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2021-01

Kolehmainen , A M , Pasanen , A M , Koivisto-Korander , R L , Bützow , R C & Loukovaara , M J 2021 , ' Molecular characterization in the prediction of disease extent in endometrial carcinoma ' , European Journal of Obstetrics, and Gynecology ,and Reproductive Biology , vol. 256 , pp. 478-483 . https://doi.org/10.1016/j.ejogrb.2020.10.031

http://hdl.handle.net/10138/341335
https://doi.org/10.1016/j.ejogrb.2020.10.031

European Journal of Obstetrics & Gynecology and Reproductive Biology xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

European Journal of Obstetrics & Gynecology and Reproductive Biology

journal homepage: www.elsevier.com/locate/ejogrb

Full length article

Molecular characterization in the prediction of disease extent in endometrial carcinoma

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ARTICLE INFO

Article history:
Received 29 July 2020
Received in revised form 12 October 2020
Accepted 14 October 2020
Available online xxx

Keywords:
Endometrial carcinoma
Lymphadenectomy
Stage The Cancer Genome Atlas

ABSTRACT

Objective: Patients with endometrial carcinoma are usually triaged to staging lymphadenectomy selectively based on estimated risk of lymphatic spread. The risk is generally assessed by the presence of uterine risk factors, but their preoperative and intraoperative identification remain a challenge. The objective of this study was to assess the capability of molecular classification, described by The Cancer Genome Atlas (TCGA), to predict the stage of endometrial carcinoma.

Study design: Sequencing of polymerase-ε (*POLE*) and immunohistochemistry of mismatch repair (MMR) proteins and p53 were performed to stratify endometrial carcinomas into subgroups of *POLE* exonuclease domain mutation (EDM), MMR deficiency, abnormal p53 (p53 abn) and 'no specific molecular profile' (NSMP). NSMP was the reference subgroup for comparisons. Associations of molecular subgroups and uterine risk factors with stage were examined in univariable and multivariable analyses.

Results: Six hundred and four patients were included in the study. None of the *POLE* EDM tumours extended beyond the uterine cervix. In an unadjusted analysis, p53 abn was associated with increased risk for stage IIIC–IV disease [odds ratio (OR) 4.6, 95% confidence interval (CI) 2.3–9.2; $p < 0.0005$]. When controlling for uterine risk factors (histotype and grade, depth of myometrial invasion, tumour size, lymphovascular space invasion), p53 was not an independent predictor of advanced disease. In contrast, *POLE* EDM independently predicted local disease (OR 0.12, 95% CI 0.015–0.99; $p = 0.049$ for stage II–IV cancer). Of the molecular subgroups, p53 abn was most strongly associated with the presence of high-risk uterine factors (ORs between 2.2 and 19; $p \leq 0.010$).

Conclusion: Of the TCGA-based molecular subgroups, *POLE* EDM independently predicted early-stage endometrial carcinoma. Although p53 abn was not an independent predictor of advanced disease, its association with uterine risk factors could allow utilization of molecular data in deciding the type of staging surgery if knowledge of uterine factors is deficient.

1 Introduction

Tumour stage plays an important role in determining the prognosis of patients with endometrial cancer. Compared with the 5-year survival rate of 78–90% for stage I disease, the survival rate is 74% for stage II disease and only 21–57% for advanced stages (III–IV) [1]. Due to the favourable outcome, patients with stage I endometrial cancer can generally forgo adjuvant therapies, especially if hysterectomy and bilateral salpingo-oophorectomy have been complemented with regional lymphadenectomy to confirm disease stage. If lymphadenectomy has not been performed, adjuvant therapy decisions rest on the presence of uterine pathological factors (i.e. histotype and grade, depth of myometrial invasion, tumour size, lymphovascular space invasion). These factors are not only associated with higher risk for extrauterine disease, but also independently predict recurrence and poor survival in early-stage cancers [2,3].

