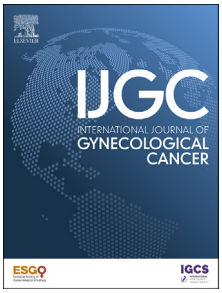
ORIGINAL RESEARCH

Improving pre-operative binary grading: relevance of p53 and PR expression in grade 2 endometrioid endometrial carcinoma



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ABSTRACT

Objective: This study aimed to evaluate the association between pre-operative progesterone receptor (PR) and p53 expression and prognosis in pre-operative grade 2 endometrioid endometrial carcinoma compared with grade 1 and grade 3 carcinomas.

Methods: Three European endometrial carcinoma cohort studies were included. Patients with pre-operative grade 2 endometrioid carcinoma and known pre-operative PR and p53 status were included ($n = 400$), as were patients with pre-operative grade 1 ($n = 602$) or grade 3 ($n = 148$) endometrioid carcinomas. Kaplan-Meier and Cox regression analyses were performed to analyze disease-specific and disease-free survival.

Results: Patients with pre-operative grade 2 endometrial carcinoma and wild-type p53 plus PR-positive expression showed a similar 7-year disease-specific survival to grade 1 endometrial carcinoma patients (95.8% vs 97.5%, $p = .13$), while the 7-year disease-specific survival of patients with grade 2 endometrial carcinoma with p53 aberrant and/or negative PR expression (83.5%) was significantly lower ($p < .001$). The combination of these markers was an independent prognostic factor in multivariate Cox regression analyses.

Conclusions: The prognostic impact of pre-operative p53 and PR expression in patients with grade 2 endometrioid endometrial carcinoma supports a modified binary grading system in which grade 2 patients should be pre-operatively classified as low- or high-grade depending on p53 and PR expression.

WHAT IS ALREADY KNOWN ON THIS TOPIC

Overall, 14% of grade 2 endometrioid endometrial carcinomas are upgraded post-operatively, putting them at risk of (surgical) undertreatment. Therefore, pre-operative risk stratification can be improved for pre-operative grade 2 endometrial carcinomas.

WHAT THIS STUDY ADDS

Pre-operative grade 2 endometrial carcinomas with wild-type p53 plus positive PR expression showed similar survival to grade 1 endometrial carcinomas, whereas pre-operative grade 2 endometrial carcinomas with aberrant p53 and/or negative PR expression showed survival similar to grade 3 endometrial carcinomas.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE, OR POLICY

A low-cost, widely available, two-step immunohistochemical approach involving p53 and PR improves risk stratification in pre-operative grade 2 endometrial carcinoma.

Keywords:
Endometrioid Endometrial Carcinoma; p53; Progesterone Receptor; Grade 2

INTRODUCTION

In Europe, endometrial carcinoma is the most common gynecologic malignancy.[1] Endometrial carcinoma is classified into different histologic subtypes, of which the endometrioid subtype accounts for > 75% of cases.[1,2] The extent of surgical treatment for endometrial carcinoma varies from simple hysterectomy to staging procedures, including lymphadenectomy/sentinel lymph node (SLN) biopsy with or without omentectomy.[1] SLN biopsy can be considered in patients with low- or intermediate-risk disease, while lymphadenectomy is recommended in patients who are pre-

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operatively stratified in high-intermediate- or high-risk groups, for which SLN biopsy is an alternative in stage I or II disease.[1]

In this risk stratification, clinical stage, histologic subtype, and grade were considered. As recommendations for surgical procedures are based on this stratification, it is of great importance that they are accurate.[1] Endometrioid endometrial carcinoma is currently graded from 1 to 3, according to the International Federation of Gynecology and Obstetrics (FIGO) grading criteria. All non-endometrioid endometrial carcinomas are considered high grade.[1,3] To simplify and improve the reproducibility of grading, a binary classification was proposed in the most recent international guidelines.[1,4] In this classification, grade 2 endometrioid endometrial carcinomas are lumped together with grade 1 into a low-grade category because their current management is similar.[1,2,4] However, pre- and post-operative agreement in grade 2 endometrioid endometrial carcinomas was only 0.61 (95% CI 0.53 to 0.69, $n = 3027$) in a meta-analysis of Visser and colleagues,[5] with post-operative upgrading in 14%. This issue was not solved using the proposed binary approach and underscores the need for a more accurate pre-operative classification system.

