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Molecular classification of endometrial carcinoma applied to endometrial biopsy specimens: Towards early personalized patient management

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HIGHLIGHTS

- Endometrial carcinoma is difficult to accurately classify using histomorphologic and grade systems.
- Molecular classifications, however, is more reproducible and accurate.
- Clinically applicable methods are proposed for molecularly classifying these cancers.
- Biopsy serves as an early option to accurately manage patients.
- Applying the molecular classification to these samples results in more personalized and individualized patient care.

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ABSTRACT

Background. The current risk stratification systems used to guide management of endometrial cancer are based on irreproducible post surgical pathological information, hence the need for more reliable classification systems. Using microarray and sequencing technologies, TCGA recently identified four prognostically significant endometrial carcinoma subtypes, which subsequently proved reproducible using clinically applicable surrogate tests. Using these tests, we sought to determine the level of concordance between endometrial biopsies and subsequent hysterectomy specimens in assessing the molecular classification of endometrial carcinoma.

Materials and methods. Fifty biopsies with corresponding hysterectomy specimens for endometrial carcinomas were collected. Additionally, 10 cases of biopsy proven atypical hyperplasia/EIN who were found to have endometrial carcinoma on resection were included. IHC for mismatch repair (MMR) proteins (MLH1, PMS2, MSH2 and MSH6) and P53 was performed. Microsatellite instability analysis was performed by PCR and Sanger sequencing was performed to detect mutations in exons 9 and 13 of the *POLE* gene. The level of concordance for tumor grade, histologic subtype, immunohistochemical and molecular profile in both specimens was determined using Cohen's kappa estimates.

Results. A high level of concordance was achieved for MMR-loss, MSI-high, P53-wild and abnormal types. In contrast, grade and histologic subtype showed only moderate levels of agreement. *POLE* gene mutation was detected in two patients. For both cases, mutations were detected only in resection specimens. When comparing atypical hyperplasia/EIN with subsequent hysterectomy tumor, the profile was identical to that of endometrial carcinoma.

Conclusion. In our cohort of endometrial carcinoma, a high level of concordance was achieved between biopsy and hysterectomy specimens for MMR-loss, MSI-high, P53-wild and abnormal types, superior to that of grade and histologic subtype, providing earlier and more reliable prognostic information to inform management. Similar concordance could not be achieved for *POLE* mutation, given the low frequency of this mutation in our study.

1. Background

Endometrial carcinoma is the most common gynecological malignancy in the United States [1]. Stratification of patients with

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endometrial carcinoma into several risk groups is currently based on post surgical pathological information including histologic subtype, tumor grade, stage, lymphovascular and myometrial invasion [2,3]. However, high-grade endometrial carcinomas cannot be reliably classified by histomorphologic criteria with moderate to poor inter-observer agreement for histotype and tumor grade [4]. In addition, 8–10% of early stage endometrial carcinomas develop recurrence and distant metastasis, hence the need for more reliable systems to categorize and classify endometrial carcinomas to inform clinical management. Using a combination of whole exome and whole genome sequencing and copy number analysis, The Cancer Genome Atlas (TCGA) classified endometrial carcinomas into four prognostically distinct molecular subtypes: *POLE* ultramutated, hypermutated with microsatellite instability (MSI), copy number-low and copy number-high subtypes [5]. Molecular classification of endometrial carcinoma has shown great promise, proving to be reproducible, demonstrating associations with clinical outcomes and providing valuable prognostic and predictive information.

These molecular subtypes subsequently proved to be reproducible using clinically applicable surrogate tests [6]. The *POLE* ultramutated phenotype was identified on basis of targeted sequencing for *POLE* gene mutation, the microsatellite instable subtype was evaluated by immunohistochemical staining for mismatch repair protein (MMR) expression and finally the copy number status was determined by P53 immunostain; low copy number tumors showed normal p53 (wild-type) expression while high copy number tumors had abnormal (aberrant) p53 expression.

Using these tests, we sought to determine the level of concordance between endometrial biopsies and subsequent hysterectomy specimens in assessing the molecular classification of endometrial carcinoma. If endometrial biopsies reflect disease state and are consistent with information available from hysterectomies, physicians and patients will be in a position to make early treatment decisions more confidently.

