared with the fact that 14 of 85 (16%) $ER_{CYT}-/ER_{IHC}-$ samples and 15 of 153 (10%) $PgR_{CYT}-/PgR_{IHC}-$ samples showed the presence of necrosis. The percentages of patients with remarks were similar for the pre- and postmenopausal subgroups (ER: 38% vs. 33%; PgR: 42% vs. 39%). As it has been suggested that IHC is better than CYT in the identification of rare positive tumour cells in lobular carcinomas (13), the histological

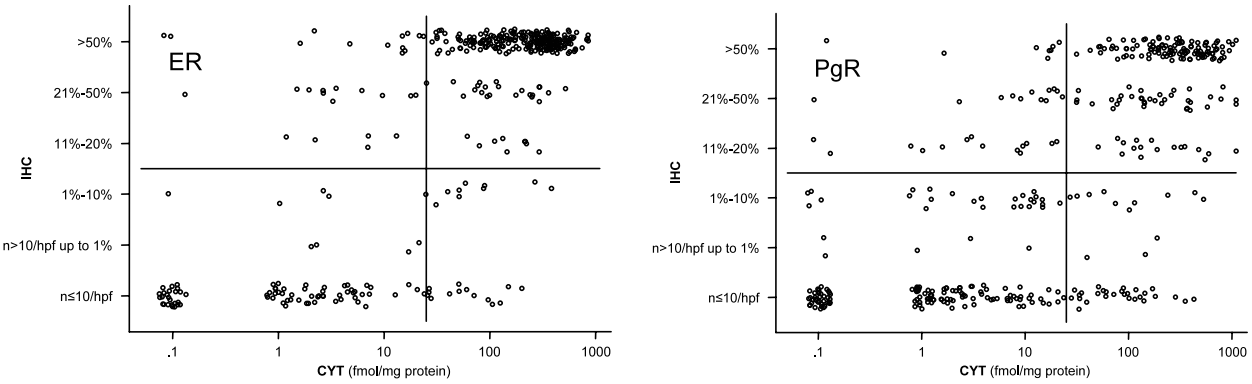

Fig. 1. Scatterplot of receptor class (IHC) vs. receptor content (CYT) for ER (left) and PgR (right). A logarithmic scale was used for the cytosol content and all values below 0.1 fmol/mg protein were set to 0.1. Furthermore, a small amount of spherical random noise has been added to each point to keep the data from overplotting. The horizontal and vertical lines represent the cut-offs used for receptor positivity ($\geq 10\%$ stained nuclei for IHC and ≥ 25 fmol/mg protein for CYT).

types were considered (Table 3). The percentage of ER+ and PgR+ cases for pure lobular cases were similar with both techniques (ER+: 85% (CYT) and 91% (IHC) and PgR+: 59% and 56%, respectively), thus indicating no major influence on receptor status.

Comparison between IHC and CYT with regard to clinical outcome

Results based on the analyses of one primary tumour; progression-free survival (PFS). The median follow-up for those patients still alive and progression-free is 5.7 (ER) and 5.6 (PgR) years. One hundred and twenty-one ER (113 distant and 8 locoregional) and 122 PgR (114 distant and 8 locoregional) patients had developed progressions. Univariate analyses showed that ER (positive vs. negative) was significantly associated with PFS with both IHC ($p < 0.001$) and CYT ($p = 0.02$). When the results obtained with the two methods were combined, the worst prognosis was found for the $ER_{CYT}-/ER_{IHC}-$ subgroup (Fig. 2). The $ER_{CYT}+/ER_{IHC}+$ and $ER_{CYT}-/ER_{IHC}+$ subgroups had a significantly better PFS than the $ER_{CYT}-/ER_{IHC}-$ subgroup ($p < 0.001$ and $p = 0.007$, respectively). The PFS for the remaining group, $ER_{CYT}+/ER_{IHC}-$, was not significantly different from that of the $ER_{CYT}-/ER_{IHC}-$ group ($p = 0.31$). When the two discordant groups were compared, a tendency towards a better PFS was found for patients who were $ER_{CYT}-/ER_{IHC}+$ than for those showing the opposite pattern ($p = 0.10$). The prognostic importance of PgR was less pronounced in this series with both methods ($p = 0.22$ [IHC] and 0.06 [CYT], Fig. 2). The $PgR_{CYT}+/PgR_{IHC}-$ subgroup had a significantly better PFS than the $PgR_{CYT}-/PgR_{IHC}-$ subgroup ($p = 0.03$; Fig. 2). The PFS for the remaining two subgroups, $PgR_{CYT}+/PgR_{IHC}+$ or $PgR_{CYT}-/PgR_{IHC}+$, did not differ significantly from that for $PgR_{CYT}-/PgR_{IHC}-$ ($p = 0.08$ and $p = 0.19$, respectively). No difference in PFS was found between the two discordant groups ($PgR_{CYT}+/PgR_{IHC}-$ *vs.* $PgR_{CYT}-/PgR_{IHC}+$; $p = 0.57$).