The molecular classification of endometrial carcinomas in the Cancer Genome Atlas was introduced in the most recent guidelines for endometrial carcinoma.[6,7] To date, this molecular classification has been demonstrated to be mainly helpful in high-grade and/or advanced-stage endometrial carcinoma to guide adjuvant treatment in the post-operative setting but not necessarily in the risk stratification of low-grade endometrial carcinomas in the pre-operative setting.[1,4,8-10]

Several immunohistochemical markers may be useful for this purpose. *TP53* mutated endometrial carcinomas have the worst clinical outcomes in the Cancer Genome Atlas molecular classification of endometrial carcinomas and are considered high-risk according to recent guidelines.[1,6,7] Aberrant p53 immunohistochemical staining, a surrogate marker for *TP53* mutations, is known to be associated with the non-endometrioid subtype, unfavorable clinicopathologic factors, and worse outcomes.[6,7,11-18] Hormone receptor expression has been extensively studied in relation to outcomes. Low or absent progesterone receptor (PR) expression is associated with lymph node metastasis and unfavorable outcomes.[19-23] Furthermore, Visser and colleagues[24] demonstrated in a cohort of cases pre-operatively classified as grade 2 endometrioid carcinoma on hematoxylin and eosin staining that post-operative upgrading was reduced by 6% when cases with p53 aberrant and PR-negative immunohistochemical expression were designated as high-grade in the pre-operative setting.

This leads to the question of whether a combination of these markers improves the prognosis of patients with pre-operative grade 2 endometrioid endometrial carcinoma. It has been hypothesized that in patients with grade 2 endometrioid endometrial carcinoma, wild-type p53, and PR-positive expression are associated with improved survival compared to grade 2 endometrioid endometrial carcinoma with a p53 aberrant and/or PR-negative expression profile. Thus, our primary outcome was disease-specific survival in grade 2 endometrioid carcinoma when patients were stratified according to their p53 and PR status. The secondary outcomes were disease-free survival, correlation of clinicopathologic features with p53 and PR status, and differences in post-operative discordance between the 2 pre-operative classification methods.

METHODS

Study Cohort

For this retrospective study, data from 3 European endometrial cancer databases were merged[24-27] (Table S1). Cohort 1 consisted of 763 patients treated between 1995 and 2013 in 10 collaborating centers associated with the European Network for Individualized Treatment of Endometrial Cancer; ethical approval was given in Nijmegen, The Netherlands (Institutional Study Protocol 2015-2101).[25] Cohort 2 consisted of 432 patients who were prospectively included in 9 Dutch hospitals between 2011 and 2013. Ethical approval was provided by the local medical ethical committee, St. Elisabeth Hospital Tilburg, the Netherlands (registered in the Netherlands Trial Register, number NTR3503).[24,27] Cohort 3 consisted of 235 patients treated at the University Hospital of Brno, Czech Republic between 2012 and 2019, ethical approval was granted by the ethics committee of the University Hospital Brno, Czech Republic (approval number 06-151221/EK) (Table S1).[26] All variables in the 3 data sets were recoded when necessary such that the variable names, labels, and label values were identical in all data sets. All patients with pre-operative grade 1 to 3 endometrioid endometrial carcinoma were included in the merged data set. Patients with pre-operative grade 2 disease without known pre-operative PR or p53 status were excluded.

Histologic and Immunohistochemical Analysis

Details of both the pathologic examination and immunohistochemical analyses are provided in the Table S1 and the corresponding articles.[24-27] Examples of immunohistochemical staining are provided in the Figure S1.

Outcome Measures

The biomarkers of interest were the pre-operative grade, p53, and PR status. Patients were grouped into pre-operative grade 1 (irrespective of p53 and PR status), pre-operative grade 2 (stratified by p53 and PR status), or pre-operative grade 3 (irrespective of p53 and PR status). Patients were subsequently stratified into 4 groups ([1] P53 wild-type, PR-positive, [2] P53 aberrant, PR-positive; [3] P53 wild-type, PR-negative, and [4] P53 aberrant, PR-negative), or 2 categories ([1] P53 wild-type, PR-positive and [2] all other).