In 2013, The Cancer Genome Atlas (TCGA) Research Network proposed four subgroups of endometrial carcinoma based on their genomic architecture [4]: polymerase-ε (*POLE*) ultramutated, microsatellite instability hypermutated, copy-number low, and copy-number high. The TCGA analysis focused on endometrioid

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https://doi.org/10.1016/j.ejogrb.2020.10.031
0301-2115/

and serous carcinomas, and demonstrated an association between molecular subgroups and patient outcome. *POLE* ultramutated and copy-number high subgroups were associated with the best and worst outcomes, respectively [4]. These findings have subsequently been recapitulated in two classifiers based on surrogate markers that are clinically more feasible than the original genome-wide TCGA analysis: one in stage I endometrioid carcinomas [5] and the other in a sample that was unselected with regard to stage and histology [6].

The TCGA study [4] was a landmark that provided a new platform for the prognostication of endometrial cancers. Although the stage distribution has been found to differ across molecular subgroups [6–10], molecular subgroups have not been studied as predictors of disease extent in the context of uterine risk factors. This study examined the association of molecular subgroups with disease extent in patients with endometrial cancer, alone and in conjunction with uterine risk factors.

2 Materials and methods

Patients who underwent primary surgical treatment for stage I–IV endometrial carcinoma at the Department of Obstetrics and Gynaecology, Helsinki University Hospital between 1 January 2007 and 31 December 2012 were identified. Those patients with a tissue microarray tumour sample available for immunohistochemistry were eligible for inclusion in the study. The construction of the tissue microarray has been described previously [11]. To improve the sensitivity of immunohistochemistry, four duplicate 0.8-mm cores from the corresponding area of the paraffin blocks were drawn and analysed. The final cohort consisted of patients with successful molecular characterization of their primary tumours. The study was approved by the institutional review board and the National Supervisory Authority for Welfare and Health.

The following monoclonal antibodies were used for chromogenic immunohistochemistry on multicore tissue microarray slides: MLH1 (ES05; Dako, Santa Clara, CA, USA), MSH2 (G219-1129; BD Biosciences, San Jose, CA, USA), MSH6 (EPR3945; Abcam, Cambridge, UK), PMS2 (EPR3947; Epitomics, Burlingame, CA, USA) and p53 (DO-7, Dako). Tissue microarray slides were scanned with a three-dimensional Histech Pannoramic 250 Flash II scanner (Fimmic Oy, Helsinki, Finland). Slide images were managed and analysed with WebMicroscope software (Fimmic Oy). Virtual slides were scored by a pathologist blinded to the clinical data. A second investigator examined equivocal cases and a consensus was reached. Mismatch repair (MMR) protein status was considered deficient (MMR-D) when a complete loss of nuclear expression was observed in carcinoma cells of one or more MMR proteins (MLH1, MSH2, MSH6, PMS2) detected by immunohistochemistry. Aberrant p53 staining (p53 abn) was defined as strong and diffuse nuclear staining or completely negative ('null') staining in carcinoma cells. Weak and heterogeneous staining was classified as wild-type (wt) expression. Stromal cells and inflammatory cells served as an internal control for MMR protein and p53 staining. Samples with scarce carcinoma cells or completely negative staining of the internal control (when applicable) were discarded.

For DNA extraction, representative areas of formalin-fixed paraffin-embedded tumour sections were macrodissected as identified by pathologist assessment. DNA was extracted using the proteinase K/phenol-chloroform method. *POLE* exonuclease domain mutation (EDM) screening of hot spots in exons 9, 13 and 14 was performed by direct sequencing [12]. Only samples with a high-quality sequence for all of the four *POLE* hot spots examined were included in the study.

Conforming to the Proactive Molecular Risk Classifier for Endometrial Cancer (ProMisE) [6], cases that showed p53 abn staining were excluded from the MMR proficient, *POLE* wt cases, and this subgroup was termed 'no specific molecular profile'

Table 1
Clinicopathologic data (n = 604).

