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Original contribution

The cutoff for estrogen and progesterone receptor expression in endometrial cancer revisited: a European Network for Individualized Treatment of Endometrial Cancer collaboration study☆,☆☆

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Summary There is no consensus on the cutoff for positivity of estrogen receptor (ER) and progesterone receptor (PR) in endometrial cancer (EC). Therefore, we determined the cutoff value for ER and PR expression with the strongest prognostic impact on the outcome. Immunohistochemical expression of ER and PR was scored as a percentage of positive EC cell nuclei. Cutoff values were related to disease-specific survival (DSS) and disease-free survival (DFS) using sensitivity, specificity, and multivariable regression analysis. The results were validated in an independent cohort. The study cohort ($n = 527$) included 82% of grade 1−2 and 18% of grade 3 EC. Specificity for DSS and DFS was highest for the cutoff values of 1−30%. Sensitivity was highest for the cutoff values of 80−90%. ER and PR expression were independent markers for DSS at cutoff values of 10% and 80%. Consequently, three subgroups with distinct clinical outcomes were identified: 0−10% of ER/PR expression with, unfavorable outcome (5-year DSS = 75.9−83.3%); 20−80% of ER/PR expression with, intermediate outcome (5-year DSS = 93.0−93.9%); and 90−100% of ER/PR expression with, favorable outcome (5-year DSS = 97.8−100%). The association between ER/PR subgroups and outcomes was confirmed in the validation cohort ($n = 265$). We propose classification of ER and PR expression based on a high-risk (0−10%), intermediate-risk (20−80%), and low-risk (90−100%) group.

1. Background

Estrogen receptor (ER) and progesterone receptor (PR) are frequently present in endometrial cancer (EC) and are important biomarkers for outcome [1,2]. ER and PR belong to the superfamily of steroid receptors and mediate the activity of estrogen and progesterone in the endometrium [3,4]. Binding to its ligand leads to translocation of the ligand-receptor complex to the nucleus, where receptor dimers bind specific hormone-responsive DNA elements of target genes [5,6]. In the endometrium, estrogen results in proliferation, whereas progesterone inhibits estrogen-induced endometrial proliferation [7]. Excess estrogen that is insufficiently opposed by progesterone can result in endometrial hyperplasia, which can ultimately lead to development of endometrioid-type endometrial cancer (EEC) [8,9]. EEC is the most common subtype of EC and is characterized by the presence of ER and PR expression and a favorable prognosis [9,10]. In contrast, nonendometrioid EC (NEEC) subtypes such as serous and clear cell carcinomas develop independently from estrogens, often lack ER and PR expression, and have a poor prognosis [10]. The presence of ER and PR in tumor tissue is routinely evaluated by immunohistochemical analysis in EC. Immunohistochemical loss of ER and PR expression in tumor tissue is associated with a higher risk of lymph node metastases, reduced disease-free

survival (DFS) and disease-specific survival (DSS), and lack of response to hormonal therapy [1,11−14]. However, the cutoff value for ER and PR positivity that differentiates best between favorable and unfavorable outcomes is unclear [1,15,16]. Most scoring systems used in EC define receptor positivity based on the percentage of tumor cells exhibiting positive nuclear expression, although combinations of percentages and intensity of staining (scoring indices) are used frequently in research as well [2,17,18]. Currently used cutoff values for receptor positivity in EC are adopted from breast cancer studies in which cutoff values of 1% or 10% are most frequently used [19,20]. To define relevant thresholds for ER and PR expression in EC, we performed analysis in a large retrospectively collected multicenter cohort to determine the cutoff values with the strongest prognostic impact for clinical outcomes in EC.

2. Methods

2.1. ENITEC cohort

2.1.1. Patients

A retrospective multicenter study was performed. The study cohort included patients that were surgically treated for early-stage (FIGO stage I−II) EEC, advanced-stage (FIGO stage III−IV) EEC, or NEEC at one of the European Network for Individualized Treatment of Endometrial Cancer (ENITEC) centers [21]. Patients with complete clinical and pathological data and follow-up of at least 36 months were included, which yielded a cohort containing 1199 patients. From this cohort, 573 postmenopausal patients did not use hormonal substitution therapy and had preoperative biopsies available for analysis. As endometrial biopsies are used to guide primary surgical treatment, this study was performed using preoperative material rather than hysterectomy specimens. After pathological review, 46 patients were excluded because of insufficient amount of tumor tissue ($n = 30$) or only premalignant or benign endometrium in the whole slide ($n = 16$), leaving 527 patients for analysis. The available clinical and pathological characteristics included age at diagnosis, date of diagnosis, body mass index (BMI), CA125 serum levels, postoperative tumor grade and histology, lymphovascular space invasion (LVSI), myometrial invasion (MI), FIGO stage, treatment, recurrence, and outcome (DFS and DSS). Tumor grade was categorized as low grade (grade 1−2) and high grade (grade 3). This study was performed in accordance with the Declaration of Helsinki and was approved by the Institutional Review Board at the Radboud University Medical Center (reference number = 2015−2101).

