

Prognosis Stratification Tools in Early-Stage Endometrial Cancer: Could We Improve Their Accuracy?

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Abstract

Background

There are 3 prognostic stratification tools used in clinics for endometrial cancer: ESMO-ESGO-ESTRO 2016, ProMisE, and ESGO-ESTRO-ESP 2020. However, these methods are not sufficiently accurate to address the prognosis and adjuvant therapy. Some other previously explored biomarkers have also shown prognostic relevance. The aim of this study was to investigate whether the integration of molecular classification and other biomarkers could be used to refine the prognosis in early-stage endometrial cancer.

Methods

This was a retrospective single-institution cohort of patients with early-stage endometrial cancer to evaluate these stratification tools. Relapse-free survival (RFS) and overall survival of each classifier were analysed, and the c-index was employed to assess accuracy. Other biomarkers were explored to improve the precision of risk classifiers.

Results

We analysed 294 patients: 88% had endometrioid histology, 69% stage Ia, and 15% had a relapse. A comparison between the 3 classifiers showed a slightly improved accuracy in ESGO-ESTRO-ESP 2020 when RFS was evaluated (c-index = 0.79), although we did not find differences between intermediate prognostic groups. However, the inclusion of *CTNNB1* status to stratify patients of intermediate groups allowed a better discrimination between the intermediate prognostic groups, resulting in a c-index of 0.82. Therefore, we propose a novel classifier based on ESGO-ESTRO-ESP 2020 and *CTNNB1*, which achieved statistically significant and clinically relevant differences in 5-year RFS: 93.4% for low risk, 79.6% for intermediate merged group/*CTNNB1* wild type, and 37.3% for high risk (including patients from the merged intermediate groups with *CTNNB1* mutation).

Conclusions

The incorporation of molecular classification in risk stratification of endometrial cancer resulted in better discriminatory capability, which was improved even further with the addition of *CTNNB1* mutational evaluation.

Background

Endometrial cancer (EC) is the most common gynaecological malignancy in developed countries. Most cases are diagnosed at a localised stage, reaching 5-year survival rates of over 95% in some series [1, 2]. Despite such a good prognosis, approximately 15% of patients with early stages (I and II) of EC will recur [3]. Therefore, the identification of patients with an increased risk of relapse remains a challenge for clinicians.

There are well-defined characteristics associated with prognosis, including age, lympho-vascular space invasion (LVSI), myometrial infiltration, differentiation grade and International Federation of Gynecology and Obstetrics (FIGO) stage. During the past 2 decades, these variables were integrated in multiple classifiers to stratify the prognosis. In 2016, the European Society of Medical Oncology (ESMO)-European Society of Gynecologic Oncology (ESGO)-European Society for Radiotherapy and Oncology (ESTRO) Consensus established a 4-group classification (low, intermediate, high-intermediate and high risk) based on clinicopathological features, with the aim of prognosis stratification, but also to help with the indication for adjuvant therapy [2].

The Tumor Cancer Genome Atlas (TCGA) performed a comprehensive genomic profiling of over 300 EC samples, resulting in a molecular classification with prognosis implications [4]. In search of a more cost effective and applicable method for group assignment in routine practice, the Proactive Molecular Risk Classifier for Endometrial Cancer (ProMisE) reproduced the TCGA molecular classification using surrogate biomarkers by targeted sequencing and immunohistochemistry (IHC) on formalin-fixed paraffin-embedded (FFPE) tumour samples [5–7]. The group named POLE, composed by cases with mutations in the exonuclease domain (EDM) of polymerase- ϵ gene, has an excellent prognosis. In contrast, patients with the poorest prognosis harbour tumour mutations in the *TP53* gene. This group is named p53 abnormal (p53abn) due to aberrant immunohistochemical p53 expression. The other 2 groups with intermediate risk were also established. The first encompasses mismatch repair deficient (MMRd) cases, defined by loss of expression of at least one of the mismatch repair proteins (MLH1, PMS2, MSH2 and MSH6). The remaining cases are included in the group named p53 wild type (p53wt) or non-specific molecular profile (NSMP).

In addition, other potential prognostic biomarkers have been described in EC, although most have not yet been implemented in the clinic. For example, it is reported that oestrogen and progesterone receptors (ER and PR) play a significant role in endometrial carcinogenesis. Their expressions are associated with well-differentiated tumours and correlate with earlier tumour stages and better survival [8]. L1-cell adhesion molecule (L1CAM) overexpression has been associated with a poorer outcome [9]. Amplification and increased expression of human epidermal growth factor receptor 2 (HER2) has been correlated with poor prognosis and more aggressive tumour behaviour [10]. Those with EC harbouring catenin beta 1 (*CTNNB1*) mutation encompass a more aggressive subset within low-grade early-stage endometrioid EC [11]. Other biomarkers such as phosphatase and tensin homolog (PTEN), AT-rich interactive domain-containing protein 1A (ARID1A) or E-cadherin (ECAD) have also had a possible impact on prognosis in some studies [12]. The integration of clinicopathological features and molecular subgroups is currently a reality based on the recent publication of ESGO-ESTRO-European Society of Pathology (ESP) 2020 guidelines. These guidelines still recommend a 4-risk group classification, incorporating ProMisE molecular markers with clinical characteristics and suggesting a possible improvement in the accuracy of the risk prognosis stratification [13].

Our aim with this study was to analyse and compare the 3 above-mentioned risk stratification tools in the same cohort of early-stage EC, and to identify additional biomarkers with an impact on prognosis that

could improve the precision of these classifiers.

