European Journal of Surgical Oncology 50 (2024) 108436

Contents lists available at ScienceDirect

European Journal of Surgical Oncology

journal homepage: www.ejso.com

# Application of novel algorithm on a retrospective series to implement the molecular classification for endometrial cancer

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ARTICLE INFO

*Keywords:*
Endometrial cancer
Molecular classification
Guidelines
Risk stratification

ABSTRACT

*Introduction:* The study aimed to validate the Betella algorithm, focusing on molecular analyses exclusively for endometrial cancer patients, where molecular classification alters risk assessment based on ESGO/ESTRO/ESP 2020 guidelines.

*Materials and methods:* Conducted between March 2021 and March 2023, the retrospective research involved endometrial cancer patients undergoing surgery and comprehensive molecular analyses. These included p53 and mismatch repair proteins immunohistochemistry, as well as DNA sequencing for POLE exonuclease domain. We applied the Betella algorithm to our population and evaluated the proportion of patients in which the molecular analysis changed the risk class attribution.

*Results:* Out of 102 patients, 97 % obtained complete molecular analyses. The cohort exhibited varying molecular classifications: 10.1 % as POLE ultra-mutated, 30.3 % as mismatch repair deficient, 11.1 % as p53 abnormal, and 48.5 % as non-specified molecular classification. Multiple classifiers were present in 3 % of cases. Integrating molecular classification into risk group calculation led to risk group migration in 11.1 % of patients: 7 moved to lower risk classes due to POLE mutations, while 4 shifted to higher risk due to p53 alterations. Applying the Betella algorithm, we can spare the POLE sequencing in 65 cases (65.7 %) and p53 immunochemistry in 17 cases (17.2 %).

*Conclusion:* In conclusion, we externally validated the Betella algorithm in our population. The application of this new proposed algorithm enables assignment of the proper risk class and, consequently, the appropriate indication for adjuvant treatment, allowing for the rationalization of the resources that can be allocated otherwise, not only for the benefit of settings with low resources, but of all settings in general.

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https://doi.org/10.1016/j.ejso.2024.108436
Received 21 April 2024; Received in revised form 19 May 2024; Accepted 22 May 2024
Available online 23 May 2024

## 1. Introduction

Endometrial cancer (EC) is the most common gynaecological tumour in developed countries, with rising incidence and mortality [1].

Its increased incidence has been attributed at least in part to the overall rise in obesity [2]. The increase in mortality, instead, could be caused by inaccurate risk stratification which does not allow a proper adjuvant treatment [3,4]. The European Society for Medical Oncology (ESMO), European Society for Radiotherapy & Oncology (ESTRO) and European Society of Gynecological Oncology (ESGO) consensus conference classified EC in four categories, defined as low, intermediate, high-intermediate, and high-risk based on clinicopathological features [5,6]. However, pathologists are often unable to reproducibility diagnose morphologic risk factors such as histotype and grade, especially in high-grade tumours, and lymphovascular space invasion [3,4,7].

Finally, in 2020 the European Society of Gynecological Oncology/European Society for Radiotherapy and Oncology/European Society of Pathology (ESGO/ESTRO/ESP) recommended in their guidelines to integrate molecular classification into the risk stratification for EC, thus introducing a new everyday standard in the evaluation of these patients [8]. Risk grouping has prognostic value and guides clinicians with adjuvant therapy. In current practice four classes are identified based on molecular characterization: p53 abnormal (p53abn), mismatch repair deficient (MMRd), Polimerase Epsylon (POLE) mutated and non-specific molecular profile (NSMP). The addition of molecular classification to clinicopathological features can dramatically modify the risk class of a patient and consequently the adjuvant treatment.