The primary outcome of interest was disease-specific survival, which was defined as the time between the date of surgery and death due to endometrial carcinoma or censoring. The secondary outcome of interest was disease-free survival, defined as the time between the date of surgery and an event or censoring. Residual disease, local (vaginal vault), regional (regional lymph nodes/pelvis), or distant recurrences were all considered events. Furthermore, the correlations between clinicopathologic features and p53 and PR status were evaluated. Finally, post-operative discordances were compared between the 2 methods of pre-operative classification, considering all patients with pre-operative histologic grade 2 disease as low grade, versus the algorithm of Visser and colleagues,[24] in which patients with pre-operative grade 2 endometrioid endometrial carcinoma with p53 aberrant and PR-negative expression were considered high grade. Because this

algorithm was originally tested in cohort 2, only patients with pre-operative grade 2 disease from cohorts 1 and 3 were used to validate the algorithm.

Statistical Analyses

Statistical analyses were performed using SPSS software (IBM SPSS Statistics 28). The χ^2 or Fisher exact tests with Bonferroni correction were used to compare categorical variables. Analysis of variance or Kruskal-Wallis tests were used for continuous variables. Kaplan-Meier plots were constructed (capped at 7 years), and log-rank tests and Cox regression analyses were performed. Differences were considered statistically significant with a p value of $\leq$.05; significance levels were adjusted accordingly in post hoc comparisons with Bonferroni correction.[28]

In accordance with the journal's guidelines, we will provide our data for independent analysis for the purpose of additional data analysis or for the reproducibility of this study in other centers if requested.

RESULTS

In total, 1150 patients were included: 602 with pre-operative grade 1 (52.3%), 400 with grade 2 (34.8%), and 148 with grade 3 endometrioid endometrial carcinoma (12.9%) (Table 1). Negative prognostic parameters such as advanced FIGO-stage, lymph node metastases, and lymphovascular space invasion were more frequently present when the pre-operative grade increased. Similarly, the recurrence and disease-related mortality increased when the pre-operative grade was higher. Among patients with pre-operative grade 2 endometrioid carcinoma, there were 99 (24.8%) post-operative discordant cases: 52 (52.5%) were reclassified as grade 1, 32 (32.3%) as grade 3, and 15 (15.2%) as non-endometrioid endometrial carcinoma (data not shown). The differences between cohorts are presented in the Supplementary Files (Table S2).

With increasing pre-operative grade, significantly more frequent p53 aberrant and/or negative PR expression were observed ($p <$ .001) (Table 1). Only 1.4% ($n = 8$) of the patients showed a p53 aberrant/negative PR expression profile in the pre-operative grade 1 group, compared to 17.2% ($n = 25$) in the pre-operative grade 3 group (Table 1). There were significantly more distant ($p <$ .001) and total ($p =$ .048) recurrences and disease-related deaths ($p <$ .001) in pre-operative grade 2 with p53 aberrant and/or negative PR expression status than in grade 2 endometrioid endometrial carcinoma with wild-type p53 and positive PR expression (Table 2). In patients with pre-operative grade 2 endometrioid carcinoma who underwent lymphadenectomy ($n = 148$), more lymph node metastases were observed in the p53 wild-type/negative PR (17.6%, $n = 3$) and p53 aberrant/negative PR (33.3%, $n = 1$) subgroups than in the p53 wild-type/PR-positive subgroup (8.7%, $n = 10$). No lymph node metastases in the p53 aberrant/positive PR subgroup. These differences were not statistically significant ($p =$ .19).