2. Material and methods

After approval by the Institutional Review Board (IRB), we identified fifty patients (n = 50) diagnosed with endometrial carcinoma where both diagnostic (endometrial biopsy) and their corresponding resection (hysterectomy) specimens were available. Additionally, ten cases (n = 10) of biopsy proven atypical hyperplasia/EIN and found to have endometrial carcinoma on subsequent resection specimens were included. Electronic medical records and pathology reports were reviewed to analyze clinical parameters and pathological variables (age at diagnosis, tumor size, histologic subtype, FIGO grade and treatment modality).

Representative Hematoxylin and Eosin (H&E) stained sections of each tumor (biopsy and corresponding resections) were reviewed by two gynecological pathologists (R.A and S.B.) to confirm the diagnosis as per the 2014 World Health Organization (WHO) classification/criteria; atypical hyperplasia/EIN, endometrioid carcinoma, serous carcinoma, clear cell carcinoma and mixed carcinomas. Mixed carcinomas were defined as carcinomas with one or more other components comprising at least 10% of the tumor volume. For cases with confirmed diagnosis of endometrioid carcinoma, tumor grade was assigned according to the FIGO grading system. Additionally, representative full sections from each biopsy and corresponding resection specimen were obtained for: 1. Immunohistochemical (IHC) testing for mismatch repair proteins (MMR) MLH1, MSH2, MSH6 and PMS2, 2. Microsatellite instability (MSI) status assessment by PCR 3. Sanger sequencing for polymerase epsilon (*POLE*) exonuclease domain mutations and 4. P53 IHC stain.

2.1. Immunohistochemistry for MMR proteins

IHC evaluation of MMR protein expression was performed on full sections. The antibodies used were MLH1 (clone M1; Ventana, BenchMark XT/Ultra; Ready-To-Use), MSH2 (clone G219–1129; Ventana/Cell Marque, BenchMark GX/XT/Ultra; Ready-To-Use), MSH6 (clone 44; Ventana, BenchMark XT; Ready-To-Use) and PMS2 (clone EPR3947; Ventana; Ready-To-Use). Immunostaining was performed according to previously described protocols [7].

Evaluation of the MMR protein expression status was performed by two pathologists. Cases with complete absence of nuclear staining and positive nuclear staining in internal control cells (eg. stromal fibroblasts, non-neoplastic epithelium and lymphocytes) were considered to demonstrate MMR protein loss.

2.2. P53 IHC

P53 IHC staining was performed on full sections, according to previously published protocols [6]. P53 was interpreted as abnormal if there was complete negative staining or strong/diffuse staining in >70% of tumor cells (aberrant expression pattern) [8].

2.3. DNA isolation

Genomic DNA isolation, microsatellite analysis and *POLE* mutation analysis were performed in the Detroit Medical Center University Laboratory Molecular Genetics Laboratory (Detroit, MI). Genomic DNA was isolated from 20 μm of formalin fixed paraffin embedded tissue using the QuickGene DNA Tissue S kit and the QuickGene 910 nucleic extraction machine (Fujiflim, Tokyo, Japan) according to manufacturer's recommendations.

2.4. Microsatellite instability (MSI) status analysis

10 ng of genomic DNA isolated from tumor and adjacent normal tissue and microsatellite analysis was performed using the Promega MSI Analysis System (Madison, Wisconsin) followed by capillary electrophoresis on a 3130xl Genetic Analyzer (Thermo Fisher Scientific, Waltham, MA). PCR products from normal and tumor tissues were compared to determine the stability of each mononucleotide repeat marker in the tumor tissue. The tumor tissue was classified as MSI high when at least two repeat markers demonstrated instability. Tumor tissue was classified as MSI low when one marker demonstrated instability. Tumors in which no markers demonstrated instability were classified as microsatellite stable (MSS).

2.5. POLE mutation analysis

Exons 9 and 13 of the *POLE* gene were assessed for mutations using PCR amplification and Sanger sequencing. Genomic DNA (10 ng) was amplified with 0.8 μM of M13 tagged forward and reverse primers and the BigDye™ Terminator Direct Cycle Sequencing kit (Thermo Fischer Scientific). Exon 9 was amplified with the following primers: 5′-GGCA GATGCTGCTGTAGTATGG-3′ and 5′-GGTGTTCAGGGAGGCCTAATG-3′. A portion of exon 13 containing codon 411 was amplified with the following primers: 5′-GCCCAGTTTTGCCAGTTCTC-3′ and 5′-GGTCTAGCTCC ACGGGATCA-3′. PCR products were sequenced with BigDye Terminator™ (Thermo Fisher Scientific) chemistry on a 3130xl Genetic Analyzer. Sequencing files were analyzed with Mutation Surveyor v5.0.0 (SoftGenetics, State College, PA).