Materials And Methods

Study cohort

A retrospective cohort was collected including patients diagnosed with early-stage (I and II by FIGO) EC between 2003 and 2015 at La Paz University Hospital (Madrid, Spain), with a minimum follow-up of 5 years. Patients were consecutive. The study was approved by the local Ethics Committee (HULP#PI3778) and was conducted in accordance with ethical standards of the Helsinki Declaration of the World Medical Association.

We analysed clinical and pathological variables, such as age, histological subtype, FIGO stage, tumour size, LVSI, grade of differentiation, and myometrial infiltration. Clinical data on treatment and follow-up were obtained from the electronic medical records database and were subsequently updated, allowing for an evaluation of relapse-free survival (RFS) and disease-specific overall survival (OS).

Sample selection

Optimal tissue blocks were selected by an expert gynaecological pathologist on haematoxylin and eosin (H&E) slides. DNA was extracted from selected tumour rich regions with the Qiamp DNA FFPE Tissue Kit (Qiagen, Germany), and used for polymerase chain reaction (PCR) purposes. Additionally, representative tumour non-necrotic areas of each case were selected for tissue microarray (TMA) construction. Two representative cores of 1.2 mm in diameter were taken and arrayed into a receptor block using a TMA workstation (Beecher Instruments, Silver Spring, MD, USA) as previously described [14].

Risk stratification tools

The ESMO-ESGO-ESTRO 2016 risk stratification groups were established as follows: low, intermediate, high-intermediate, and high risk. For simplicity, hereafter this classifier will be referred to as the '2016 Classifier' [2].

We also stratified patients by the ProMisE risk groups: POLE, MMRd, p53wt/NSMP and p53abn [5]. First, specific PCR and Sanger sequencing was performed to identify mutations in exons 9, 13 and 14 of *POLE*. These exons code for part of the EDM and account for most of the described mutations [15, 16]. Second, we used 4 µm sections of the TMA for IHC purposes. The expression of MLH1, PMS2, MSH2, MSH6 and p53 was evaluated with specific antibodies (p53, #IR616; MLH1, #IR079; PMS2, #IR087; MSH2, #IR084 and MSH6, #IR086 respectively), all from Agilent (Denmark), as previously described [17].

Lastly, a combination of clinicopathological and molecular variables employed in the previous risk stratification tools were used following the ESGO-ESTRO-ESP 2020 guidelines to establish a new 4-group classification: low, intermediate, high-intermediate and high risk. Henceforth, this will be named the '2020 Classifier' [13].

Biomarker analysis

Additionally, other molecular markers previously studied in EC were explored. Expressions ER, PR, ECAD, HER2, ARID1A, PTEN and L1CAM were evaluated. Specific antibodies and cut-off categories were applied to each marker to simplify their evaluation as much as possible. A detailed description can be found in Supplementary Table 1. PCR and Sanger sequencing were also performed to explore *CTNNB1* exon 3, which contains key protein phosphorylation sites.

Statistical analysis

Descriptive statistics included clinicopathological and biomarker frequencies. Qualitative variables are presented as number of cases and frequency percentages. Continuous variables are presented as median value and range. Missing values in the ProMisE and 2020 Classifier groups were imputed, taking the most frequent values from a total of 1000 runs of the predictive mean matching method provided in the mice R package [18].

The primary endpoint was to evaluate RFS, defined as the time from surgery to the time of first recurrence or death from disease. As a secondary endpoint, disease-specific OS was analysed, defined as time from the surgery to death related to disease. All relapses and deaths were considered as events. Differences in RFS, and OS were compared using Kaplan–Meier (K-M) curves.

The Goodman-Kruskal concordance index (c-index) is used as a metric to assess the models' performance. It ranges between 0 and 1; however, a value of 0.5 indicates that a model does not perform better than random. The c-index is designed to estimate the concordance probability of independent and identically distributed data of size n comparing the rankings of 2 independent survival time and hazard values [19, 20]. Therefore, this index indicates the discriminatory properties and stratification accuracy. The precision of each risk classifier for RFS and OS (censored data) was evaluated using the Cox Proportional Hazards (PH) Model. The statistical analysis was based on Student's t-test and the Mann–Whitney test for parametric and nonparametric continuous variables, respectively, and the chi-squared or Fisher's exact test, as appropriate, for categorical variables. Statistical significance was considered when $p < 0.05$. Data were managed with an Excel database (Microsoft, Redmond, WA, USA) and statistical analyses were performed using R 4.0.3 software, available online at <https://cran.r-project.org/>.

Results

Description of clinical characteristics

A total of 294 patients were included, with a median follow-up of 75 months. The clinicopathological characteristics of the entire cohort and their univariate analysis for RFS and OS are summarised in Table 1. The majority of patients had tumours with endometrioid histological subtype (88.1%), low grade (79.9%) and FIGO stage Ia (69%). Adjuvant radiation therapy and chemotherapy were administered to 36.7% and 5.1% patients, respectively. Relapse was identified in 44 (15.1%) patients, with a location

pattern divided into locoregional (34.1%) and distant metastases (65.9%). Twenty-seven (9.2%) deaths due to EC were recorded. All clinicopathological variables had a statistically significant correlation with RFS and OS (with the exception of LVSI in OS).

