Estrogen receptor and progesterone receptor are prognostic factors in soft tissue sarcomas

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Abstract. Estrogen (ER) and progesterone receptor (PgR) regulate growth and cell differentiation upon ligand-dependent and ligand-independent activation. In breast cancer and gynecological tumors their expression are known predictors of endocrine therapy benefits and a favourable therapy-independent prognosis. In soft tissue sarcomas, their expression profile is poorly defined and their significance is uncertain. We investigated the prognostic impact of ER and PgR in non-gastrointestinal stromal tumor soft tissue sarcomas (non-GIST STSs). Tumor samples and clinical data from 249 patients with non-GIST STS were obtained, and tissue microarrays (TMAs) were constructed for each specimen. Immunohistochemistry (IHC) was used to evaluate marker expression in tumor cells. In univariate analyses, the expression of neither ER nor PgR (P=0.333 and 0.067, respectively) were significant prognosticators in the total cohort. However, measured separately for each gender, ER positivity was a significant favourable indicator for disease specific survival (DSS) in women (P=0.017) while PgR positivity had inverse impact in men (P=0.001). Among the four possible coexpression profiles, ER^-/PgR^+ was significantly least favourable for survival in the univariate analysis (P<0.001). In the multivariate analysis, the ER^-/PgR^+ phenotype was an independent negative prognostic factor for DSS (HR=1.9, 95% CI=1.2-3.1, P=0.008) in addition to patient's nationality, tumor depth, histological entity, malignancy grade, metastasis at diagnosis, surgery and positive resection margins. The present findings indicate that ER and PgR have significant gender dependent impact on DSS in non-GIST STSs.

Introduction

Soft tissue sarcomas (STS) are malignant tumors arising from extraskeletal mesenchymal tissues. They are heterogeneous neoplasms, consisting of more than 50 subtypes, but comprise only 0.5% of adult malignancies (1). Approximately 50% of the STS patients will succumb to their disease because of metastasis or local relapse (2). The prognostic factors determining tumor progression and ultimately the patients' fate include tumor grade, size, location, depth, histological entity, positive resection margins and presence of local recurrence (3-9).

We have recently shown (unpublished data) that transforming growth factor-beta 1 (TGF-β1) is a strongly independent negative prognostic marker in women in separate uni- and multivariate analyses, but not significant in men. This prompted us to investigate whether expression of the sex-related steroid hormone receptors, ER and PgR, could have prognostic impact on STS associated DSS.

ER is a group of receptors activated by the hormone 17β-estradiol (estrogen). There are two separate, but highly homologous isoforms of ER, ERα and ERβ, which have completely different tissue distribution (10). They are encoded by two separate genes, ESR1 and ESR2, respectively. Like ER, PgR protein exists as two receptor isoforms, called in this case A and B, but these are product of the same gene.

ER, mostly in α isoform, mediates the action of estrogens and is responsible for growth and differentiation of target cells. PgR is considered as the ER's antagonist. However, selective ablation of PgR-A in a mouse model, resulted in exclusive production of PgR-B indicating that PR-B contributes to, rather than inhibits, epithelial cell proliferation both in response to estrogen alone and in the presence of progesterone (11).

Both ER and, to a lesser degree, PgR are well known prognosticators of endocrine therapy success in breast cancer (12,13). They are also shown to have a slight positive prognostic effect irrelative of endocrine therapy (14). However, their expression in STSs, especially those outside the gynaecological sphere, is scarcely investigated. Moreover, the prognostic value of such expression still remains unknown.

In this study, we investigate the prognostic impact of ER and PgR in 249 non-GIST STS patients. To our knowledge this is the first prognostic evaluation of these biomarkers in non-GIST STSs.

Patients and methods

Patients and clinical samples. Primary tumor tissue from anonymized patients diagnosed with non-GIST STS at the

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University Hospital of Northern Norway (UNN) 1973-2006 and The Hospitals of Arkhangelsk region, Russia, were used in this retrospective study. In total, 496 patients were registered from the hospital databases. Of these, 247 patients were excluded due to missing clinical data (n=86) or inadequate material for histological examination (n=161). Thus, 249 STS patients with full clinical records and adequate paraffin-embedded tissue blocks were eligible.

This report includes follow-up data as of September 2009. The median follow-up was 38 months (range 0.1-392). Formalin-fixed and paraffin-embedded tumor specimens were obtained from the archives of the Departments of Pathology at UNN and the Arkhangelsk hospitals. The tumors were graded according to the French Fèdèration Nationales des Centres de Lutte Contre le Cancer (FNCLCC) (15).