2.1.2. Hormone receptor analysis

Blank 4-μm sections from formalin-fixed, paraffin-embedded tissue blocks with the preoperative endometrial biopsy specimen were sent to the Radboud university medical center. The endometrial biopsy material was fixed in buffered formalin right after the material was obtained, thereby limiting the cold ischemia time. For each case, one slide was stained with hematoxylin and eosin. Subsequent slides were stained for ER and PR. ER and PR antibodies were generously provided by Dako (Agilent Technologies, Santa Clara, CA, USA). For immunohistochemical staining, antigen retrieval (97 °C for 30 min in Tris/EDTA buffer, pH 9 [Envision FLEX Target Retrieval Solution High pH; DAKO, Agilent Technologies, Santa Clara, CA, USA]) and subsequent blocking of endogenous peroxidase using hydrogen peroxide were performed. Then, slides were incubated with ER antibody (clone SP1 GA084; DAKO, Agilent Technologies, Santa Clara, CA, USA) or PR antibody (clone PgR 1294 GA090; DAKO, Agilent Technologies, Santa Clara, CA, USA). Envision FLEX/HRP (DAKO, Agilent Technologies, Santa Clara, CA, USA) was used, and visualization was performed using Envision FLEX DAB + Chromogen (DAKO, Agilent Technologies, Santa Clara, CA, USA).

Scoring of ER and PR staining in percentages was determined by eyeballing in a semiquantitative manner. The percentage of the whole examined invasive tumor area was estimated by two of the five assessors (C.R., J.B., H.V.N.K.-V., N.C.M.V., and K.v.d.V.) blinded to pathological and clinical characteristics. The percentage of tumor cells exhibiting positive nuclear expression was subsequently categorized into the following categories: ≤1%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, and 100%. Discrepancies in scoring were reviewed in a consensus meeting attended by all assessors.

2.2. Vancouver cohort

2.2.1. Patients

A selection of patients with available clinicopathological findings and tissue microarrays (TMAs) stained for ER and PR expression treated at the Vancouver General Hospital, a tertiary cancer center in Canada, was analyzed [22,23].

2.2.2. Immunohistochemistry

Immunohistochemistry was performed on previously constructed TMAs for ER and PR expression as described by Karnezis et al [24]. In brief, previously constructed TMAs were immunohistochemically stained for ER (1 h at 37 °C, ER antibody clone SP1, RM-9101, diluted 1:25; Thermo, Thermo Fisher Scientific, Waltham, Massachusetts, USA) or PR (16 min at 36 °C, PR antibody clone 1E2 790−2223, undiluted; Ventana, Ventana Medical Systems, Oro Valley, Arizona, USA) expression via the Ventana Discovery Ultra protocol. Antigen retrieval was performed using cell conditioning 1 for 64 min. The slides were incubated. Visualization was performed using the DABmap kit (Ventana Medical Systems, Oro Valley, Arizona, USA). For each patient, two digitalized TMA cores for both ER and PR expression were scored semiquantitatively, defined

Table 1 Overview of clinicopathological findings of the ENITEC cohort.

Characteristic	Number (%), $n = 527$	ER expression in %, mean (SD)	PR expression in %, mean (SD)
Mean age (SD)	65.9 (9)		
Mean BMI (SD)	30.4 (7)		
CA125 levels			
35 and lower	267 (51)	72 (25)	63 (30)[a]
>35	79 (15)	67 (28)	46 (34)
Unknown	181 (34)		
Postoperative grade			
Low-grade (grade 1 or 2)	430 (82)	75 (22)[a]	63 (30)[a]
High-grade (grade 3)	97 (18)	56 (35)	42 (35)
Histology			
Endometrioid	502 (95)	73 (25)[a]	61 (31)[a]
Nonendometrioid	25 (5)	42 (36)	19 (25)
Serous	13 (3)	49 (35)	23 (27)
Clear cell	5 (1)	35 (46)	20 (26)
Other	7 (1)	33 (36)	11 (22)
LVSI			
Yes	81 (15)	73 (25)[a]	50 (36)[a]
No	397 (75)	65 (31)	61 (31)
Unknown	49 (9)		
MI			
<50%	323 (61)	73 (25)	61 (31)
>50%	201 (38)	70 (27)	57 (32)
Unknown	3 (1)		
FIGO stage			
Early (stage I or II)	478 (91)	72 (25)	60 (31)[a]
Advanced (stage III or IV)	49 (9)	65 (32)	47 (35)
Treatment			
Surgery	527 (100)	72 (26)	59 (27)
Adjuvant radiotherapy	259 (51)	71 (27)	57 (32)
Adjuvant chemotherapy	35 (7)	57 (36)	44 (35)
Lymph node metastasis			
Yes	25 (5)	65 (34)	51 (28)
No	271 (51)	71 (25)	59 (37)
Unknown	231 (44)		
Recurrence			
Yes	63 (12)	62 (34)[a]	49 (36)[a]
Local	19 (4)	73 (30)	67 (33)[b]
Regional	9 (2)	73 (30)	52 (37)[b]
Distant	40 (8)	57 (34)	43 (34)
No	462 (88)	73 (26)	61 (32)
Unknown	2 (0)		
Death			
Yes	67 (13)	61 (34)[a]	37 (32)[a]
EC-related	37 (7)	52 (34)	37 (32)
No	449 (85)	73 (25)	61 (32)
Unknown	11 (2)		

Abbreviations: BMI, body mass index; ER, estrogen receptor; PR, progesterone receptor; LVSI, lymphovascular space invasion; MI, myometrial invasion; EC, endometrial cancer; SD, standard deviation; ENITEC, European Network for Individualized Treatment of Endometrial Cancer.