Patients with a pre-operative increase in tumor grade had significantly shorter disease-specific survival (Fig. A). Patients with pre-operative grade 1 endometrioid carcinoma ($n = 596$) showed a 7-year disease-specific survival of 97.5%, which was comparable to the disease-specific survival of patients with pre-operative grade 2 endometrial carcinoma with wild-type p53 and PR-positive expression ($n = 312$) of 95.8% ($p =$ .13) (Fig. B). The 7-year disease-specific survival of grade 2 endometrial carcinoma patients with p53 aberrant and/or negative PR expression ($n = 85$) was 83.5%, which was comparable to that of grade 3 endometrioid endometrial carcinoma patients ($n = 146$) with a 7-year disease-specific survival of 78.1% ($p =$ .24) (Fig. B). Both differed significantly from the disease-specific survival of patients with pre-operative grade 1 and grade 2 endometrial carcinoma with wild-type p53 and PR-positive expression ($p <$ .001) (Fig. B). Similarly, the disease-free survival curves showed an overall significant difference ($p <$ .001) (Fig. C). Disease-specific and disease-free survival was significantly shorter in patients with pre-operative grade 2 endometrioid carcinoma with p53 aberrant and/or PR expression profiles (Table 2). Kaplan-Meier disease-specific survival curves for grade 2 tumors divided into 4 subgroups based on p53 and PR expression are shown in the Supplementary Files (Fig. S2).

Subsequently, univariate and multivariate Cox regression analyses were performed to study variables affecting the HRs. The variable "data set" refers to the original cohorts of which cases were extracted and were added in view of observed differences between the original cohorts (Table S2). In both univariate and multivariate analyses of disease-specific survival in pre-operative grade 2 endometrioid carcinoma, FIGO-stage, p53 aberrant and/or negative PR expression, and data set were shown to have an independent prognostic impact (Table 3). Lymphovascular space invasion showed a prognostic impact only in univariate Cox regression analysis. Both p53 aberrant and negative PR status as individual markers affected the HR in univariate disease-specific survival Cox regression analysis, but only PR status remained an independent prognosticator in multivariate regression analyses (data not shown). P53 aberrant and/or negative PR expression status was also an independent prognosticator in Cox regression analyses for disease-free survival, together with FIGO stage and lymphovascular space invasion (Table S4).

In addition to the primary and secondary outcomes, the algorithm proposed by Visser and colleagues was applied to a subgroup of patients with pre-operative grade 2 endometrioid carcinoma in our merged cohort ($n = 304$).[24] The pre-operative and post-operative concordance with the usual binary grading (all pre-operative grade 2 endometrioid carcinomas were considered low-grade, omitting p53 and PR status) was determined, which resulted in 31 post-operative discordant cases. Application of the algorithm of Visser and colleagues (pre-operative grade 2 endometrioid carcinomas with p53 aberrant and negative PR expression were high grade) did not result in a reduction in discordant cases ($n =$ 33) (Table S5).[24]

DISCUSSION

Summary of Main Results

The combination of pre-operative p53 and PR immunohistochemical expression status in pre-operative grade 2 endometrioid endometrial carcinomas is an important prognosticator regarding disease-specific and disease-free survival in both survival and regression analyses.

Results in the Context of Published Literature

Patients with pre-operative grade 2 endometrioid carcinoma, with wild-type p53 plus PR-positive immunohistochemical expression,

Table 1 Patient- and Clinical Characteristics, Divided in Pre-Operative Grade 1 to 3 Endometrioid Endometrial Carcinoma

