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The cut-off for estrogen and progesterone receptor in endometrial cancer revisited: an ENITEC collaboration study

Short title: The cut-off for ER and PR in endometrial cancer

Willem Jan van Weelden [1]*, Casper Reijnen [1,2]*, Heidi V.N. Küsters-Vandevelde [3], Johan Bulten [4], Peter Bult [4], Samuel Leung [5], Nicole C.M. Visser [6], Maria Santacana [7], Peter Bronsert [8], Marc Hirschfeld [9,10], Eva Colas [11], Antonio Gil-Moreno [11,12], Armando Reques [13], Gemma Mancebo [14], Jutta Huvila [15], Martin Koskas [16], Vit Weinberger [17], Marketa Bednarikova [18], Jitka Hausnerova [19], Marc P.L.M. Snijders [2], Xavier Matias-Guiu [7], Frédéric Amant [20,21], ENITEC-consortium, Camilla Krakstad [22,23], Koen van de Vijver [24, 25], Jessica McAlpine [26], Johanna M.A. Pijnenborg [1]

Affiliations

[1] Department of Obstetrics and Gynaecology, Radboud university medical center, Nijmegen, the Netherlands; [2] Department of Obstetrics and Gynaecology, Canisius-Wilhelmina Hospital, Nijmegen, the Netherlands; [3] Department of Pathology, Canisius-Wilhelmina Hospital, Nijmegen, The Netherlands; [4] Department of Pathology, Radboud university medical center, Nijmegen, the Netherlands; [5] Genetic Pathology Evaluation Center, Vancouver General Hospital, Vancouver, British Columbia, Canada; [6] Foundation Laboratory for Pathology and Medical Microbiology (PAMM), Eindhoven, The Netherlands; [7] Department of Pathology and Molecular Genetics and Research Laboratory, Hospital Universitari Arnau de Vilanova, University of Lleida, IRBLleida, CIBERONC, Lleida, Spain; [8] Institute of Pathology, University Medical Center, Freiburg, Germany; [9] Department of Obstetrics and Gynecology, University Medical Center, Freiburg; German Cancer Consortium, German Cancer Research Center, Heidelberg, Germany; [10] Institute of Veterinary Medicine, Georg-August-University, Goettingen, Germany, [11] Biomedical Research Group in Gynecology, Vall Hebron Institute of Research, Universitat Autònoma de Barcelona, CIBERONC, Barcelona, Spain; [12] Gynecological Department, Vall Hebron University Hospital, CIBERONC, Barcelona, Spain; [13] Pathology

Department, Vall Hebron University Hospital, CIBERONC, Barcelona, Spain; [14] Department of Obstetrics and Gynecology, Hospital del Mar, PSMAR, Barcelona, Spain; [15] Department of Pathology, University of Turku, Turku, Finland; [16] Obstetrics and Gynecology Department, Bichat-Claude Bernard Hospital, Paris, France; [17] Department of Gynecology and Obstetrics, Faculty of Medicine, Masaryk University, Brno, Czech Republic; [18] Department of Internal medicine, oncology and hematology, Faculty of Medicine, Masaryk University, Brno, Czech Republic; [19] Institute of Pathology, Faculty of Medicine, Masaryk University, Brno, Czech Republic; [20] Department of Oncology, KU Leuven, Leuven, Belgium; [21] Center for Gynaecologic Oncology, Netherlands Cancer Institute and Amsterdam University Medical Center, Amsterdam, the Netherlands; [22] Department of Obstetrics and Gynecology, Haukeland University Hospital, Bergen, Norway; [23] Centre for Cancer Biomarkers, Department of Clinical Science, University of Bergen, Bergen, Norway; [24] Department of Pathology, Ghent University Hospital, Cancer Research Institute Ghent (CRIG), Ghent, Belgium; [25] Department of Pathology, University Hospital Antwerp, Antwerp, Belgium; [26] Obstetrics and Gynecology, University of British Columbia, Vancouver, British Columbia, Canada.

*These authors contributed equally to this work

Declaration of interest

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Corresponding author

Willem Jan van Weelden

Radboud university medical center

791 Department of Obstetrics and Gynaecology

P.O. Box 9101, 6500HB, Nijmegen, The Netherlands

E-mail: willemjan.vanweelden@radboudumc.nl

Telephone Number: +31643274577

Abstract

Background: there is no consensus on the cut-off for positivity of estrogen receptor (ER) and progesterone receptor (PR) in endometrial cancer (EC). Therefore we determined the cut-off value for ER and PR with the strongest prognostic impact on outcome.

Methods: immunohistochemical expression of ER and PR was scored as a percentage of positive EC cell nuclei. Cut-off values were related to disease-specific (DSS) and disease-free survival (DFS) using sensitivity, specificity and multivariable regression analysis. The results were validated in an independent cohort.

Results: the study cohort (*n*=527) included 82% grade 1-2 and 18% grade 3 ECs. Specificity for DSS and DFS was highest for the cut-off values 1-30%. Sensitivity was highest for the cut-offs 80-90%. ER and PR expression were independent markers for DSS at cut-off values of 10% and 80%. Consequently, three subgroups with distinct clinical outcome were identified: ER/PR 0-10%: unfavorable outcome (5-year-DSS 75.9-83.3%); ER/PR 20-80%: intermediate outcome (5-year-DSS 93.0-93.9%) and ER/PR 90-100%: favorable outcome (5-year-DSS 97.8-100%). The association between ER/PR subgroups and outcome was confirmed in the validation cohort (*n*=265).

Conclusions: we propose classification of ER and PR expression according to a high risk (0-10%), intermediate risk (20-80%) and low risk (90-100%) group.

Keywords: endometrial cancer, estrogen receptor, progesterone receptor, cut-off, prognostic biomarker

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, while 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, non-endometrioid EC (NEEC) subtypes like 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 with 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 cut-off value for ER and PR positivity that differentiates best between favorable and unfavorable outcome, 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 cut-off values for receptor positivity in EC are adopted from breast cancer studies in which cut-off values of 1% or 10% are most frequently used (19, 20). In order to define relevant thresholds for ER and PR expression in EC, we performed analysis in a large retrospectively collected multicenter cohort to determine the cut-off values with the strongest prognostic impact for clinical outcome in EC.

Methods

ENITEC cohort

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. Available clinical and pathological characteristics included age at diagnosis, date of diagnosis, body mass index, CA125 serum level, 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 to the Declaration of Helsinki and was approved by the Institutional Review Board at the Radboud university medical center (reference number 2015-2101).