[a] Significant at $P < 0.05$.

[b] Significant at $P < 0.05$ for comparison of local/regional with distant recurrence.

Table 2 Test characteristics of different cutoffs for estrogen and progesterone receptor expression in relation to disease-specific survival.

Cutoff	Sensitivity	Specificity	PPV	NPV	AUC
The value of different cutoffs for estrogen receptor expression in prediction of disease-specific survival					
ER, 1%	18%	95%	24%	94%	0.571
ER, 10%	27%	94%	25%	94%	0.603
ER, 20%	30%	92%	23%	94%	0.611
ER, 30%	30%	90%	19%	94%	0.600
ER, 40%	35%	86%	17%	94%	0.608
ER, 50%	38%	82%	14%	94%	0.601
ER, 60%	46%	76%	15%	95%	0.611
ER, 70%	57%	68%	12%	95%	0.625
ER, 80%	89%	37%	10%	98%	0.632
ER, 90%	100%	8%	8%	100%	0.541
The value of different cutoffs for progesterone receptor expression in prediction of disease-specific survival					
PR, 1%	27%	91%	20%	94%	0.591
PR, 10%	43%	83%	17%	95%	0.631
PR, 20%	49%	79%	16%	95%	0.639
PR, 30%	49%	76%	14%	95%	0.621
PR, 40%	57%	72%	14%	95%	0.641
PR, 50%	57%	68%	12%	95%	0.623
PR, 60%	70%	60%	12%	96%	0.652
PR, 70%	86%	50%	12%	98%	0.683
PR, 80%	97%	25%	9%	99%	0.613
PR, 90%	100%	4%	8%	100%	0.518

Abbreviations: ER, estrogen receptor; PR, progesterone receptor; PPV, positive predictive value; NPV, negative predictive value; AUC, area under the curve.

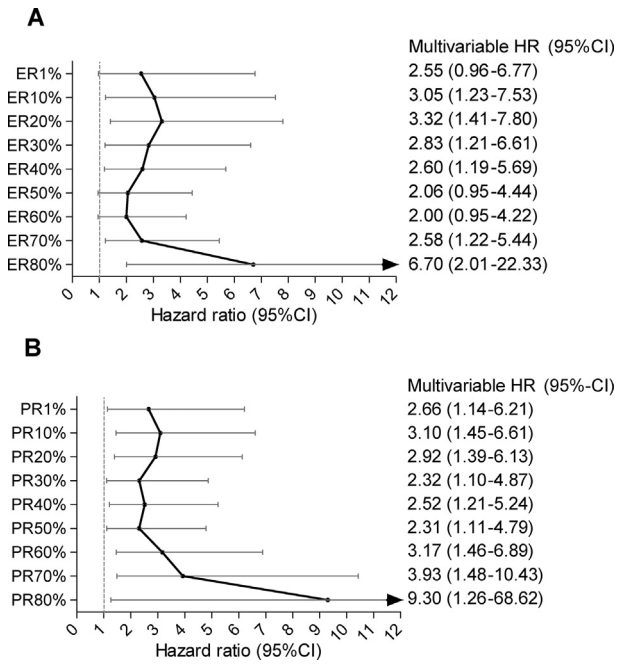

Fig. 1 Multivariable Cox regression analysis of association between estrogen receptor (A) and progesterone receptor (B) expression at different cutoff values with disease-specific survival. The other covariates in multivariable regression analysis are age, grade, histology, lymphovascular space invasion, myometrial invasion, and FIGO stage. HR, hazard ratio; CI, confidence interval; ER, estrogen receptor; PR, progesterone receptor.

as 0%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, and 100%, by two assessors (W.J.v.W. and C.R.) by estimating the percentage of positive nuclei in the whole invasive tumor area by eyeballing. As the average of two TMA scores of two reviewers was assessed, resulting scores could be outside the predefined scores (such as 12% or 83%). These scores were rounded off into the nearest category (e.g., 15% was categorized as 20%). Both assessors were blinded for clinical characteristics. Discrepancies were discussed with an expert gynecological pathologist (J.B.), with whom consensus was reached.

2.2.3. Statistical analysis

The relation between ER and PR expression and established prognostic factors was analyzed using the Student t-test. For different categories of ER and PR expression, ranging from $\leq$1% to 90%, sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and area under the curve (AUC) were calculated for the prediction of DSS and DFS. The association between the different cutoff values for ER and PR expression and DSS and DFS was investigated using multivariable Cox regression analysis. The length of DSS was calculated from the date of diagnosis to the date of death caused by EC or, for surviving patients, to the date of last follow-up. The length of DFS was calculated from the date of diagnosis to the date of recurrence or to the date of last follow-up for patients with no sign of disease recurrence. Known risk factors including age at diagnosis, date of diagnosis, BMI, CA125 serum levels, postoperative tumor grade and histology, LVSI, MI, and FIGO stage were included in the analyses. Variables identified by univariable regression analysis with $P < 0.10$ were used for multivariable regression analysis. For the cutoff values with the strongest associations with outcomes, Kaplan-Meier curves were constructed. The interobserver variability for scoring ER and PR expression was evaluated using the Cohen's κ-value. P-values <0.05 were considered to indicate a significant difference. SPSS version 25 (SPSS IBM, New York, NY, USA) statistical software was used to perform the statistical analyses.