2.6. Statistical analysis

The level of concordance for tumor grade, histologic subtype, immunohistochemical and molecular profile in both specimens was determined using Cohen's kappa estimates. Kappa statistics of 0.86 (95% CI) is consistent as “near perfect” level of agreement.

3. Results

A total of 60 patients qualified for analyses. The demographics and clinicopathologic features of these patients and their tumors are detailed in Table 1. The median age at diagnosis was 60 years (range 27 to 85 years). The median tumor size at the time of resection was 4.0 cm (range 0.1 to 11 cm). Endometrial biopsies included 65% endometrioid carcinomas (n = 39/60), 17% atypical hyperplasia/EIN (n = 10/60), 15% serous carcinomas (n = 9/60), 1.5% clear cell carcinoma (n = 1/60) and 1.5% mixed carcinoma (1/60). Of the 39 endometrioid carcinoma cases, 18 were FIGO grade 1 (46%), 19 were FIGO grade 2 (49%) and 2 were FIGO grade 3 tumors (5%). Mixed carcinoma included histologic features of both endometrioid and serous carcinoma. All patients underwent definite surgical treatment in the form of either total abdominal, robotic assisted or vaginal hysterectomy. Lymphadenectomy was performed as part of the surgical staging in 59% (n = 35/60) of the patients. Radiotherapy was part of the adjuvant therapy in 15% (n = 9/60), chemotherapy in 18% (n = 11/60), while 15% (n = 9/60) of the patients received both. Histologic diagnosis on resection specimens included 85% endometrioid carcinoma (n = 51/60), 10% serous carcinoma (n = 6/60), 3.5% mixed carcinoma (n = 2/60) and 1.5% clear cell carcinoma (n = 1/60). Mixed carcinomas on resection specimens also included histologic features of both endometrioid and serous carcinomas. Of the 51 endometrioid carcinomas diagnosed on resection, 19 were FIGO grade 1 (37%), 21 were FIGO grade 2 (42%) and 10 were FIGO grade 3 tumors (14%).

Table 1
Clinicopathologic features of 60 endometrial carcinoma patients included in our study.

Patient ID	Age	Biopsy diagnosis	Resection diagnosis	FIGO grade biopsy	FIGO grade resection	Size	Stage
1	53	ACH	EC	NA	2	6.5	IA
2	72	EC	SC	1	NA	3.2	IA
3	61	EC	EC	1	1	5	IIA
4	75	EC	EC	2	3	5.7	IB
5	57	EC	EC	2	2	7	IB
6	35	ACH	EC	NA	1	0.2	IA
7	63	EC	EC	2	3	4.2	IA
8	82	EC	EC	3	3	3	IB
9	61	EC	EC	2	2	2	IA
10	76	SC	MIXED	NA	NA	7.5	IIIC
11	55	MIXED	MIXED	NA	NA	11	IA
12	49	EC	EC	2	2	6.5	II
13	41	EC	EC	1	2	5.5	II
14	85	EC	EC	2	2	5.5	IB
15	68	EC	EC	2	2	5.5	IA
16	51	EC	EC	1	2	7.5	IA
17	50	ACH	EC	NA	1	0.1	IA
18	66	EC	EC	1	1	2	IA
19	41	EC	EC	2	3	6.1	IIIC
20	60	EC	EC	2	1	1	IA
21	52	ACH	EC	NA	1	0.8	IA
22	31	EC	EC	2	2	2.5	IIIC
23	27	EC	EC	2	1	0.5	IA
24	62	EC	EC	2	2	0.3	IA
25	56	ACH	EC	NA	1	0.1	IA
26	46	ACH	EC	NA	1	0.1	IA
27	59	SC	SC	NA	NA	8	IIIC
28	63	EC	EC	1	1	7	IA
29	66	EC	EC	2	2	7	IA
30	68	SC	EC	NA	3	2.8	IA
31	72	EC	EC	1	2	5.6	IA
32	57	EC	EC	1	1	7	IA
33	77	SC	EC	NA	3	5.2	IIIC
34	66	EC	EC	1	1	2.5	IA
35	74	SC	SC	NA	NA	3.5	IIIC
36	67	EC	EC	1	2	9	IA
37	59	EC	EC	2	2	8	IIIA
38	67	SC	EC	NA	3	4	IIIC
39	64	SC	SC	NA	NA	2	IA
40	64	EC	EC	1	2	6.6	IA
41	76	SC	SC	NA	NA	2	IA
42	59	EC	EC	1	2	5.5	II
43	58	EC	EC	1	3	2.5	IA
44	51	ACH	EC	NA	1	0.1	IA
45	59	EC	EC	1	1	0.1	IA
46	70	ACH	EC	NA	1	1.6	IA
47	51	ACH	EC	NA	1	1.9	IA
48	58	EC	EC	1	2	2	IA
9	73	ACH	EC	NA	2	5	IA
50	85	EC	EC	2	2	8	IA
51	56	EC	EC	2	2	3.5	IA
52	63	EC	EC	2	2	8	II
53	52	EC	EC	2	2	4	IA
54	48	EC	EC	1	1	4.5	IA
55	69	SC	SC	NA	NA	9.5	IIIA
56	64	CC	CC	NA	NA	6	IA
57	49	EC	EC	2	3	4	IA
58	76	EC	EC	3	3	2	IB
59	33	EC	EC	1	1	0.1	IA
60	49	EC	EC	1	1	4	IA