Microarray construction. All sarcomas were histologically reviewed by two trained pathologists (Sveinung Sorbye and Andrej Valkov) and the most representative areas of viable tumor cells (neoplastic cells) were carefully selected and marked on the hematoxylin and eosin (HE) slides and sampled for the tissue microarray blocks (TMAs). The TMAs were assembled using a tissue-arraying instrument (Beecher Instruments, Silver Springs, MD). The Detailed methodology has been previously reported (16). Briefly, we used a 0.6-mm diameter stylet, and the study specimens were routinely sampled with two replicate core samples (different areas) of neoplastic tissue. To include all core samples, 12 tissue array blocks were constructed. Multiple 4-μm sections were cut with a Micron microtome (HM355S) and stained by specific antibodies for immunohistochemistry (IHC) analyses.

Immunohistochemistry (IHC). The applied antibodies were subjected to in-house validation by the manufacturer for IHC analysis on paraffin-embedded material. Estrogen (ERα) receptor (mouse monoclonal; SP1; Ventana Medical Systems; prediluted) and progesterone (PgRA+PgRB) receptor (rabbit monoclonal; 1E2; Ventana Medical Systems; prediluted).

Sections (4 μm) were deparaffinized with EZ prep at 75˚C and stained using Ventana Benchmark XT (Ventana Medical Systems Inc.), procedure iViewDAB. Antigen retrieval was CC1 mild for PgR and CC1 standard for ER. Primary antibodies against ER and PgR were incubated at 37˚C for 32 min for ER and 24 min for PgR. As secondary antibodies biotinylated goat anti-mouse IgG and IgM and goat anti-rabbit IgG were used for, correspondingly, SP1 and 1E2. This was followed by application of liquid diaminobenzidine and substrate-chromogen, yielding a brown reaction product at the site of the target antigen (iView DAB® procedure). Finally, slides were counterstained with hematoxylin to visualize the nuclei. For each antibody, include negative controls, all TMA staining were performed in a single experiment.

Scoring of IHC. The ARIOL imaging system (Genetix, San Jose, CA) was used to scan the slides with immunohisto-chemically stained TMAs. The specimens were scanned at a low resolution (1.25x) and high resolution (20x) using Olympus BX 61 microscope with an automated platform (Prior). The slides were loaded in the automated slide loader (Applied Imaging SL 50). Representative and viable tissue sections were scored semiquantitatively on the computer screen for nuclear staining. To measure the grade of staining, we adapted the Allred Score (17) system for STS as shown (Table I). Examples of scoring assessment are shown in Fig. 1. All samples were anonymized and independently scored by two pathologists (A. Valkov and S. Sorbye). In case where score difference was exceeding 1, the slides were re-examined and a consensus was reached by the observers. When assessing a score for a given core, the observers were blinded to the scores of the other variables and to outcome. Mean score for duplicate cores from each individual was calculated.

Statistical methods. All statistical analyses were done using the statistical package SPSS (Chicago, IL), version 16. The IHC scores from each observer were compared for interobserver reliability by use of a two-way random effect model with absolute agreement definition. The intraclass correlation coefficient (reliability coefficient) was obtained from these results. The χ^2 test and Fisher's exact test were used to examine the association between molecular marker expression and various clinicopathological parameters. Univariate analyses were done by using the Kaplan-Meier method, and statistical significance between survival curves was assessed by the log-rank test. Disease-specific survival (DSS) was determined from the date of histological confirmed STS diagnosis to the time of STS death. To assess the independent value of different pretreatment variables on survival, in the presence of other variables, a multivariate analysis was performed using the Cox proportional hazards model. Only variables of significant value from the univariate analysis were entered into the Cox regression analysis. Probability for stepwise entry and removal was set at 0.05 and 0.10, respectively. The significance level used was $P<0.05$.

Ethical clearance. The National Cancer Data Inspection Board and The Regional Committee for Research Ethics approved the study.