3. Results

3.1. ENITEC cohort

A total of 527 patients with EC were included in the analysis. The clinicopathological findings of this cohort and the correlations with mean ER/PR expression are

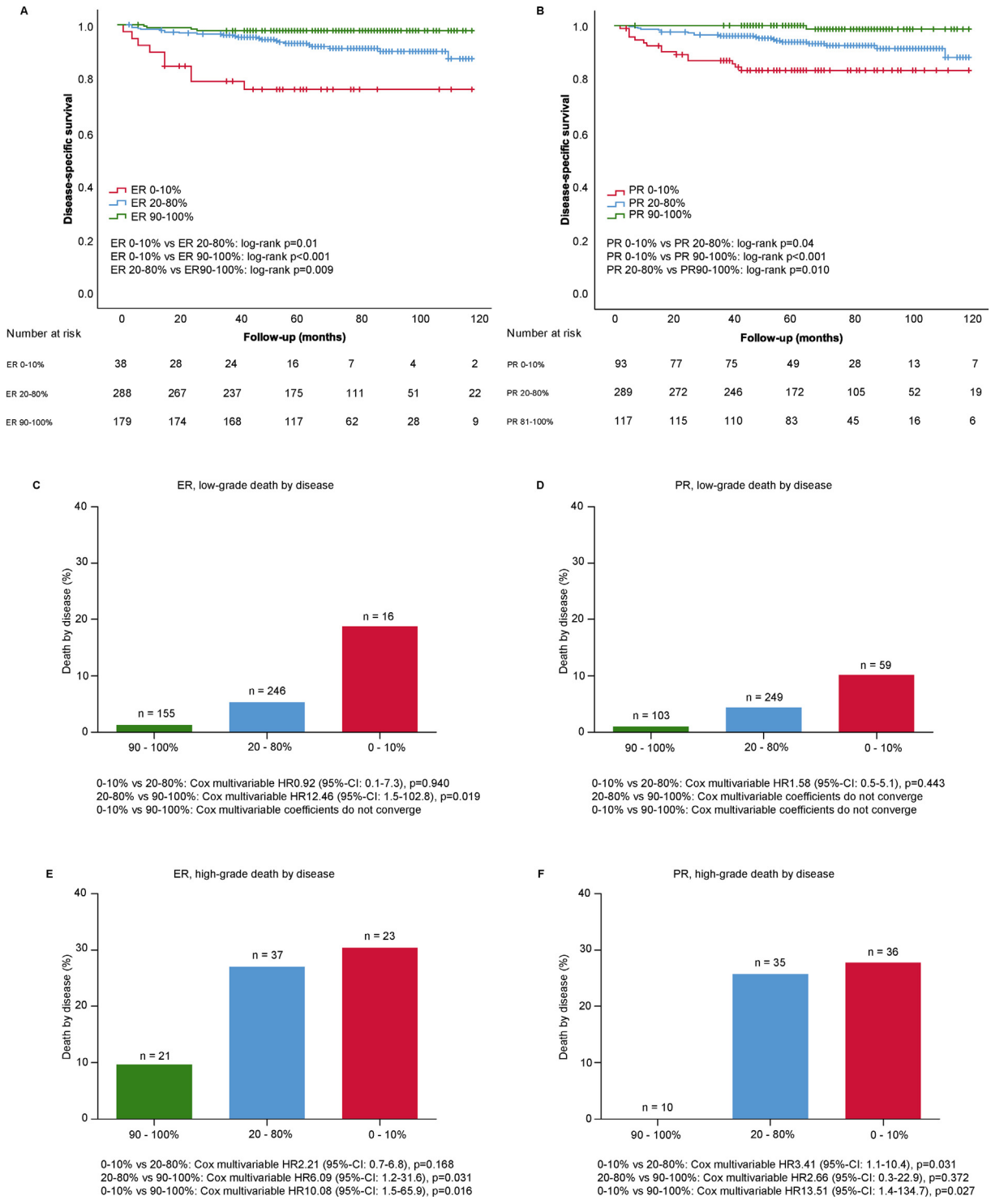

Fig. 2 Association between ER expression (A) and PR expression (B) as per high-risk (0−10%), intermediate-risk (20−80%), and low-risk (90−100%) groups with disease-specific survival in the complete ENITEC cohort and in low-grade (C−D) and high-grade subgroups (E−F). NEEC was included in the high-grade subgroup. The other variables in Cox variable regression analysis are age, histology, lymphovascular space invasion, myometrial invasion, and FIGO stage. HR, hazard ratio; CI, confidence interval; ER, estrogen receptor; PR, progesterone receptor; NEEC, nonendometrioid endometrial cancer; ENITEC, European Network for Individualized Treatment of Endometrial Cancer.

Table 3 Overview of clinicopathological findings of the Vancouver cohort.