A visual inspection of Fig. 2 indicates that the difference in PFS between receptor positive and receptor negative patients was restricted to the first years after the primary operation. This was also demonstrated by Schoenfeld's test

Table 2

Distribution of remarks (%) for subgroups based on ER and PgR status when analysed with different methods (CYT/IHC)[1]

Remark	ER				PgR			
	−/− (n = 85)	−/+ (n = 28)	+/− (n = 22)	+/+ (n = 267)	−/− (n = 153)	−/+ (n = 33)	+/− (n = 40)	+/+ (n = 168)
Non-invasive cells (> 25%)	13[2]	14	18	10	12	9	20	9
Sclerosis	9	0	5	13	10	15	5	12
Necrosis	16	11	5	0	10	6	0	0
Heterogeneous receptor content	1	36	18	6	1	52	10	11
Technical artefacts	7	7	5	5	22	9	5	4
Any remark[3]	38	54	41	30	43	67	35	32

[1] Cut-offs: CYT: < 25 fmol/mg protein (−); ≥ 25 fmol/mg protein (+). IHC: < 10% stained nuclei (−); ≥ 10% stained nuclei (+).
[2] Percentage.
[3] For ER 113 cases had one remark, 21 had two, and 2 had three remarks. For PgR the corresponding figures were 120, 32, and 3, respectively.

Table 3

Distribution (%) of histological types for subgroups based on ER and PgR status when analysed with different methods (CYT/IHC)[1]

Histological Type	ER				PgR			
	−/− (n = 85)	−/+ (n = 28)	+/− (n = 22)	+/+ (n = 267)	−/− (n = 153)	−/+ (n = 33)	+/− (n = 40)	+/+ (n = 168)
Ductal	78[2]	54	86	74	74	61	78	76
Lobular	4	25	14	19	12	21	22	17
Lobular + ductal	1	4	0	4	2	6	0	5
Medullary[3]	16	11	0	1	9	9	0	1
Others	1	7	0	2	3	3	0	1

[1] Cut-offs: CYT: < 25 fmol/mg protein (−); ≥ 25 fmol/mg protein (+). IHC: < 10% stained nuclei (−); ≥ 10% stained nuclei (+).
[2] Percentage.
[3] Including atypical medullary.

(19), which revealed grossly violated proportional hazards assumptions for both ER status (IHC: $p = 0.01$, and CYT: $p < 0.0001$) and PgR status (IHC: $p = 0.03$, and CYT: $p = 0.004$). It is thus not appropriate to fit Cox models and estimate one hazard ratio (HR) per receptor and method. To describe the time-dependent effects, separate Cox models were estimated for each of the first five years after primary surgery (see Table 4). The differences in PFS between