### 1.1. Betella alghorithm

In current practice, when molecular classification is applied, every specimen from every patient is tested for the 3 potential molecular characterizations: tumours protein 53 and the proteins belonging to the mismatch repair system are tested through immunohistochemistry, while there is a direct analysis of POLE via next generation sequencing. The systematic routine testing of molecular characteristics of all samples is expensive, and it is not necessarily useful. An algorithm has been proposed by Betella et al. [9]: rather than evaluating the whole molecular spectra in every single patient, the specimen for mutations that would change the risk group and consequently the adjuvant treatment could be tested only. The algorithm first step is a mandatory analysis for mismatch repair system. Actually, it is the only molecular signature helpful for target therapy and for identifying potential familiar cluster (i.e. patients with Lynch Syndrome). The second step divides the patients according to the International Federation of Gynecology and Obstetrics (FIGO) stage 2009 of the disease. The management of FIGO stages III and IV is not influenced by molecular classification, so further testing would be wasteful unnecessary. Then, the immunohistochemistry evaluation of p53 is obtained in FIGO stages I and II. Finally, POLE sequencing is only performed when the presence of a POLE mutation results in class migration i.e. when p53 is abnormal, when the specimen is a FIGO grade 3, when there is involvement of the outer half of the myometrium (FIGO stage IB), of the cervical stroma (FIGO stage II), when lymphovascular spaces are involved. If p53 is wild type or if one of the other 3 characteristics is missing, POLE would not change the management of the patient, since it would already be a low-risk patient. The authors retrospectively studied 278 patients who underwent surgery and complete molecular assessment. Risk class was modified by molecular classification in 6.8 % of the cases. The application of Betella algorithm resulted in a reduction of 67 % in the number of POLE sequencing tests. The DNA sequencing of POLE is the most time-consuming and costly technique, which means that, applying the proposed algorithm, facilities could save resources and time, allowing physicians to work with essential-only information, streamlining the start of adjuvant therapy of patients with EC.

To date, no external validation article has been found. The aim of our study was to validate the Betella algorithm in our population, evaluating the proportion of patients in which the molecular analysis was able to change the risk class attribution. In addition, we compared our population to Milan cohort.

## 2. Materials and methods

This retrospective study was approved by our institutional review board (IRB: 96/2023). We routinely introduced molecular classification for EC in our institution in March 2021: from that time to March 2023, 102 patients underwent surgical staging for endometrial cancer. All patients gave written consent to use their data for research purposes and we pooled all data from the clinical software in our institution. All women underwent a thoraco-abdominal CT scan and pelvic ultrasonography [10] prior to surgery, and blood sample as usual (blood count, renal and hepatic function). Surgical staging performed according to international guidelines [11], either through laparoscopy, robotic assisted laparoscopy or laparotomy.

We included all patients with EC who underwent surgery during the study period and who had given consent for their data to be used. According to the manuscript by Betella et al., we included all histotypes except the mucinous one.

For every patient the following information was gathered: age at time of surgery, body mass index, histotype, myometrial invasion, lymphovascular invasion, FIGO grading, FIGO staging (2009) and, according to ESGO/ESTRO/ESP guideline, risk group. Formalin-fixed, paraffin-embedded tumours tissues were analyzed by a dedicated pathologist for molecular analyses. Molecular classification was achieved by immunohistochemical staining for p53 and mismatch repair proteins (MSH6, PMS2, MSH2, and MLH1 proteins) and by gene sequencing for POLE for the whole cohort of patients. All cases were classified into one of four categories: POLE-mutated; MMR-d; p53abn; NSMP. Tumours harbour more than one molecular classifying feature, defined as “multiple classifier” EC, POLE-mutated–p53abn were categorized as POLE, MMR-d–p53abn as MMR-d, and POLE-mutated–MMR-d–p53abn as POLE [12]. All cases were classified under one of five risk groups according to the ESGO/ESTRO/ESP guidelines [8]. Subsequently, the Betella algorithm was applied (Fig. 1).

### 2.1. Statistical analysis

We tested the null hypothesis, which is expected to improve by 7 % (risk class migration by applying molecular classification) [9], with a margin of error of no more than 0.05. The sample size was calculated according to the study design by Simon [13], using an alpha-error of 0.05 and a Beta-error of 0.80. Considering a patient dropout of approximately 10 %, the study was planned to enroll 102 patients.

The sample was described in its clinicopathological and demographic characteristics using descriptive statistics techniques. Qualitative variables will be summarized as frequencies and percentages. Quantitative variables will be presented as mean (std.dev). The $\chi 2$ analysis or Fisher’s exact test were utilized, when appropriate, for categorical variables and the Student t-test and Mann–Whitney test, when appropriate, for continuous variables. Differences between the groups were considered statistically significant at $p < 0.05$ (95 % confidence interval). The NCSS statistical software program, version 11.0 (NCSS Statistical Software, Kaysville, UT), was utilized.

## 3. Results

Since March 2021, 102 patients with endometrial cancer have been submitted to surgical staging at our hospital. Among them, in 99 cases (97 %) a complete molecular analysis was obtained.