Characteristic	Number (%), $n = 265$	ER expression in %, mean (SD)	PR expression in %, mean (SD)
Mean age (SD)	65.5 (12)		
Mean BMI (SD)	31.3 (10)		
Grade			
Low-grade (grade 1 or 2)	95 (36)	77 (18)[a]	56 (31)[a]
High-grade (grade 3)	170 (64)	40 (35)	21 (29)
Histology			
Endometrioid	182 (69)	63 (32)[a]	43 (34)[a]
Nonendometrioid	79 (30)	33 (33)	12 (23)
Serous	67 (25)	34 (33)	12 (22)
Clear cell	1 (0)	2 (0)	1 (0)
Other	11 (4)	31 (35)	17 (24)
Undifferentiated	4 (2)	33 (42)	24 (44)
LVSI			
Yes	116 (44)	46 (34)[a]	23 (29)[a]
No	130 (49)	60 (34)	43 (40)
Unknown	19 (7)		
MI			
<50%	145 (55)	61 (33)[a]	41 (36)[a]
>50%	113 (43)	44 (35)	25 (31)
Unknown	7 (3)		
FIGO Stage			
Early (stage I or II)	181 (68)	57 (35)[a]	38 (35)[a]
Advanced (stage III or IV)	79 (30)	44 (35)	22 (30)
Unknown	5 (2)		
Treatment			
Surgery	289 (100)		
Adjuvant radiotherapy	34 (13)	51 (34)	34 (36)
Adjuvant chemotherapy	35 (13)	42 (35)	22 (30)
Adjuvant chemoradiotherapy	67 (25)	41 (34)	20 (26)
No adjuvant treatment	123 (46)	64 (32)	44 (35)
Recurrence			
Yes	75 (28)	44 (34)[a]	22 (31)[a]
No	178 (67)	58 (35)	39 (35)
Unknown	12 (5)		
Death			
Yes	96 (36)	48 (34)[a]	27 (33)[a]
EC-related	63 (25)	43 (34)	22 (28)
No	169 (64)	57 (35)	37 (35)

Abbreviations: SD, standard deviation; BMI, body mass index; ER, estrogen receptor; PR, progesterone receptor; LVSI, lymphovascular space invasion; MI, myometrial invasion; EC, endometrial cancer.

[a] Significant at $P < 0.05$.

summarized in Table 1. The mean age was 65.9 years, and the mean BMI was 30.4. Most patients had early stage disease (91%), low-grade disease (82%) with EEC histology (95%). Among patients with early EC, 54.8% underwent lymphadenectomy. Recurrences occurred in 12% of patients, and 7% of patients died owing to EC. The mean ER expression was 72% (standard deviation [SD] = 26%), and the mean PR expression was 59% (SD = 27%). A significantly higher mean ER and PR expression was found in low-grade compared with high-grade tumors (ER: 75% vs. 56%, respectively; PR: 63% vs. 42%, respectively). In addition, a significantly higher PR expression was found in early-stage than in advanced-stage EC (60% vs. 47%, respectively). ER and PR expression were significantly lower in patients with recurrence than in nonrecurrent cases. PR expression was significantly higher in patients with local/regional recurrences than in patients with distant recurrence; for ER expression, the difference was not significant ($P = 0.087$). ER and PR expression were significantly lower in patients who died owing to EC than in those who died owing to other causes.

3.1.1. ER and PR expression at different cutoff values

Different categories of ER and PR expression cutoff values, starting at 1% and 10%, with subsequent increases of 10%, were defined. An overview of the sensitivity, specificity, PPV, NPV, and AUC for each cutoff value is provided for DSS in Table 2 and for DFS in Appendix A.

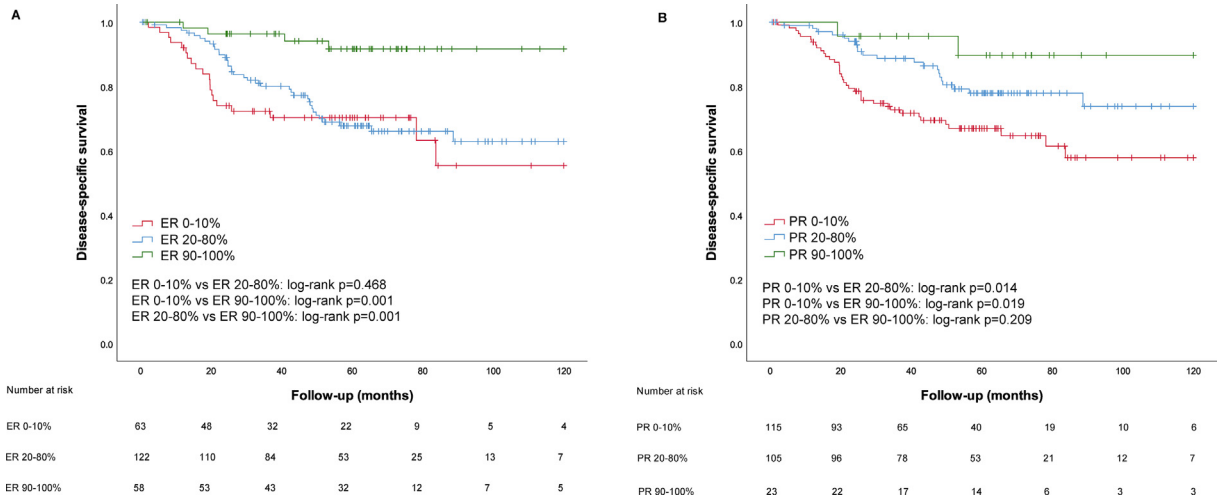

Fig. 3 Kaplan-Meier analysis of association between ER and PR expression as per high-risk (0−10%), intermediate-risk (20−80%), and low-risk (90−100%) groups with disease-specific survival in the Vancouver cohort. ER, estrogen receptor; PR, progesterone receptor.