Results

Clinicopathological variables. The clinicopathological variables are summarized in Table II. Median age was 59 (range, 0-91) years and 56% were female. The non-GIST STS comprised 249 tumors including undifferentiated pleomorphic sarcoma (n=68), leiomyosarcoma (n=67), liposarcoma (n=34), malignant fibroblastic/myofibroblastic tumors (n=20), rhabdomyosarcoma (n=16), synovial sarcoma (n=16), angiosarcoma (n=13), malignant peripheral nerve sheath tumor (MPNST) (n=11) and other types of sarcoma (n=4). The tumors were localized in the extremities (n=89), trunk (n=47), retroperitoneum (n=37), head/neck (n=18) and viscera (n=58). The first line treatment modality was surgery (n=228), 120 patients received surgery alone, 55 patients received surgery and radiotherapy, 40 patients received surgery and chemotherapy, 13 patients received surgery, radiotherapy and chemotherapy. Of the 21 non-operated patients (inoperable, n=11; advanced age/other serious disease, n=5, STS diagnosis confirmed post mortem, n=3; patient refusal, n=2) seven received chemotherapy and/or radiotherapy. Fourteen patients did not obtain any treatment.

Table I. Modified Allred score system, adapted for STS showing score 0-3 related to staining intensity and percent positive cells.

	% positivity			
Intensity	<1%	1-10%	11-33%	34% and more
Weak	0	0	1	2
Moderate and strong	0	1	2	3

Interobserver variability. Interobserver scoring agreement was tested for both markers. The intraclass correlation coefficients were 0.92 for ER (P<0.001) and 0.96 for PgR (P<0.001).

Expression pattern and correlations with clinicopathological variables. The ER and PgR demonstrated nuclear positivity in tumor cells and the positivity threshold was taken as 1% for both ER and PgR (Table I). The most intensively ER- and PgR-positive tumors were leiomyosarcomas in the uterus. However, the moderately and especially weakly positive tumors were distributed relatively equally between genders and histological entities (Table III).

The expressions of ER and PgR correlated strongly with each other. Among the PgR-positive tumors, 53% were also ER-positive, while 32% of PgR-negative tumors showed some grade of ER-expression (r=0.206, P=0.002). Fifty-three percent of STSs expressed at least one of the steroid hormone receptors.

Women had PgR-positive tumors significantly more often than men (P=0.025), and STSs of younger patients (<60) expressed PgR more frequently compared to the older age group, 37 and 24% (P=0.008) respectively. No such relations were seen for ER expression.

ER expression correlated significantly with STS location and size. While visceral tumors expressed ER in 50% of cases, the tumors located on extremities did so in 30% and retroperitoneal STS in 24% (P=0.008). Further, tumors <5 cm in diameter were ER-positive in 52%, while larger tumors showed ER-positivity in 33% (P=0.007). None of the steroid hormone receptors correlated significantly with histological diagnosis, tumor depth, grade of malignancy, or distant relapse rate.

Univariate analyses. Data are presented in Table II. Patient nationality (P=0.011), tumor size (P=0.027), histological grade (P<0.001), tumor depth (P<0.001), metastasis at time of diagnosis (P<0.001), surgery (P<0.001) and resection margins (P<0.001) were all significant prognostic variables for DSS.

As shown in Table IV, ER and PgR showed no prognostic impacts on DSS by analysing the whole cohort. However, separate analyses of each gender revealed that ER expression was a significant positive prognostic factor in women (P=0.017), while PgR expression was associated with a poor prognosis in men (P=0.001). Moreover, ER positivity in men and women tended towards opposite prognostic effects as shown in Fig. 2A and B. Among the four possible coexpression patterns of ER and PgR, the ER^-/PgR^+ profile for the whole cohort, which was seen in 14% of the patients (n=34), was associated with a dismal prognosis (P<0.001) (Fig. 2E).

Multivariate Cox proportional hazards analyses. Only variables which were significant in univariate analyses were entered into the multivariate analysis, which was carried out for all patients and separately for men and women (Table V). Neither ER nor PgR expression influenced significantly on prognosis alone taken in the Cox regression analysis. However, ER^-/PgR^+ phenotype was an independent negative prognostic factor for DSS (HR=1.9, 95% CI=1.2-3.1, P=0.008) in addition to tumor depth, malignancy grade, metastasis at diagnosis, presence of surgery and positive resection margins.