Variable	Grade 1[a]	Grade 2[a]	Grade 3	*p* Value[b]
Number of patients	602	400	148	
Mean age in years ± SD[c]	64.5 ± 10.1	65.5 ± 9.5	67.4 ± 10.0	**.021**
Median BMI [IQR][d]	29.4 [25.7-34]	31 [26.6-35]	28 [24.7-33.4]	**.005**
Post-menopausal status[e]	56 (9.4)	25 (6.3)	7 (4.7)	.07
Diabetes mellitus[f]	94 (17.3)	97 (24.8)	29 (20.6)	**.020**
Cardiovascular disease[g]	230 (42.4)	203 (51.9)	65 (46.1)	**.016**
Lymph node dissection	217 (36)	148 (37)	82 (55.4)	**< .001**
of which positive	14 (6.5)	14 (9.5)	16 (20.0)	**.002**
Adjuvant treatment[h]	201 (33.4)	188 (47.0)	110 (74.3)	**< .001**
of which radiotherapy	185 (92.0)	164 (87.2)	88 (80)	
of which chemotherapy	6 (3.2)	14 (7.4)	11 (10)	
of which chemoradiation	9 (4.9)	10 (5.3)	11 (10)	
Lymphovascular space invasion[i]	54 (11.5)	51 (14.2)	46 (33.6)	**< .001**
FIGO-stage				**< .001**
Ia	401 (66.6)	219 (54.8)	59 (39.9)	
Ib	150 (24.9)	117 (29.3)	42 (28.4)	
II	23 (3.8)	34 (8.5)	15 (10.1)	
IIIa	8 (1.3)	14 (3.5)	5 (3.4)	
IIIb	4 (0.7)	0 (0)	3 (2)	
IIIc	11 (1.8)	11 (2.8)	16 (10.8)	
IVa	1 (0.2)	0 (0)	1 (0.7)	
IVb	4 (0.7)	5 (1.3)	7 (4.7)	
p53 and PR status[j]				**< .001**
p53 wild-type, PR positive	480 (84.7)	313 (78.3)	67 (46.2)	
p53 aberrant, PR positive	33 (5.8)	43 (10.8)	30 (20.7)	
p53 wild-type, PR negative	46 (8.1)	33 (8.3)	23 (15.9)	
p53 aberrant, PR negative	8 (1.4)	11 (2.8)	25 (17.2)	
Post-operative discordances	199 (33.1)	99 (24.8)	50 (33.8)	**.012**
Median follow-up in mos [IQR][k]	59.8 [49.1-78.5]	58.4 [45.8-71.9]	61.4 [52.1-78.0]	**.028**
Total recurrences[l]	36 (6.0)	59 (14.8)	43 (29.3)	**< .001**
Local	13 (33.3)	26 (43.4)	14 (32.6)	
Regional	5 (12.8)	11 (18.3)	5 (11.6)	
Distant	22 (56.4)	31 (51.7)	35 (81.4)	
Deceased[m]	67 (11.2)	58 (14.6)	54 (36.5)	**< .001**
Endometrial carcinoma-related mortality	17 (25.4)	28 (48.3)	33 (61.1)	

Abbreviations: ANOVA, analysis of variance; BMI, body mass index; FIGO, International Federation of Gynecology and Obstetrics; IQR, interquartile range; SD, standard deviation. Percentages are calculated from total number of patients in vertical columns. FIGO-stage is according to 2009 FIGO staging system for endometrial cancer. All values are *n* (%) unless otherwise specified.

[a] A total of 4 grade 1 and 2 grade 2 patients had residual disease after surgical treatment.

[b] *p* Value for the ANOVA- or Kruskal-Wallis test for continuous variables and χ^2-test for categorical variables. Bold variables indicate statistical significance.

[c] A total of 291 missing values.

[d] A total of 84 missing values.

[e] A total of 8 missing values.

[f] A total of 75 missing values

[g] A total of 76 missing values.

[h] A total of 1 missing value. One patient received an aromatase inhibitor as adjuvant therapy.

[i] A total of 186 missing values.

[j] A total of 38 missing values (in grade 1 and 3 subgroups).

[k] Median follow-up excluding deceased patients, $n = 961$.

[l] A total of 2 missing values.

[m] A total of 9 missing values regarding variable deceased yes or no. Cause of death is unknown in 27 patients.

Table 2 Tumor- and Patient Characteristics in Pre-Operative Grade 2 Endometrioid Endometrial Carcinoma, Divided in Subgroups According to p53 and PR Status ($N = 400$)