	NSMP (n = 218)	*POLE* EDM (n = 30)	MMR-D (n = 287)	p53 abn (n = 69)	p-value
Age (years), median (interquartile range)	66 (60–73)	55.5 (52.5–67.5)	70 (61–77)	72 (65.5–78)	<0.0005
Body mass index (kg/m^2), median (interquartile range)	28.5 (24.3–33.2)	25.1 (22.9–27.6)	27.3 (23.5–32.5)	27.3 (24.4–30.5)	0.028
Pelvic lymphadenectomy, number of cases (%)	129 (59.2%)	18 (60.0%)	165 (57.5%)	32 (46.4%)	0.292
Pelvic-aortic lymphadenectomy, number of cases (%)	19 (8.7%)	4 (13.3%)	50 (17.4%)	22 (31.9%)	<0.0005
Histology, number of cases (%)					<0.0005
Endometrioid carcinoma	206 (94.5%)	29 (96.7%)	264 (92.0%)	36 (52.2%)	
Clear cell carcinoma	5 (2.3%)	0 (0%)	7 (2.4%)	13 (18.8%)	
Serous carcinoma	2 (0.9%)	1 (3.3%)	4 (1.4%)	11 (15.9%)	
Carcinosarcoma	2 (0.9%)	0 (0%)	4 (1.4%)	7 (10.1%)	
Undifferentiated carcinoma	3 (1.4%)	0 (0%)	8 (2.8%)	2 (2.9%)	
Grade, number of cases (%), for endometrioid carcinoma alone, n=535					<0.0005
1	141 (68.4%)	20 (69.0%)	127 (48.1%)	5 (13.9%)	
2	52 (25.2%)	5 (17.2%)	83 (31.4%)	15 (41.7%)	
3	13 (6.3%)	4 (13.8%)	54 (20.5%)	16 (44.4%)	
Myometrial invasion ≥50%	83 (38.1%)	6 (20.0%)	120 (41.8%)	40 (58.0%)	0.002
Tumour diameter >5 cm	44 (21.8%) [a]	4 (13.8%) [b]	64 (23.9%) [c]	26 (37.7%)	0.027
Lymphovascular space invasion	49 (22.5%)	4 (13.3%)	80 (27.9%)	27 (39.1%)	0.015
Stage, number of cases (%)					<0.0005
IA	123 (56.4%)	24 (80.0%)	140 (48.8%)	22 (31.9%)	
IB	42 (19.3%)	5 (16.7%)	66 (23.0%)	18 (26.1%)	
II	23 (10.6%)	1 (3.3%)	22 (7.7%)	1 (1.4%)	
IIIA	9 (4.1%)	0 (0%)	19 (6.6%)	5 (7.2%)	
IIIB	1 (0.5%)	0 (0%)	4 (1.4%)	1 (1.4%)	
IIIC1	13 (6.0%)	0 (0%)	24 (8.4%)	3 (4.3%)	
IIIC2	1 (0.5%)	0 (0%)	8 (2.8%)	9 (13.0%)	
IVA	0 (0%)	0 (0%)	0 (0%)	0 (0%)	
IVB	6 (2.8%)	0 (0%)	4 (1.4%)	10 (14.5%)	

NSMP, no specific molecular profile; MMR-D, mismatch repair protein deficiency; *POLE* EDM, polymerase-ε exonuclease domain mutation; p53 abn, abnormal p53.
[a] Data missing for 16 patients.
[b] Data missing for one patient.
[c] Data missing for 19 patients.

(NSMP). The NSMP subgroup can also be referred to as 'p53 wt' [6]. p53 abn and NSMP/p53 wt correspond to the copy-number high and copy-number low subgroups, respectively, in the TCGA classification system, while MMR-D is a surrogate to the microsatellite instability hypermutated subgroup [5,6].

Clinicopathological data were abstracted from institutional medical and pathology records. Stage was determined according to the International Federation of Gynecology and Obstetrics guidelines revised in 2009 [13]. Primary tumour diameter was defined as the largest dimension of the tumour. If more than one lesion was present, the lesion with the largest diameter was considered. Primary tumour diameter was not available for 36 patients. The choice of 5 cm as a determinant for the analysis of tumour size was based on earlier literature and the authors' experience [14,15]. Lymphovascular space invasion was defined as the presence of adenocarcinoma, of any extent, in endothelium-lined channels of uterine specimens outside the tumour.