In our cohort, 10 patients (10.1 %) have been classified as POLE ultra-mutated, 30 (30.3 %) as mismatch repair deficient (no one was germinal variant), 11 (11.1 %) as p53 abnormal and finally 48 patients

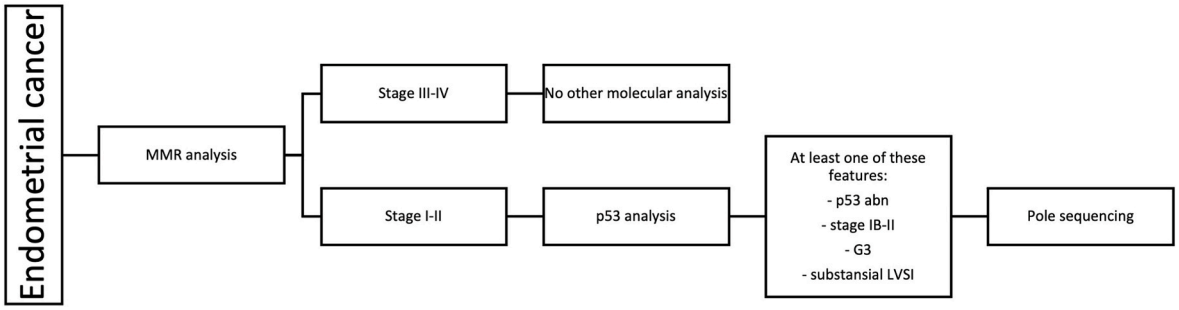


**Fig. 1.** Betella algorithm.

(48.5 %) belonged to a non-specified molecular classification. 3 cases (3 %) of multiple classifiers were found. Specifically, one was POLE-mutated and p53 abnormal, 2 were MMR-d and p53-abnormal. The average BMI is 29 and the average age at the time of diagnosis is 65 years. The overwhelming majority of histotype is endometrioid endometrial cancer, accounting for 88 out of 99 patients (88.9 %); 2 women were affected by clear cell carcinoma (2 %), 7 serous adenocarcinoma (7.1 %), 1 carcinosarcoma and 1 undifferentiated tumours (1 % each). Considering myometrial invasion, only 1 patient had a tumours confined within the endometrial lining (1 %), in 57 patients only the inner half of the myometrium was involved (57.6 %) and in 41 cases the tumours was already spreading to the outer half of the myometrium (41.4 %). Lymphovasculars spaces had been invaded in 27 (27.3 %). Considering FIGO grading: G1 in 29 patients (29.3 %), G2 40 (40.4 %), G3 29 (29.3 %); 1 specimen was undifferentiated (1.2 %). At the time of surgery 73 patients had a disease still in stage I (IA 51 patients 51.5 % and IB 22 patients 22.2 %). Stage II accounted for 9 patients (9.1 %). Finally, 17 patients were in stages III and IV at the time of surgery: stage IIIA 7 (7.1 %), IIIB 1 (1 %), IIIC1 5 (5.1 %), IIIC2 3 (3 %), IVB 1 (1 %). The risk classification, according to ESGO/ESTRO/ESP guidelines, is reported in Table 1. When molecular classification is integrated in the calculation of risk group, we can observe the risk group migration of 11 patients (11.1 %): specifically, 7 patients migrate to lower classes thanks to a POLE mutation, while 4 patients move to a higher risk because of a p53 alteration (Table 2).

Our population (i.e: Udine's population) was compared to European Institute of Oncology (IEO) of Milan cohort (Table 1 and Fig. 3) (i.e.: Milan's population). Clinical, histopathological and molecular features were similar between the two groups.

Applying Betella algorithm, we can spare the POLE sequencing in 65 cases (65.7 %) and p53 immunochemistry in 17 cases (17.2 %) (Fig. 2). Comparing our cohort to IEO population, no statistically significant difference was recorded as to the number of POLE sequencings spared (65.7 % vs 67 %, $p = 0.770$) and of p53 immunochemistry treatments spared (17.2 vs 27, $p = 0.051$).

## 4. Discussion

Since 2021, molecular classification has been routinely applied to all EC in our center. Our molecular data (10.1 % POLE mutated, 30.3 % MMRd, 11.1 %, p53abn and 48.5 % NSMP) were comparable with the results shown by Kommoss et al. [14]: Pole exonuclease domain mutations have been reported in about 10 % of ECs, p53abn in about 11 % of tumours, MMRd and NSMP type, respectively, in 28 % in 51 % of cases. In our cohort 3 cases (3 %) of multiple classifiers were found, consistent with literature data in which around 2–4% of ECs had double features [3,12,14].