The sensitivity of ER expression for DSS and DFS showed a substantial increase from the 70% to the 80% cutoff (57% and 41%, respectively, at 70% cutoff versus 89% and 70%, respectively, at 80% cutoff), indicating that patients with an ER expression of 90−100% have a lower risk of adverse outcomes than those with lower cutoff values. The AUC was similar for 70% and 80% cutoff values.

Similar results were found for PR expression: a cutoff of 80% resulted in a sensitivity of 97% for DSS and 86% for DFS compared with 86% and 65%, respectively, at a 70% cutoff. The AUC was similar for DFS and lower for the 80% cutoff value than for the 70% cutoff value for DSS.

The specificity for identification of patients with impaired DSS and DFS was highest at a range of cutoff values from 1% to 30% for ER and PR expression.

3.1.2. Values of ER and PR expression in multivariable analysis

The association between different cutoff values of ER and PR expression and outcomes was analyzed using multivariable Cox regression analyses including, age, grade, histology, LVSI, FIGO stage, CA125 levels, and ER or PR expression. As shown in Fig. 1A, ER was an independent marker for DSS at cutoff values of 1−40% and 70−80%. The association with DSS was strongest at the 80% cutoff value, indicating that the ratio of disease-specific mortality is highest when applying the cutoff of ≤80% expression. PR was an independent marker for DSS at all cutoff values (Fig. 1B). ER expression was an independent marker for DFS at the cutoff values 10−30%, with the strongest association at the 10% cut-off value (Appendix B). PR was an independent marker for DSS at all cutoff values and for DFS at cutoff values of 10−20% (Fig. 1B and Appendix B). A cutoff value of ER 1% was not significantly associated with DSS or DFS.

3.1.3. Risk groups

Based on the results for sensitivity, specificity, and multivariable regression analysis, three risk groups were defined using the 10% and 80% cutoff value as both cutoff values showed consistent significant associations with outcomes, and the 80% cutoff value also had a high sensitivity for DSS and DFS. Cases with 0−10% of ER/PR expression had a high risk for adverse outcomes, cases with 20−80% of ER/PR expression had an intermediate risk, and cases with ER/PR expression of 90−100% had a low risk (Fig. 2). Patients with 0−10% of ER expression had a 5-year DSS of 75.9% (95% confidence interval [CI]: 62.5−89.3), which was significantly lower than for patients with an ER expression of 20−80% (5-year DSS = 93.0% [95% CI: 90.0−95.9], $P = 0.01$) and an ER expression of 90−100% (5-year DSS = 97.8% [95% CI: 95.7−99.9], $P < 0.001$, Fig. 2A). The 5-year DSS of patients with 20−80% of ER expression was also significantly lower than in patients with an ER expression of 90−100% ($P = 0.009$). Similarly, patients with 0−10% of PR expression had a lower 5-year DSS (83.3% [95% CI: 75.8−90.8]) than patients with a PR expression of 20−80% (93.9% [95% CI: 91.1−96.7], $P = 0.04$) and 90−100% (100%, $P < 0.001$, Fig. 2B). The 5-year DSS of patients with a PR expression of 20−80% was also significantly lower than in patients with 90−100% of PR expression ($P = 0.01$). The 5-year DFS for 0−10% of ER and PR expression was 67.5% (95% CI: 53.0−82.0) and 78.8% (95% CI: 70.6−87.0), respectively, which was significantly lower than that for 20−80% (ER: 89.9% [95% CI: 86.4−93.3], PR: 89.5 [95% CI: 86.0−93.0]) and 90−100% of ER and PR expression (ER: 90.4% [95% CI: 86.1−94.7], PR: 92.3% [95% CI: 87.6−97.0]). The DFS for the 20−80% and 90−100% risk groups were similar (see Appendix C). Fig. 2C−F and Appendix C show DSS and DFS in low- and high-grade carcinomas including Cox

multivariable regression analysis with the high-, intermediate-, and low-risk groups. Most recurrences and deaths are observed in carcinomas with 0−10% of ER/PR expression, whereas the group with 90−100% of ER/PR expression had the lowest proportion of cases with adverse outcomes. The 0−10% ER/PR group has a significantly shorter DSS and DFS than the 90−100% group with respect to high-grade EC. Regarding low-grade EC, analysis is hampered by limited numbers of events in the groups.

The Cohen's κ value for scoring ER/PR expression as per the three risk groups was 0.703.