Hormone receptor analysis

Blank 4µm sections from formalin-fixed, paraffin-embedded (FFPE) 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 (H&E). 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 ^{o}C for 30 minutes in Tris/EDTA buffer pH 9 [Envision FLEX Target Retrieval Solution High pH, DAKO, Agilent Technologies, Santa Clara, CA, United States]) and subsequent blocking of endogenous peroxidase with hydrogen peroxide were performed. Then, slides were incubated with ER antibody (clone SP1 GA084, DAKO, Agilent Technologies, Santa Clara, CA, United States) or PR antibody (clone, PgR 1294 GA090, DAKO, Agilent Technologies, Santa Clara, CA, United States). Envision FLEX/HRP (DAKO, Agilent Technologies, Santa Clara, CA, United States) was used and visualization was performed with Envision FLEX DAB+ Chromogen (DAKO, Agilent Technologies, Santa Clara, CA, United States).

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 five assessors (C.R., J.B., H.K-V., N.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.

Vancouver cohort

Patients

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

Immunohistochemistry

Immunohistochemistry was performed on previously constructed tissue microarrays (TMAs) for ER and PR as described before (24). In brief, previously constructed TMAs were immunohistochemically stained for ER (ER antibody clone SP1, RM-9101 diluted 1:25 Thermo, 1 h at 37 ^{o}C) or PR (PR antibody clone 1E2 790-2223 undiluted Ventana, 16 minutes at 36 ^{o}C) with the Ventana Discovery Ultra protocol. Antigen retrieval was performed using cell conditioning 1 (CC1) for 64 minutes. The slides were incubated. Visualization was performed with the DABmap kit. For each patient, two digitalized TMA cores for both ER and PR expression were scored semi-quantitively, defined as 0%, 1%, 10%, 20%, 30%, 40%, 50%, 60%, 70% ,80%, 90% and 100%, by two assessors (W.W. and C.R.) by estimating the percentage of positive nuclei in the whole invasive tumor area through eyeballing. As the average of two TMA scores of two reviewers was assessed, resulting scores could be outside the predefined scores (like 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.

Statistical analysis

The relation between ER and PR expression and established prognostic factors was analyzed with the student T-test. For different categories of ER and PR expression, ranging from ≤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 cut-off 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, body

mass index, CA125 serum level, 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 cut-off values with the strongest associations with outcome, 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.

Results

ENITEC cohort

A total of 527 EC patients were included in the analysis. The clinicopathological findings of this cohort and the correlations with mean ER/PR expression are summarized in **Table 1**. The mean age was 65.9 years and the mean BMI was 30.4. Most patients had stage I-II (91%), low-grade disease (82%) with EEC histology (95%). Among patients with stage I-II EC, 54.8% underwent lymphadenectomy. Recurrences occurred in 12% of patients and 7% of patients died due 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 to 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 compared to advanced stage EC (60% vs. 47%, respectively). ER and PR expression were significantly lower in patients with recurrence compared to non-recurrent cases. PR expression was significantly higher in patients with local/regional recurrences compared to 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 due to EC compared to non-EC related deaths.

ER and PR at different cut-off values

Different categories for ER and PR expression cut-off 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 cut-off value is provided for DSS in **Table 2** and for DFS in **Appendix A.**

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

Similar results were found for PR expression; a cut-off of 80% resulted in a sensitivity of 97% for DSS and 86% for DFS compared to 86% and 65%, respectively at a 70% cut-off. The AUC was similar for DFS and lower for 80% cut-off than 70% for DSS.

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

Value of ER and PR expression in multivariable analysis

The association between different cut-off values of ER and PR and outcome was analyzed using multivariable Cox regression analyses including, age, grade, histology, lymphovascular space invasion, FIGO stage, CA125 level and ER or PR expression. As is shown in **Figure 1A,** ER was an independent marker for DSS at cut-off values of 1-40% and 70-80%. The association with DSS was strongest at the 80% cut-off value, indicating that the ratio of disease-specific mortality is highest when applying the cut-off of ≤80% expression. PR was an independent marker for DSS at all cut-off values (**Figure 1B**). ER expression was an independent marker for DFS at the cut-off values 10-30%, with the strongest association at the 10% cut-off value (**Appendix B**). PR was an independent marker for DSS at all cut-off values and for DFS at cut-off values 10-20% (**Figure 1B and Appendix B**). A cut-off value of ER 1% was not significantly associated with DSS nor DFS.

Risk groups

Based on the results for sensitivity, specificity and multivariable regression analysis three risk groups were defined using the 10% and 80% cut-off value as both cut-off values showed consistent significant associations with outcome and the 80% cut-off value also had a high sensitivity for DSS and DFS. Cases with 0-10% ER/PR expression had a high risk for adverse outcome, cases with 20-80% ER/PR expression had an intermediate risk, and cases with ER/PR expression of 90-100% had a low risk (**Figure 2**). Patients with 0-10% ER expression had a 5-year DSS of 75.9% [95%-CI: 62.5-89.3], which was significantly lower compared to patients with an ER expression of 20-80% (5-years 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, **Figure 2A**). The 5-year DSS of patients with 20-80% ER expression was also significantly lower than in patients with an ER expression of 90-100% (p=0.009). Similarly, patients with 0-10% PR expression had a lower 5-year DSS (83.3% [95%-CI 75.8-90.8]) compared to 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, **Figure 2B**). The 5-year DSS of patients with a PR expression of 20-80% was also significantly lower than in patients with 90-100% PR expression (p=0.010). The 5-year DFS for 0-10% ER and PR expression was 67.5% [95%-CI 53.0-82.0] respectively 78.8% [95%-CI 70.6-87.0], which was significantly lower compared to 20-80% (ER: 89.9% [95%-CI 86.4-93.3], PR: 89.5 [95%-CI 86.0-93.0]) and 90-100% 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 was similar (see **Appendix C**). **Figure 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% ER/PR expression, while the 90-100% had the lowest proportion of cases with adverse outcome. The 0-10% ER/PR group has a significantly shorter DSS and DFS compared to the 90-100% group in high grade EC. In low grade EC, analysis is hampered by limited numbers of events in the groups.

The Cohen's κ for scoring ER/PR expression according to the three risk groups was 0.703.

Combination of ER and PR

A combination marker for ER and PR expression was analyzed in relation to outcome. A combined ER and PR analysis, in which both ER and PR 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. Application of the 80% cut-off value, in which both ER and PR 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.