EC = endometrioid carcinoma, SC = serous carcinoma, CC = clear cell carcinoma, Mixed = mixed carcinoma.

The descriptive analysis of endometrial carcinomas diagnosed on biopsies and resection specimens with respect to the molecular subgroups are detailed in Tables 2 and 3, respectively. Endometrial carcinomas with loss of MMR protein expression were most commonly FIGO grade 2 (53% and 58%) and of the endometrioid type (43.5% and 51%) while tumors with aberrant p53 expression were most commonly of the serous type (67% and 100%). Furthermore, tumors with loss of MMR and wild type p53 expression were FIGO stage I (68% and 80%, respectively) while aberrant p53 tumors were FIGO stage III (67%). *POLE* gene mutations (p. S297F in exon 9 and p.V411M in exon 13 of the *POLE* gene) were detected in two patients, respectively (Fig. 1). Both patients had FIGO grade 3 endometrioid carcinomas (3.5% of the entire cohort and 14% of FIGO grade 3 endometrioid adenocarcinomas) and were FIGO stage I.

3.1. Concordance of the molecular classification between biopsy and resection specimens

Concordance between biopsy and resection specimens is detailed in Table 4. A high level of concordance was achieved for MMR-loss, MSI-high, p53-wild and abnormal types. *POLE* gene mutations were only detected in resection specimens. MSI-high status correlated highly with the MMR IHC profile, which revealed loss of expression of one or more of the 4 MMR proteins.

3.2. Concordance of histotype and tumor grade between biopsy and resection specimens

Concordance between biopsy and resection specimens is detailed in Table 5. Grade and histologic subtype showed only moderate levels of agreement. Despite the discordant results between biopsy and resection specimens for both histotype and tumor grade, a very high level of

Table 2
Descriptive analysis of endometrial hyperplasia/carcinoma diagnosed on biopsy according to the molecular subgroups.

	MMR-loss	MSI high	p53 wild	p53 mut
Histologic subtype				
ACH	2 (20%)	2 (20%)	10 (100%)	0
Endometrioid	17 (43.5%)	12 (31%)	38 (97.5%)	2 (5%)
Serous	1 (11%)	1(11%)	3 (33.3%)	6 (67%)
Clear cell	0	0	1 (100%)	0
Mixed	0	0	1 (100%)	1 (100%)
FIGO grade				
1	6 (35%)	4 (31%)	16 (43%)	2 (100%)
2	9 (53%)	8 (61.5%)	19 (51%)	0
3	2 (12%)	1 (7.5%)	2 (6%)	0

Table 3
Descriptive analysis of endometrial carcinomas diagnosed on resection according to the molecular subgroups.

	MMR-loss	MSI High	p53 wild	p53 mut	*POLE* mut
Histologic subtype					
Endometrioid	20 (51%)	15 (29%)	47 (92%)	1 (2%)	2 (100%)
Serous	0	0	0	6 (100%)	0
Clear cell	0	0	1(100%)	0	0
Mixed	0	0	0	2 (100%)	0
FIGO grade					
1	3 (16%)	1 (7%)	19 (36.5%)	0	0
2	11 (58%)	10 (71%)	22 (42.5%)	1 (100%)	0
3	5 (26%)	3 (21%)	11 (21%)	0	2 (100%)
FIGO stage					
I	13 (68%)	10 (67%)	41 (80%)	2 (22%)	2 (100%)
II	4 (21%)	3 (20%)	6 (12%)	0	0
III	2 (11%)	2 (13%)	4 (8%)	6 (67%)	0
IV	0	0	0	1 (11%)	0

concordance was seen in these cases for MMR and p53 protein expression pattern and MSI status (kappa 1.0) (Table 6) (Figs. 2 and 3).