3.1.4. Combination of ER and PR expression

A combination marker for ER and PR expression was analyzed in relation to the outcomes. A combined ER and PR analysis, in which both ER and PR expressions were ≤10% to be defined as negative, showed a sensitivity of 22% for DSS and 14% for DFS and a specificity of 96% for DSS and 95% for DFS (see Appendix D). ER and PR expression was discordant in 63 cases: 61 cases with positive ER and negative PR and 2 cases with negative ER and positive PR expression. Application of the 80% cutoff value, in which both ER and PR expression had to be >80% to be defined as positive, resulted in a sensitivity of 100% for DSS and 89% for DFS and a specificity of 20% for DSS and DFS. Discordances between ER and PR occurred in 115 cases: 89 cases with positive ER and negative PR and 26 cases with negative ER and positive PR expression.

3.2. Vancouver cohort

In total, 265 patients with EC were included in the validation cohort. The clinicopathological findings of this cohort and the correlations with mean ER/PR expression are shown in Table 3. Compared with the ENITEC cohort, the validation cohort included a higher proportion of patients with high-grade tumors (64% in the Vancouver cohort, 18% in the ENITEC cohort) and more advanced-stage tumors (30% in the Vancouver cohort, 9% in the ENITEC cohort). Recurrences occurred in 29% patients, and EC-related deaths occurred in 25% patients. The mean ER expression was 53% (SD = 35%), and the mean PR expression was 34% (SD = 34%). ER and PR expression were significantly lower in patients with a recurrence than in nonrecurrent cases. Patients who died owing to EC had a significantly lower ER and PR expression than in patients with non−EC-related mortality or patients who were alive at the end of follow-up.

3.2.1. Validation

The risk classification for ER and PR expression showed that patients with an ER expression of 0−10% had a significantly lower 5-year DSS (70.8% [95% CI: 59.0−81.6]) than patients with an ER expression of 90−100% (91.6% [95% CI: 83.8−99.4], Fig. 3A). There was no difference in DSS between the group with 0−10% of ER expression and the group with 20−80% of ER expression (5-year DSS = 67.7% [95% CI: 58.7−76.7]). For PR expression, the 0−10% group had a significantly lower 5-year DSS (66.9% [95% CI: 57.7−76.1]) than patients with a PR expression of 90−100% (5-year DSS = 89.7% [95% CI: 76.0−100.0]) and 20−80% (77.9% [95% CI: 69.3−86.5], Fig. 3B). The 5-year DFS for ER expression of 0−10% and 20−80% was 62.6% [95% CI: 50.7−74.5] and 62.3% [95% CI: 53.8−70.8], respectively, which was significantly lower than that for 90−100% of ER expression (89.6% [95% CI: 82.1−97.1], Appendix E). The 5-year DFS for a PR expression of 0−10% was 58.2% (95% CI: 49.3−67.1), which was significantly lower than that for a PR expression of 20−80% and 90−100% (76.1% [95% CI: 68.0−84.2] and 88.8% [95% CI: 76.3−100.0], respectively). The Cohen's κ value for scoring ER/PR expression as per the three risk groups in this cohort was 0.796.

4. Discussion

In the present study, we have confirmed the prognostic value of ER and PR expression and determined the cutoff values with the strongest prognostic value for clinical outcomes in EC. Based on our results, we propose an EC-specific classification for ER and PR expression into three groups: a high-risk group with ER and PR expression between 0 and 10% and unfavorable outcomes, an intermediate-risk group with ER/PR expression between 20 and 80%, and a low-risk group with ER/PR expression between 90 and 100% with a favorable outcome. The validity of this EC-specific classification was confirmed in an independent validation cohort consisting of predominantly high-grade EC. The low- and high-risk groups were consistently identified in low- and high-grade cancers, whereas the intermediate group showed a variable outcome depending on the tumor grade.

The results of our study indicate that patients with ER/PR expression >10% exhibit different clinical behavior and can be further stratified in intermediate- and low-risk groups. This highlights the relevance of reporting semi-continuous values for ER/PR expression as opposed to dichotomous values (eg, positive, negative). Previous studies have focused on one cutoff value (eg, 1% or 10% of positive tumor nuclei or a staining intensity index cutoff value of 3 [on a 0−9 scale]) to differentiate between favorable and unfavorable prognosis [25−28]. To our knowledge, this is the first study that identified two cutoff values for ER/PR expression. The cutoff values of 1% and 10% are most frequently used for ER/PR expression in endometrial and breast cancer worldwide [19,20]. In this study, the ≤10% cutoff value was shown to be superior to the ≤1% cutoff value, as the ≤1% cutoff value lacked