Analysis of variance and Kruskal–Wallis test were used for comparison of continuous variables after testing for normality by Shapiro–Wilk test. Chi-squared test was used for comparison of categorical variables. Odds ratios (ORs) along with 95% confidence intervals (CIs) were computed for the associations between risk variables and stage. Logistic regression analyses were used to identify variables that predicted stage independently. Statistical significance was set at $p < 0.05$. Data were analysed using Statistical Package for the Social Sciences Version 25 (IBM Corp., Armonk, NY, USA).

3 Results

Of the 965 patients who underwent primary surgery for endometrial carcinoma during the study period, 123 were excluded because a tissue microarray tumour sample was not available for immunohistochemistry. Fifty-two cases were excluded due to failed immunohistochemistry of MMR proteins or p53, 72 cases were excluded due to insufficient formalin-fixed paraffin-embedded tissue for *POLE* sequencing, and 114 cases were excluded due to failed *POLE* sequencing. Thus, the final cohort consisted of 604 patients with successful molecular characterization of their primary tumours. Of these, 287 (47.5%) were classified as MMR-D, 218 (36.1%) as NSMP, 69 (11.4%) as p53 abn, and 30 (5.0%) as *POLE* EDM. Twenty cases (3.9%) displayed multiple molecular features. Three cases displayed *POLE* EDM and either MMR-D or p53 abn, and one case had all three molecular alterations. These were classified as *POLE* EDM tumours. Sixteen cases, classified as MMR-D tumours, displayed both MMR-D and p53 abn.

Pertinent patient characteristics are shown in Table 1. *POLE* EDM was associated with younger age and lower body mass index, whereas p53 abn was associated with older age.

Of the 604 patients, 439 (72.7%) underwent pelvic or pelvic-aortic lymphadenectomy. Of the patients who were recommended to receive lymphadenectomy according to the joint guidelines by the European Society for Medical Oncology (ESMO), European Society of Gynaecological Oncology (ESGO) and European Society for Radiotherapy and Oncology (ESTRO) [16] – i.e. those with grade 3 endometrioid carcinoma, non-endometrioid carcinoma or grade 1–2 carcinoma with deep (≥50%) myometrial invasion – 77.0% (234/304) underwent pelvic or pelvic-aortic lymphadenectomy. Comprehensive lymphadenectomy was most frequently performed in the p53 abn subgroup of patients (Table 1). Stage distribution varied among subgroups; most notably, none of the *POLE* EDM tumours extended beyond the uterine cervix (Table 1).

Of the molecular subgroups, with NSMP as reference, p53 abn was associated with increased risk for stage IIIC–IV disease in an unadjusted analysis (Table 2). Grade 3 endometrioid and non-endometrioid histology, deep myometrial invasion, large tumour size and lymphovascular space invasion were associated with higher risk for advanced disease (Table 2).

Table 2
Univariable analyses for advanced (stage IIIC–IV) endometrial cancer (n = 604).

	n	OR (95% CI)	p-value
Molecular subgroup			<0.0005
NSMP	218 (36.1%)	1	
POLE EDM	30 (5.0%)	–	0.998
MMR-D	287 (47.5%)	1.4 (0.80–2.5)	0.234
p53 abn	69 (11.4%)	4.6 (2.3–9.2)	<0.0005
Histology			<0.0005
Endometrioid grade 1–2	448 (74.2%)	1	
Endometrioid grade 3	87 (14.4%)	4.0 (2.2–7.5)	<0.0005
Non-endometrioid	69 (11.4%)	8.6 (4.7–16)	<0.0005
Myometrial invasion ≥50%	249 (41.2%)	7.0 (3.9–13)	<0.0005
Tumour diameter >5 cm[a]	138 (24.3%)	5.6 (3.4–9.4)	<0.0005
Lymphovascular space invasion	160 (26.5%)	6.3 (3.8–10)	<0.0005

NSMP, no specific molecular profile; MMR-D, mismatch repair protein deficiency; *POLE* EDM, polymerase-ε exonuclease domain mutation; p53 abn, abnormal p53; OR, odds ratio; CI, confidence interval.
[a] Data missing for 36 patients.