Additionally, when comparing atypical hyperplasia/EIN with subsequent hysterectomy tumor, the profile was identical to that of endometrial carcinoma (Table 6). All but one case showed intact MMR, MSI stable and P53-wild type patterns.

4. Discussion

Current risk stratification systems used to guide adjuvant therapy for endometrial cancer patients are based on irreproducible histomorphologic features. The histologic classification of endometrial carcinoma presents a challenge, even to subspeciality gynecologic pathologists. The reported rates of interobserver disagreement in endometrial carcinoma histotype assignment range from 10% to 20% [9,10] and reaches 26% to 37% in high-grade tumors [11]. Additionally, tumor stage can only be assigned after definitive surgery and therefore for young women who may benefit from fertility-sparing alternatives, this information comes too late. There is a need for improved classification of endometrial carcinoma. In 2013, TCGA applied array-based and sequencing methodologies on a large series of endometrial carcinomas and identified four molecular subgroups that are associated with differences in outcomes: 1.ultramutated polymerase epsilon (*POLE*) group, 2. hypermutated microsatellite instability group, 3. Copy number abnormalities – low group and 4. Copy number abnormalities – high group. The ultramutated group had the best progression-free survival, copy number- high the worst, whereas the hypermutated and copy number- low had intermediate survival curves [5]. Molecular classification of endometrial carcinoma has proven to be more reproducible and provided valuable prognostic and predictive information. However, the methodologies applied were costly, complex to analyze and labor-intensive, making this approach unsui\ for wider clinical application. Surrogate methods for the genomic classification of endometrial carcinoma into four molecular subgroups modeled after the TCGA have therefore been developed [6,12]. Talhouk and colleagues designated their approach as the Proactive Molecular Classification tool for Endometrial cancers (ProMisE) [13,14]. It consisted of IHC for MMR proteins to identify the hypermutated group, sequencing the exonuclease domain mutations of *POLE* gene to identify the ultramutated group and using p53 IHC to separate the remaining cases into p53 wildtype and p53 abnormal groups, as a surrogate for the assessment of somatic copy number changes to identify the copy number-low and copy number-high tumors, respectively [6]. Using these surrogate markers, the four molecular subgroups and survival curves in both TCGA and Vancouver cohorts were reproduced [6]. However, these studies were applied to hysterectomy specimens which might be late in the decision making. Young women with *POLE* mutated endometrial carcinomas for example, may not need aggressive treatnent, given the good prognosis associated with these mutations despite the high tumor grade, and may be managed with less aggressive chemotherapy/surgical approaches, whereas, women identified as having an abnormal mismatch repair system and who tests positive for Lynch syndrome would be encouraged to have a definitive surgery. Therefore, performing molecular tests on diagnostic specimens (biopsy/curettage) would provide the patients and their physicians valuable information that would guide and influence decision making at the time of diagnosis.

In this study, we utilized the surrogate molecular markers to determine the level of concordance between biopsy and hysterectomy specimens in assessing the molecular classification of endometrial carcinoma. Additionally, we compared the level of concordance of histotype and tumor grade between both specimens and finally, we compared the molecular profile of atypical complex hyperplasia with that of endometrial carcinoma detected on subsequent hysterectomy specimens.

Our results confirm the previously reported lack of reproducibility of histopathological assessment between biopsy and hysterectomy specimens. We have also confirmed the distribution of different histotypes across the four molecular subtypes of endometrial carcinoma; most p53-wild type being FIGO grade 1 or 2 endometrioid carcinomas, most p53-abnormal tumors being serous carcinomas, *POLE* mutated being high-grade endometrioid carcinomas and MMR-lost tumors being an admixture of grades and histotypes, but consisting predominantly of endometrioid type. Conversely, a high level of concordance was observed for MMR-loss, MSI-high, p53-wild and abnormal types. Similarly, previous studies have shown [8] that MMR assessment could be reliably performed on endometrial biopsy specimens as they are promptly fixed, with better antigen preservation than the corresponding hysterectomy specimen. This was further supported by a recent study [15] that showed that IHC for MMR protein deficiency in endometrioid endometrial carcinoma yields equivalent results when performed on endometrial biopsy/curettage or hysterectomy specimens. Discordant cases will be encountered, as seen in our study, which may be related to intratumoral heterogeneity given the small portion of tumor obtained by biopsy/curettage.

