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Prognostic significance of oestrogen receptor alpha (ERα) and beta (ERβ), progesterone receptor A (PR-A) and B (PR-B) in endometrial carcinomas

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ARTICLE INFO

Article history:
Received 29 March 2007
Received in revised form
2 August 2007
Accepted 7 August 2007

Keywords:
Endometrium
Cancer
Immunohistochemistry
Oestrogen receptor alpha (ERα)
Oestrogen receptor beta (ERβ)
Progesterone receptor A (PR-A)
Progesterone receptor B (PR-B)
Survival
Prognostic significance

ABSTRACT

The expression of the classic steroid receptors ERα and PR-A has been correlated with stage, histological grade and survival in endometrial cancer. Endometrial cancer samples (293) were immunohistochemically analysed with monoclonal antibodies against the four steroid receptors. The loss of ERα, PR-A and PR-B resulted in a poorer survival in endometrial cancer patients, while ERβ expression did not demonstrate any correlations with several analysed clinicopathological characteristics and did not affect survival. Additionally, multivariate survival analysis demonstrated that PR-B was a significant independent prognostic factor for cause-specific survival. In contrast, although ERα and PR-A showed a significant association between different endometrial histological subtypes and grading, both receptors were not independent factors with survival in endometrial carcinoma patients. Therefore, the PR-B immunostaining might be used as an easy, simple and highly efficient marker to identify high-risk patients and may aid in the selection of patients for a more aggressive adjuvant therapy.

1. Introduction

Endometrial cancer is becoming the most common gynaecologic malignancy in the Western World and occurs in reproductive and postmenopausal women.[1] It mostly appears in women with conditions resulting in unopposed oestrogens (i.e. oestrogen-only hormone replacement therapy, obesity, and oestrogen-producing tumours or anovulation).[1] However, the current used diagnostic technology is quite insufficient to identify endometrial cancer patients with poor prognosis. Therefore, immunohistochemistry of different specific markers might be an interesting alternative to select high-risk patients.[2]

Oestrogen receptor (ER) and progesterone receptor (PR) are ligand-dependent transcriptional factors belonging to the nuclear steroid receptor superfamily. For several years it was

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0959-8049/$ - see front matter
doi:10.1016/j.ejca.2007.08.014

generally believed that only single ER and PR receptors existed. However, the discovery of a new ER (ERβ) and PR (PR-B) has induced new insights in the steroid receptor signalling system.[3–5] The ER and PR expressions and distribution patterns might play an important role in normal endometrial function and pathogenesis and the expression and relationship of the two distinct ER's and PR's could be of essential clinical implications.[6,7] The expression of the classic steroid receptors ERα and PR-A has been correlated with stage, histological grade and survival in several studies. Additionally, it is thought that the ER and PR status constitutes an independent prognostic factor.[8–10] Therefore, the National Cancer Institute of the USA has recommended an incorporation of these parameters in the evaluation of endometrial cancer patients with stage I and II. However, PR, in contrast to ER, is suggested to be a more predictive factor of disease-free survival,[11,12] while some authors favour the fact that the expression of steroid receptors does not constitute an independent prognostic factor for endometrial cancer.[13,14] Therefore, the usefulness of the determination of receptor status in endometrial cancer patients is still controversially discussed. Additionally, there are limited data on the prognostic significance of the recently discovered ERβ and PR-B in endometrial cancer. Therefore, the aims of this study were the evaluation of the expression patterns of these steroid receptors (ERα, ERβ, PR-A and PR-B) and the assessment of their prognostic significance in endometrial cancer patients.

2. Materials and methods

2.1. Tissue samples

Hysterectomy specimens (293) containing endometrial carcinoma were obtained from the pathological archives that were operated at the 1st Department of Obstetrics and Gynaecology – Ludwig-Maximilians-University Munich between the years 1990 and 2001. All haematoxylin and eosin-stained slides were re-reviewed by a gynaecological pathologist (N.S.) to verify the diagnosis, histological grade, histological type, FIGO stage, lymphangiosis and haemangiosis.[15] Women with sarcoma of the uterus were excluded from this study. Pathological stage and histological subtype were determined for each surgical specimen according to 1988 International Federation of Gynaecology and Obstetrics (FIGO) criteria.[16] Histological classification was performed according to the World Health Organization system in the well differentiated (G1; $n = 160$), the moderate differentiated (G2; $n = 80$) and the poor differentiated (G3; $n = 53$).

Patients with endometrial carcinoma received modified radical hysterectomy, salpingo-oophorectomy or selective pelvic lymphadenectomy, with or without para-aortic lymphadenectomy. Lymph node sampling or dissection was generally performed in patients having tumours with deep myometrial invasion and/or high-grade or aggressive histological features. Obesity, advanced age and excessive comorbidity were factors against full surgical staging.

Patient data were obtained from three sources: hospital tumour registry, automated database and chart review. The Munich tumour registry systematically collects baseline data, including demographic data, diagnosis, additionally diseases (i.e. obesity, diabetes, and blood pressure) and treatment information on all cancer patients who are diagnosed or treated at the 1st Department of Obstetrics and Gynaecology, Ludwig-Maximilians-University Munich. Automated records and, when available, charts for each patient were reviewed to verify diagnosis and presence or absence of radiographic or pathological evidence of disease recurrence. All cases of recurrence had radiographic evidence of disease or biopsy-proven progression of disease. Only the records of patients who died of disease were considered to be uncensored; the records of all patients who were alive at follow-up or who did not die of disease (or a related cause) were considered to be censored. Additionally, as censored cases were also those cases considered where the exact cause of death was unknown but died within two years after the diagnosis of a metastatic lesion.