Table 1
Clinicopathological characteristics univariate analysis

| Variable | Descriptive | RFS | | OS | |
|----------------------|-------------|------------------|------------------|------------------|------------------|
| | | HR (95%CI) | p value | HR (95%CI) | p-value |
| | n (%) | | | | |
| Age | 97 (33) | 1.70 (1.03–2.80) | 0.04 | 1.94 (1.08–3.47) | 0.03 |
| < 60 years | 197 (67) | | | | |
| > 60 years | | | | | |
| Histological subtype | 35 (11.9) | 0.33 (0.19–0.58) | < 0.01 | 0.37 (0.19–0.70) | < 0.01 |
| NEEC | 259 (88.1) | | | | |
| EEC | | | | | |
| Tumor grade | 235 (80) | 2.78 (1.72–4.48) | < 0.01 | 2.99 (1.76–5.07) | < 0.01 |
| Low (G1 + G2) | 59 (20) | | | | |
| High (G3) | | | | | |
| Myometrial invasion | 58 (19.7) | 3.32 (1.44–7.63) | < 0.01 | 3.05 (1.22–7.69) | 0.02 |
| No | 235 (79.9) | | | | |
| Yes | 1 (0.4) | | | | |
| NE | | | | | |
| LVSI | 239 (81.3) | 1.86 (1.12–3.09) | 0.02 | 1.39 (0.76–2.60) | 0.29 |
| No | 53 (18) | | | | |
| Yes | 2 (0.7) | | | | |
| NE | | | | | |
| FIGO stage | 203 (69) | 2.13 (1.53–2.96) | < 0.01 | 1.91 (1.31–2.78) | < 0.01 |
| Ia | 74 (25.2) | | | | |
| Ib | 17 (5.8) | | | | |
| II | | | | | |

EEC: Endometrioid endometrial carcinoma; NEEC: Non-endometrioid endometrial carcinoma; NE: No evaluable; LVSI: Lymph-vascular space invasion; FIGO: International Federation of Gynecology and Obstetrics; HR: Hazard ratio. 95%CI: 95% Confidence interval; RFS: Relapse-free survival; OS: Overall survival.

Prognosis features and accuracy of stratification tools

The distribution of prognosis risk groups, 5-year survival rate, Cox regression and c-index analysis for each classifier are detailed in Table 2 and K-M curves for RFS and OS are shown in Fig. 1.

Table 2
Risk stratification tools accuracy comparison by relapse-free survival

| Classifier | Descriptive | | RFS | | |
|--|-------------|-----------------|------------------|-----------------|---------|
| | n (%) | 5-year rate (%) | HR (95%CI) | p-value | c-index |
| 2016 Classifier | 147 (50) | 93.2 | 1.63 (1.36–1.96) | <0.01 | 0.77 |
| Low | 42 (14.3) | 77.0 | | | |
| Intermediate | 51 (17.3) | 77.5 | | | |
| High-intermediate | 54 (18.4) | 49.3 | | | |
| High | | | | | |
| ProMisE | 13 (4.4) | 92.3 | 1.19 (0.84–1.69) | 0.32 | 0.54 |
| POLE | 69 (23.5) | 75.1 | | | |
| MMRd | 180 (61.2) | 86.9 | | | |
| p53wt/NSMP | 32 (10.9) | 49.3 | | | |
| p53abn | | | | | |
| 2020 Classifier | 148 (50.3) | 93.3 | 1.90 (1.57–2.31) | <0.01 | 0.79 |
| Low | 61 (20.7) | 79.8 | | | |
| Intermediate | 50 (17.0) | 72.5 | | | |
| High-intermediate | 35 (11.9) | 36.3 | | | |
| High | | | | | |
| RFS: Relapse-free survival; POLE: Polymerase ε exonuclease domain mutation; MMRd: Mismatch repair deficiency. P53wt/NSMP: P53 wild type/Non-specific molecular profile; p53abn: p53 aberrant; HR: Hazard ratio; 95% CI: 95% Confidence interval. | | | | | |

Regarding the 2016 Classifier, the low-risk group is the most represented, accounting for half of the patients. The K-M curves showed a clear differentiation between low and high risk groups, but the intermediate groups had early overlap.

The ProMisE classification found that p53/NSMP followed by MMRd groups represented the majority of cases. The K-M curves confirmed that POLE and p53abn were the extreme prognosis groups. In the POLE

group there was only one relapse. The MMRd group showed a poorer 5-year survival rate than p53wt/NSMP, but without significant differences.

Lastly, regarding the 2020 Classifier, the low-risk group was the most frequent, with a similar proportion as the 2016 Classifier. However, there was a redistribution of the other 3 groups, with a decrease in the percentage of high-risk cases, and an increase of the intermediate and high-intermediate risk groups. As observed with the 2016 Classifier, there was not a clear separation between these intermediate groups.

Despite the lack of separation between intermediate groups in the K-M curves, the Cox PH regression model for RFS found statistically significant differences for both the 2016 and 2020 Classifiers, but not for ProMisE. Discriminative metrics in the entire cohort showed that the 2020 Classifier reached the highest c-index (0.79), closely followed by the 2016 Classifier (0.77). Although the improvement in c-index value was discrete, when looking at the 5-year survival rates, it showed that the redistribution among groups over the 2020 Classifier achieved a better RFS stratification (Table 2).

The Cox regression model was also performed for OS, again finding statistical significance for risk assessment in the 2016 and 2020 Classifiers: HR 1.55 (95% CI 1.26–1.90) and 1.82 (95% CI (1.46–2.27)), respectively; $p < 0.01$ for both. In contrast, there was still an absence of significant differences for ProMisE ($p = 0.44$).

Other biomarker assessments

The univariate statistics of other biomarkers for RFS and OS are provided in Table 3. ER expression was the only biomarker significantly correlated with a longer RFS. Additionally, PR and ECAD expression showed a correlation with a longer RFS, albeit not as significant as that of ER. ER, PR and ECAD expression were significantly associated with a longer OS.