According to FIGO 2023 staging [15], molecular classification should be encouraged to allow a better prediction of prognosis. The authors highlighted that molecular classification could be used whenever feasible, but we know that this is frequently not possible in clinical practice. It is not often implemented due to its cost, especially for POLE sequencing, and to the lack of skilled pathologists. The ESMO 2022 guidelines [6] suggested that molecular analysis should be performed as a priority for cases where the results are relevant to guide adjuvant treatment, considering that not all laboratories are able to perform molecular classification on all ECs. These guidelines emphasized the importance of applying the molecular classification in high-grade or high-stage endometrial carcinoma (FIGO stage 2009 $\geq$ II), considering the most significant clinical consequences for these patients. This, however, may lead to under-classification of p53 mutated tumours that are not in these categories. In our cohort, in 2 cases (Table 2; patient 7 and 11) if we had not applied the molecular classification, the risk category would have been underestimated.

To implement the application of molecular classification especially in resource-limited settings, Betella ed al [9] proposed an algorithm, which restricts the molecular analysis only to cases in which knowing the molecular classification results in a risk group migration, according to ESGO/ESTRO/ESP (2020) guidelines [8].

In the present study, we externally validated this algorithm in our population.

In our cohort, integrating molecular classification with clinicopathological features, more than 1 out of 10 patients (11.1 %) were reallocated to a different risk class, and consequently they experienced a change in adjuvant treatment.

By applying Betella algorithm, our facility could have spared resources: POLE analysis can be skipped in 65.7 % of the cases while p53 can be reduced by 17.2 %. These reductions come at no risk for the patients and at no loss of precision for risk class allocation. Among the molecular analysis, mismatch repair immunohistochemistry cannot be avoided: it allows the identification of patients who could be affected by Lynch syndrome [16] and, furthermore, MMR-d is a biomarker that predicts the benefit from immune checkpoint inhibitors (ICI) [17]. Recently, two randomized trials (ENGOT-en6/GOG-3031/RUBY and NRG-GY018/Keynote-868) have demonstrated significant progression-free survival benefit with the addition of ICI (dostarlimab or pembolizumab, respectively) to standard carboplatin/paclitaxel chemotherapy followed by IC in MMRd patients with primary advanced or recurrent EC [18,19]. Applying Betella algorithm, once MMR-d was assessed, all stages III and IV would be excluded from the molecular analysis and 82 patients would remain for further screening. The latest FIGO guidelines [15], in agreement with Betella algorithm, show that, at present, molecular classification does not change the therapeutic approach for advanced endometrial carcinoma; consequently, it could be spared in these cases. According to guidelines, FIGO stage IA endometrial cancer, low grade (G1-2) and without substantial lymphovascular invasion do not receive adjuvant therapy and are scheduled for follow-up when p53 is excluded, making POLE sequencing futile. In our population 60 (17 Stage III and IV plus 43 stage IA without risk factors) patients out of 99 would not have been tested for POLE. In Milan cohort, POLE sequencing is useful to risk group migration in less than half of the cases and our cohort reaches the same conclusion. Even though POLE is the least analyzed variant when Betella algorithm was applied, in our population it is the most associated with risk subgroup