2.2. Immunohistochemistry

Immunohistochemistry was performed using the mouse-IgG-Vectastain Elite ABC kit (Vector Laboratories, Burlingame, California, USA) as previously described.[6,7] Briefly, paraffin-fixed tissue sections were dewaxed using xylol for 15 min, rehydrated in an alcohol row, and subjected to antigen retrieval on a high setting for 10 min in a pressure cooker in sodium citrate buffer (pH 6.0), containing 0.1 M citrate acid and 0.1 M sodium citrate in distillate water. After cooling, the slides were washed twice in PBS. Endogenous peroxidase activity was quenched by immersion in 3% hydrogen peroxide (Merck, Darmstadt, Germany) in methanol for 20 min. Non-specific binding of the primary antibodies was blocked by incubating the sections with diluted normal serum (10 ml PBS with 150 μl horse serum, provided by Vectastain Elite ABC kit) for 20 min at room temperature. Sections were then incubated at room temperature for 60 min with the primary antibodies (Table 1). After washing with PBS, the slides were incubated in diluted biotinylated anti-serum secondary antibody for another 30 min at room temperature (10 ml PBS, 50 μl horse serum, provided by Vectastain Elite ABC kit). After incubation with the avidin–biotin peroxidase complex (diluted in 10 ml PBS, reagent ABC provided by Vectastain Elite ABC kit) for another 30 min and a repeated washing step with PBS, visualisation was performed with substrate and chromagen 3,3′-diaminobenzidine (DAB; Dako, Glostrup, Denmark) for 8–10 min. The slides were counterstained further with Mayer's acidic haematoxylin and washed in an alcohol multiple-row (50–98%). After xylol treatment the slides were covered. For positive controls sections of human breast cancer tissue and normal colon were used, while human ileum served as negative control tissue. Positive cells showed a brownish colour and negative controls as well as unstained cells were blue.

2.3. Immunohistochemical evaluation

The intensity and distribution patterns of specific steroid receptor immunohistochemical staining reaction were evaluated by two blinded, independent observers, including a gynaecological pathologist, using a semi-quantitative score as previously described and used to assess the expression pattern of steroid receptors in endometrial tissue.[7]

Table 1 – Antibodies used for immunohistochemical characterisation of endometrial glandular cells (ER = estrogen receptor, PR = progesterone receptor)

Antibody	Clone	Isotype	Dilution	Source
ERα	1D5	Mouse IgG_1	1:150 in dilution-medium	Immunotech, Hamburg, Germany
ERβ	PPG5/10	Mouse IgG_{2a}	1:50 in PBS	Serotec, Oxford, United Kingdom
PR-A	10A9	Mouse IgG_{2a}	1:50 in dilution-medium	Immunotech, Hamburg, Germany
PR-B	SAN27	Mouse IgG_1	1:50 in PBS	NovoCastra, Newcastle, United Kingdom

Dilution-medium was obtained by Dako, Glostrup, Denmark.

The IRS score was calculated by multiplication of optical staining intensity (graded as 0 = no, 1 = weak, 2 = moderate and 3 = strong staining) and the percentage of positive stained cells (0 = no staining, 1 = <10% of the cells, 2 = 11–50% of the cells, 3 = 51–80% of the cells and 4 = >81% of the cells) as previously described.[18] Sections were examined using a Leika (Tokyo, Japan) photomicroscope. Digital images were obtained with a digital camera system and were saved on a computer. The IRS scores were compared using the Kruskal–Wallis one-way analysis of variance by ranks. Correlations of steroid receptor expression levels were assessed using the Spearman rank correlation test. Significance of differences was assumed at $p \leqslant 0.05$ at the two-sided test.

2.4. *Statistical analysis*

For the purposes of statistical survival analysis, steroid receptors expression in tumour sample, was considered to be elevated if >10% positive staining was observed as previously suggested,[11,17,18] which corresponds per definition to an immunohistochemical staining score (IRS) of higher than two. For the evaluation of the ERβ staining intensity the median for all tumour samples was used (median for ERβ = 0). Increased/positive versus not increased/negative immunostaining in tumour samples was compared using the χ^2 test and the exact Fisher's test where applicable.

The outcomes analysed were progression-free survival, cause-specific survival and overall survival. Univariate analyses were performed with Kaplan–Meier life-table curves to estimate survival and were compared using the log-rank test.[19] Prognostic models used multivariate Cox regression analysis for multivariate analyses of survival. The variables were entered in a forward stepwise manner.[20] Data were adjusted for age, stage, grade, lymph nodes, lymphangiosis, haemangiosis, unfavourable histology (endometrioid versus papillary serous/clear cell), steroid receptor expression, hypertension, obesity, diabetes, anti-hormone therapy and radiation therapy. Lymph node involvement was entered as categorical variable defined as no lymph node involvement, positive lymph nodes and unknown status. Significance of differences was assumed at $p \leqslant 0.05$ at the two-sided test (SPSS version 14.0; SPSS Inc., Munich, Germany).

3. Results

3.1. *Clinicopathological characterisation*

The clinicopathological features of the endometrial carcinomas are summarised in Table 2. The median patient's age at the time of diagnosis was 64.8 years (range 35.5–87.9 years). 219 (74.7%) and 21 (7.2%) patients were diagnosed in FIGO stages I and II, respectively, while 45 (15.4%) patients had FIGO stage III and eight patients (2.7%) presented with metastatic disease (FIGO IV). Of the analysed 293 patients, 256 had an endometrioid histology (87.4%), while 37 (12.6%) patients presented with a serous/clear cell or undifferentiated carcinomas. Of the endometrioid carcinomas, 214 patients demonstrated an endometrioid adenocarcinoma (83.6%), 12 (4.7%) showed a mucinous carcinoma and 34 (13.3%) showed a mixed adenocarcinoma. Lymph node sampling or dissection was generally performed in patients having tumours with deep myometrial invasion and/or high-grade or aggressive histological features. Pelvic and/or para-aortic lymph node sampling was performed for 209 patients (71.3%) while 23 patients (7.8%) demonstrated lymph node metastasis. A low FIGO stage (FIGO Ia), obesity, advanced age and excessive comorbidity were factors against a full surgical staging in 84 patients (28.4%). Obesity was observed in 99 (33.8%) cases, while 115 (39.4%) and 33 (11.3%) patients presented with high blood pressure and diabetes, respectively. Of the analysed 293 patients, 116 patients (39.6%) received a radiation therapy, while 11 patients (3.8%) received an anti-hormone therapy. During follow-up a tumour recurrence was observed in 45 patients (15.4%), and 41 patients (14.0%) died of disease.