Table 3
Univariate biomarker analysis for relapse-free survival and overall survival

| Variable | Descriptive | RFS | | OS | |
|----------|-------------|------------------|------------------|------------------|------------------|
| | n (%) | HR (95%CI) | p-value | HR (95%CI) | p-value |
| ER | 40 (13.6) | 0.37 (0.22–0.64) | < 0.01 | 0.33 (0.18–0.58) | < 0.01 |
| Negative | 244 (83) | | | | |
| Positive | 10 (3.4) | | | | |
| NE | | | | | |
| PR | 45 (15.3) | 0.58 (0.33–1.01) | 0.05 | 0.52 (0.28–0.94) | 0.03 |
| Negative | 235 (79.9) | | | | |
| Positive | 14 (4.8) | | | | |
| NE | | | | | |
| ECAD | 47 (16) | 0.57 (0.32–1.01) | 0.05 | 0.47 (0.25–0.87) | 0.02 |
| Negative | 234 (79.6) | | | | |
| Positive | 13 (4.4) | | | | |
| NE | | | | | |
| HER2 | 290 (98.6) | 1.15 (0.16–8.29) | 0.89 | NE | NE |
| Negative | 3 (1) | | | | |
| Positive | 1 (0.3) | | | | |
| NE | | | | | |
| ARID1A | 220 (74.8) | 0.86 (0.50–1.49) | 0.58 | 0.92 (0.50–1.70) | 0.79 |
| Negative | 61 (20.8) | | | | |
| Positive | 13 (4.4) | | | | |
| NE | | | | | |
| PTEN | 193 (65.6) | 1.31 (0.83–2.06) | 0.25 | 1.29 (0.77–2.16) | 0.33 |
| Negative | 95 (32.3) | | | | |
| Positive | 6 (2) | | | | |
| NE | | | | | |

| Variable | Descriptive | RFS | | OS | |
|--|-------------|------------------|------|------------------|------|
| L1CAM | 248 (84.3) | 1.28 (0.61–2.68) | 0.51 | 1.31 (0.56–3.07) | 0.53 |
| Negative | 32 (10.9) | | | | |
| Positive | 14 (4.8) | | | | |
| NE | | | | | |
| <i>CTNNB1</i> | 257 (87.4) | 1.72 (0.86–3.44) | 0.13 | 1.32 (0.56–3.06) | 0.52 |
| Non mutated | 21 (7.1) | | | | |
| Mutated | 16 (5.4) | | | | |
| NE | | | | | |
| NE: Not evaluable; HR: Hazard ratio; 95%CI: 95% Confidence interval; RFS: Relapse-free survival; OS: Overall survival. | | | | | |

We also performed a subgroup analysis by histology and differentiation grade. Considering only the endometrioid histology subgroup, the *CTNNB1* mutation was associated with a significantly poorer RFS, whereas ER expression correlated with a better OS and a trend towards a longer RFS (Supplementary Table 2). In the non-endometrioid subgroup, L1CAM expression had a trend to a longer RFS and OS (Supplementary Table 3). In the low-grade (grade 1 and 2) subgroup, there was a trend to a longer RFS with ECAD expression, and to a shorter RFS and OS with PTEN expression (Supplementary Table 4). None of the biomarkers showed a correlation with RFS or OS in the high-grade subgroup (Supplementary Table 5).

The descriptive analysis of these biomarkers regarding their distribution by the risk classifier categories are summarised in Table 4.

Table 4
Biomarker distribution among risk classifiers

| 2016 Classifier | Low | Intermediate | High-intermediate | High |
|------------------------|-------------|---------------------|--------------------------|---------------|
| | n (%) | n (%) | n (%) | n (%) |
| ER | 141 (95.9) | 41 (97.6) | 43 (84.3) | 19 (35.2) |
| PR | 131 (89.1) | 41 (97.6) | 41 (80.4) | 22 (40.7) |
| ECAD | 132 (89.8) | 35 (83.3) | 41 (80.4) | 26 (48.2) |
| HER2 | 0 | 0 | 1 (2) | 2 (3.7) |
| ARID1A | 28 (19.1) | 6 (14.3) | 15 (29.4) | 12 (22.2) |
| PTEN | 50 (34) | 14 (33.3) | 13 (25.5) | 18 (33.3) |
| L1CAM | 3 (2) | 1 (2.4) | 7 (13.7) | 21 (38.9) |
| <i>CTNNB1</i> | 9 (6.1) | 4 (9.5) | 5 (9.8) | 3 (5.6) |
| ProMisE | POLE | MMRd | p53wt/NSMP | p53abn |
| | n (%) | n (%) | n (%) | n (%) |
| ER | 8 (61.5) | 61 (88.4) | 161 (89.4) | 14 (43.8) |
| PR | 9 (69.2) | 53 (76.8) | 158 (87.8) | 15 (46.9) |
| ECAD | 10 (76.9) | 58 (84.1) | 145 (80.6) | 21 (65.6) |
| HER2 | 0 | 0 | 1 (0.6) | 2 (6.3) |
| ARID1A | 0 | 21 (30.4) | 37 (20.6) | 3 (9.4) |
| PTEN | 6 (46.2) | 12 (17.4) | 63 (35) | 14 (43.8) |
| L1CAM | 3 (23.1) | 4 (5.8) | 8 (4.4) | 17 (53.1) |
| <i>CTNNB1</i> | 2 (15.4) | 3 (4.3) | 15 (8.3) | 1 (3.1) |
| 2020 Classifier | Low | Intermediate | High-intermediate | High |
| | n (%) | n (%) | n (%) | n (%) |
| ER | 138 (93.2) | 50 (82) | 42 (84) | 14 (40) |
| PR | 128 (86.5) | 50 (82) | 41 (82) | 16 (45.7) |
| ECAD | 131 (88.5) | 50 (82) | 35 (70) | 18 (51.4) |
| HER2 | 0 | 1 (1.6) | 0 | 2 (5.7) |
| ARID1A | 28 (18.9) | 8 (13.1) | 19 (38) | 6 (17.1) |
| PTEN | 53 (35.8) | 18 (29.5) | 11 (22) | 13 (37.1) |