The independent effects of the various risk factors on disease extent were examined in two logistic regression models (Table 3). The first model (comprehensive model) included all risk factors, while the second model was restricted to molecular subgroups and those uterine factors that are included as predictors of lymph node involvement in the ESMO–ESGO–ESTRO guidelines [16] – i.e. histology and depth of myometrial invasion (ESMO–ESGO–ESTRO–TCGA model). Histology, depth of myometrial invasion, tumour size and lymphovascular space invasion, but not molecular subgroups, were identified as independent predictors of stage IIIC–IV disease in the comprehensive model. Unlike molecular subgroups, histology and depth of myometrial invasion were significant predictors of stage IIIC–IV disease in the ESMO–ESGO–ESTRO–TCGA model.

ORs were not calculable for *POLE* EDM because there were no stage III or IV cases in this subgroup of patients (Tables 2 and 3). When the cut-off for stage was set at II in the multivariable models, *POLE* EDM predicted early-stage disease in the comprehensive model and in the ESMO–ESGO–ESTRO–TCGA model (Table 4).

With the exception of grade 3 endometrioid histotype, *POLE* EDM was associated with the lowest rates of high-risk uterine factors (Table 1). p53 abn was invariably associated with the highest rates of uterine risk factors (Table 1). ORs for the presence of uterine risk factors according to molecular tumour type are shown in Table 5. ORs for all of these risk factors were increased in the p53 abn subgroup. Moreover, ORs for high-risk histology were increased in the MMR-D subgroup.

4 Discussion

Uterine risk factors are commonly utilized as predictors of disease extent in endometrial cancer. This information is helpful in the stratification of at-risk patients to surgical staging (i.e. pelvic-aortic lymphadenectomy). Tumour size can be reliably measured intraoperatively without frozen section analysis [17], but data on histology and depth of myometrial invasion are only known with certainty after surgery. Preoperative assessment of histotype and grade is fairly reliable because clinically significant upgrading in the final histology occurs in just 2–3% of preoperative grade 1 endometrioid carcinomas [18–21]. On the other hand, the diagnostic performance of imaging techniques may not be ideal for the prediction of myometrial invasion, with reported sensitivities of 87% and 71% and specificities of 57% and 72% for magnetic resonance imaging and transvaginal ultrasound, respectively [22]. Findings on frozen section analysis in determining histologic grade

Table 3
Multivariable analyses for advanced (stage IIIC–IV) endometrial cancer.

	Multivariable (n = 568) Comprehensive		Multivariable (n = 604) ESMO–ESGO–ESTRO–TCGA	
	OR (95% CI)	p-value	OR (95% CI)	p-value
Molecular subgroup		0.507		0.682
NSMP	1		1	
POLE EDM	–	0.998	–	0.998
MMR-D	1.2 (0.59–2.4)	0.640	1.2 (0.63–2.2)	0.617
p53 abn	1.9 (0.81–4.7)	0.139	1.7 (0.73–3.8)	0.224
Histology		0.003		<0.0005
Endometrioid grade 1–2	1		1	
Endometrioid grade 3	1.8 (0.85–3.7)	0.128	2.2 (1.1–4.3)	0.020
Non-endometrioid	3.8 (1.8–8.3)	0.001	5.9 (2.9–12)	<0.0005
Myometrial invasion ≥50%	2.7 (1.4–5.4)	0.004	5.6 (3.0–10)	<0.0005
Tumour diameter >5 cm[a]	3.0 (1.7–5.4)	<0.0005		
Lymphovascular space invasion	3.5 (1.9–6.4)	<0.0005		

NSMP, no specific molecular profile; MMR-D, mismatch repair protein deficiency; *POLE* EDM, polymerase-ε exonuclease domain mutation; p53 abn, abnormal p53; ESMO, European Society for Medical Oncology; ESGO, European Society of Gynaecological Oncology; ESRTO, European Society for Radiotherapy and Oncology; TGCA, The Cancer Genome Atlas; OR, odds ratio; CI, confidence interval.
[a] Data missing for 36 patients.