3.2. *Endometrial carcinoma samples*

3.2.1. *Oestrogen receptors*

The results of the immunohistochemical analysis of endometrial carcinoma samples are summarised in Table 3. Positive ERα and ERβ immunostaining were observed in 129 (44.0%) and 40 (13.7%) of 293 endometrial carcinoma samples, respectively (Fig. 1a–d). No significant difference in ERβ expression was found among various subtypes of endometrial carcinoma, while ERα demonstrated highly significant differences ($p = 0.009$). ERα expression in endometrial carcinoma samples demonstrated a significant association with grading ($p = 0.032$) and histology ($p = 0.033$) with no statistical significance with surgical stage ($p = 0.066$) (Table 4). ERβ could not be correlated with any of the assessed clinicopathological characteristics of the patients. In addition, no correlation was found between ERβ and the other steroid receptors, while ERα demonstrated a high correlation with PR-A and PR-B ($p < 0.001$ each) (Table 5).

3.2.2. *Progesterone receptors*

A positive immunohistochemical staining reaction of PR-A and PR-B was observed in 145 (49.5%) and 189 (64.9%)

Table 2 – Clinicopathological characteristics of the analysed endometrial carcinomas

	Total (n = 293)	Endometrioid adenocarcinoma (n = 256)	Non-endometrioid (n = 37)
Age (years)			
<65	148 (50.5%)	133 (52%)	15 (40.5%)
>65	145 (49.5%)	123 (48%)	22 (59.5%)
WHO grading			
Grade 1	160 (54.6%)	151 (59%)	9 (24.3%)
Grade 2	80 (27.3%)	72 (28.1%)	8 (21.6%)
Grade 3	53 (18.1%)	33 (12.9%)	20 (54.1%)
FIGO stage			
FIGO I	219 (74.7%)	197 (77%)	22 (59.5%)
FIGO II	21 (7.2%)	19 (7.4%)	2 (5.4%)
FIGO III	45 (15.4%)	33 (12.9%)	12 (32.4%)
FIGO IV	8 (2.7%)	7 (2.7%)	1 (2.7%)
LN status			
Negative	186 (63.5%)	162 (63.3%)	24 (64.9%)
Positive	23 (7.8%)	18 (7%)	5 (13.5%)
Unknown	84 (28.7%)	76 (29.7%)	8 (21.6%)
Lymphangiosis			
Negative	265 (90.4%)	234 (91.4%)	31 (83.8%)
Positive	28 (9.6%)	22 (8.6%)	3 (16.2%)
Haemangiosis			
Negative	285 (97.3%)	249 (97.3%)	36 (97.3%)
Positive	8 (2.7%)	7 (2.7%)	1 (2.7%)
Diabetes			
Negative	260 (88.7%)	227 (88.7%)	33 (89.2%)
Positive	33 (11.3%)	29 (11.3%)	4 (10.8%)
Adipositas			
Negative	194 (66.2%)	168 (65.6%)	26 (70.3%)
Positive	99 (33.8%)	88 (34.3%)	11 (29.7%)
Hypertension			
Negative	178 (60.8%)	153 (59.8%)	25 (67.6%)
Positive	115 (39.2%)	103 (40.2%)	12 (32.4%)
Radiotherapy			
Negative	171 (58.4%)	150 (58.6%)	21 (56.8%)
Positive	116 (39.6%)	100 (39.1%)	16 (43.2%)
Denial	6 (2.0%)	6 (2.3%)	0 (0%)
Anti-hormone therapy			
Negative	282 (96.2%)	247 (96.5%)	35 (94.6%)
Positive	11 (3.8%)	9 (3.5%)	2 (5.4%)
Denial	0 (0%)	0 (0%)	0 (0%)

endometrial cancer cases, respectively (Fig. 1e–h). Significant differences in the IRS for both PRs were demonstrated among the various subtypes of endometrial carcinoma ($p = 0.006$ and $p < 0.001$ for PR-A and PR-B, respectively). By analysing positive and negative expressions, we could also observe significant differences between histological subtypes ($p = 0.038$ and $p = 0.018$, respectively). Additionally, we demonstrated a correlation between PR-A and PR-B with grading (χ^2: $p = 0.001$ and $p = 0.004$) and histology ($p = 0.008$ and $p = 0.005$) (Table 4). Additionally, PR-A was correlated with stage ($p = 0.008$) and haemangiosis ($p = 0.035$). A significant correlation between the two progesterone receptors was also observed ($p < 0.001$). In addition, both subunits showed a positive correlation with ERα expression ($p < 0.001$ each) (Table 5).

3.3. *Survival analysis*

The median time to death for the uncensored subgroup was 26.2 months (range 3.2–135.5 months), whereas the median follow-up of censored patients was 89.6 months (range 0.3–176.8 months). Univariate survival analysis revealed that patients with a ERα, PR-A and PR-B expression had a significant better progression-free survival compared with the patients with no expression (Fig. 2) ($p = 0.043$, $p = 0.004$ and $p = 0.011$ for ERα, PR-A and PR-B, respectively, log-rank test). Additionally, patients with ERα, PR-A and PR-B expressions demonstrated a highly significant poorer cause-specific survival (Fig. 3) ($p = 0.023$, $p = 0.004$ and $p = 0.002$ for ERα, PR-A and PR-B, respectively, log-rank test). However, only the lack of PR-A and PR-B demonstrated a significant poorer overall