| 2016 Classifier | Low | Intermediate | High-intermediate | High |
|---|---------|--------------|-------------------|-----------|
| L1CAM | 5 (3.4) | 9 (14.8) | 3 (6) | 15 (42.9) |
| <i>CTNNB1</i> | 9 (6.1) | 6 (9.8) | 4 (8) | 2 (5.7) |
| POLE: Polymerase ε exonuclease domain mutation; MMRd: Mismatch repair deficiency; p53wt/NSMP: p53 wild type/Non-specific molecular profile; p53abn: p53 aberrant. | | | | |

Improving patient stratification by considering other biomarkers

We found that the 2020 Classifier was a slightly better stratification tool than the 2016 and ProMisE Classifiers in our series. However, the intermediate groups (intermediate and high-intermediate) still overlapped (Fig. 1). Therefore, we merged these intermediate groups and performed a Cox regression analysis to explore the impact of the selected biomarkers. The results showed a statistically significant association between the *CTNNB1* mutation and a poorer RFS (Table 5).

Table 5
Univariate biomarker analysis over 2020 Classifier intermediate groups merged cohort

| Variable | Descriptive | RFS | OS | | |
|----------|-------------|-------------------|---------|------------------|---------|
| | n (%) | HR (95%CI) | p-value | HR (95%CI) | p-value |
| ER | 14 (12.6) | 2.60 (0.61–11.10) | 0.20 | 1.76 (0.41–7.60) | 0.45 |
| Negative | 92 (82.9) | | | | |
| Positive | 5 (4.5) | | | | |
| NE | | | | | |
| PR | 16 (14.4) | 3.22 (0.76–13.63) | 0.11 | 2.21 (0.51–9.47) | 0.29 |
| Negative | 91 (82.0) | | | | |
| Positive | 4 (3.6) | | | | |
| NE | | | | | |
| ECAD | 21 (18.9) | 0.91 (0.38–2.21) | 0.84 | 0.67 (0.25–1.82) | 0.44 |
| Negative | 85 (76.6) | | | | |
| Positive | 5 (4.5) | | | | |
| NE | | | | | |
| HER2 | 109 (98.2) | NE | NE | NE | NE |
| Negative | 1 (0.9) | | | | |
| Positive | 1 (0.9) | | | | |
| NE | | | | | |
| ARID1A | 80 (72.1) | 0.47 (0.18–1.20) | 0.11 | 0.41 (0.12–1.38) | 0.15 |
| Negative | 27 (24.3) | | | | |
| Positive | 4 (3.6) | | | | |
| NE | | | | | |
| PTEN | 80 (72.1) | 1.13 (0.55–2.32) | 0.74 | 1.50 (0.67–3.39) | 0.33 |
| Negative | 29 (26.1) | | | | |
| Positive | 2 (1.8) | | | | |
| NE | | | | | |

| Variable | Descriptive | RFS | | OS | |
|--|-------------|------------------|------------------|------------------|------|
| L1CAM | 96 (86.5) | 0.24 (0.03–1.77) | 0.16 | 0.39 (0.05–2.94) | 0.37 |
| Negative | 12 (10.8) | | | | |
| Positive | 3 (2.7) | | | | |
| NE | | | | | |
| <i>CTNNB1</i> | 97 (87.3) | 3 (1.30–6.91) | < 0.01 | 2.42 (0.90–6.52) | 0.08 |
| Non mutated | 10 (9.0) | | | | |
| Mutated | 4 (3.6) | | | | |
| NE | | | | | |
| NE: Not evaluable; HR: Hazard ratio; 95%CI: 95% Confidence interval; RFS: Relapse-free survival; OS: Overall survival. | | | | | |

The K-M plots on the merged intermediate groups by *CTNNB1* mutation status showed an improved stratification. Therefore, we substituted the 2 original intermediate 2020 Classifier groups for these new ones, while maintaining the original low and high risk groups (Figs. 2A and 2B). Subsequently, we observed that patients with tumours harbouring the *CTNNB1* mutation showed a poor prognosis, with a similar RFS to the high-risk group (Fig. 2C). Thus, we proposed a novel stratification model consisting of 3 categories instead of 4, by merging the 2020 Classifier high risk group with *CTNNB1* mutated tumours. The intermediate group was redefined as *CTNNB1* non-mutated cases (Fig. 2D). This new stratification system of 3 categories improved the c-index to 0.82 compared with 0.79 based on the 2020 Classifier. Furthermore, it achieved statistically significant and clinically relevant differences in 5-year RFS: 93.4% for low risk, 79.6% for the intermediate merged group/*CTNNB1* wild type and 37.3% for the high risk group (including patients from the merged intermediate groups with *CTNNB1* mutation). A decision-tree model based on this proposal is shown in Fig. 2E.