Table 4
Multivariable analyses for extrauterine (stage II–IV) endometrial cancer.

	Multivariable (n = 568) Comprehensive		Multivariable (n = 604) ESMO–ESGO–ESTRO–TCGA	
	OR (95% CI)	p-value	OR (95% CI)	p-value
Molecular subgroup		0.162		0.119
NSMP	1		1	
POLE EDM	0.12 (0.015–0.99)	0.049	0.10 (0.012–0.82)	0.032
MMR-D	0.87 (0.53–1.4)	0.593	0.95 (0.60–1.5)	0.828
p53 abn	0.60 (0.27–1.3)	0.195	0.64 (0.31–1.3)	0.231
Histology		<0.0005		<0.0005
Endometrioid grade 1–2	1		1	
Endometrioid grade 3	2.5 (1.4–4.6)	0.003	3.1 (1.8–5.4)	<0.0005
Non-endometrioid	4.9 (2.4–10)	<0.0005	7.1 (3.7–14)	<0.0005
Myometrial invasion ≥50%	2.9 (1.8–4.7)	<0.0005	5.0 (3.2–7.6)	<0.0005
Tumour diameter >5 cm[a]	3.6 (2.2–5.8)	<0.0005		
Lymphovascular space invasion	2.7 (1.7-4.4)	<0.0005		

NSMP, no specific molecular profile; MMR-D, mismatch repair protein deficiency; *POLE* EDM, polymerase-ε exonuclease domain mutation; p53 abn, abnormal p53; ESMO, European Society for Medical Oncology; ESGO, European Society of Gynaecological Oncology; ESRTO, European Society for Radiotherapy and Oncology; TGCA, The Cancer Genome Atlas; OR, odds ratio; CI, confidence interval.
[a] Data missing for 36 patients.

Table 5
Associations of molecular subgroups with uterine risk factors (n = 604).

	High-risk histology[a]		Myometrial invasion ≥50%		Tumour diameter >5 cm[b]		Lymphovascular space invasion	
	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
Molecular subgroup		<0.0005		0.003		0.031		0.018
NSMP	1		1		1		1	
POLE EDM	1.5 (0.54–4.4)	0.416	0.41 (0.16–1.0)	0.059	0.58 (0.19–1.7)	0.327	0.53 (0.18–1.6)	0.259
MMR-D	2.8 (1.7–4.6)	<0.0005	1.2 (0.82–1.7)	0.396	1.1 (0.73–1.7)	0.593	1.3 (0.89–2.0)	0.169
p53 abn	19 (9.7–37)	<0.0005	2.2 (1.3–3.9)	0.004	2.2 (1.2–3.9)	0.010	2.2 (1.2–4.0)	0.007

NSMP, no specific molecular profile; MMR-D, mismatch repair protein deficiency; *POLE* EDM, polymerase-ε exonuclease domain mutation; p53 abn, abnormal p53; OR, odds ratio; CI, confidence interval.
[a] Endometrioid grade 3 or non-endometrioid.
[b] Data missing for 36 patients.

and depth of myometrial invasion have been inconsistent [23–26]. It appears that large practices are needed for development of the robust technical expertise required for its successful utilization [26]. Lymphovascular space invasion is not included in frozen section protocols for endometrial cancer, and remains a constant postoperative finding. Introduction of novel, clinically applicable methods for the accurate prediction of lymphatic spread preoperatively would be desirable in the surgical management of endometrial cancer.

The advent of molecular characterization of endometrial cancer [4] has raised interest to supplement traditional risk factors with molecular subgroups in clinical practice, with the hope of achieving a more objective risk assessment. Thus far, molecular subgroups have been demonstrated to improve the prediction of

survival outcomes compared with conventional risk factors alone [5–10]. A meta-analysis [27] of individual studies [5–10] found that the prognosis of p53 abn was the worst, and was worsened further by unfavourable clinicopathologic factors; the prognosis of MMR-D overlapped with NSMP but was worsened by unfavourable clinicopathologic factors; and the prognosis of *POLE* was the best and did not seem to be significantly affected by clinicopathologic factors.