**Table 1**
Patient's characteristics of each molecular class (Udine vs IEO population).

| | Total | | | POLE | | MMR-d | | p53abn | | NSMP | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | UD n = 99 | MI N = 278 | P value | UD n = 10, 11.1 % | MI n = 27 | UD n = 30, 30.3 % | MI n = 77 | UD n = 11, 11.1 % | MI n = 49 | UD n = 48, 48.5 % | MI n = 125 |
| **Age (year), Mean ± SD** | 65.2 ± 11.1 | 61.4 ± 12.1 | **0.006** | 65.4 ± 14.2 | 56.3 ± 11.2 | 66.2 ± 10.7 | 62.7 ± 10.5 | 71.9 ± 9.05 | 65.0 ± 10.9 | 63 ± 10.8 | 60.2 ± 13.1 |
| **BMI Mean (kg/m2) ± SD** | 29 ± 7.4 | 27.4 ± 7.2 | 0.06 | 25.7 ± 4.6 | 24.8 ± 5.2 | 28 ± 6.5 | 27.3 ± 7.1 | 28 ± 6.3 | 27.5 ± 6.5 | 30.9 ± 8.1 | 28.1 ± 7.7 |
| **Histotype** | | | | | | | | | | | |
| **Endometrioid adenocarcinoma, n (%)** | 88 (88.9) | 246 (88.5) | 0.914 | 9 (90) | 25 (92.6) | 28 (93.3) | 73 (94.8) | 4 (36.4) | 26 (53.1) | 47 (97.9) | 122 (97.6) |
| **Clear cell carcinoma, n (%)** | 2 (2) | 4 (1.4) | 0.691 | 1 (10) | 1 (3.7) | 1 (3.3) | 1 (1.3) | 0 | 1 (2) | 0 | 1 (0.8) |
| **Serous adenocarcinoma, n (%)** | 7 (7.1) | 14 (5) | 0.484 | 0 | 0 | 0 | 1 (1.3) | 6 (54.4) | 12 (24.5) | 1 (2.1) | 1 (0.8) |
| **Mixed** | 0 | 4 (1.4) | / | 0 | 1 (3.7) | 0 | 0 | 0 | 3 (6.1) | 0 | 0 |
| **Undifferentiated** | 1 (1) | 3 (1.1) | 0.559 | 0 | 0 | 1 | 1 (1.3) | 0 | 2 (4.1) | 0 | 0 |
| **Carcinosarcoma (MMT)** | 1 (1) | 6 (2.2) | 0.974 | 0 | 0 | 0 | 0 | 1 | 5 (10.2) | 0 | 1 (0.8) |
| **Myometrial invasion** | | | | | | | | | | | |
| **<50 %, n (%)** | 57 (57.6) | 135 (48.6) | 0.139 | 3 (30) | 16 (59.3) | 14 (46.7) | 36 (46.8) | 2 (18.2) | 17 (34.7) | 38 (79.2) | 66 (55.8) |
| **≥50 %, n (%)** | 41 (42.4) | 93 (33.5) | 0.169 | 6 (60) | 5 (18.5) | 16 (53.3) | 25 (32.5) | 9 (81.8) | 24 (49) | 10 (20.8) | 39 (31.2) |
| **None, n (%)** | 1 (1) | 48 (17.3) | **0.000** | 1 (10) | 6 (22.2) | 0 | 15 (19.5) | 0 | 7 (14.3) | 0 | 20 (16) |
| **Lymphovascular space invasion (LVSI)** | | | | | | | | | | | |
| **No, n (%)** | 72 (72.7) | 214 (77) | 0.278 | 7 | 23 (85.2) | 18 | 57 (74) | 4 (36.4) | 28 (57.1) | 43 (89.6) | 106 (84.8) |
| **Yes, n (%)** | 27 (27.3) | 60 (21.6) | | 3 | 4 (14.8) | 12 | 19 (24.7) | 7 (63.6) | 18 (36.7) | 5 (10.4) | 19 (15.2) |
| **Grading** | | | | | | | | | | | |
| **G1, n (%)** | 29 (29.3) | 96 (34.5) | 0.342 | 1 (10) | 9 (33.3) | 6 (20) | 20 (26) | 1 (9.1) | 4 (8.2) | 21 (43.8) | 63 (50.4) |
| **G2, n (%)** | 40 (40.4) | 98 (35.3) | 0.361 | 3 (30) | 10 (37) | 14 (46.7) | 32 (41.6) | 1 (9.1) | 11 (22.5) | 22 (45.8) | 45 (36) |
| **G3, n (%)** | 29 (29.3) | 73 (26.3) | 0.559 | 6 (60) | 7 (25.9) | 9 (30) | 21 (27.3) | 9 (81.8) | 29 (59.2) | 5 (10.4) | 16 (12.8) |
| **Dedifferentiated, n (%)** | 0 | 2 (0.7) | / | 0 | 0 | 0 | 1 (1.3) | 0 | 0 | 0 | 1 (0.8) |