Vancouver cohort

In total, 265 EC patients 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 to the ENITEC cohort, the validation cohort included a higher proportion of patients with high-grade tumors (64% in Vancouver, 18% in ENITEC cohort) and more advanced stage, (30% in Vancouver, 9% in ENITEC cohort). Recurrences occurred in 29% and EC-related death in 25%. 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 compared to non-recurrent cases. Patients that died due to EC had a significantly lower ER and PR expression compared to patients with non EC-related mortality or patients that were alive at the end of follow-up.

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]) compared to patients with an ER expression of 90 – 100% (91.6% [95%-CI 83.8-99.4], **Figure 3A**). There was no difference in DSS between the group with 0-10% ER expression and the group with 20-80% 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]) compared to 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], **Figure 3B**). The 5-year DFS for ER expression of 0-10% and 20-80% was 62.6% [95%-CI 50.7-74.5] respectively 62.3% [95%-CI 53.8-70.8] which was significantly lower compared to 90-100% 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 compared to a PR expression of 20-80% and 90-100% (76.1% [95%-CI: 68.0-84.2]

283 and 88.8% [95%-CI: 76.3-100.0]). The Cohen's κ for scoring ER/PR expression according to the three

284 risk groups in this cohort was 0.796.

Discussion

In the present study we have confirmed the prognostic value of ER and PR expression and determined the cut-off values with the strongest prognostic value for clinical outcome 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 outcome, 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 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 semicontinuous values for ER/PR expression as opposed to dichotomous values (e.g. positive, negative). Previous studies have focused on one cut-off value (e.g. 1% or 10% of positive tumor nuclei, or a staining-intensity index cut-off 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 cut-off values for ER/PR expression. The cut-off 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% cut-off value was shown to be superior to ≤1% cut-off value, as the ≤1% cut-off value lacked significant associations with outcome in multivariable regression analysis. These findings are supported by results of the study in breast cancer of Yi et al. in which cut-off values of 1% and 10% were compared among 9 639 patients (19). Patients with an ER expression of 1-9% and <1% had a similar outcome, while patients with an expression ≥10% had a better outcome compared to those with 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% cut-off value for ER, also in relation to prediction of response to adjuvant endocrine treatment (20). In our study, the cut-off values of 10% and 20% for ER and PR positivity performed similarly in terms of sensitivity and specificity and associations with outcome in multivariable analysis. We selected the 10% cut-off value because it is a highly reproducible cut-off value and it is consistent with currently used cut-off 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 are present in around 40% of NEEC, in line with the results of this study (30, 31).

The cut-off value of 80% showed a higher sensitivity compared to 70% while the AUCs were mostly similar between the two cutoffs. Therefore, the cut-off 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 outcome. These findings are in line with the results of Weinberger et al., in which cut-off values of 78% for ER and 88% for PR provided optimal cut-off values to stratify EC-patients into low and high risk groups based on preoperative biopsies (32). To our knowledge, there are no other studies available that explored the 80% cut-off value in relation to prognosis in EC.

The results of this study suggest that ER and PR have complementary value in identifying high-risk and low-risk populations. At the cut-off value 10%, ER had a higher specificity than PR, indicating that ER ≤10% could be applied to identify high-risk cases. At the cut-off value 80%, PR had a higher sensitivity than ER, suggesting that PR is, more than ER, able to identify a low-risk population. Based on this data no superiority for ER or PR could be found, supporting the routine performance of both ER and PR in all EC patients.

For ER and PR expression assessment in EC, pre-analytic, analytic and post-analytic 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 testing in breast cancer (20). In the present study we expect no problems in the pre-analytic 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 and 1E2 in the Vancouver cohort). As is known for breast cancer, different clones for the ER and PR can give different results for the 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 post-analytic factor is the interpretation of the ER and PR expression by the pathologist. We reached a Cohen's κ of 0.703 and 0.796 for scoring ER/PR expression according to the three risk groups in the ENITEC and Vancouver cohort, respectively. This is in line with results of recent studies in 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 cut-off values identified in this study could guide both manual and digital evaluation of immunohistochemical analysis for ER and PR.

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 due to application of multiple cut-off values for ER/PR expression. (24, 36) Further integration of ER/PR expression, using the updated cut-off 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 non-endometrioid 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 according to 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 between biopsy and surgical specimen of at least 85%, 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 cut-off 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). Also, data on molecular subgroups were lacking (e.g. *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 tissue microarray, as used in our study, is supported by a recent study from Visser et al. in which discordant expression was found in just 6% of cases (39).

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

Additional information

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None

Authors' contributions

WW and CR: study concept, data curation, formal analysis, manuscript writing and review, HKV, JB, NV, KvdV: formal analysis , manuscript review and editing, PB: manuscript editing and review, SL, MS, PB, MH, EC, AGM, AR, GM, JH, MK, VW, MB, JH, MS, XMG, FA, CK, JM: investigation and manuscript review, JM: investigation and manuscript editing, JP: study concept, manuscript editing and review.