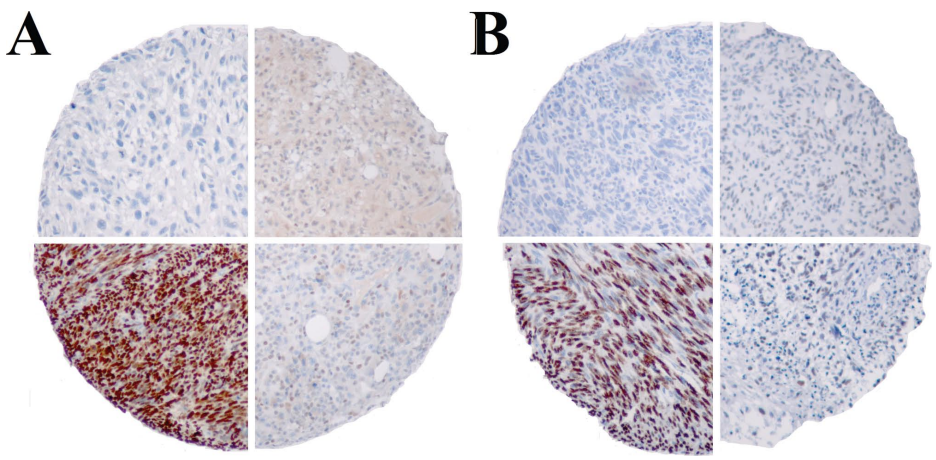

Figure 1. IHC analysis of TMA of non-GIST STS representing different scores of tumor cell expression of ER and PgR. (A) Upper left quadrant, AS, ER, negative staining, score 0; upper right quadrant, LS, ER, weak staining, score 1; lower right quadrant, CCS, ER, moderate staining, score 2; lower left quadrant, LMS, ER, strong staining, score 3. (B) Upper left quadrant, MPNST, PgR, negative staining, score 0; upper right quadrant, LS, PgR, weak staining, score 1; lower right quadrant, UPS, PgR, moderate staining, score 2; lower left quadrant, LMS, PgR, strong staining, score 3. IHC, immunohistochemistry; TMA, tissue microarray; non-GIST STS, non gastrointestinal stromal tumor soft-tissue sarcoma; ER, estrogen receptor; PgR, progesterone receptor; AS, angiosarcoma; LS, liposarcoma; CCS, clear cell sarcoma; LMS, leiomyosarcoma; MPNST, malignant peripheral nerve sheath tumor; UPS, undifferentiated pleomorphic sarcoma.

Table II. Prognostic clinicopathological variables as predictors for disease-specific survival (univariate analyses, log-rank test) in 249 non-GIST STSs, for all patients and separately for men and women.

	Patients, n (%)			Median survival (months)			5-year survival (%)			P-value		
Characteristic	A	M	W	A	M	W	A	M	W	A	M	W
Age												
≤20	20 (8)	10 (50)	10 (50)	15	15	11	40	30	0	0.126	0.341	0.310
21-60	113 (45)	54 (48)	59 (52)	68	67	59	52	54	49			
>60	116 (47)	46 (40)	70 (60)	30	29	32	40	40	40			
Gender												
Male	110 (44)			41			46			0.390		
Female	139 (56)			45			45					
Nationality												
Norwegian	167 (67)	74 (44)	93 (56)	63	NR	57	51	57	42	0.011	0.030	0.145
Russian	82 (33)	36 (44)	46 (56)	22	22	26	34	28	40			
Histological type												
UPS	68 (27)	35 (51)	33 (49)	29	37	22	40	41	39	0.102	0.402	0.073
LMS	67 (27)	14 (20)	53 (80)	45	26	48	46	43	47			
LS	34 (14)	18 (53)	16 (47)	NR	NR	NR	67	67	67			
MF/MFT	20 (8)	7 (35)	13 (65)	43	43	120	50	38	54			
AS	13 (5)	8 (62)	5 (38)	10	15	5	31	0	20			
RMS	16 (6)	8 (50)	8 (50)	17	15	32	38	25	50			
MPNST	11 (5)	5 (45)	6 (55)	49	NR	14	45	80	0			
SS	16 (6)	11 (69)	5 (31)	31	31	31	29	34	20			
Other STS	4 (2)	4 (100)	0 (0)	NR	41	45	75	75	-			
Site												
Extremities	89 (36)	45 (51)	44 (49)	100	67	100	53	53	54	0.348	0.487	0.688
Trunk	47 (29)	25 (53)	22 (47)	32	37	17	44	42	46			
Retroperitoneum	37 (25)	15 (41)	22 (59)	25	21	36	38	31	42			
Head and neck	18 (7)	12 (67)	6 (33)	15	12	15	41	36	0			
Viscera	58 (23)	13 (22)	45 (78)	30	NR	29	42	59	37			
Tumor size												
<5 cm	74 (30)	33 (45)	41 (55)	127	NR	127	57	53	60	0.027	0.588	0.019
5-10 cm	91 (37)	38 (42)	53 (58)	44	41	45	45	47	44			
>10 cm	81 (32)	37 (46)	44 (54)	28	38	23	36	41	33			
Missing	3 (1)	2 (67)	1 (33)									
Histological grade												
1	61 (25)	32 (52)	29 (48)	NR	NR	NR	74	70	79	<0.001	0.001	<0.001
2	98 (39)	41 (42)	57 (58)	41	41	45	45	45	45			
3	90 (36)	37 (41)	53 (59)	16	21	15	26	28	25			
Tumor depth												
Superficial	17 (7)	10 (59)	7 (41)	NR	NR	NR	93	88	100	<0.001	0.022	0.006
Deep	232 (93)	100 (43)	132 (57)	36	40	30	42	43	42			
Metastasis at the time of diagnosis												
Yes	43 (17)	21 (49)	22 (51)	10	11	9	10	0	9	<0.001	<0.001	<0.001
No	206 (83)	89 (43)	117 (57)	76	NR	75	53	56	51			