Discussion

In this study, the 3 main risk classifiers described in the last decade (ESMO-ESGO-ESTRO 2016, ProMisE and ESGO-ESTRO-ESP 2020) were evaluated in a large early-stage EC cohort. The results showed that all of these classifiers differentiate RFS between high and low-risk groups, but there was an overlap between the intermediate and high-intermediate risk groups. Similar findings have been observed in other studies. For example, regarding the 2016 Classifier, 2 retrospective cohorts reported no differences between the intermediate and high-intermediate group, one of them with overlapping K-M OS curves [21, 22]. In terms of the ProMisE Classifier, there are other publications that also showed no significant differences between the 2 intermediate molecular subtypes, although it performed well on the 2 extreme groups: the POLE group, with a very low incidence of relapses, and the p53abn group, with a high risk of recurrence [23, 24]. In our study, we observed similar results, with only 1 relapse in the POLE group.

The recently published 2020 Classifier has incorporated the molecular variables of the ProMisE classification into the prognostic stratification carried out in the 2016 Classifier, with the aim of improving its accuracy and thus making better therapeutic recommendations. In this new classification, stage I-II *POLE* mutated tumours are included in the low-risk group, for which adjuvant treatment is not recommended, whereas most of the p53abn tumours (except those without myometrial invasion) have been incorporated into the high-risk group, for which adjuvant chemotherapy is strongly recommended.

In this study, we have provided one of the first evaluations of this new risk classification in a cohort of patients and, to our knowledge, the first comparison of the 3 classifiers focused on early-stage EC. Two recent publications have evaluated the 2020 Classifier in 2 large patient cohorts, including those with advanced disease [25, 26]. Similarly to our results, Ortoft et al described fewer patients allocated to the high risk group using the 2020 Classifier and reported a poorer RFS for this group than that achieved with the 2016 Classifier [25]. These findings suggest that the 2020 Classifier achieves a better redistribution of the 4 risk groups that impact the 5-year survival rates. However, in terms of c-index values, we found only a discrete improvement over the 2016 Classifier. Furthermore, in our experience this classifier is still not good enough to separate the 2 intermediate groups, and following this classification, different adjuvant treatments would be recommended to patients with a similar prognosis (intermediate and high-intermediate groups). In the same way, Imboden et al found significant differences in RFS using the 2020 Classifier, but with an overlap of K-M curves of both intermediate-risk groups [26]. These results reaffirm the unmet need for an accurate stratification system and motivate us to explore the potential of other biomarkers that could improve the current options.

To improve the precision of the 2020 Classifier, we focused on the molecular biomarkers previously explored in EC, with potential prognostic value but not yet included in the main risk classifiers. We first evaluated their association with prognosis in our entire cohort. Among them, only ER showed a significant correlation with RFS, and ER, PR and ECAD with OS. These results are in agreement with previous publications [27, 28]. There are several reports on HER2 amplification, specifically in non-endometrioid histologies and a subset of high-grade endometrioid tumours. We had almost no HER2 overexpression, so no correlations with the prognosis could be established [29]. Loss of ARID1A has been linked to shorter progression-free survival in EC, and loss of PTEN might be a good prognostic factor [30, 31]. Our results are similar in terms of the positive proportion of cases for both biomarkers, but we did not find any statistical significance related to survival.

Among the remaining analysed markers, probably the most intriguing results concern L1CAM, which has frequently been associated with distant recurrence and OS. We have used a previously established cutoff for IHC to achieve the best correlation with prognosis [32]. Our results are similar regarding positivity rates to those published for the PORTEC-1 trial samples, but do not reach significance, probably because of the lower positivity of the marker and the smaller size of our cohort [33]. The other biomarker frequently associated with prognosis is *CTNNB1*. In our cohort, it showed significance only when intermediate risk groups were merged, and for this reason it was subsequently considered for their inclusion in the risk classifier.

The impact of the *CTNNB1* mutation and other biomarkers (like *POLE*, *MMRd*, *p53*, *L1CAM*, or *LVSI*) prompted the design of the PORTEC-4 trial. This phase III study, including patients with high-intermediate risk EC, randomises patients between a standard arm with adjuvant vaginal brachytherapy and an experimental arm with an adjuvant radiation therapy tailored by a molecular-integrated risk profile. In this trial, patients with *p53wt/NSMP* and no mutation in *CTNNB1* are considered to be in the same low-risk group as those with the *POLE* mutation [34]. However, in our study, patients initially classified in the intermediate or high-intermediate groups with no mutation in *CTNNB1* have a poorer prognosis than those of the low-risk group (which included patients with the *POLE* mutation).

The *CTNNB1* mutation leads to the over-activation of beta catenin, which results in the aberrant signalling of the Wnt pathway, contributing to tumour progression [35]. The poorer prognosis associated with the *CTNNB1* mutation in exon 3 has been shown in other studies, mainly in grade 1–2 endometrioid or *NSMP* cohorts [36, 37], suggesting that this mutation is more likely to be functional, and not a passenger event [38]. The ESGO-ESTRO-ESP 2020 guidelines mention that the *CTNNB1* mutation might be potentially useful in the group of low-grade *p53wt/NSMP* EC, but they did not include it in the risk stratification proposal. In our study, the *CTNNB1* status was significantly associated with RFS in the intermediate and high-intermediate risk groups.

Further, the *CTNNB1* mutational analysis over both intermediate groups could reallocate some patients into the high-risk group (those with the *CTNNB1* mutation), while the remaining patients would be considered to be within the intermediate risk group. Moreover, by including the *CTNNB1* status in the 2020 Classifier, we simplified the 4-group classification into 3 groups. Based on this proposal, adjuvant treatment recommendations could be made for each novel group; for example, patients allocated as intermediate or high-intermediate by the 2020 Classifier with the *CTNNB1* mutation can be considered for adjuvant chemotherapy.