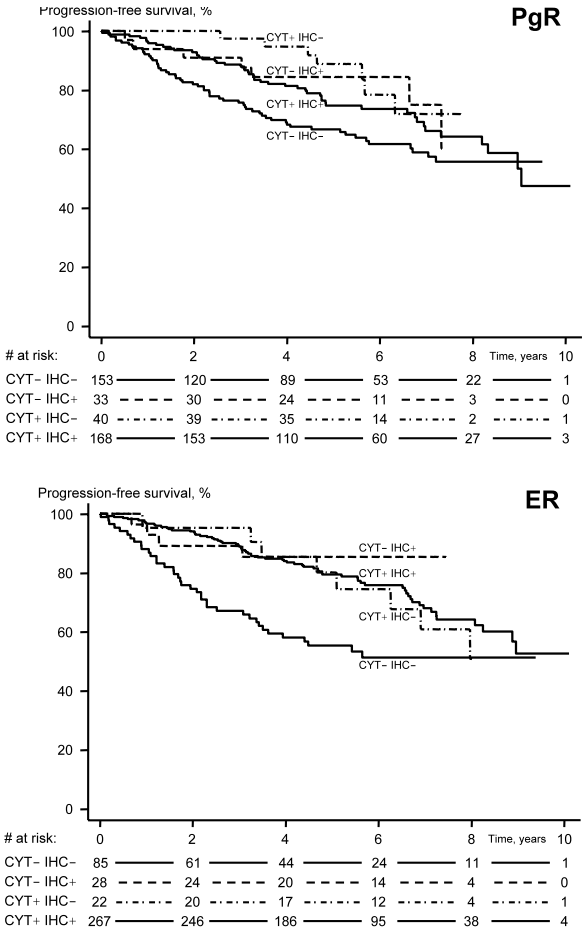

Fig. 2. Progression-free survival according to combinations of the receptor status, when measured with CYT and IHC.

receptor negative and receptor positive patients were, for both methods, most pronounced during the first 2 years after operation.

Overall survival. After a median follow-up of 8.0 (ER) and 8.1 (PgR) years for those patients still alive, 127 (ER) and 128 (PgR) patients had died. Univariate analyses showed that ER (positive vs. negative) was significantly associated with overall survival both with IHC ($p < 0.001$) and CYT ($p = 0.002$). As for PFS, the prognostic importance of PgR was less pronounced in this series with both methods ($p = 0.25$ [IHC] and $p = 0.21$ [CYT]).

Two primary synchronic tumours in the same breast. Eighteen patients had two synchronic tumours. All these pairs showed concordant ER status (15 positive and 3 negative). For PgR, all but one of the 17 analysed pairs showed concordant PgR status (14 positive and 2 negative).

Primary vs. tumour-involved lymph node metastasis. ER status was evaluated in 251 lymph nodes. In 233 of these the same ER status was obtained as in the primary tumour (93%; 52 negative and 181 positive). Twelve cases were positive in the primary tumour and negative in the lymph node, whereas six showed the opposite pattern. The number of progressions was three in both these discordant groups. For PgR, the concordance was somewhat lower [205/246, 98 negative and 107 positive (83%)]. Twenty-seven cases (four progressions) were PgR positive in the primary tumour but negative in the lymph node and 14 (four progressions) showed the opposite pattern.

DISCUSSION

Concordance in ER and PgR status between IHC and CYT was obtained in 88% and 81% of the cases, respectively, figures similar to those obtained in other investigations (7–13). The main reason for discordant results was heterogeneity in the distribution of receptor positive and receptor negative cells within a sample, besides values near cut-off. The importance of receptor heterogeneity can be explained by the fact that with both techniques only a small proportion of a tumour is analysed, i.e. if the tumour is

Table 4

Time-dependent effects of ER and PgR status during the first five years after primary surgery

Time interval	ER_{CYT}		ER_{IHC}		PgR_{CYT}		PgR_{IHC}	
	HR[1]	CI[2]	HR	CI	HR	CI	HR	CI
0–1 years	3.0	1.3–7.0	3.2	1.4–7.6	2.3	0.93–5.7	1.4	0.60–3.4
1–2 years	5.5	2.2–14	3.9	1.6–9.3	4.0	1.5–11	2.8	1.1–7.3
2–3 years	1.5	0.56–3.9	1.6	0.60–4.2	1.5	0.59–3.6	1.3	0.52–3.2
3–4 years	1.8	0.75–4.3	2.3	0.99–5.4	1.3	0.57–3.0	1.1	0.49–2.6
4–5 years	0.61	0.13–2.8	1.1	0.30–4.1	0.25	0.05–1.12	0.53	0.16–1.8

[1] Hazard ratio: receptor negative vs. receptor positive.
[2] 95% confidence interval.