Table II. Continued.

Characteristic	Patients, n (%)			Median survival (months)			5-year survival (%)			P-value		
	A	M	W	A	M	W	A	M	W	A	M	W
Surgery												
Yes	228 (92)	98 (43)	130 (57)	5	67	54	50	53	48	<0.001	<0.001	<0.001
No	21 (8)	12 (57)	9 (43)	59	5	4	0	0	0			
Resection margins												
Free	178 (71)	77 (43)	101 (57)	127	NR	75	66	64	51	<0.001	<0.001	<0.001
Not free or no surg.	71 (29)	33 (46)	38 (54)	10	10	10	18	0	28			
Chemotherapy												
No	191 (77)	88 (46)	103 (54)	52	67	48	47	51	44	0.424	0.023	0.396
Yes	58 (23)	22 (38)	36 (62)	29	15	38	40	27	47			
Histological grade												
Radiotherapy												
No	176 (71)	75 (43)	101 (57)	48	41	48	46	47	47	0.590	0.991	0.389
Yes	73 (29)	35 (48)	38 (52)	38	41	22	43	45	42			

Non-GIST STS, non-gastrointestinal stromal tumor soft-tissue sarcoma; A, all; M, men; W, women; UPS, undifferentiated pleomorphic sarcoma; LMS, leiomyosarcoma; LS, liposarcoma; MF/MFT, malignant fibroblastic/myofibroblastic tumors; AS, angiosarcoma; RMS, rhabdomyosarcoma; MPNST, malignant peripheral nerve sheath tumor; SS, synovial sarcoma; NR, not reached; NOS, not otherwise specified.

Discussion

Expression of ER and PgR is a routinely investigated indicator of endocrine therapy success in breast cancer (12,13) and a modest, but significantly better overall survival of anti-estrogen receptor therapy is documented (14). ER and PgR are also reported to be positive prognosticators of leiomyosarcomas in uterus (18). However, extrauterine sarcomas have barely been explored in this context. The distribution and prognostic value of expression of these steroid hormone receptors in STSs are therefore of great scientific interest.

In our large-scale retrospective study, ER showed significantly favourable influence on survival in female patients, while PgR was an unfavourable prognosticator for men in the univariate analyses. The coexpression of ER^-/PgR^+ was a significantly independent negative prognostic indicator of DSS. To our knowledge this is the first prognostic evaluation of these biomarkers in whole-array non-GIST STSs.

Steroid hormones, especially estrogens, but also progestins, are known to stimulate the progression of breast cancer as well as other gynaecological tumors. The expression of ER and PgR, as docking sites for the corresponding hormones, can therefore serve as a predictor of tumor response to both surgical and medical endocrine therapy. For more than 3 decades, ER has been the most important biomarker measured for the management of breast cancer, due to considerable benefit of hormone-ablation therapy for ER-positive in contrast to ER-negative breast cancers (12,13).

Due to possible toxicity and adverse effects of endocrine therapy it was essential to establish the cut-off point for ER and PgR expression. For ligand-binding assays (LBA) which was used until about early 1990s, this threshold value varied from 3 to 20 femtomol/l. After the establishment of IHC methods, the corresponding value fluctuated between 1 and 10% positive cells (17,19). Several studies showed that even low steroid hormone expression may subsequently have importance for endocrine therapy (20). The recent issued guideline recommendations by the American Society of Clinical Oncology/College of American Pathologists determine 1% positivity as a cut-off value in breast cancer both for ER and PgR based on multiple clinical trials (21).