Table 3 – Summary of immunohistochemical analysis of endometrial carcinomas

	Total (n = 293)	Endometrioid adenocarcinoma			Non-endometrioid		
		Endometrioid (n = 214)	Mucinous (n = 12)	Mixed (n = 34)	Serous (n = 23)	Clear cell (n = 5)	Undifferentiated (n = 5)
ERα							
Median	2	2	3	2	2	0	6
Mean ± SD	3.00 ± 2.89	3.07 ± 2.91	4.17 ± 2.59	2.85 ± 2.78	1.96 ± 2.03	0.4 ± 0.89	5.8 ± 4.92
Negative	164 (56.0%)	160 (74.8%)	10 (83.3%)	29 (85.3%)	17 (73.9%)	4 (80%)	5 (100%)
Positive	129 (44.0%)	54 (25.2%)	2 (16.7%)	5 (14.7%)	6 (26.1%)	1 (20%)	0 (0%)
Kruskal–Wallis	0.009						
χ^2	0.578						
ER β							
Median	0	0	0	0	0	0	0
Mean ± SEM	0.56 ± 0.96	0.28 ± 0.91	0.08 ± 0.29	0.12 ± 0.48	0.74 ± 0.69	5.6 ± 2.04	1.6 ± 3.05
Negative	253 (86.3%)	179 (84%)	12 (100%)	31 (91.2%)	15 (65.2%)	4 (80%)	4 (80%)
Positive	40 (13.7%)	34 (16.3%)	0 (0%)	3 (8.8%)	8 (34.8%)	1 (20%)	1 (20%)
Kruskal–Wallis	0.071						
χ^2	0.084						
PR-A							
Median	3	3	2.5	2	2	0	0
Mean ± SEM	3.22 ± 3.04	3.53 ± .05	3.08 ± 2.47	2.68 ± 2.99	2.3 ± 3.15	0.2 ± 0.45	1.4 ± 1.17
Negative	145 (49.5%)	114 (53.3%)	7 (58.3%)	23 (67.6%)	19 (82.6%)	5 (100%)	4 (80%)
Positive	148 (50.5%)	100 (46.7%)	5 (41.7%)	11 (32.4%)	4 (17.4%)	0 (0%)	1 (20%)
Kruskal–Wallis	0.006						
χ^2	0.038						
PR-B							
Median	4	4	6	3	2	0	0
Mean ± SEM	4.53 ± 3.58	4.95 ± 3.56	5.17 ± 3.51	3.65 ± 3.26	2.7 ± 3.1	0.2 ± 0.45	3.6 ± 4.93
Negative	104 (35.5%)	116 (54.2%)	2 (16.7%)	18 (52.9%)	17 (73.9%)	5 (100%)	2 (40%)
Positive	189 (64.5%)	98 (45.8%)	10 (83.3%)	16 (47.1%)	6 (26.1%)	0 (0%)	3 (60%)
Kruskal–Wallis	0.001						
χ^2	0.018						

survival (Fig. 4) ($p = 0.014$, $p = 0.013$ for PR-A and PR-B respectively, log-rank test). In contrast, ERβ expression did not demonstrate any significant differences in progression-free survival ($p = 0.907$), cause-specific survival ($p = 0.369$) and overall survival ($p = 0.815$). Other clinical parameters, including advanced surgical stage (stage I/II versus stage III/IV) and advanced histological grade (G3 versus G1 or G2), which are known prognostic factors of endometrial cancer, significantly affected the survival rates in our patients, demonstrating the validity of the patient group enrolled in this study (p in log rank was <0.001 for both analysed parameters for progression-free, cause-specific and overall survival, respectively).

Prognostic factors were also analysed by the multivariate Cox proportional-hazard model. For the stepwise logistic regression models, we included the following variables: age, FIGO stage, tumour grading, tumour type, lymph node status, lymphangiosis, haemangiosis, obesity, high blood pressure, diabetes, radiotherapy, anti-hormone therapy, ERα, ERβ, PR-A and PR-B status. Forward stepwise elimination according to Cox regression results led to a model containing four independent terms that were predictive of progression-free survival: WHO grading ($p < 0.001$), FIGO stage ($p < 0.001$), lymph node involvement ($p = 0.010$) and obesity ($p = 0.043$) (Table 6). Independent prognostic factors for cause-specific survival were age ($p < 0.001$), FIGO stage ($p < 0.001$), grade ($p = 0.022$), obesity ($p = 0.039$) and PR-B expression ($p = 0.019$). The overall survival was influenced by age ($p < 0.001$), FIGO stage ($p < 0.001$), tumour grade ($p = 0.008$), lymph node involvement ($p < 0.001$), haemangiosis ($p = 0.016$) and high blood pressure ($p = 0.047$) (Table 6).

4. Discussion

Although more than 50% of patients with endometrial carcinomas are diagnosed with FIGO stage I, as many as 20% die as a result of their disease.[21] This is an unusual situation, compared to other solid tumours, and may reflect the failure of current diagnostics to identify pre-malignant stages and endometrial cancer patients with a poor prognosis. There is a clinical need for a simple and efficient marker of the activity of this tissue, especially in regard to the endometrial carcinogenesis and prognostic factors.

In this large series of 293 analysed cases, the lack of ERα and PR-A expressions was associated with poor differentiation in endometrial cancer patients as previously suggested for these steroid receptors.[8,9] Additionally, we demonstrated for the first time an association between the newly identified PR-B and histological differentiation. Interestingly, only the expression of PR-A was associated with surgical staging. Moreover, we could not demonstrate any significant correlation of the newly cloned ERβ with any clinicopathological