Variable	p53 wild-type, PR positive ($n = 313$)	p53 aberrant, PR positive ($n = 43$)	p53 wild-type, PR negative ($n = 33$)	p53 aberrant, PR negative ($n = 11$)	*p* Value[a]
FIGO-stage					.09
Ia	175 (55.9)	24 (55.8)	16 (48.5)	4 (36.4)	
Ib	93 (29.7)	11 (25.6)	11 (33.3)	2 (18.2)	
II	27 (8.6)	3 (7)	2 (6.1)	2 (18.2)	
IIIa	8 (2.6)	3 (7)	1 (3)	2 (18.2)	
IIIc	8 (2.6)	0 (0)	2 (6.1)	1 (9.1)	
IVb	2 (0.6)	2 (4.7)	1 (3)	0 (0)	
Lymphovascular space invasion[b]	39 (13.7)	5 (13.9)	6 (20)	1 (11.1)	.81
Lymph node metastases[c]	10 (8.7)	0 (0)	3 (17.6)	1 (33.3)	.19
Total recurrences	38 (12.1)	10 (23.3)	8 (24.2)	3 (27.3)	**.048**
Local recurrences[d]	21 (6.7)	0 (0)	3 (9.4)	2 (18.2)	.12
Regional recurrences[e]	6 (1.9)	2 (4.7)	2 (6.3)	1 (9.1)	.22
Distant recurrences[f]	17 (5.4)	7 (16.3)	4 (12.5)	3 (27.3)	**.004**
Endometrial carcinoma-related mortality[g]	14 (4.5)	6 (14.3)	6 (18.8)	2 (18.2)	**< .001**
7-year disease-free survival[h]	273 (87.5)	33 (78.6)	24 (75)	8 (72.7)	**.008**
7-year disease-specific survival[i]	299 (95.8)	36 (85.7)	26 (81.3)	9 (81.8)	**0.001**

Abbreviations: ANOVA, analysis of variance; FIGO, International Federation of Gynecology and Obstetrics; PR, progesterone receptor.
Percentages are calculated from total number of patients in vertical columns unless stated otherwise. FIGO-stage is according to 2009 FIGO staging system for endometrial cancer. All values are *n* (%).

[a] *p* Value for the ANOVA or Kruskal-Wallis test for continuous variables, χ^2-test for categorical variables and log-rank test for survival. Bold variables indicate statistical significance.

[b] 41 missing values.

[c] Percentages are calculated in the subgroups of patients who received lymphadenectomy ($n = 115$ in pre-operative grade 2 endometrioid endometrial carcinoma with p53 wild-type, PR positive; $n = 13$ in pre-operative grade 2 endometrioid endometrial carcinoma with p53 aberrant, PR positive; $n = 17$ in pre-operative grade 2 endometrioid endometrial carcinoma with p53 wild-type, PR negative; $n = 3$ in pre-operative grade 2 endometrioid endometrial carcinoma with p53 aberrant, PR negative).

[d] A total of 1 missing value.

[e] A total of 3 missing values.

[f] A total of 1 missing value.

[g] Survival status is unknown in 3 patients. Cause of death is unknown in 7 patients.

[h] A total of 3 missing values.

[i] A total of 3 missing values.

showed similar survival to that of pre-operative grade 1 patients, while the survival of patients with aberrant p53 and/or negative PR expression was comparable to that of patients with grade 3 endometrioid carcinoma. The importance of both p53 and PR as individual prognostic markers is already known from previous studies. The Cancer Genome Atlas reported that prognosis was worse in the copy number-high endometrial carcinoma subgroup, which is considered a high-risk subgroup according to recent guidelines.[1,6,7] In the high copy number group, 92% showed *TP53* mutations, for which p53 aberrant immunohistochemical staining was used as a surrogate marker.[6,7]

Aberrant immunohistochemical p53 expression is an established prognostic biomarker associated with non-endometrioid histologic subtypes, unfavorable clinicopathologic factors, and worse outcomes in many studies.[11-18] In particular, the latter was underscored in this study (eg, patients with pre-operative grade 2 carcinoma showed shorter disease-specific and disease-free survival when there was aberrant p53 expression). Interestingly, although hormone receptor expression profiles have been well-established biomarkers for decades, estrogen receptor (ER) and PR expression are not part of the current molecular classification of endometrial carcinoma. However, several studies have suggested the prognostic value of ER and PR status in endometrial carcinoma molecular subgroups with 'no specific molecular profile.'[7,16,19,20,22,23,29-31] Recently, Huvila and colleagues[21] demonstrated that negative PR expression was an independent risk factor for relapse in patients with stage I and II endometrioid carcinoma. In this study, p53 aberrant expression only significantly affected the HR in univariate analyses, which is similar to our results and underlines the importance of PR as an independent prognostic marker.[21] Similarly, in a study by Trovik and colleagues,[23]