A diversity of soft tissue tumors express both ER and PgR (22,23), but there is also uncertainty concerning steroid hormone receptor expression value in the mesenchymal tumors. Klemi *et al* showed effect of hormone-ablation therapy in aggressive intraabdominal fibromatosis (24). Leithner and colleagues found ER positivity in a minority of 80 fibromatosis patients, while PgR was invariably negative, and concluded that the published effects of antioestrogens in the treatment of aggressive fibromatoses may not be attributable to estrogen receptor (25). However, the established positivity cut-off in this study was 10%, which could have distorted the result.

Leiomyomatous tumors of the uterus are most studied in the context of steroid hormone receptor expression. Generally, it is agreed that the rate of ER and PgR expression rises with the grade of differentiation of malignant tumors (18,26).

Table III. Hormone receptor expression grade in relation to clinicopathological variables in 249 non-GIST STSs.

	ER[a]				PGR[a]			
Characteristic	0 (n=143)	1 (n=56)	2 (n=20)	3 (n=12)	0 (n=166)	1 (n=55)	2 (n=15)	3 (n=13)
Age								
≤20	14	5	1	0	9	6	4	0
21-60	61	24	11	9	74	21	5	10
>60	68	27	8	3	83	18	6	3
Gender								
Male	66	26	11	0	81	23	2	0
Female	77	30	9	12	85	22	13	13
Nationality								
Norwegian	87	43	19	12	110	30	10	11
Russian	56	13	1	0	56	15	5	2
Histological type								
UPS	39	16	7	0	49	14	2	1
LMS	36	11	4	12	41	8	4	11
LS	21	7	3	0	26	6	1	0
MF/MFT	13	4	1	0	11	7	0	0
AS	8	4	1	0	12	1	0	0
RMS	7	6	2	0	7	4	4	0
MPNST	9	1	1	0	8	1	2	0
SS	8	6	0	0	9	3	2	1
Other STS	2	1	1	0	3	1	0	0
Site								
Extremities	56	19	5	0	62	16	5	2
Trunk	25	9	10	0	30	13	3	1
Retroperitoneum	26	7	0	1	27	7	1	0
Head and neck	8	7	2	0	11	3	2	0
Viscera	28	14	3	11	36	6	4	10
Tumor size								
<5 cm	33	21	10	5	46	11	3	8
5-10 cm	58	19	5	6	60	18	6	5
>10 cm	50	15	5	1	57	16	4	0
Histological grade								
1	32	16	7	4	44	7	3	6
2	56	23	8	5	64	24	1	5
3	55	17	5	3	58	14	11	2
Tumor depth								
Superficial	12	3	2	0	15	1	0	1
Deep	131	53	18	12	151	44	14	13
Metastasis at the time of diagnosis								
Yes	116	48	18	10	140	35	10	12
No	27	8	2	2	26	10	5	1
Surgery								
No	8	5	1	1	12	3	2	0
Yes	135	51	19	11	154	42	13	13

Table III. Continued.

Characteristic	ER[a]				PGR[a]			
	0 (n=143)	1 (n=56)	2 (n=20)	3 (n=12)	0 (n=166)	1 (n=55)	2 (n=15)	3 (n=13)
Resection margins								
Not free or no surgery	36	15	8	2	39	18	6	2
Free	107	41	12	10	127	27	9	11
Chemotherapy								
No	117	38	14	5	138	32	9	5
Yes	26	18	6	7	28	13	6	8
Radiotherapy								
No	100	37	17	11	117	28	12	11
Yes	43	19	3	1	49	17	3	2

Non-GIST STS, non-gastrointestinal stromal tumor soft-tissue sarcoma; UPS, undifferentiated pleomorphic sarcoma; LMS, leiomyosarcoma; LS, liposarcoma; MF/MFT, malignant fibroblastic/myofibroblastic tumors; AS, angiosarcoma; RMS, rhabdomyosarcoma; MPNST, malignant peripheral nerve sheath tumor; SS, synovial sarcoma; NR, not reached; NOS, not otherwise specified. [a]Hormone receptor expression grade designated as: 0, negative; 1, weak; 2, intermediate; 3, strong.