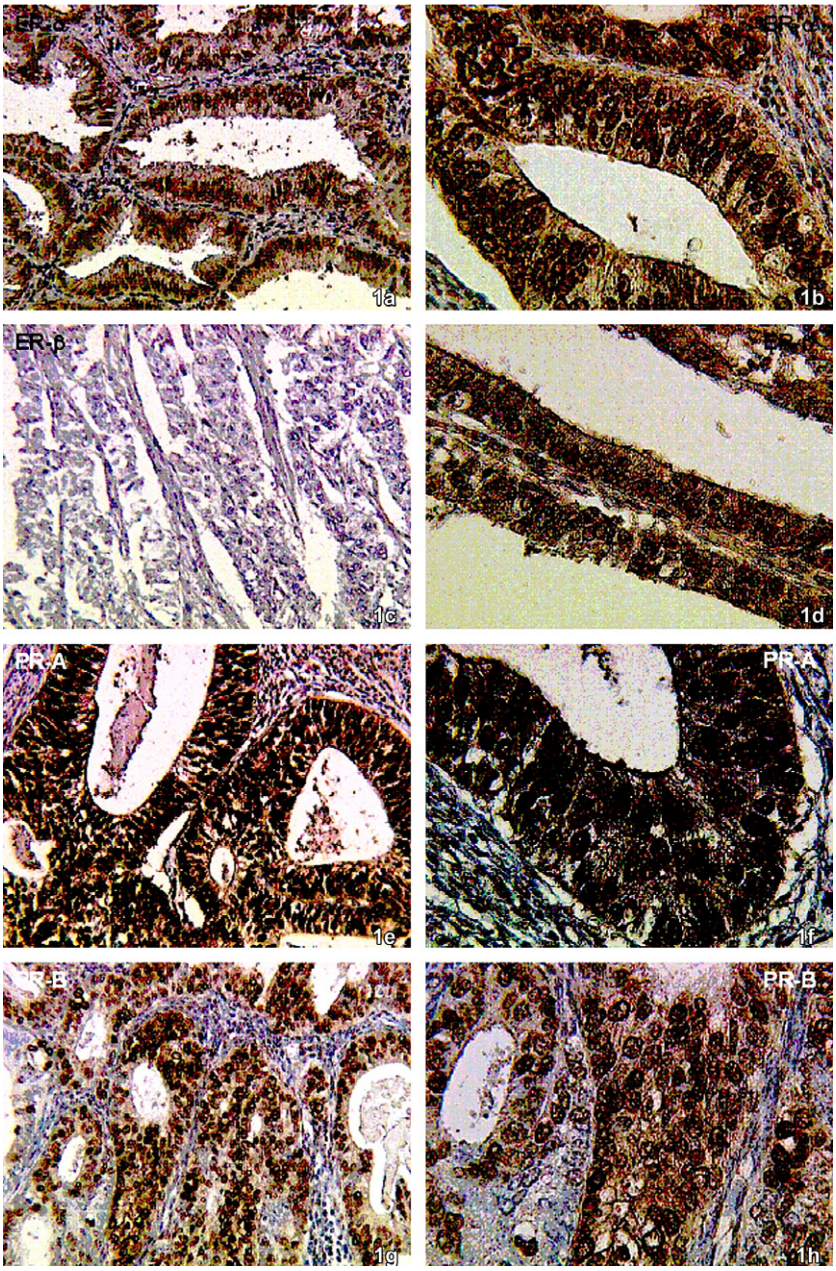

Fig. 1 – Expression of oestrogen (ERα and ERβ) and progesterone (PR-A and PR-B) in malignant human endometrial tissue. ERα showed moderate expression in well-differentiated endometrial cancer tissue (a, 150×) with a characteristic nuclear staining reaction (b, 250×), whereas in most cases endometrioid adenocarcinomas did not demonstrate an ERβ immunoreactivity (c, 150×). A positive staining reaction of ERβ is demonstrated in d (250×). The staining reaction against PR-A was more intense compared to ERα (e, 250×) being also mainly localised in the nucleoli (f, 400×). The PR-B immunoreactivity was similar to the PR-A, being also mainly localised within the nucleoli (g and h, 150× and 250×, respectively).

characteristics. Interestingly, ERα, PR-A and PR-B constitute a significant prognostic marker regarding progression-free and cause-specific survival. However, we determined that receptor status did not constitute an independent prognostic factor confirming previous results.[13,14,22] Interestingly, only the immunohistochemical expression of PR-B was an independent prognostic factor for cause-specific survival.

The ERα status is believed to provide prognostic information independent of tumour stage and grade in women with endometrial carcinoma.[9,17] Interestingly, the ERβ/ERα mRNA ratio was high in advanced invasive carcinoma, suggesting that ERβ is important in the progression of myometrial invasion.[23] The intact synchronised expression of ERβ interacting with ERα might be disrupted in malignant endometrium,[24] playing an important role in endometrial cancer pathogenesis. However, we neither could demonstrate any significant association between the immunohistochemical expression of ERβ and several clinicopathological characteristics nor an

Table 4 – Univariate statistical analysis for positive oestrogen receptor alpha (ERα), oestrogen receptor beta (ERβ), progesterone receptor A (PR-A) and progesterone receptor B (PR-B) according to various clinicopathological features

	Total (n = 293)	ERα	ERβ	PR-A	PR-B
Age (years)					
<65	148 (50.5%)	64 (43.2%)	19 (13.1%)	76 (51.3%)	96 (64.9%)
>65	145 (49.5%)	65 (44.8%)	21 (14.5%)	72 (49.7%)	93 (64.1%)
χ^2		NS	NS	NS	NS
WHO grading					
Grade 1 + 2	240 (81.9%)	113 (47.1%)	30 (12.5%)	135 (56.3%)	164 (68.3%)
Grade 3	53 (18.1%)	16 (30.2%)	10 (18.9%)	13 (24.5%)	25 (47.2%)
χ^2		0.032	NS	<0.001	0.004
FIGO stage					
FIGO I + II	240 (81.9%)	112 (46.7%)	33 (13.8%)	132 (55.0%)	161 (67.1%)
FIGO III + IV	53 (18.1%)	17 (29.1%)	7 (17.0%)	16 (30.2%)	28 (52.8%)
χ^2		0.066	NS	0.001	0.058
Histology					
Endometrioid	256 (87.4%)	119 (46.5%)	31 (12.1%)	137 (53.5%)	173 (67.6%)
Non-endometrioid	37 (12.6%)	10 (27.1%)	9 (24.3%)	11 (29.7%)	16 (43.2%)
χ^2		0.033	0.068	0.008	0.005
LN status					
Negative	186 (63.5%)	84 (45.2%)	38 (20.4%)	99 (53.2%)	123 (66.1%)
Positive	23 (7.8%)	7 (30.4%)	1 (2.7%)	8 (34.8%)	11(47.8%)
Unknown	84 (28.7%)	38 (45.2%)	12 (14.3%)	41 (48.8%)	55 (65.5%)
χ^2		NS	NS	NS	NS
Lymphangiosis					
Negative	265 (90.4%)	119 (44.9%)	38 (14.3%)	136 (51.3%)	172 (64.9%)
Positive	28 (9.6%)	10 (35.7%)	2 (7.1%)	12 (42.9%)	17 (60.7%)
χ^2		NS	NS	NS	NS
Haemangiosis					
Negative	285 (97.3%)	126 (44.2%)	40 (14.1%)	147 (51.6%)	185 (64.9%)
Positive	8 (2.7%)	3 (37.5%)	0 (0%)	1 (12.5%)	4 (50%)
χ^2		NS	NS	0.035	NS
Diabetes					
Negative	260 (88.7%)	116 (44.6%)	34 (13.1%)	130 (50%)	165 (63.5%)
Positive	33 (11.3%)	13 (39.4%)	6 (18.2%)	18 (54.4%)	24 (72.7%)
χ^2		NS	NS	NS	NS
Adipositas					
Negative	194 (66.2%)	86 (44.3%)	30 (15.5%)	92 (47.4%)	121 (62.4%)
Positive	99 (33.8%)	46 (46.5%)	10 (10.1%)	56 (56.6%)	68 (68.7%)
χ^2		NS	NS	NS	NS
Hypertension					
Negative	178 (60.6%)	76 (42.7%)	24 (13.5%)	90 (50.6%)	113 (63.4%)
Positive	115 (39.4%)	53 (46.1%)	15 (13.0%)	58 (50.4%)	76 (66.1%)
χ^2		NS	NS	NS	NS
Radiotherapy					
Negative	177 (60.4%)	82 (46.3%)	25 (14.1%)	96 (54.2%)	115 (65%)
Positive	116 (39.6%)	47 (40.5%)	15 (12.9%)	52 (44.8%)	74 (63.8%)
χ^2		NS	NS	NS	NS
Anti-hormone therapy					
Negative	282 (96.2%)	124 (44%)	40 (14.2%)	142 (50.4%)	183 (64.9%)
Positive	11 (3.8%)	5 (45.5%)	0 (0%)	6 (54.5%)	6 (54.5%)
χ^2		NS	NS	NS	NS