Notably, molecular classification can be achieved on diagnostic endometrial samples and is highly concordant with hysterectomy specimens [28]. Thus, it has been suggested that preoperative molecular classification could be used to triage patients to different types of staging surgery [29]. To test this hypothesis, the capability of molecular classification to predict disease stage in 604 women with endometrial cancer was assessed. The ProMisE classification system [6] that recapitulates the molecular subgroups described by the TCGA was used [4]. The ProMisE classifier is a decision tree analysis where molecular analyses are performed sequentially in the order of MMR protein immunohistochemistry, *POLE* sequencing (in MMR-proficient cases) and p53 immunohistochemistry (in *POLE* wt cases). After its development, the ProMisE classifier was confirmed [7] and validated [10] according to the Institute of Medicine Guidelines for the development of omics-based biomarkers. As a modification to the ProMisE algorithm, the authors attempted to perform comprehensive molecular characterization on all primary tumour samples. As a consequence, 20 cases with multiple molecular features were identified. Based on clinical outcomes associated with the multiple classifiers, *POLE* EDM − MMR-D and *POLE* EDM − p53 abn tumours were classified as *POLE* EDM tumours, and MMR-D − p53 abn tumours were classified as MMR-D tumours [30,31].

None of the *POLE* EDM tumours extended beyond the uterine cervix. *POLE* EDM was an independent predictor of local (stage I) endometrial cancer. Together with the excellent prognosis associated with *POLE* EDM [4–10,27], the data suggest that these patients may not benefit from surgical lymph node assessment.

In an unadjusted analysis, p53 abn was associated with increased risk for advanced (stage IIIC–IV) endometrial cancer. In a multivariable analysis, uterine risk factors independently predicted advanced disease but the effect of p53 was no longer significant. However, p53 abn was invariably associated with increased ORs for the presence of high-risk uterine factors. It could be suggested that molecular data, when examined preoperatively, could aid in lymphadenectomy decisions when data on traditional risk factors are inconsistent or unavailable.

Similar to previous studies [6,7,9], demographic characteristics varied among the subgroups; *POLE* EDM was associated with younger age and lower body mass index, and p53 abn was associated with older age. Approximately 50% of *POLE* mutant tumours were grade 3 endometrioid carcinomas in the original TCGA study [4] and in the ProMisE confirmation cohort [7]. The proportion was up to 35% in subsequent studies [5,9,10], which is more comparable to the present finding (13%). This variation may be due to under-representation of the more common low-grade carcinomas in the earlier studies [4,7]. Of the other uterine risk factors, associations of deep myometrial invasion and lymphovascular space invasion with molecular subgroups were validated in the later cohorts [5,9,10]. Although significant differences among molecular subgroups were demonstrated, p53 abn did not stand out as a consistently unique subgroup, especially in the NRG Oncology/Gynecologic Oncology Group study [9]. The possibility that the replication of the present multivariable analyses in patient samples with disparate features might yield different results cannot be excluded.

The study population was obtained from a single tertiary referral centre, which can be construed as a weakness of this study. Molecular classification could not be performed on 238 cases in the tissue microarray cohort, mainly due to limited yields of high-quality DNA from formalin-fixed paraffin-embedded samples and stringent inclusion criteria for *POLE* sequencing data. Despite the excluded cases, the stage distribution and proportion of non-endometrioid carcinomas remained comparable with a typical unselected cohort of patients with endometrial cancer, such as the Gynecologic Oncology Group 210 surgical pathological staging study of 5866 patients, of whom the vast majority had early endometrioid carcinoma [32].

5 Conclusion

In this unselected cohort of patients with endometrial carcinoma, *POLE* EDM independently predicted local disease, suggesting that molecular characterization, when available preoperatively, will allow these patients to safely forgo surgical lymph node staging. The association of p53 abn with high-risk uterine factors suggests that molecular data could be used in lymphadenectomy decisions if data on uterine factors are incomplete or difficult to interpret.

Funding

This study was supported by Helsinki University Hospital research funds.

Declaration of Competing Interest

The authors report no declarations of interest.

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