$10 \times 10 \times 10$ mm^3 in size, 5% (50 mg) is used in the cytosol preparation for CYT, while a section evaluated with IHC corresponds to 0.05% of the whole tumour ($0.005 \times 10 \times 10$ mm^3 in size). The remaining remarks could only explain discordances in a very limited number of cases. Suboptimal fixation condition has been suggested to be one reason (9), but in our investigation, very few cases had this remark (nine for ER and eight for PgR). Technical artefacts, including not only suboptimal fixation but also cytoplasmic and/or background staining and artefacts caused by dehydration or microwave treatment, were not more common in the discordant than in the concordant subgroups. An extensive stromal component has also been suggested to explain discrepancies (7), but this could not be confirmed in our investigation. In the study by Zafrani and co-workers, it was demonstrated that IHC was better than CYT in the identification of rare positive tumour cells in lobular carcinomas (13). In our study this seems to explain only a small proportion of the discrepancies, since the percentage of receptor positive cases in lobular carcinoma was similar with both techniques. Similarly, the presence of a high proportion ($>25\%$) of non-invasive cells (benign and/or DCIS) seems only to a minor extent to explain discordant results in our study. In agreement with our findings, the latter two explanations did not turn out to be of any major importance in an extensive study by Harvey and co-workers including almost 2 000 patients (12). The concordance in ER status was better for postmenopausal than for premenopausal patients. Similar results have previously been obtained by Alberts and co-workers (9). They explained this difference by the masking of ER by endogenous estrogens. Estrogen-bound ER can be detected by IHC, but not with CYT. The percentage of remarks for the evaluation of IHC was similar for the pre- and postmenopausal subgroups.

ER was significantly associated with prognosis after adjuvant tamoxifen, with both CYT and IHC, the latter method yielding somewhat stronger associations. Because of the low number of cases with discordance in receptor status, the present study was not able to demonstrate whether one method was significantly better than the other. This was also what we expected when planning the design of the present study (see Statistics). Investigations of similar size, claiming that one method is better than the other, may therefore not have had the statistical power for such a conclusion (Barnes et al.: 33 discordances, total 170 cases (10); Blomqvist et al.: 44 discordances, total 142 cases (15)). The more extensive study by Harvey and co-workers concluded that IHC has an equivalent or better ability to predict response to adjuvant endocrine therapy ($n = 517$) than CYT (12). PgR was not significantly associated with PFS, either with CYT ($p = 0.06$) or with IHC ($p = 0.22$). The results for PgR are in contrast with others from our group, showing that PgR is a better marker than ER for predicting sensitivity to tamoxifen treatment (3, 6). It should be noted that receptor status was a prognostic indicator only during the first two years after the primary operation. Thereafter, no difference in PFS could be demonstrated between receptor positive and receptor negative subgroups. Similar results have previously been obtained in other studies (20, 21). It should furthermore be mentioned that as all patients have been treated with adjuvant tamoxifen the observed difference in PFS between receptor positive and negative cases can be explained either by an intrinsic difference in prognosis between these two subgroups, difference in treatment response, or both. Most data suggest that the second explanation probably is the most important one, i.e. that steroid receptors are strong treatment predictive factors and that their prognostic importance is very limited (22).

As far as IHC was concerned, the number of cases around the cut-off of 10% stained was too low for evaluating the importance of different percentages of receptor stained nuclei for the prognosis after adjuvant tamoxifen (ER: 14 below [1%–10%] with 6 recurrences and 14 above [11%–20%] with 5 recurrences; PgR: 36 below [11 recurrences] and 31 above [8 recurrences]). The importance of choosing another cut-off level has previously been demonstrated in a large (almost 2 000 cases) study by Harvey and co-workers (12). They concluded that tumours with as few as 1%–10% weakly ER positive tumour cells as detected by their IHC assay were associated with better disease-free and overall survival compared with ER negative

breast cancer. The effect of adjuvant therapy for patients with very low levels of receptor positivity (1%–10%) needs to be studied further.

Owing to highly concordant receptor status between the primary tumour and the lymph node metastases, and between two synchronic ipsilateral primary tumours, these additional analyses should be of limited clinical importance. However, in the distant metastatic setting, ER and/or PgR status was changed in 36% (18/50) in a study by Kuukasjärvi and co-workers, and they conclude that response to tamoxifen seems to be more associated with ER content in the metastases than in the primary tumour (23).

In conclusion, we have demonstrated that IHC and CYT give similar associations between ER and PgR status and prognosis after adjuvant endocrine therapy for patients with primary breast cancer. It can therefore be concluded that IHC is a suitable method for routine analysis of ER and PgR.

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