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

References

1. Trovik J, Wik E, Werner HM, Krakstad C, Helland H, Vandenput I, Njolstad TS, Stefansson IM, Marcickiewicz J, Tingulstad S, Staff AC, MoMa TECsg, Amant F, Akslen LA, Salvesen HB. Hormone receptor loss in endometrial carcinoma curettage predicts lymph node metastasis and poor outcome in prospective multicentre trial. Eur J Cancer 2013; 49, 3431-3441.
2. Jongen V, Briet J, de Jong R, ten Hoor K, Boezen M, van der Zee A, Nijman H, Hollema H. Expression of estrogen receptor-alpha and -beta and progesterone receptor-A and -B in a large cohort of patients with endometrioid endometrial cancer. Gynecol Oncol 2009; 112, 537-542.
3. Conneely OM, Mulac-Jericevic B, Lydon JP. Progesterone-dependent regulation of female reproductive activity by two distinct progesterone receptor isoforms. Steroids 2003; 68, 771-778.
4. Mylonas I, Jeschke U, Shabani N, Kuhn C, Kriegel S, Kupka MS, Friese K. Normal and malignant human endometrium express immunohistochemically estrogen receptor alpha (ER-alpha), estrogen receptor beta (ER-beta) and progesterone receptor (PR). Anticancer Res 2005; 25, 1679-1686.
5. Evans RM. The steroid and thyroid hormone receptor superfamily. Science 1988; 240, 889-895.
6. Tsai MJ, O'Malley BW. Molecular mechanisms of action of steroid/thyroid receptor superfamily members. Annual review of biochemistry 1994; 63, 451-486.
7. Fritz M.A., Speroff L. Clinical Gynecologic Endocrinology and Infertility. Philadelphia: Lippincott Williams & Wilkins, 2011.
8. Ellenson HL, Ronnett BM, Soslow RA, Zaino RJ, Kurman RJ. Endometrial carcinoma. In: Kurman RJ, Ronnett BM, Ellenson HL, eds. Blaustein's Pathology of the Female Genital Tract. New York: Springer, 2011, p. 393-452.
9. Sherman ME. Theories of endometrial carcinogenesis: a multidisciplinary approach. Mod Pathol 2000; 13, 295-308.
10. Bokhman JV. Two pathogenetic types of endometrial carcinoma. Gynecol Oncol 1983; 15, 10-17.
11. van der Putten LJM, Visser NCM, van de Vijver K, Santacana M, Bronsert P, Bulten J, Hirschfeld M, Colas E, Gil-Moreno A, Garcia A, Mancebo G, Alameda F, Trovik J, Kopperud RK, Huvila J, Schrauwen S, Koskas M, Walker F, Weinberger V, Minar L, Jandakova E, Snijders M, van den Berg-van Erp S, Matias-Guiu X, Salvesen HB, Werner HMJ, Amant F, Massuger L, Pijnenborg JMA. Added Value of Estrogen Receptor, Progesterone Receptor, and L1 Cell Adhesion Molecule Expression to Histology-Based Endometrial Carcinoma Recurrence Prediction Models: An ENITEC Collaboration Study. Int J Gynecol Cancer 2018; 28, 514-523.
12. Zannoni GF, Monterossi G, De Stefano I, Gargini A, Salerno MG, Farulla I, Travaglia D, Vellone VG, Scambia G, Gallo D. The expression ratios of estrogen receptor alpha (ERalpha) to estrogen receptor beta1 (ERbeta1) and ERalpha to ERbeta2 identify poor clinical outcome in endometrioid endometrial cancer. Hum Pathol 2013; 44, 1047-1054.
13. van Weelden WJ, Massuger L, Enitec, Pijnenborg JMA, Romano A. Anti-estrogen Treatment in Endometrial Cancer: A Systematic Review. Front Oncol 2019; 9, 359.
14. Ethier JL, Desautels DN, Amir E, MacKay H. Is hormonal therapy effective in advanced endometrial cancer? A systematic review and meta-analysis. Gynecol Oncol 2017; 147, 158-166.
15. Wang Y, Ma X, Wang Y, Liu Y, Liu C. Comparison of Different Scoring Systems in the Assessment of Estrogen Receptor Status for Predicting Prognosis in Endometrial Cancer. Int J Gynecol Pathol 2019; 38, 111-118.
16. Palmer DC, Muir IM, Alexander AI, Cauchi M, Bennett RC, Quinn MA. The prognostic importance of steroid receptors in endometrial carcinoma. Obstet Gynecol 1988; 72, 388-393.
17. Krakstad C, Trovik J, Wik E, Engelsen IB, Werner HM, Birkeland E, Raeder MB, Oyan AM, Stefansson IM, Kalland KH, Akslen LA, Salvesen HB. Loss of GPER identifies new targets for therapy among a subgroup of ERalpha-positive endometrial cancer patients with poor outcome. Br J Cancer 2012; 106, 1682-1688.

18. Singh M, Zaino RJ, Filiaci VJ, Leslie KK. Relationship of estrogen and progesterone receptors to clinical outcome in metastatic endometrial carcinoma: a Gynecologic Oncology Group Study. Gynecol Oncol 2007; 106, 325-333.
19. Yi M, Huo L, Koenig KB, Mittendorf EA, Meric-Bernstam F, Kuerer HM, Bedrosian I, Buzdar AU, Symmans WF, Crow JR, Bender M, Shah RR, Hortobagyi GN, Hunt KK. Which threshold for ER positivity? a retrospective study based on 9639 patients. Ann Oncol 2014; 25, 1004-1011.
20. Allison KH, Hammond MEH, Dowsett M, McKernin SE, Carey LA, Fitzgibbons PL, Hayes DF, Lakhani SR, Chavez-MacGregor M, Perlmutter J, Perou CM, Regan MM, Rimm DL, Symmans WF, Torlakovic EE, Varella L, Viale G, Weisberg TF, McShane LM, Wolff AC. Estrogen and Progesterone Receptor Testing in Breast Cancer: ASCO/CAP Guideline Update. J Clin Oncol 2020, JCO1902309.
21. van der Putten LJ, Visser NC, van de Vijver K, Santacana M, Bronsert P, Bulten J, Hirschfeld M, Colas E, Gil-Moreno A, Garcia A, Mancebo G, Alameda F, Trovik J, Kopperud RK, Huvila J, Schrauwen S, Koskas M, Walker F, Weinberger V, Minar L, Jandakova E, Snijders MP, van den Berg-van Erp S, Matias-Guiu X, Salvesen HB, Amant F, Massuger LF, Pijnenborg JM. L1CAM expression in endometrial carcinomas: an ENITEC collaboration study. Br J Cancer 2016; 115, 716-724.
22. Talhouk A, McConechy MK, Leung S, Yang W, Lum A, Senz J, Boyd N, Pike J, Anglesio M, Kwon JS, Karnezis AN, Huntsman DG, Gilks CB, McAlpine JN. Confirmation of ProMisE: A simple, genomics-based clinical classifier for endometrial cancer. Cancer 2017; 123, 802-813.
23. Talhouk A, McConechy MK, Leung S, Li-Chang HH, Kwon JS, Melnyk N, Yang W, Senz J, Boyd N, Karnezis AN, Huntsman DG, Gilks CB, McAlpine JN. A clinically applicable molecular-based classification for endometrial cancers. Br J Cancer 2015; 113, 299-310.
24. Karnezis AN, Leung S, Magrill J, McConechy MK, Yang W, Chow C, Kobel M, Lee CH, Huntsman DG, Talhouk A, Kommoss F, Gilks CB, McAlpine JN. Evaluation of endometrial carcinoma prognostic immunohistochemistry markers in the context of molecular classification. J Pathol Clin Res 2017; 3, 279-293.
25. Wik E, Raeder MB, Krakstad C, Trovik J, Birkeland E, Hoivik EA, Mjos S, Werner HM, Mannelqvist M, Stefansson IM, Oyan AM, Kalland KH, Akslen LA, Salvesen HB. Lack of estrogen receptor-alpha is associated with epithelial-mesenchymal transition and PI3K alterations in endometrial carcinoma. Clin Cancer Res 2013; 19, 1094-1105.
26. Guan J, Xie L, Luo X, Yang B, Zhang H, Zhu Q, Chen X. The prognostic significance of estrogen and progesterone receptors in grade I and II endometrioid endometrial adenocarcinoma: hormone receptors in risk stratification. J Gynecol Oncol 2019; 30, e13.
27. Huvila J, Talve L, Carpen O, Edqvist PH, Ponten F, Grenman S, Auranen A. Progesterone receptor negativity is an independent risk factor for relapse in patients with early stage endometrioid endometrial adenocarcinoma. Gynecol Oncol 2013; 130, 463-469.
28. Mylonas I. Prognostic significance and clinical importance of estrogen receptor alpha and beta in human endometrioid adenocarcinomas. Oncol Rep 2010; 24, 385-393.
29. Chebil G, Bendahl PO, Ferno M. Estrogen and progesterone receptor assay in paraffin-embedded breast cancer--reproducibility of assessment. Acta Oncol 2003; 42, 43-47.
30. Tangen IL, Werner HM, Berg A, Halle MK, Kusonmano K, Trovik J, Hoivik EA, Mills GB, Krakstad C, Salvesen HB. Loss of progesterone receptor links to high proliferation and increases from primary to metastatic endometrial cancer lesions. Eur J Cancer 2014; 50, 3003-3010.
31. Peevey JF, Seagle BL, Maniar KP, Kim JJ. Association of body mass index with ER, PR and 14-3-3sigma expression in tumor and stroma of type I and type II endometrial carcinoma. Oncotarget 2017; 8, 42548-42559.
32. Weinberger V, Bednarikova M, Hausnerova J, Ovesna P, Vinklerova P, Minar L, Felsinger M, Jandakova E, Cihalova M, Zikan M. A Novel Approach to Preoperative Risk Stratification in Endometrial Cancer: The Added Value of Immunohistochemical Markers. Front Oncol 2019; 9, 265.
33. Troxell ML, Long T, Hornick JL, Ambaye AB, Jensen KC. Comparison of Estrogen and Progesterone Receptor Antibody Reagents Using Proficiency Testing Data. Arch Pathol Lab Med 2017; 141, 1402-1412.