| **Undifferentiated, n (%)** | 1 (1) | 6 (2.2) | 0.467 | 0 | 1 (3.7) | 1 (3.3) | 2 (2.6) | 0 | 3 (6.1) | 0 | 0 |
| **Unknown, n (%)** | 0 | 3 (1.1) | / | 0 | 0 | 0 | 1 (1.3) | 0 | 2 (4.1) | 0 | 0 |
| **Staging** | | | | | | | | | | | |
| **IA, n (%)** | 51 (51.5) | 152 (54.7) | 0.588 | 4 (40) | 22 (81.5) | 12 (40) | 47 (61) | 2 (18.2) | 14 (28.6) | 33 (68.7) | 69 (55.2) |
| **IB, n (%)** | 22 (22.2) | 31 (11.2) | **0.009** | 5 (50) | 4 (14.8) | 8 (26.6) | 8 (10.4) | 2 (18.2) | 5 (10.2) | 7 (14.6) | 14 (11.2) |
| **II, n (%)** | 9 (9.1) | 20 (7.2) | 0.543 | 0 | 0 | 2 (6.7) | 4 (5.1) | 3 (27.2) | 3 (6.1) | 4 (8.3) | 13 (10.4) |
| **IIIA, n (%)** | 7 (7.1) | 19 (6.8) | 0.936 | 1 (10) | 0 | 2 (6.7) | 4 (5.1) | 2 (18.2) | 7 (14.3) | 2 (4.2) | 8 (6.4) |
| **IIIB, n (%)** | 1 (1) | 6 (2.2) | 0.467 | 0 | 0 | 0 | 0 | 1 (9.1) | 3 (6.1) | 0 | 3 (2.4) |
| **IIIC1, n (%)** | 5 (5.1) | 25 (9) | 0.213 | 0 | 0 | 4 (13.3) | 10 (13) | 0 | 2 (4.1) | 1 (2.1) | 13 (10.4) |
| **IIIC2, n (%)** | 3 (3) | 11 (4) | 0.675 | 0 | 0 | 2 (6.7) | 3 (3.9) | 1 (9.1) | 5 (10.2) | 0 | 3 (2.4) |
| **IVA, n (%)** | 0 | 2 (0.7) | / | 0 | 1 (3.7) | 0 | 0 | 0 | 1 (2) | 0 | 0 |
| **IVB, n (%)** | 1 (1) | 12 (4.3) | 0.122 | 0 | 0 | 0 | 1 (1.3) | 0 | 9 (18.4) | 1 (2.1) | 2 (1.6) |
| **ESGO/ESTRO/ESP (2020) molecular classification unknown** | | | | | | | | | | | |
| **Low** | 44 (44.4) | 124 (44.6) | 0.978 | 2 (20) | 18 (66.7) | 8 (26.7) | 34 (44.2) | 1 (9.1) | 6 (12.2) | 33 (68.7) | 66 (52.8) |
| **Intermediate** | 13 (13.1) | 36 (13) | 0.936 | 3 (30) | 5 (18.5) | 6 (20) | 16 (20.8) | 1 (9.1) | 4 (8.16) | 3 (6.3) | 11 (8.8) |
| **High-Intermediate** | 19 (19.2) | 31 (11.2) | **0.043** | 3 (30) | 1 (3.7) | 7 (23.3) | 9 (11.7) | 2 (18.2) | 5 (10.2) | 7 (14.6) | 16 (12.8) |
| **High** | 22 (22.2) | 73 (26.3) | 0.427 | 2 (20) | 3 (11.1) | 9 (30) | 17 (2.1) | 7 (63.6) | 24 (49) | 4 (8.3) | 29 (23.2) |
| **Advanced/metastatic** | 1 (1) | 14 (5) | 0.078 | 0 | 0 | 0 | 1 (1.3) | 0 | 10 (20.4) | 1 (2.1) | 3 (2.4) |
| **ESGO/ESTRO/ESP (2020) molecular classification known** | | | | | | | | | | | |
| **Low** | 50 (50.5) | 124 (44.6) | 0.312 | 9 (90) | 24 (89) | 8 (26.7) | 34 (44.2) | 0 | 0 | 33 (68.7) | 66 (52.8) |
| **Intermediate** | 9 (9.1) | 32 (11.5) | 0.501 | 0 | 0 | 6 (20) | 16 (20.8) | 0 | 5 (10.2) | 3 (6.3) | 11 (8.8) |
| **High-Intermediate** | 14 (14.1) | 25 (9) | 0.149 | 0 | 0 | 7 (23.3) | 9 (11.7) | 0 | 0 | 7 (14.6) | 16 (12.8) |
| **High** | 25 (25.3) | 83 (29.9) | 0.384 | 1 (10) | 3 (11.1) | 9 (30) | 17 (22.1) | 11 (100) | 34 (71.4) | 4 (8.3) | 29 (23.2) |
| **Advanced/metastatic** | 1 (1) | 14 (5) | 0.078 | 0 | 0 | 0 | 1 (1.6) | 0 | 10 (20.4) | 1 (2.1) | 3 (2.4) |