NS, not significant.

association with survival. An isolated evaluation of the ERβ expression regarding surgical staging or prognostic marker might not be useful in endometrial cancer patients. Additionally, we could not demonstrate a correlation between ERα and both PR receptors with ERβ, suggesting different regulation and signalling mechanisms of ERβ in endometrial cancer. We therefore suggest that ERβ is only useful if it is evaluated with the ERα expression, since the ratio of the two steroid receptors is probably more useful than evaluating each receptor separately.

Table 5 – Correlation between oestrogen receptor alpha (ERα), oestrogen receptor beta (ERβ), progesterone receptor A (PR-A) and progesterone receptor B (PR-B) in all endometrial carcinoma samples

	ERα	ERβ	PR-A	PR-B
ERα				
Correlation coefficient	–	0.012	0.352	0.390
p	–	NS	<0.001	<0.001
ERβ				
Correlation coefficient	0.012	–	0.014	0.045
p	NS	–	NS	NS
PR-A				
Correlation coefficient	0.352	0.014	–	0.537
p	<0.001	NS	–	<0.001
PR-B				
Correlation coefficient	0.390	0.045	0.537	–
p	<0.001	NS	<0.001	–

NS, not significant.

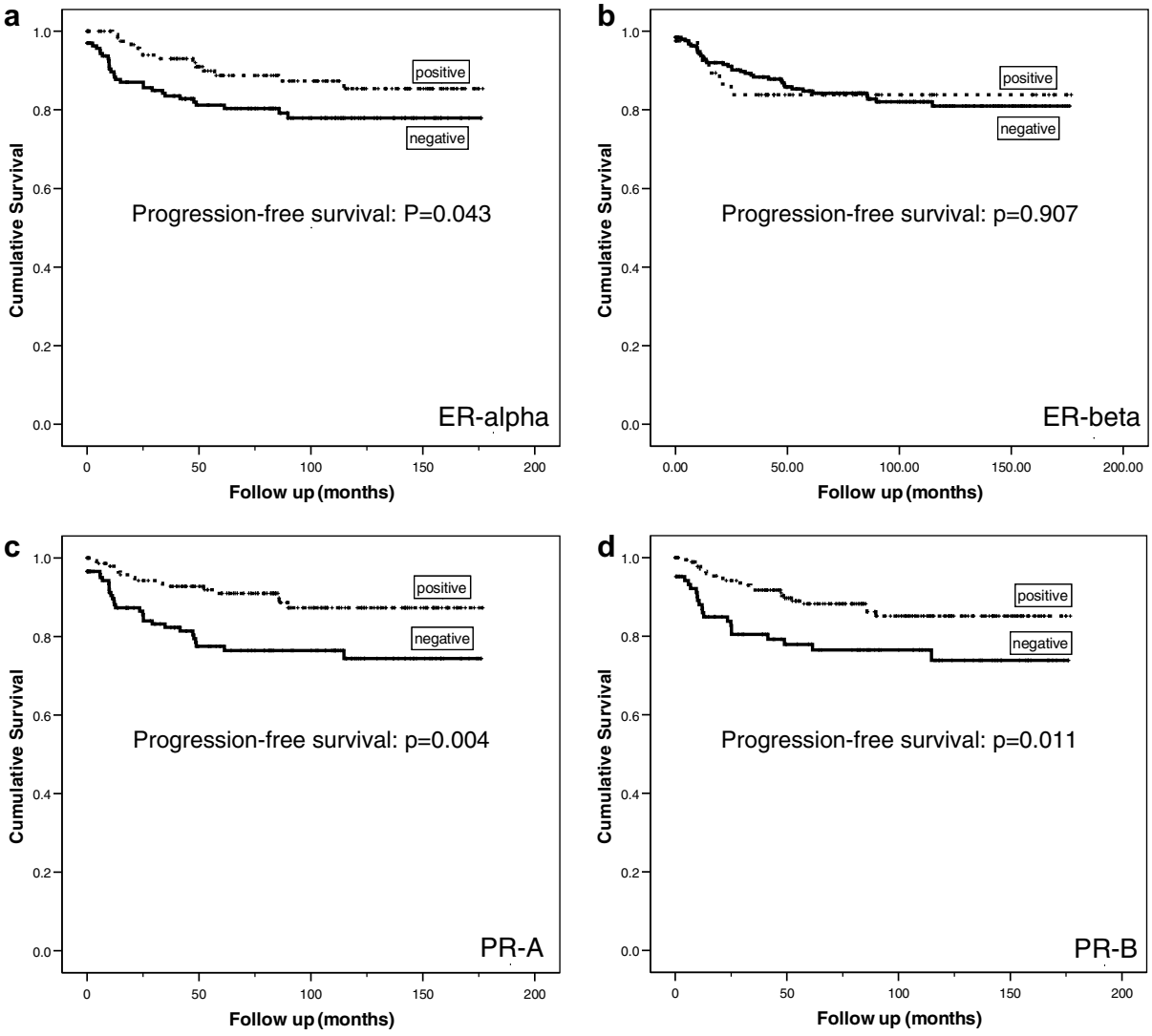

Fig. 2 – Kaplan–Meier curves of clinical outcome regarding ERα (a), ERβ (b), PR-A (c) and PR-B (d) for progression-free survival. ERα, PR-A and PR-B demonstrated a significant association with survival (log rank: $p = 0.043$, $p = 0.004$ and $p = 0.011$, respectively).