significant associations with outcomes in multivariable regression analysis. These findings are supported by results of the study on breast cancer by Yi et al. [19], in which cutoff values of 1% and 10% were compared among 9639 patients. Patients with an ER expression of 1−9% and <1% had a similar outcome, whereas patients with an expression ≥10% had a better outcome than those with an expression of 1−9% and <1%. The recently updated American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) guideline on ER and PR testing in breast cancer endorsed the clinical importance of the 10% cutoff value for ER, also in relation to prediction of response to adjuvant endocrine treatment [20]. In our study, the cutoff values of 10% and 20% for ER and PR positivity performed similarly in terms of sensitivity and specificity and associations with outcomes in multivariable analysis. We selected the 10% cutoff value because it is a highly reproducible cutoff value and it is consistent with currently used cutoff values in EC and breast cancer [29]. ER and PR expression was observed both in EEC and NEEC. Although NEECs are considered to develop independent of estrogen, ER and PR expressions are present in around 40% of NEEC cases, which is in line with the results of this study [30,31]. The cutoff value of 80% showed a higher sensitivity than the 70% cutoff value, whereas the AUCs were mostly similar between the two cutoffs. Therefore, the cutoff value of 80% was selected in the EC-specific classification, indicating that patients with an ER or PR expression of 90−100% have a low risk for adverse outcomes. These findings are in line with the results of Weinberger et al. [32], in which cutoff values of 78% for ER and 88% for PR provided optimal cutoff values to stratify patients with EC into low- and high-risk groups based on preoperative biopsies. To our knowledge, there are no other studies available that explored the 80% cutoff value in relation to prognosis in EC. The results of this study suggest that ER and PR expressions have complementary value in identifying high-risk and low-risk populations. At the cutoff value of 10%, ER expression had a higher specificity than PR expression, indicating that ER expression ≤10% could be applied to identify high-risk cases. At the cutoff value of 80%, PR expression had a higher sensitivity than ER expression, suggesting that PR expression is, more than ER expression, able to identify a low-risk population. Based on these data, no superiority for ER or PR expression could be found, supporting the routine performance of both ER and PR expression in all patients with EC.

For ER and PR expression assessment in EC, preanalytic, analytic, and postanalytic factors play a role, as in breast cancer. In breast cancer, these factors have been addressed by the ASCO/CAP guidelines for ER and PR expression testing in breast cancer [20]. In the present study, we expect no problems in the preanalytic phase as the biopsy material was fixed in buffered formalin as soon as it was acquired. In the analytic phase, the type of antibody used plays an important role. The ER and PR antibodies we used in the present study for EC are also used in breast cancer [33]. In both cohorts (ENITEC and Vancouver), we used the same clone SP1 for ER and two different clones for PR (PgR 1294 in the ENITEC cohort and 1E2 in the Vancouver cohort). As is known for breast cancer, different clones for the ER and PR can give different results for ER and PR expression, and this should be appreciated in interpreting results. This is of importance as different pathology laboratories may use different antibodies [33]. An important postanalytic factor is the interpretation of ER and PR expression by the pathologist. We reached a Cohen's κ value of 0.703 and 0.796 for scoring ER/PR expression based on the three risk groups in the ENITEC and Vancouver cohort, respectively. This is in line with results of recent studies on EC [1,11]. Immunohistochemical analysis for ER and PR expression is currently performed manually. Digital image analysis can also assist in scoring biomarkers and can contribute to a more objective and reproducible evaluation. Interestingly, in prostate cancer, digital image analysis was shown to significantly improve interobserver variability for scoring of ER expression [34]. The cutoff values identified in this study could guide both manual and digital evaluation of immunohistochemical analysis for ER and PR expression.

In 2013, The Cancer Genome Atlas (TCGA) suggested a new classification of EC subgroups based on four prognostic subgroups with distinct molecular signatures [35]. Available evidence on the prognostic value of ER/PR expression within these subgroups has shown contradictory results possibly owing to application of multiple cutoff values for ER/PR expression [24,36]. Further integration of ER/PR expression, using the updated cutoff values, with the TCGA classification is relevant to better identify the prognostic value of ER and PR expression within the TCGA subgroups.

The strengths of this study include confirmation of the results in an independent study cohort and the use of a large number of patients in both cohorts. Validation of the results in a cohort with a substantial number of nonendometrioid tumors indicates that the EC-specific classification for ER/PR expression can be applied in EEC and NEEC, although the prognostic relevance appears most pronounced in low-grade EC. In addition, scoring for ER and PR as per this EC-specific system is easy to use and adds relevant prognostic information to current clinical practice. Finally, ER and PR are affordable immunohistochemical markers that are available in pathological laboratories worldwide, and thus, this scoring system could be easily implemented in routine practice. However, there are also some limitations to address. First, lymphadenectomy was not performed in a substantial number of cases, possibly affecting tumor staging. Second, the correlation between ER and PR expression in preoperative and postoperative material has not been investigated in EC. However, in breast cancer, multiple studies have reported concordance rates of at least 85% between biopsy and surgical specimens, indicating

that validation of findings in preoperative material can be performed in tumors from surgical specimens [37,38]. Third, we did not relate the reported cutoff values to staining intensity scores. However, the percentage score is more relevant than staining intensity scores as confirmed by a recent study that compared a percentage score with staining intensity scores in EC [15,20]. In addition, data on molecular subgroups were lacking (eg, *POLE* and mismatch repair status), and therefore, it was not possible to investigate the prognostic value of ER and PR expression within the TCGA molecular subgroups. Finally, the agreement between ER and PR expression in whole slide and TMA, as used in our study, is supported by a recent study from Visser et al. [39], in which discordant expression was found in just 6% of cases.

In conclusion, we have identified prognostic groups based on ER and PR expression, and we propose classification based on a high-risk (0−10%), intermediate-risk (20−80%), and low-risk (90−100%) group.

Ethics approval and consent to participate

This study was performed in accordance to the Declaration of Helsinki and was approved by the Institutional Review Board at the Radboud University Medical Center (reference number = 2015−2101). The need to obtain consent was waived based on the code of conduct for responsible use of human tissue in medical research [40].

Consent for publication

Not applicable.

Research data availability

The data can be made available on reasonable request from the authors.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.humpath.2020.12.003.

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