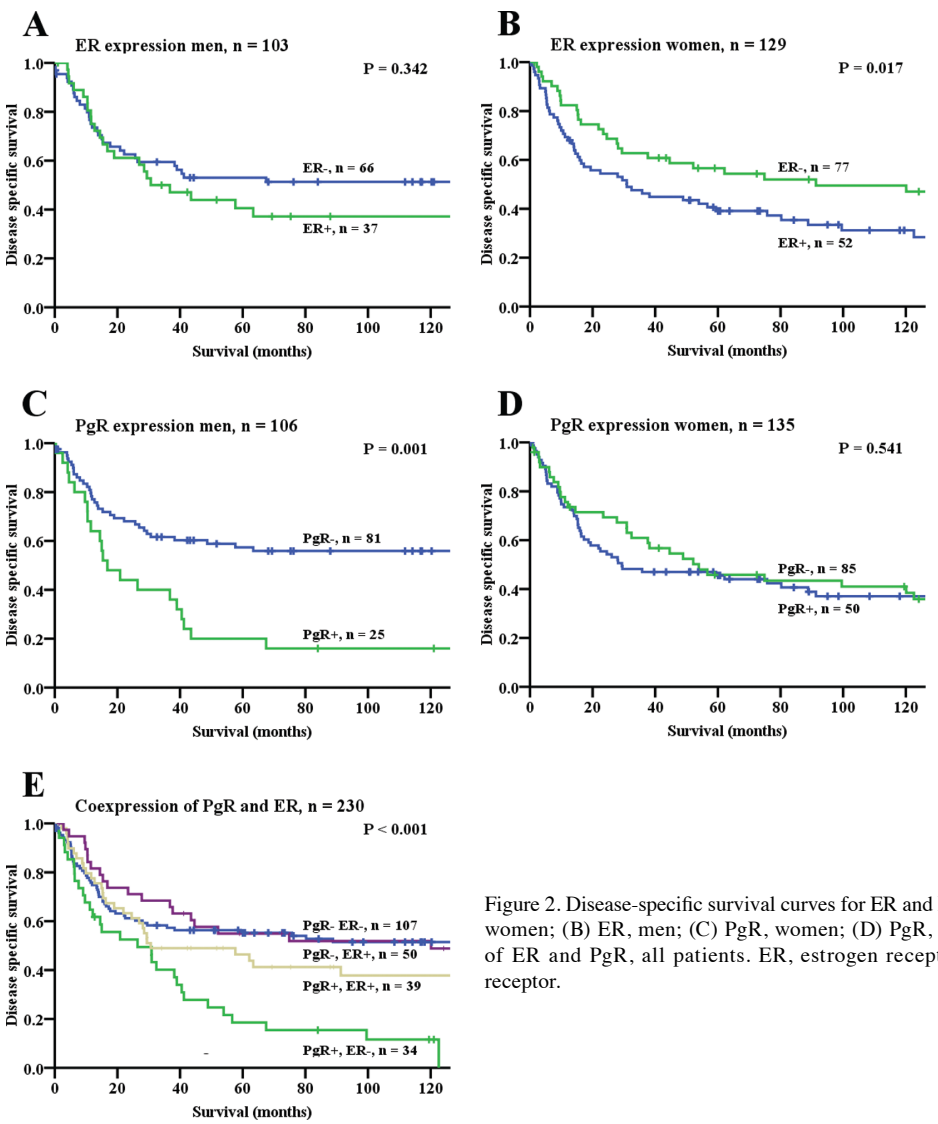

Figure 2. Disease-specific survival curves for ER and PgR expression. (A) ER, women; (B) ER, men; (C) PgR, women; (D) PgR, men; (E) Coexpression of ER and PgR, all patients. ER, estrogen receptor; PgR, progesterone receptor.

Table IV. Tumor expression of ER and PgR and their prognostic impact on disease-specific survival in patients with non-GIST STSs (univariate analyses; log-rank test, n=249), for all patients, separately for men and women and for coexpression of ER and PgR.