a: UD = Udine's population; MI = Milan's population.

**Table 2**
Characteristic of patients reallocated in a different risk class according to the ESGO/ESTRO/ESP 2020 guidelines (molecular classification known).

| | Age | BMI | Histotype | Myometrial invasion | Grading | LVSI | Staging | ESGO/ESTRO/ESP (2020) molecular classification unknown | Molecular Group | ESGO/ESTRO/ESP (2020) molecular classification known |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 58 | 23 | endometrioid | >50 % | 2 | Negative | IB | INTERMEDIATE | POLE mut | LOW |
| 2 | 70 | 20 | endometrioid | <50 % | 3 | Negative | IA | INTERMEDIATE | POLE mut | LOW |
| 3 | 68 | 22 | endometrioid | >50 % | 3 | Negative | IB | HIGH/INT | POLE mut | LOW |
| 4 | 83 | 25 | endometrioid | >50 % | 2 | Negative | IB | INTERMEDIATE | POLE mut | LOW |
| 5 | 52 | 24 | endometrioid | >50 % | 3 | Positive | IB | HIGH/INTERMEDIATE | POLE mut | LOW |
| 6 | 67 | 25.6 | endometrioid | >50 % | 3 | Positive | IB | HIGH/INTERMEDIATE | p53 abn | HIGH |
| 7 | 79 | 35 | endometrioid | <50 % | 1 | Negative | IA | LOW | p53 abn | HIGH |
| 8 | 85 | 24 | endometrioid | >50 % | 3 | Positive | II | HIGH/INTERMEDIATE | p53 abn | HIGH |
| 9 | 43 | 22 | endometrioid | <50 % | 3 | Positive | IA | HIGH/INTER | POLE mut | LOW |
| 10 | 65 | 34 | clear cell | >50 % | 3 | Negative | IB | HIGH | POLE mut | LOW |
| 11 | 66 | 36 | Endometrioid | >50 % | 2 | Negative | IB | INTERMEDIATE | P53 abn | HIG |