The main limitation of our study is related to its retrospective design and the absence of a validation cohort. Second, the study is based on TMA and not on whole tissue sections, which might not completely reflect the heterogeneity of some tumours. On the other hand, as strengths, the large number of patients with a long follow-up, and the high homogeneity of the series should be highlighted, given it encompasses only early stages (FIGO I-II). Furthermore, it is the first study to evaluate and compare the 3 most important risk classifiers in EC, including the recent ESGO-ESTRO-ESP Classification, focused on early-stage disease.

Conclusions

None of the main published risk classifiers developed in EC achieved a significant difference in RFS between their intermediate groups. The 2020 ESGO-ESTRO-ESP classification showed a slightly better discriminatory capacity than the other classifications. The incorporation of additional biomarkers, such as *CTNNB1*, into the 2020 Classifier could improve the accuracy of the stratification, especially in terms

of redefining the intermediate prognostic groups. A validation of this proposal in additional series will be necessary.

Abbreviations

EC, endometrial cancer; ProMiSe, Proactive Molecular Risk Classifier for Endometrial; LVSI, lymphovascular space invasion, TCGA, The Cancer Genome Tumor Atlas; FFPE, formalin-fixed paraffin-embedded; EDM, exonuclease domain of polymerase- β gene, polymerase; MMRd, mismatch repair deficient; NSMP, non-specific molecular profile; RFS, relapse free survival; OS, disease-specific overall survival; K-M, Kaplan Meier; NE, not evaluable.

Declarations

Ethics approval and consent to participate

The study was approved by the local Ethics Committee (HULP#PI3778) and was conducted in accordance with ethical standards of the Helsinki Declaration of the World Medical Association.

Consent for publication

Not applicable.

Availability of data and materials

The datasets are available from the corresponding authors on reasonable request.

Competing interests

AG reports honoraria (Clovis, MSD, AstraZeneca, GSK, PharmaMar and Roche) and travel/accommodation/expenses (Merck Sharp & Dohme, PharmaMar, Roche, Eisai, Pfizer, Pierre-Fabre and Tesaro-A GSK Company), outside the submitted work.

MM reports honoraria (MSD, AstraZeneca and GSK), research grant/funding to her institution (Eisai and PharmaMar), travel/accommodation/expenses (AstraZeneca, GSK, PharmaMar, Roche and Pfizer), outside the submitted work.

AR reports honoraria and advisory/consultancy (MSD, AstraZeneca, Roche, GSK, Clovis, PharmaMar, Lilly, Amgen), research grant/funding to his institution (Eisai, PharmaMar, Roche), travel/accommodation/expenses (AstraZeneca, Tesaro: A GSK Company, PharmaMar, Roche), and speakers bureau (MSD, AstraZeneca, Roche, GSK, Clovis, PharmaMar), outside the submitted work.

The remaining authors declare no conflicts of interest.

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Authors' Contributions

Conceptualisation: JLR-P, IR-C, VH-S, MM, AR.; Methodology: JLR-P, IR-C, VH-S, LEG, BZ, YW, AB, AL-J, MMi, JE, AG, LY, BC, LY, AP-G, JT, MM, AR; Formal analysis: JLR-P, IR-C, BZ, YW, JT; Resources: AH, JF, JT, DH, MM, AR; Visualisation: BZ, YW, JT; Supervision: AR, MM, JT; Project administration: AR, MM; Writing—original draft preparation: JLR-P, JT, MM, AR; Writing—review and editing: All authors.