34. Rizzardi AE, Zhang X, Vogel RI, Kolb S, Geybels MS, Leung YK, Henriksen JC, Ho SM, Kwak J, Stanford JL, Schmechel SC. Quantitative comparison and reproducibility of pathologist scoring and digital image analysis of estrogen receptor beta2 immunohistochemistry in prostate cancer. Diagn Pathol 2016; 11, 63.
35. Cancer Genome Atlas Research N, Albert Einstein College of M, Analytical Biological S, Barretos Cancer H, Baylor College of M, Beckman Research Institute of City of H, Buck Institute for Research on A, Canada's Michael Smith Genome Sciences C, Harvard Medical S, Helen FGCC, Research Institute at Christiana Care Health S, HudsonAlpha Institute for B, Ilsbio LLC, Indiana University School of M, Institute of Human V, Institute for Systems B, International Genomics C, Leidos B, Massachusetts General H, McDonnell Genome Institute at Washington U, Medical College of W, Medical University of South C, Memorial Sloan Kettering Cancer C, Montefiore Medical C, NantOmics, National Cancer I, National Hospital AN, National Human Genome Research I, National Institute of Environmental Health S, National Institute on D, Other Communication D, Ontario Tumour Bank LHSC, Ontario Tumour Bank OIfCR, Ontario Tumour Bank TOH, Oregon H, Science U, Samuel Oschin Comprehensive Cancer Institute C-SMC, International SRA, St Joseph's Candler Health S, Eli, Edythe LBIoMIoT, Harvard U, Research Institute at Nationwide Children's H, Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins U, University of B, University of Texas MDACC, University of Abuja Teaching H, University of Alabama at B, University of California I, University of California Santa C, University of Kansas Medical C, University of L, University of New Mexico Health Sciences C, University of North Carolina at Chapel H, University of Oklahoma Health Sciences C, University of P, University of Sao Paulo RaPMS, University of Southern C, University of W, University of Wisconsin School of M, Public H, Van Andel Research I, Washington University in St L. Integrated genomic and molecular characterization of cervical cancer. Nature 2017; 543, 378-384.
36. Stelloo E, Nout RA, Osse EM, Jurgenliemk-Schulz IJ, Jobsen JJ, Lutgens LC, van der Steen-Banasik EM, Nijman HW, Putter H, Bosse T, Creutzberg CL, Smit VT. Improved Risk Assessment by Integrating Molecular and Clinicopathological Factors in Early-stage Endometrial Cancer-Combined Analysis of the PORTEC Cohorts. Clin Cancer Res 2016; 22, 4215-4224.
37. Tamaki K, Sasano H, Ishida T, Miyashita M, Takeda M, Amari M, Tamaki N, Ohuchi N. Comparison of core needle biopsy (CNB) and surgical specimens for accurate preoperative evaluation of ER, PgR and HER2 status of breast cancer patients. Cancer Sci 2010; 101, 2074-2079.
38. Mann GB, Fahey VD, Feleppa F, Buchanan MR. Reliance on hormone receptor assays of surgical specimens may compromise outcome in patients with breast cancer. J Clin Oncol 2005; 23, 5148-5154.
39. Visser NCM, van der Wurff AAM, Pijnenborg JMA, Massuger L, Bulten J, Nagtegaal ID. Tissue microarray is suitable for scientific biomarkers studies in endometrial cancer. Virchows Arch 2018; 472, 407-413.
40. FEDERA. Human Tissue and Medical Research: Code of conduct for responsible use 2011.

Legends

Figure 1: Multivariable Cox regression analysis of association between estrogen receptor (A) and progesterone receptor (B) at different cut-off values with disease-specific survival. The other covariates in multivariable regression analysis are: age, grade, histology, lymphovascular space invasion, myometrial invasion, FIGO stage.

Figure 2: Association between ER (A) and PR expression (B) according to high (0-10%), intermediate (20-80%) and low risk (90-100%) groups with disease specific survival in the complete ENITEC cohort and in low (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.

Figure 3: Kaplan Meier analysis of association between ER and PR expression according to high (0-10%), intermediate (20-80%) and low risk (90-100%) groups with disease specific survival in Vancouver cohort.