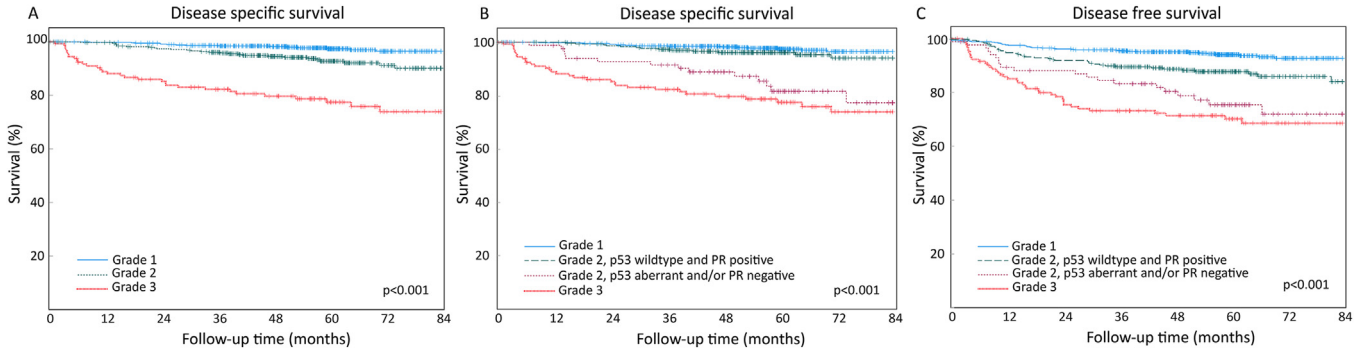

Figure (**A**) Kaplan-Meier plots of 7-year DSS of pre-operative grade 1 to 3 endometrioid endometrial carcinoma; 74 events. (**B**) Kaplan-Meier plots of 7-year DSS of pre-operative grade 1 to 3 endometrioid endometrial carcinoma, with grade 2 further specified regarding PR and p53 status; 74 events. (**C**) Kaplan-Meier plots of 7-year disease-free survival of pre-operative grade 1 to 3 endometrioid endometrial carcinoma, with grade 2 further specified regarding PR and p53 status; 136 events. DSS, disease survival specific; PR, progesterone receptor.

Table 3 Univariate and Multivariate Cox Regression Analyses of Disease-Specific Survival in Pre-Operative Grade 2 Endometrioid Endometrial Carcinoma ($N = 400$)

Variable	Univariate Cox regression			Multivariate Cox regression		
	HR	95% CI	*p* Value	Adjusted HR	95% CI	*p* Value
FIGO-stage[a]	4.5	2.1 to 9.6	**<.001**	3.3	1.3 to 8.2	**.012**
p53 aberrant and/or PR negative[b]	3.8	1.8 to 7.9	**<.001**	3.3	1.5 to 7.5	**.004**
Lymphovascular space invasion[c]	3.7	1.6 to 8.4	**.002**	2.2	0.9 to 5.8	.11
Data set[d]						
Data set 2	2.9	1.3 to 6.8	**.013**	3.0	1.2 to 7.3	**.016**
Data set 3	0.7	0.3 to 2.2	.679	0.9	0.3 to 2.6	.78

Abbreviations: FIGO, International Federation of Gynecology and Obstetrics; PR, progesterone receptor.
Events: 25. FIGO-stage is according to 2009 FIGO staging system for endometrial cancer. Bold variables indicate statistical significance.
[a] Reference category is FIGO I; 7 missing values.
[b] Reference category is p53 wild-type and PR positive; 7 missing values.
[c] Reference category is number 48 missing values.
[d] Reference category is data set 1. 7 missing values.

both p53 aberrant expression and loss of ER/PR expression in curettage specimens were associated with shorter disease-specific survival and lymph node metastasis. ER/PR loss was also an independent predictor in multivariate Cox regression.