Such intra-tumoral heterogeneity may interfere with correct risk prediction. However, recent studies [16] on intra-tumoral heterogeneity pertaining to the prognostic molecular markers in endometrial carcinoma showed low intra-tumoral heterogeneity which facilitated the implementation of the molecular risk assignment, advocating its use in clinical decision making.

Importantly, incorrect assignment appears to be very unlikely in the subgroup with the worst outcomes (p53-abnormal), suggesting that we are unlikely to miss patients who may require more comprehensive surgery and/or additional chemotherapy and/or radiotherapy. An interesting result was particularly observed in discordant case 2, where the initial diagnostic specimen showed histologic features of low-grade endometrioid adenocarcinoma, however the final hysterectomy diagnosis was consistent with high grade serous carcinoma. P53 immunostain on this case revealed aberrant p53 expression on both biopsy and resection specimens. Another striking finding was that the two mixed carcinomas included in our study showed aberrant p53 expression in both serous and endometrioid components suggesting that these tumor foci may be clonally related. These findings are in parallel with those published by Espinosa et al. [17] who found that the highest number of mixed carcinoma diagnoses were p53-abnormal, suggesting that mixed carcinomas are high grade endometrial carcinomas with morphologic heterogeneity.

POLE gene mutations (p. S297F in exon 9 and p. V411M in exon 13 of the *POLE* gene) were detected in two patients, respectively. These were only detected on hysterectomy specimens. Discordant results may be related to low tumor cellularity/inadequate DNA yield. However, a study [8] has previously shown a high level of concordance level of *POLE* mutation detection between biopsy and hysterectomy specimens.