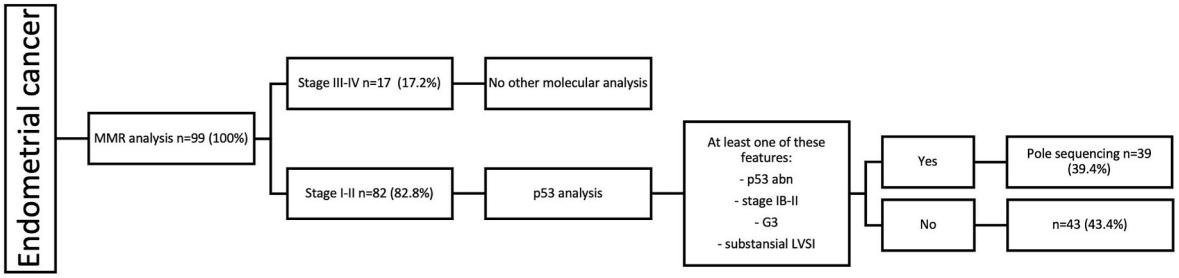


**Fig. 2.** Application of Betella algorithm to Udine population.

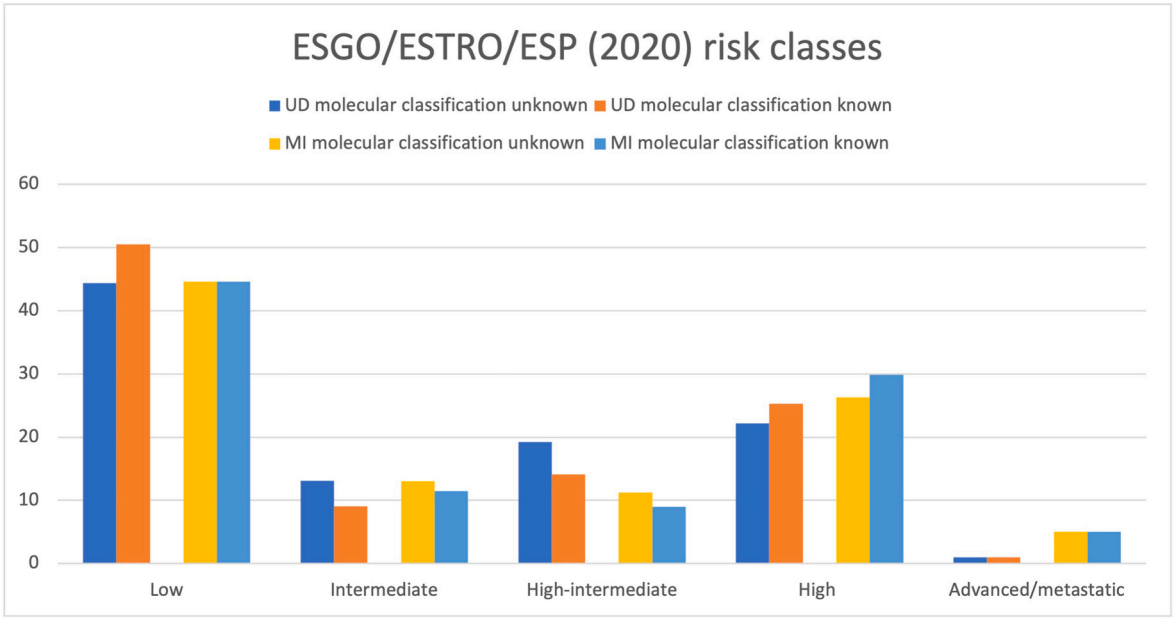


**Fig. 3.** Risk classes.

switch and consequently, according to guidelines [6,8], these patients can avoid adjuvant therapy and its related toxicity, improving their quality of life at zero risk (if appropriately staged by surgery).

The application of this algorithm, while saving resources, could extend the number of centres that could apply the molecular classification. In our institution, the estimated cost of POLE sequencing is about EUR 55,00 per sample, while that of p53 immunochemistry is about EUR 35,00. These costs estimated are only for materials, but the most important costs are those related to the need for a next-generation sequencing system and a dedicated pathologist, not available in all hospitals, and the cost of work time. The application of the Betella algorithm can save in our cohort about EUR 4170.00 but remarkably cut down working time; moreover, it will put hospitals with limited financial resources equipped with scrupulous health care professionals in a position to send parts of tumours' sample to centres in which pathologists with specific expertise operate. However, this could only be possible if patients with EC are treated according to the guidelines and by experienced surgeons because the algorithm and molecular classification are only applicable if the patient has been correctly staged. Centres with a low volume of patients and which cannot guarantee care

standards should refer patients to gynecology oncology departments. This is necessary to improve the prognosis of the only gynecological cancer that has shown worsening survival rates in recent years [1].

The proposed algorithm is very useful but also has some limits. Currently, we do not know if the presence of POLE can change the treatment in the advanced stages, but this may be important in the future. Actually, it could be relevant when counselling patients on their prognoses. The data of ongoing prospective studies will answer this question and give more precise indication on treatment based on the molecular profile [20–22].

The limitations of our study are the retrospective nature of the study and the limited number of cases. However, our study is the first external validation of the Betella algorithm and presents the clinicopathological and molecular characteristics of a homogeneous population of ECs in which molecular classification has always been performed.

In conclusion, we believe that this new proposed algorithm could be included in the guidelines as a minimum molecular evaluation that all centres, even those with scarce resources, should perform.

## 5. Conclusions

The application of this new proposed algorithm appears safe for the patients while rationalizing resources that could thus be allocated otherwise, not only for the benefit of settings with low resources, but of all settings in general. However, it must be stressed that the application of the algorithm has one fundamental precondition: staging surgery must be properly executed by surgeons with the appropriate expertise.

## Ethics approval and consent to participate

Not applicable.

## Consent for publication

Not applicable.

## Availability of data and materials

All data generated or analyzed during this study is included in this published article.

## Competing interests

The authors have no conflicts of interest.

## Funding

None.

## CRediT authorship contribution statement

**Martina Arcieri:** Conceptualization, Data curation, Writing – original draft, preparation, Formal analysis. **Giuseppe Vizzielli:** Conceptualization, Data curation, Writing – original draft, preparation. **Tommaso Occhiali:** Data Collection. **Cristina Giorgiutti:** Data Collection. **Veronica Tius:** Data Collection. **Sara Pregnolato:** Data Collection. **Laura Mariuzzi:** Data curation, Writing – original draft, preparation. **Maria Orsaria:** Data curation, Writing – original draft, preparation. **Angelica Tulisso:** Data Collectio. **Giuseppe Damante:** Visualization. **Angela Valentina D'Elia:** Visualization. **Giuseppe Cucinella:** Validation. **Vito Chiantera:** Methodology, Formal analysis. **Francesco Fanfani:** Methodology. **Alfredo Ercoli:** Methodology, Formal analysis. **Lorenza Driul:** Supervision. **Giovanni Scambia:** Supervision. **Stefano Restaino:** Conceptualization, Writing – review & editing, Supervision.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgements

All authors want to thank Cecilia Rizzotti for her contribution to the English translation of the original manuscript.

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