Characteristic	Patients (n)	Patients (%)	Median survival (months)	5-year survival (%)	P-value
ER, all patients					
Negative	143	57	41	46	0.333
Positive	89	36	62	50	
Missing	17	7			
ER, men					
Negative	66	60	NR	53	0.342
Positive	37	34	31	41	
Missing	7	6			
ER, women					
Negative	77	55	31	39	0.017
Positive	52	37	91	57	
Missing	10	8			
PgR, all patients					
Negative	166	67	63	51	0.079
Positive	75	30	38	37	
Missing	8	3			
PgR, men					
Negative	81	74	NR	57	0.001
Positive	25	23	17	20	
Missing	4	3			
PgR, women					
Negative	85	61	29	46	0.541
Positive	50	36	54	46	
Missing	4	3			
ER/PgR coexpression, all patients					
ER^-/PGR^-	107	43	127	55	<0.001
ER^-/PgR^+	34	14	26	18	
ER^+/PgR^-	50	20	31	46	
ER^+/PgR^+	39	16	120	55	
Missing	19	7			

Non-GIST STS, non-gastrointestinal stromal tumor soft-tissue sarcoma; ER, estrogen receptor; PgR, progesterone receptor; NR, not reached.

However, the information concerning steroid hormone receptor expression in soft tissue tumors outside the gynaecological area is scarce and controversial. Indeed, the extent of ER and PgR expression shows a surprising discrepancy in different studies (18,22,23,25). This may also be due to different cut-off points used by the authors. Most of the studies utilise established scoring system for breast cancer. Anyway, we do not know whether a scoring system elaborated for gynaecological cancers, which are mostly ER and PgR positive, is valid in sarcomas.

We have modified the Allred score (17) for STSs and used 1% positivity as cut-off value. The strong and moderate (score 3 and 2, respectively) hormone receptor expression occurred mostly in sarcomas of uterus, pelvis and breast, while the weak (score 1) expression of both ER and PgR was surprisingly evenly distributed among location, gender and age. Generally,

Table V. Results of the Cox regression analysis summarizing significant independent prognostic factors in the overall material.

Factor	Hazard ratio	95% CI	P-value
Tumor depth			
Superficial	1.0		
Deep	12	1.7-88	0.013
Malignancy grade			<0.001[a]
1	1.0		
2	2.9	1.7-5.1	<0.001
3	3.3	1.9-6.0	<0.001
Metastasis at the time of diagnosis			
No	1.0		
Yes	2.2	1.4-3.5	0.001
Surgery			
Yes	1.0		
No	5.9	2.9-12	<0.001
Resection-margins			
Free	1.0		
Non-free	2.6	1.7-4.0	<0.001
ER/PgR coexpression			0.007[a]
ER^-PGR^-	1.0		
ER^-PGR^+	1.9	1.2-3.1	0.008
ER^+PGR^-	1.4	0.86-2.3	0.183
ER^+PGR^+	0.73	0.43-1.3	0.938

UPS, undifferentiated pleomorphic sarcoma; MF/MFT, malignant fibroblastic/myofibroblastic tumors; MPNST, malignant peripheral nerve sheath tumor; NOS, not otherwise specified; ER, estrogen receptor; PgR, progesterone receptor. [a]Overall significance as a prognostic factor.

36% of the tumors expressed ER and 30% expressed PgR in our material, which is partly in agreement with findings of Chaudhuri *et al* (22) who found ER to be positive in 24% of 29 investigated STS. PgR, however, was positive in just 1 of 29 (4%) tumors in their cytosol assay-based study with a cut-off value 10 femtomol/l. In our study, ER expression had positive impact on survival in women in univariate analysis, but failed to show any significant value in the Cox proportional hazards analysis.

The prognostic value of PgR with regard to antiestrogen therapy effect is controversial (27), but the same criteria and positivity threshold for PgR as for ER is recommended (21). PgR expression is shown to have a positive prognostic impact in meningeomas (28). In our study, PgR expression showed a clearly negative impact on DSS in men and slightly positive, but not significant influence on survival in women.

The value of ER/PgR coexpression profiles is well studied in breast carcinoma. Shortly, any hormone receptor positivity gives better prognosis for success of antihormonal therapy (29,30). We were not able to find any available published investigation of the prognostic impact of ER/PgR coexpression profiles on DSS and overall survival without relation to endocrine therapy. In our study, the ER^-/PgR^+ profile was a significantly unfavourable factor for the whole patient cohort both in univariate and in multivariate analysis. Interestingly, such a profile occurred in only 2% of patients in one large-scale study, based on 3000 breast cancer cases (29), while in our STS study this profile was seen in 14% of the tumors.

In conclusion, we have characterized occurrence, distribution and prognostic value of ER and PgR in non-GIST STS. ER was a positive prognosticator in women, while PGR was a negative prognosticator in men. The ER^-/PgR^+ profile was a negative prognosticator for the whole patient cohort. Pointing out aggressive phenotypes of sarcomas may help to identify patients who may have benefit from endocrine therapy.

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