Table 1: Overview of clinicopathological findings of ENITEC cohort

	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			
35 and lower	267 (51)	72 (25)	63 (30)*
>35	79 (15)	67 (28)	46 (34)
Unknown	181 (34)		
Postoperative grade			
Low grade (grade 1 or 2)	430 (82)	75 (22)*	63 (30)*
High grade (grade 3)	97 (18)	56 (35)	42 (35)
Histology			
Endometrioid	502 (95)	73 (25)*	61 (31)*
Non-endometrioid	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)*	50 (36)*
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)*
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)*	49 (36)*
Local	19 (4)	73 (30)	67 (33)**
Regional	9 (2)	73 (30)	52 (37)**
Distant	40 (8)	57 (34)	43 (34)
No	462 (88)	73 (26)	61 (32)
Unknown	2 (0)		
Death			
Yes	67 (13)	61 (34)*	37 (32)*
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, * significant at $p<0.05$, ** significant at $p<0.05$ for comparison local/regional to distant recurrence

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

A: The value of different cutoffs for estrogen receptor in prediction of disease-specific survival

Cutoff	Sensitivity	Specificity	PPV	NPV	AUC
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

B: The value of different cutoffs for progesterone receptor in prediction of disease-specific survival

Cutoff	Sensitivity	Specificity	PPV	NPV	AUC
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

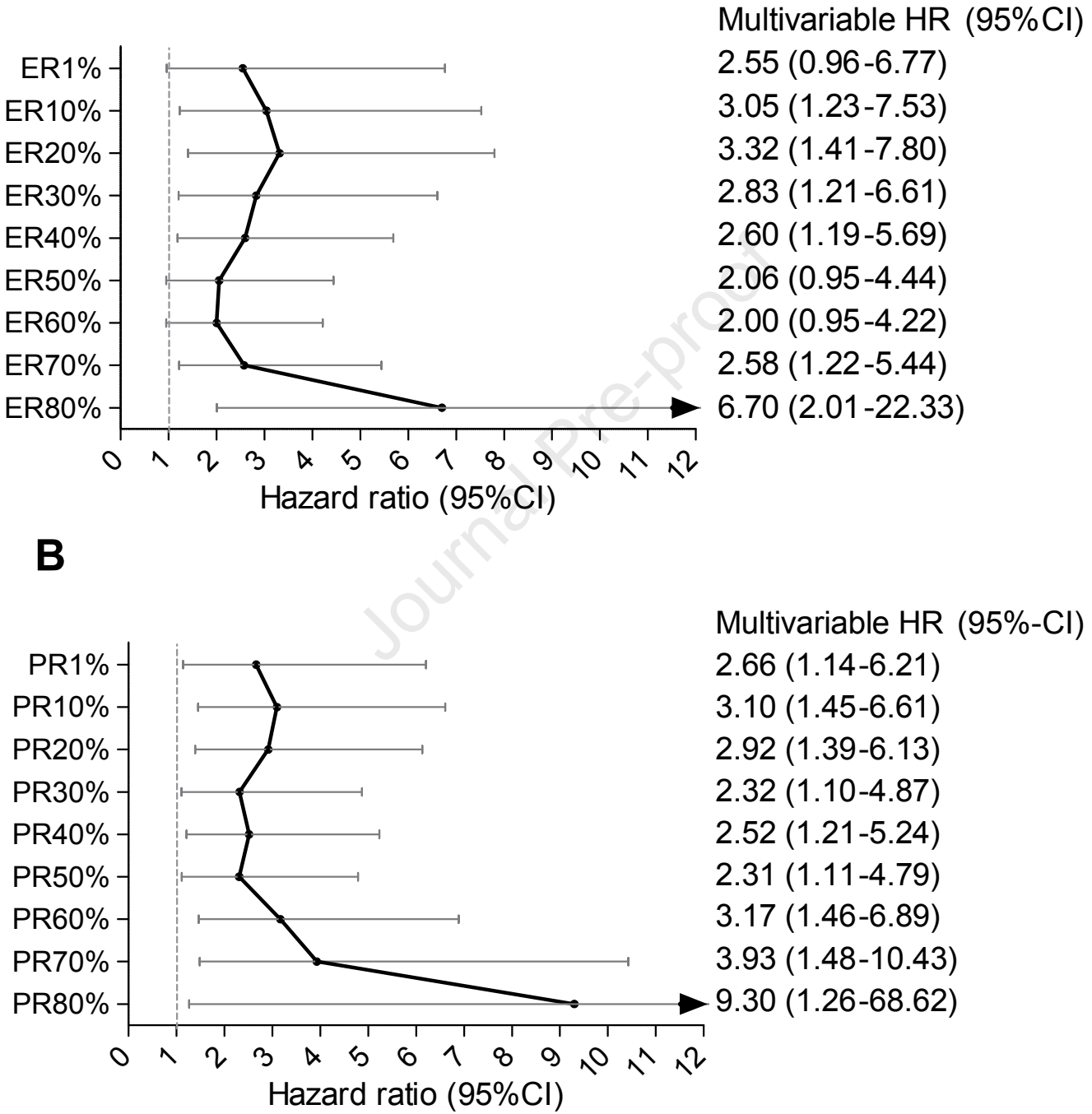
Abbreviations: ER: estrogen receptor, PR: progesterone receptor, PPV: positive predictive value, NPV: negative predictive value, AUC: area under the curve.