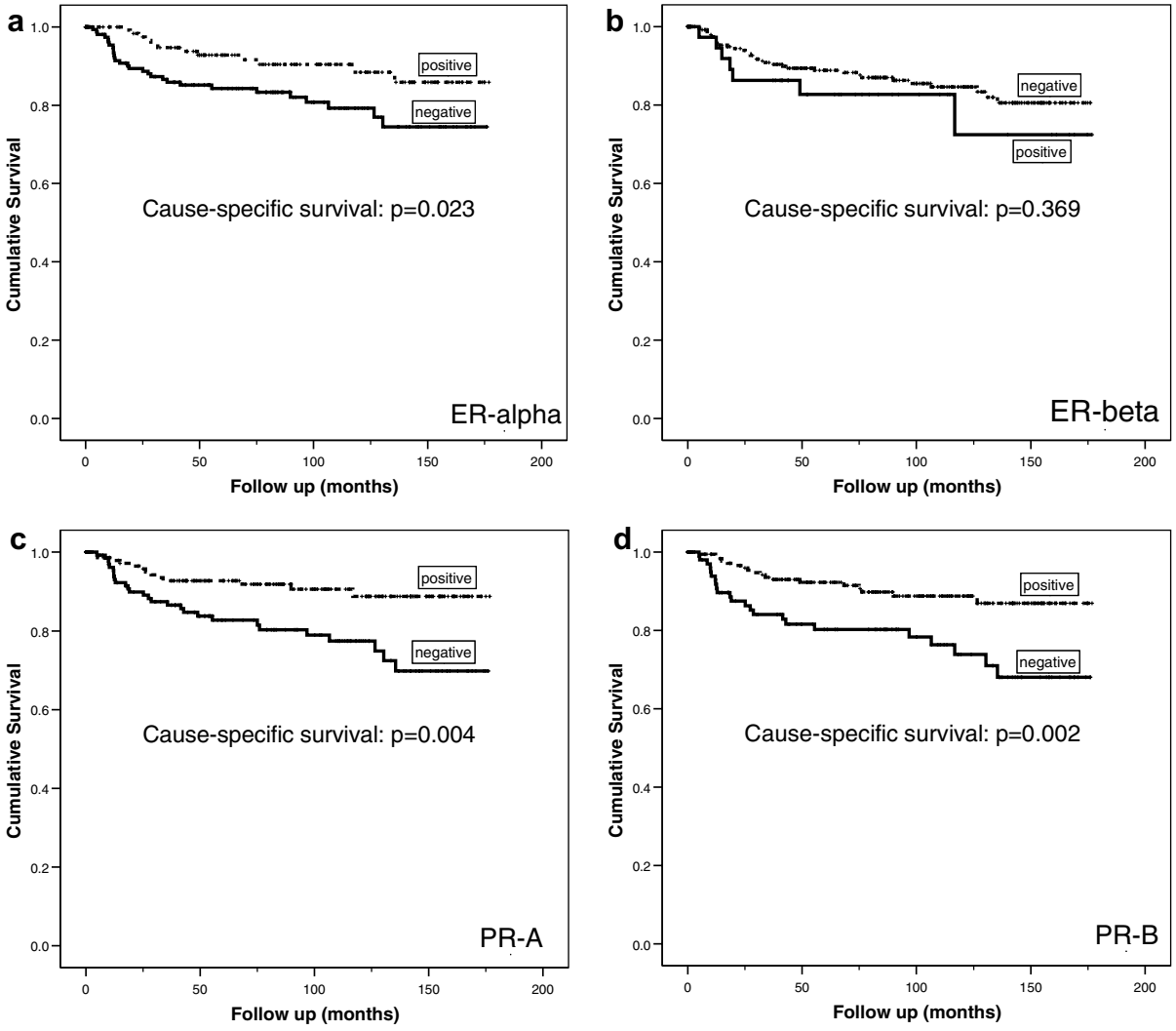

Fig. 3 – Kaplan–Meier curves of clinical outcome regarding ERα (a), ERβ (b), PR-A (c) and PR-B (d) for cause-specific survival. ERα, PR-A and PR-B demonstrated a significant association with survival (log rank: $p = 0.023$, $p = 0.004$ and $p = 0.002$, respectively).

PR has also been implicated in the development of endometrial cancer, exerting its effects by its two receptors PR-A and PR-B. A decrease of PR-B has been observed in poorly differentiated endometrial cancer cell-lines.[5] In PR-A knockout-mice model the PR-B induces cell growth,[25] suggesting endometrial growth through PR-B in the absence of PR-A.[26] In the present study PR-A was more predictive than ERs of disease-free survival, which has been suggested by some authors.[11,12] However, several conflicting results have been reported, where loss of ER expression – and not PR expression – has been associated with poorer survival,[17,27] leading to controversial discussions with regard to the usefulness of the determination of the specific receptor in endometrial cancer patients. Interestingly, Palmer and colleagues[28] confirmed that PR/ER status was significantly related to survival and demonstrated that, when only one receptor could be obtained, PR provided the most helpful information for the greatest number of patients. Nevertheless, the statistical technique and the large series of analysed cases in this study clearly distinguished between the importance of PR and ER immunohistochemistry. Additionally, we demonstrated a significant association of PR-B expression and patients' survival. Recently, a drastic decrease of PR-B mRNA, but not PR-A mRNA, was associated with poor prognosis in endometrial cancer patients.[29] Our results seem additionally to be confirmed by a recent study in 141 patients, where PR-A and PR-B expressions were significantly correlated with biologically malignant potential.[30] Especially, the PR-B expression was suggestive of a useful prognostic indicator of endometrial adenocarcinoma.[30] In the present study, PR-B was the only steroid receptor which was an independent prognostic factor in cause-specific survival. Therefore, we suggest that PR-B is more suitable in assessing an individual risk profile and selecting high-risk patients than ERα, ERβ and PR-A.