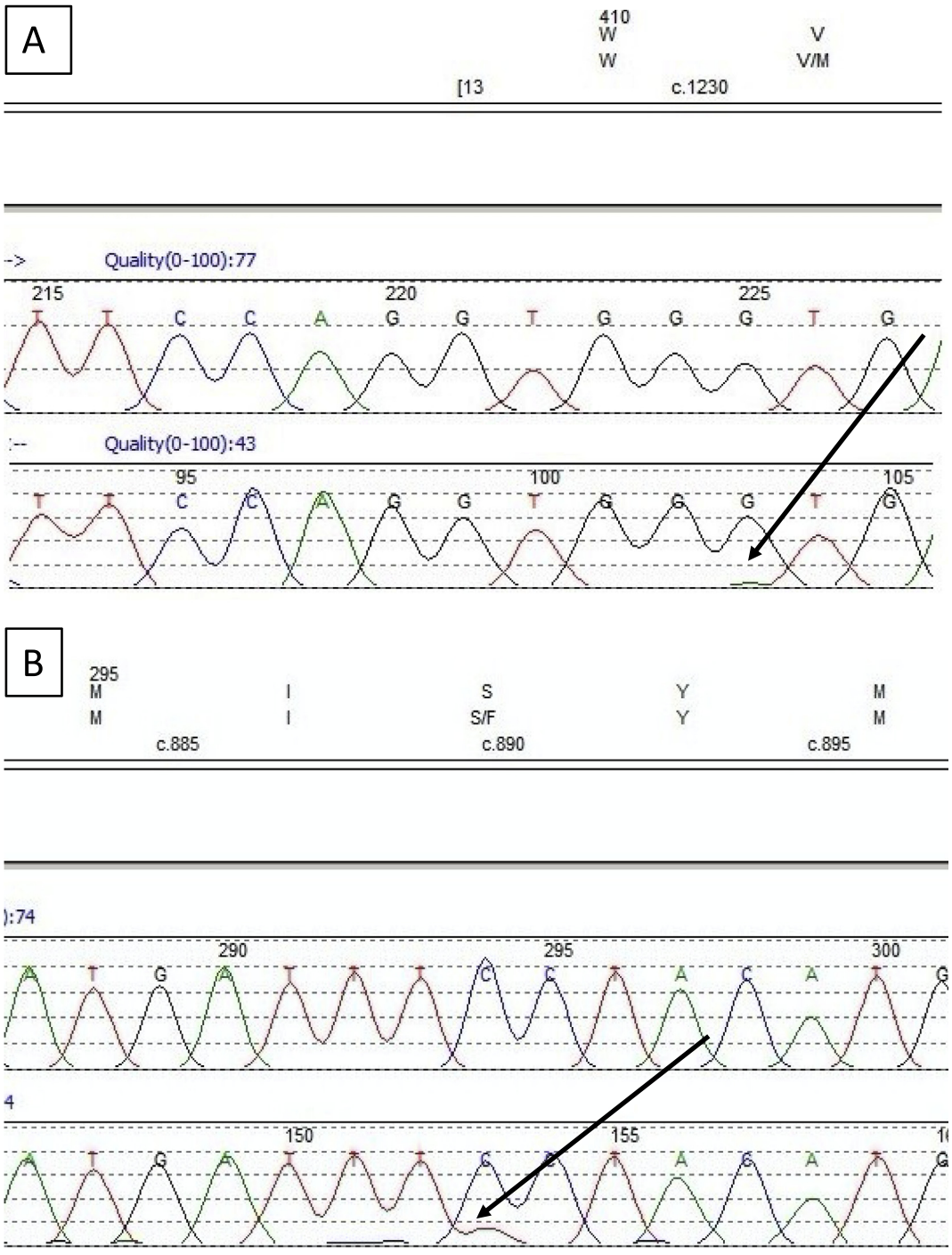

Fig. 1. Capillary electrogram demonstrating point mutations: A. p.V411 M in exon 13 and B. p. S297F in exon 9 of the *POLE* gene.

Table 4
Levels of concordance between biopsy and resection specimens for each molecular subgroup.

	Biopsy	Resection	Kappa (95% CI)
MMR loss			
MLH-1	1	1	1.0 (0.0–7.74)
PMS-2	16	14	0.91 (0.61–7.08)
MSH-2	2	2	1.0 (0.0–7.74)
MSH-6	16	18	0.83 (0.79–6.49)
MSI high	15	15	0.91 (0.63–6.86)
p53 wild type	51	51	1.0 (0.0–7.74)
p53 abnormal	9	9	1.0 (0.0–7.74)
POLE mutation	0	2	0

Table 5
Levels of concordance between biopsy and resection specimen for histotype and FIGO grade.

	Biopsy	Resection	Kappa (95% CI)
Histologic subtype			
Endometrioid	39	51	0.51 (0.10–6.70)
Serous	9	6	
Clear cell	1	1	
Mixed	1	2	
FIGO grade			0.52 (0.97–5.34)
1	18	19	
2	19	22	
3	2	10	

Table 6
Examples of cases showing discordant histology and/or FIGO grade between biopsy and resection specimens.

Discordant results	Biopsy histology and grade	Resection histology and grade	MMR loss biopsy	MMR loss resection	MSI High biopsy	MSI High resection	p53 wild type biopsy	p53 wild type resection	p53 mut biopsy	p53 mut resection
1	ACH	EC, G1	Intact	Intact	NO	NO	YES	YES	NO	NO
2	EC, G1	SC	Intact	Intact	NO	NO	NO	NO	YES	YES
3	ACH	EC, G1	Intact	Intact	NO	NO	YES	YES	NO	NO
4	EC, G1	EC, G2	Intact	Intact	NO	NO	YES	YES	NO	NO
5	ACH	EC, G1	Intact	Intact	NO	NO	YES	YES	NO	NO
6	EC, G1	EC, G2	Loss	Loss	YES	YES	YES	YES	NO	NO
7	ACH	EC, G1	Intact	Intact	NO	NO	YES	YES	NO	NO
8	EC, G2	EC, G1	Intact	Intact	NO	NO	YES	YES	NO	NO
9	EC, G1	EC, G3	Intact	Intact	NO	NO	YES	YES	NO	NO
10	ACH	EC, G2	Loss	Loss	YES	YES	YES	YES	NO	NO
11	SC	EC, G2	Loss	Loss	YES	YES	YES	YES	NO	NO

ACH = atypical complex hyperplasia, EC = endometrioid carcinoma, SC = serous carcinoma, G1 = grade, G2 = grade 2, G3 = grade 3.

Both patients with *POLE* mutations identified in our study were diagnosed with early stage FIGO grade 3 endometrioid endometrial carcinoma, which is in line with what have been previously published in the literature regarding the favorable prognosis of *POLE* mutated endometrial cancers despite their association with higher grade tumors [18–21].