Table 3: Overview of clinicopathological findings of Vancouver cohort

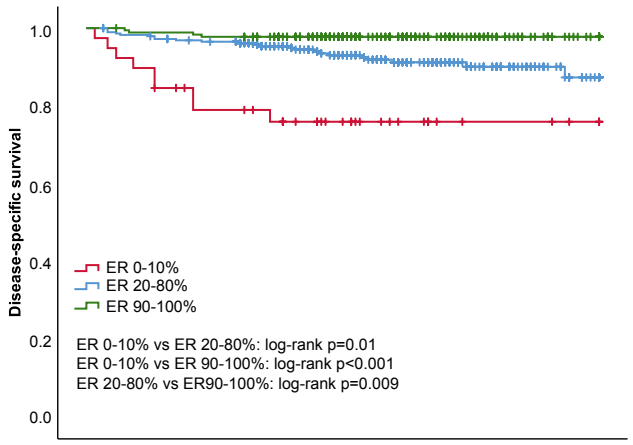
	Number (%) *n*=265	ER expression in %, mean (SD)	PR expression in %, mean (SD)
Mean age (SD)	65.5 (12)		
Mean BMI (SD)	31.3 (10)		
Grade			
Low grade (grade 1 or 2)	95 (36)	77 (18)*	56 (31)*
High grade (grade 3)	170 (64)	40 (35)	21 (29)
Histology			
Endometrioid	182 (69)	63 (32)*	43 (34)*
Non-endometrioid	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)*	23 (29)*
No	130 (49)	60 (34)	43 (40)
Unknown	19 (7)		
MI			
<50%	145 (55)	61 (33)*	41 (36)*
>50%	113 (43)	44 (35)	25 (31)
Unknown	7 (3)		
FIGO Stage			
Early (stage I or II)	181 (68)	57 (35)*	38 (35)*
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)
Adj chemoradiotherapy	67 (25)	41 (34)	20 (26)
No adjuvant treatment	123 (46)	64 (32)	44 (35)
Recurrence			
Yes	75 (28)	44 (34)*	22 (31)*
No	178 (67)	58 (35)	39 (35)
Unknown	12 (5)		
Death			
Yes	96 (36)	48 (34)*	27 (33)*
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, * significant at $p<0.05$.

Figure 1



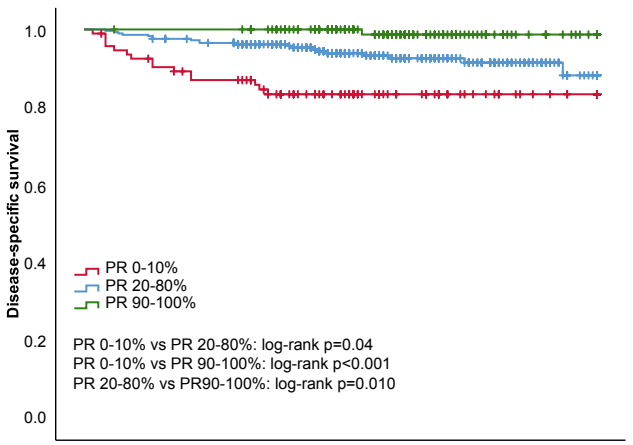
A



Number at risk

ER 0-10%	38	28	24	16	7	4	2
ER 20-80%	288	267	237	175	111	51	22
ER 90-100%	179	174	168	117	62	28	9

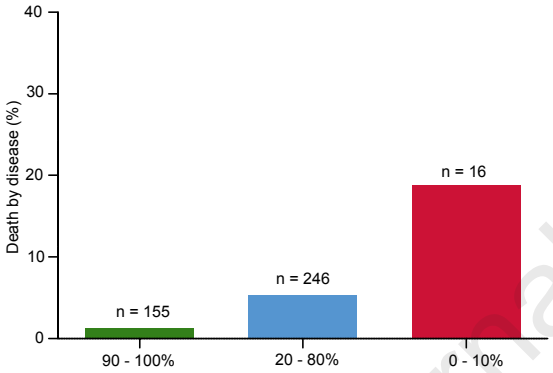
B



Number at risk

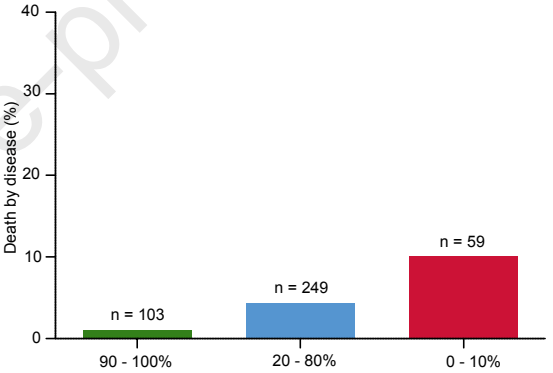
PR 0-10%	93	77	75	49	28	13	7
PR 20-80%	289	272	246	172	105	52	19
PR 81-100%	117	115	110	83	45	16	6

C



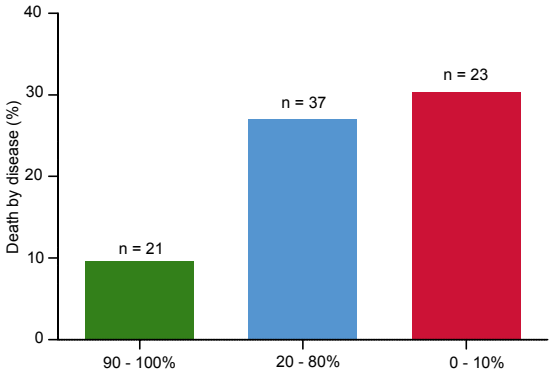
0-10% vs 20-80%: Cox multivariable HR0.92 (95%-CI: 0.1-7.3), p=0.940
20-80% vs 90-100%: Cox multivariable HR12.46 (95%-CI: 1.5-102.8), p=0.019
0-10% vs 90-100%: Cox multivariable coefficients do not converge

D



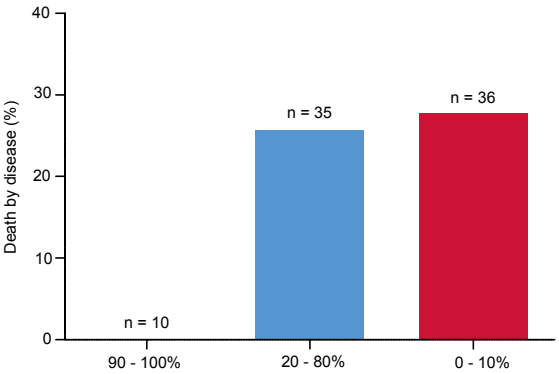
0-10% vs 20-80%: Cox multivariable HR1.58 (95%-CI: 0.5-5.1), p=0.443
20-80% vs 90-100%: Cox multivariable coefficients do not converge
0-10% vs 90-100%: Cox multivariable coefficients do not converge