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Figures

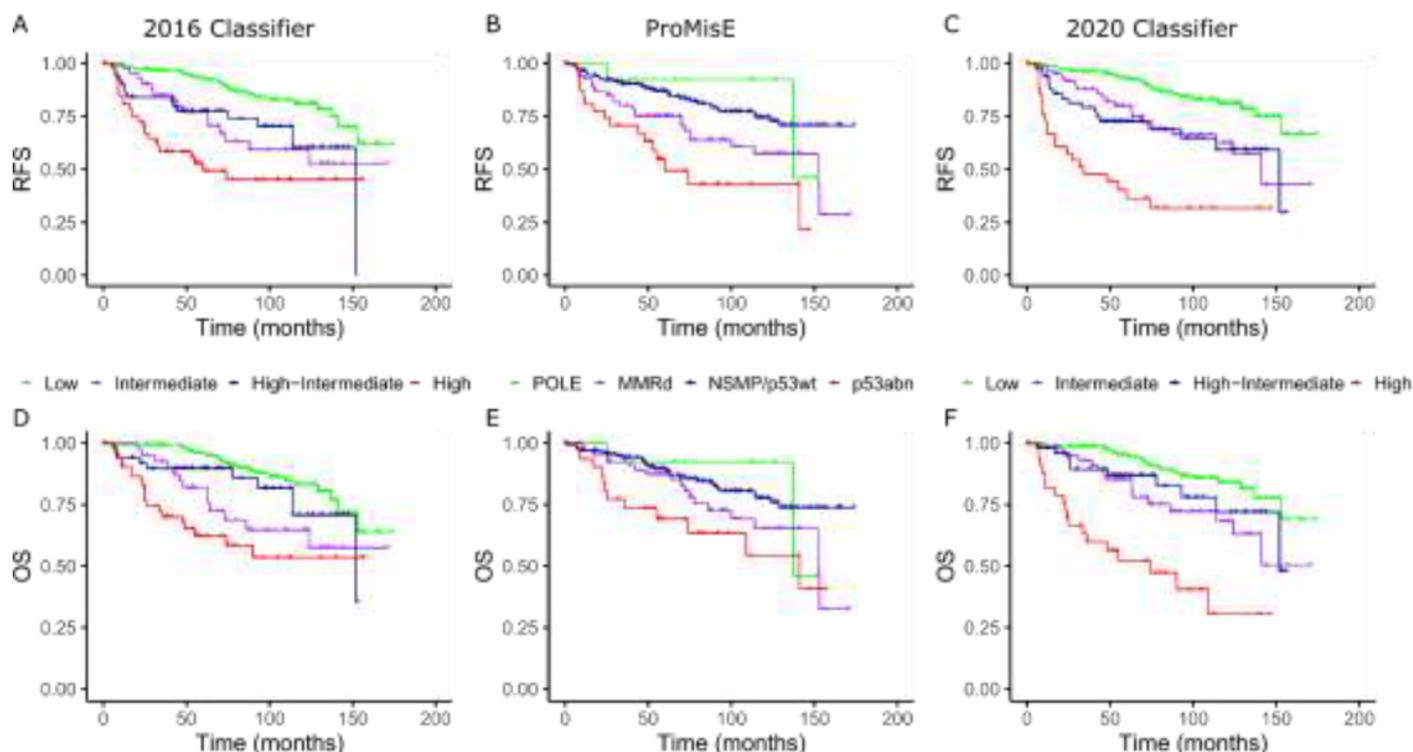


Figure 1

Relapse-free survival (RFS) and overall survival (OS) curves estimation by stratification prognosis tools. Upper row illustrates RFS for the 2016 Classifier (A), ProMisE (B), 2020 Classifier (C). Lower row illustrates OS for the 2016 Classifier (D), ProMisE (E), 2020 Classifier (F). The 2016 Classifier refers to the ESMO-ESGO-ESTRO classification, ProMisE to the Proactive Molecular Risk Classifier for Endometrial Cancer, and the 2020 Classifier to ESGO-ESTRO-ESP.

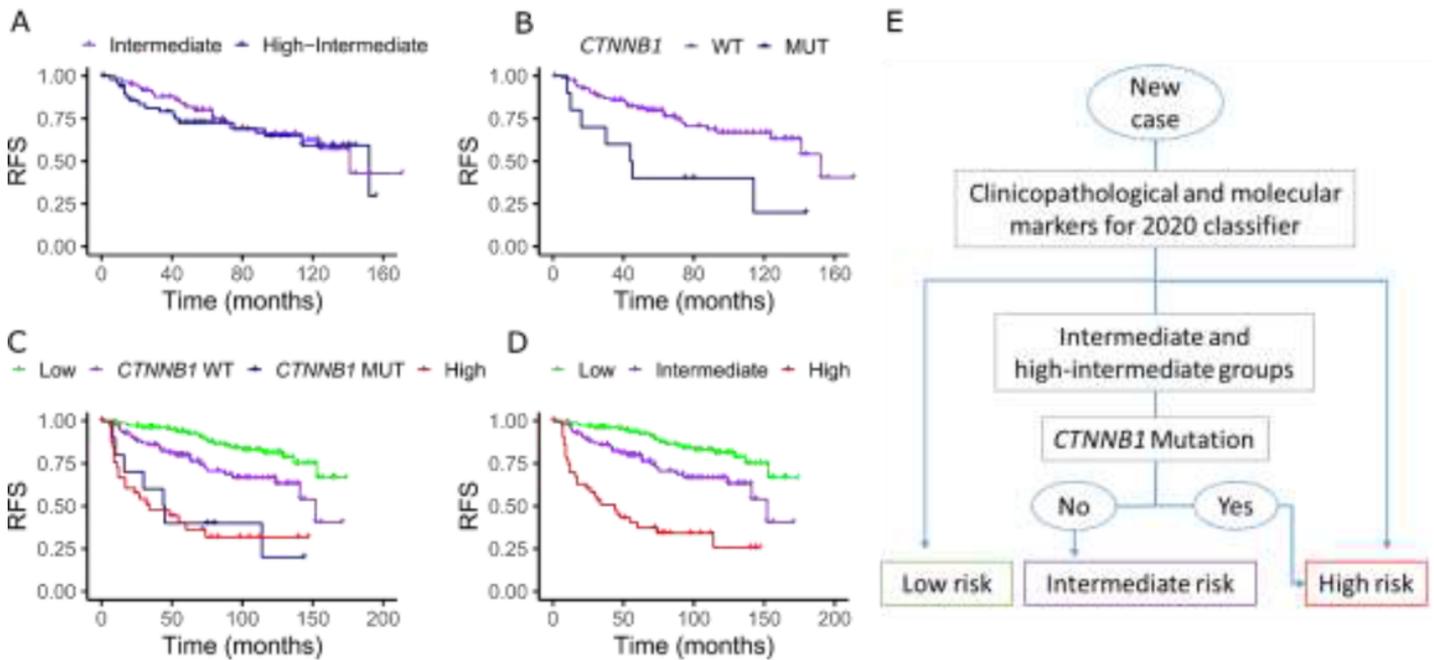


Figure 2

KM plots for the new risk stratification proposal. Survival estimation curves for the 2020 Classifier intermediate and high-intermediate groups merged, with the 2020 Classifier criteria (A), and stratified by CTNNB1 status (B). Survival plots for the entire series, including high and low-risk groups based on 2020 Classifier criteria and intermediate and high-intermediate risk groups stratified by CTNNB1 status, in 4 groups (C), and in 3 groups, by adding those with CTNNB1 mutation to the high-risk group (D). Decision tree based on this proposal (E)

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