Summarising, we showed an expression of both oestrogen receptors (ERα and ERβ) as well as both progesterone receptors (PR-A and PR-B) in malignant endometrial tissue. The loss of receptor positivity for ERα, PR-A and PR-B resulted in a poorer survival in endometrial cancer patients, while ERβ did not affect survival. Additionally, survival analysis demonstrated that PR-B immunoreactivity was a significant independent prognostic factor for cause-specific survival. In contrast, ERα

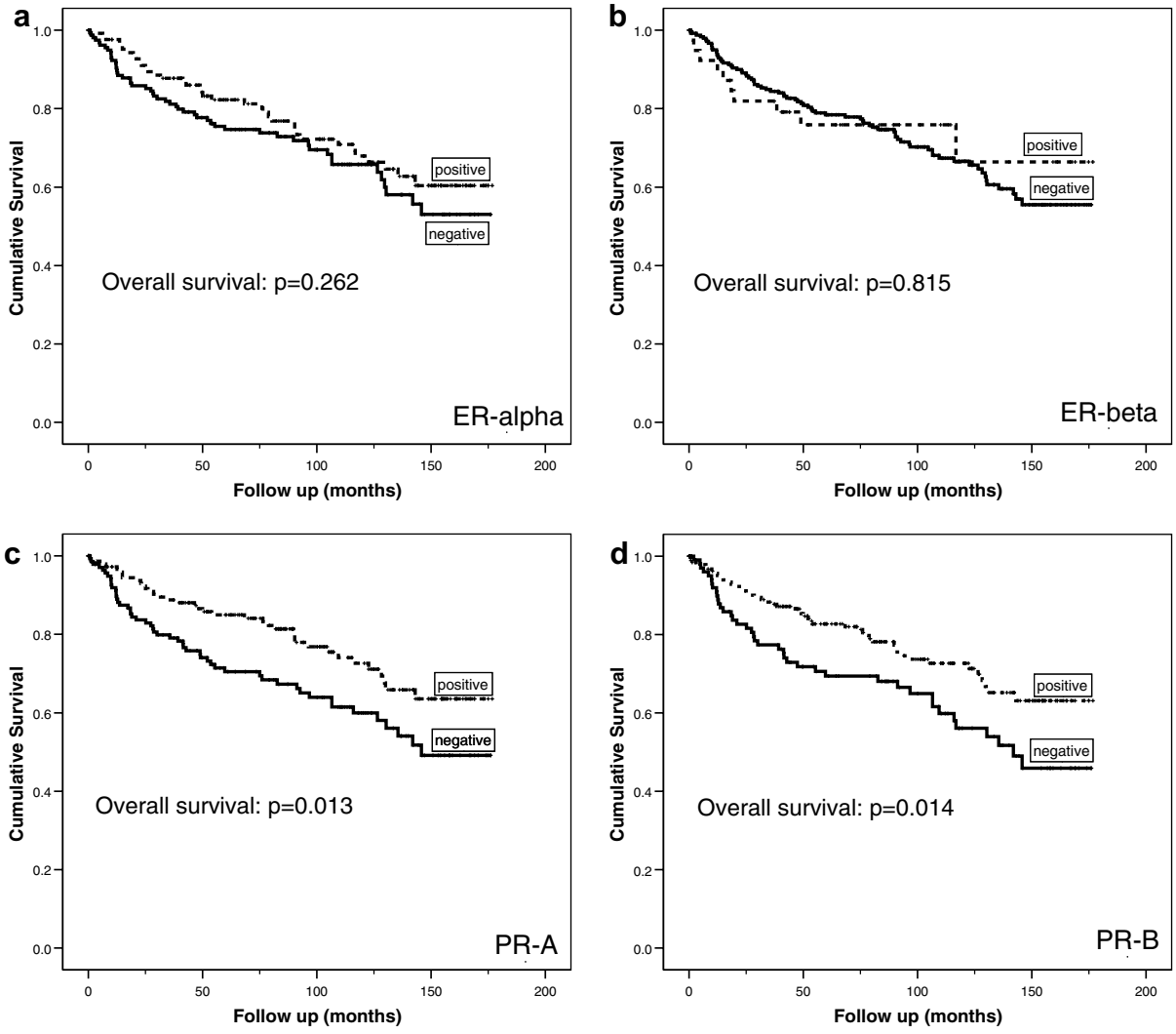

Fig. 4 – Kaplan–Meier curves of clinical outcome regarding ERα (a), ERβ (b), PR-A (c) and PR-B (d) for cause-specific survival. PR-A and PR-B demonstrated a significant association with survival (log rank: $p = 0.013$, $p = 0.014$, respectively).

Table 6 – Hazard ratios by multivariate Cox regression analysis

	Progression-free survival			Cause-specific survival			Overall survival		
	RR	CI (5–95%)	*p*	RR	CI (5–95%)	*p*	RR	CI (5–95%)	*p*
Age (>65years)	–	–	–	3.948	1.97–7.93	<0.001	3.698	2.18–6.28	<0.001
WHO grading (G1/G2 vs. G3)	3.332	1.72–6.44	<0.001	2.279	1.12–4.62	0.022	2.017	1.22–3.34	0.008
FIGO stage (I/II vs. III/IV)	13.79	6.8–27.95	<0.001	6.756	3.3–13.82	<0.001	4.328	2.34–8.02	<0.001
LN status	0.414	0.21–0.81	0.010	–	–	–	0.362	0.23–0.58	<0.001
Haemangiosis	–	–	–	–	–	–	3.481	1.27–9.56	0.016
Hypertension	–	–	–	–	–	–	0.628	0.397–0.995	0.047
Obesity	0.449	0.21–0.97	0.043	0.446	0.21–0.96	0.039	–	–	–
PR-beta (positive vs. negative)	–	–	–	0.458	0.24–0.88	0.019	–	–	–

and PR-A were not independent factors with survival in endometrial cancer patients. Therefore, the PR-B immunostaining might be used as an easy, simple and highly efficient marker to identify high-risk patients and may aid in the selection of patients for a more aggressive adjuvant therapy.

Conflict of interest statement

All authors do not have any financial and personal relationships with other people or organisations that could inappropriately influence this work.

Acknowledgements

We would like to thank Mrs. I. Wiest for her excellent work with the endometrial samples. We also express our gratitude to Prof. D. Hölzel – Institute of Medical Informatics, Biometry and Epidemiology, Ludwig-Maximilians-University Munich and Mr. M. Schmidt of the Munich Tumour Registry for supplying the survival data. This study was partially supported by the FöFoLe programme of the Ludwig-Maximilians-University Munich (297/03), the Friedrich-Baur-Institute and the Weigland Stipendium Programme of the Ludwig-Maximilians-University Munich for I. Mylonas.

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