E



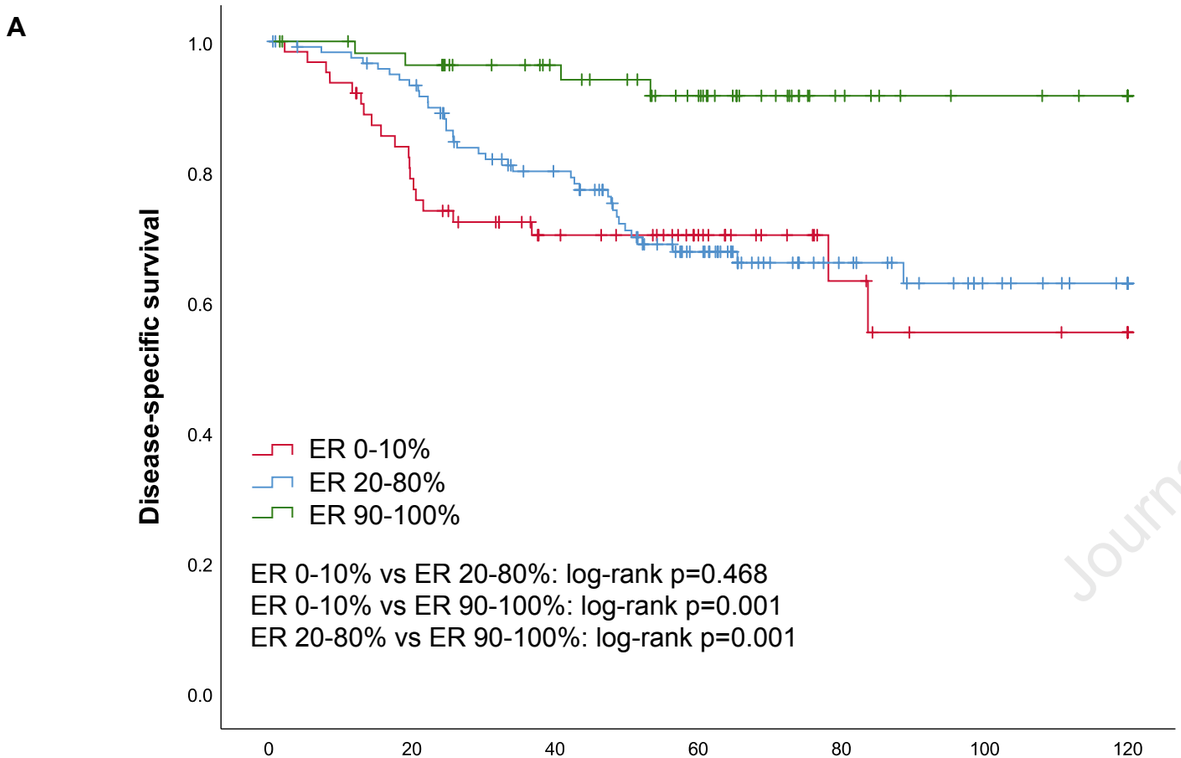
0-10% vs 20-80%: Cox multivariable HR2.21 (95%-CI: 0.7-6.8), p=0.168
20-80% vs 90-100%: Cox multivariable HR6.09 (95%-CI: 1.2-31.6), p=0.031
0-10% vs 90-100%: Cox multivariable HR10.08 (95%-CI: 1.5-65.9), p=0.016

F

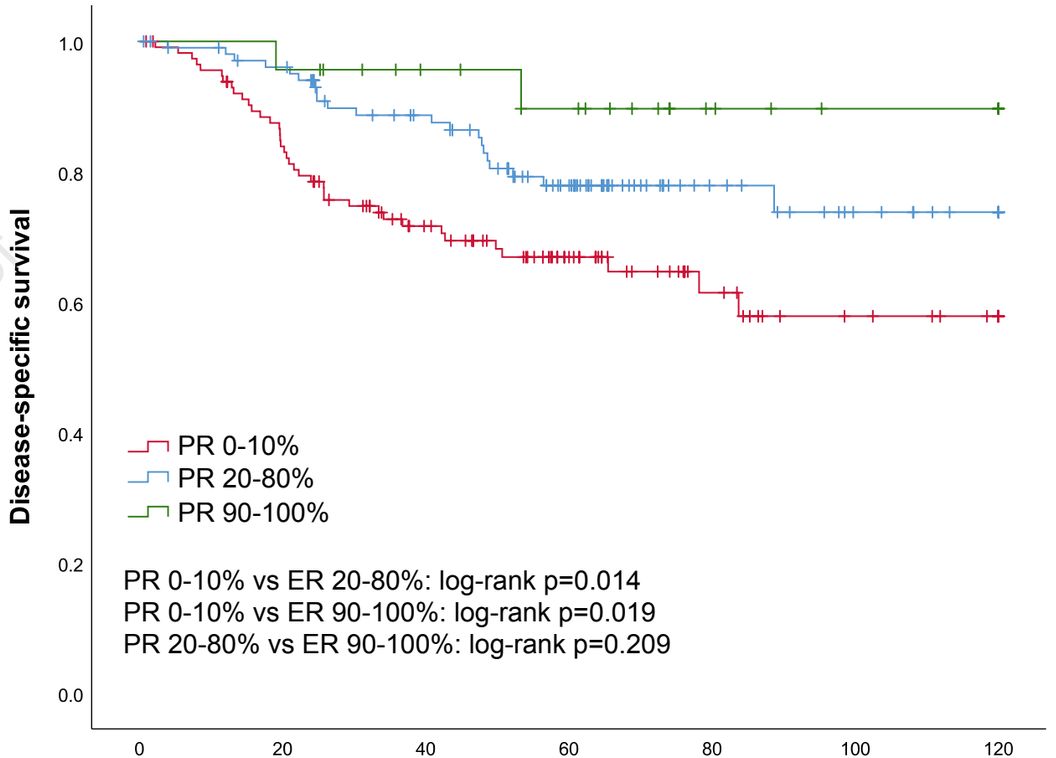


0-10% vs 20-80%: Cox multivariable HR3.41 (95%-CI: 1.1-10.4), p=0.031
20-80% vs 90-100%: Cox multivariable HR2.66 (95%-CI: 0.3-22.9), p=0.372
0-10% vs 90-100%: Cox multivariable HR13.51 (95%-CI: 1.4-134.7), p=0.027

Figure 3



Number at risk							
ER 0-10%	63	48	32	22	9	5	4
ER 20-80%	122	110	84	53	25	13	7
ER 90-100%	58	53	43	32	12	7	5



Number at risk							
PR 0-10%	115	93	65	40	19	10	6
PR 20-80%	105	96	78	53	21	12	7
PR 90-100%	23	22	17	14	6	3	3

Highlights

Estrogen (ER) and progesterone receptor (PR) are prognosticators in endometrial cancer

However, the optimal cut-off for ER and PR expression is unclear

Three ER/PR subgroups with distinct clinical outcome were identified

ER/PR 0-10% had adverse outcome and 90-100% ER/PR had favorable outcome

We propose classification according to the 0-10%, 20-80% and 90-100% subgroups