It is important to note that we specifically explored the combination of PR/p53, based on the broad analysis by Visser and colleagues[24] favoring the use of PR and p53 over ER. The abovementioned studies by Vermij and colleagues[30] and Jamieson and colleagues[29] which evaluated ER as a prognostic marker in the subgroup with 'no specific molecular profile,' likely highlighted a different part of the discussion, demonstrating that ER-positive expression is a favorable marker in this specific molecular class. However, to highlight their relatedness, in the study by Jamieson and colleagues[29] PR was an equally strong marker as ER, albeit only in the univariate analysis. Furthermore, Vermij and colleagues[30] showed that within the ER-positive group, PR-negative expression further identified patients with unfavorable pathologic characteristics (eg, high-grade or non-endometrioid subtypes), underscoring the importance of PR.

The results reported by Visser and colleagues regarding the decrease in post-operative discordance when p53 and PR expression were considered in pre-operative grade 2 patients could not be replicated in the 2 independent cohorts.[24] Although this information could not be retrieved, the pre-operative p53 and/or PR status had already been considered in these 2 other cohorts when pre-operatively assessing the grade or histologic subtype.

STRENGTHS AND WEAKNESSES

The major strengths of this study are the multicenter setting, the large size of the study population, and the focus on the pre-operative setting, analogous to routine clinical practice. Another strength is that even after correcting for data set differences in multivariate analyses, PR and p53 status remained independent prognosticators of disease-specific survival, emphasizing the validity of these markers. The retrospective nature of this study remains a limitation, leading to possible heterogeneity in some variables, including the assessment of lymphovascular space

invasion and uniformity in lymph node dissection indications between the cohorts. However, this was unlikely to have compromised our primary results. Another limitation was the heterogeneity of the 3 cohorts, including differences in staining methods. We evaluated the differences between the 2 scoring methods (staining index vs 10% cutoff value for PR) in 50 cases, which showed 100% concordance. Also, it is known from previous literature that tissue microarrays are a high-quality alternative for whole-slide immunohistochemical analyses in endometrial carcinoma for these markers.[32,33] Furthermore, the use of different antibodies is consistent with daily clinical practice and underscores the robustness of our data. There was a strikingly low post-operative discordance in cohort 3 compared to the other cohorts and the literature. This might be partially explained by the fact that only cases with known pre-operative p53 and PR status were included in this cohort, which was performed only when sufficient tissue was available.

Implications for Practice and Future Research

Despite previously published evidence showing that both p53 and PR are relevant prognostic markers, their exact roles in routine practice remain unclear. We studied their value in the prediction of prognosis in patients with pre-operative grade 2 endometrioid carcinoma and showed that not all morphologic grade 2 carcinomas behave as low-grade tumors. Our data support the hypothesis that pre-operative PR and p53 immunohistochemistry in grade 2 carcinomas improve risk assessment in the pre-operative setting. Although we fully support the binary classification system proposed by the most recent guidelines, based on our data, we would recommend that p53 and PR status should be considered when pre-operatively stratifying morphologic grade 2 endometrioid carcinomas.

Taking this a step further, such an approach may eventually help in the decision regarding the surgical approach and the consideration of whether staging procedures should be performed. This requires further exploration in prospective studies with specific attention to lymph node status and the impact of surgical staging on oncologic outcomes. Furthermore, our results should be interpreted in the increasingly important field of the molecular classification of endometrial carcinomas.[6,7] Our results confirm that p53 is a valuable prognostic marker for grade 2 endometrial carcinoma in the pre-operative setting. In addition, our results underscore the importance of PR expression in pre-operative risk stratification, which is not included in the current molecular classifications. Our proposed, low-cost, and widely available dual p53 and PR immunohistochemical approach may be an attractive alternative to reflex molecular testing for pre-operative morphologic grade 2 endometrioid endometrial carcinomas.[34,35]

CONCLUSION

We demonstrated that the combination of 2 simple immunohistochemical staining methods, p53 and PR, was an important prognosticator regarding survival. Our data support a modified binary classification system in which grade 2 endometrioid endometrial carcinomas are considered low- or high-grade, depending on the p53 and PR expression status.

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