Although we note the limitations of our study, namely a retrospective review and a limited sample size, yet our molecular analysis was performed on whole sections which ensures a high quality of DNA extraction and more reliable results. Future studies including rare tumor types such as dedifferentiated carcinomas are required to assess the applicability of the molecular classification to these tumor subtypes.

5. Conclusion

The molecular classification of endometrial carcinomas applied to diagnostic biopsy specimens through practical clinically applicable assays points towards a paradigm shift in endometrial carcinoma pathology practice in the near future. The integrated clinicopathologic and molecular classification approach will provide endometrial carcinoma

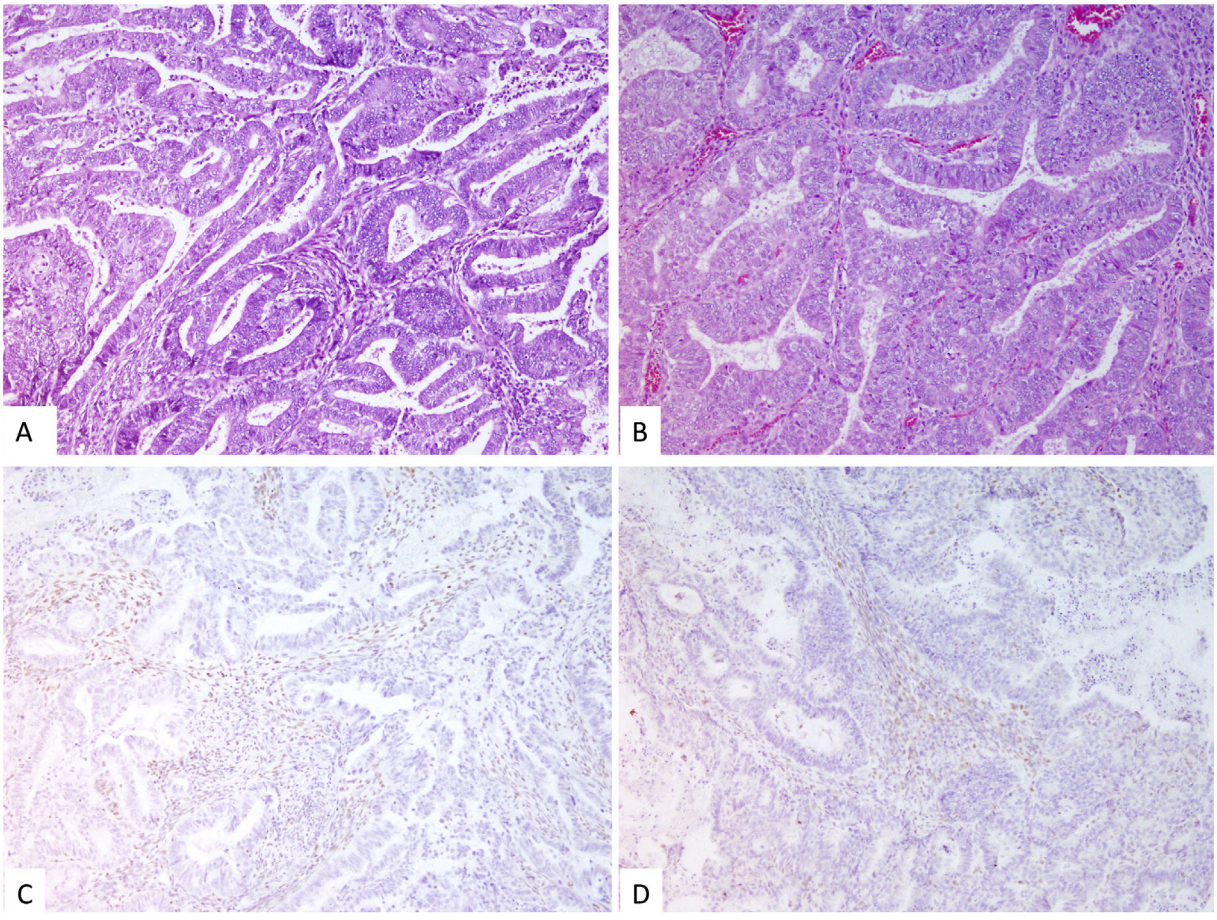

Fig. 2. A. and B. H&E images of endometrioid adenocarcinoma diagnosed on biopsy and hysterectomy specimens, respectively (200×). C. and D. IHC demonstrating loss of MLH1 on both biopsy and hysterectomy specimens, respectively (200×).

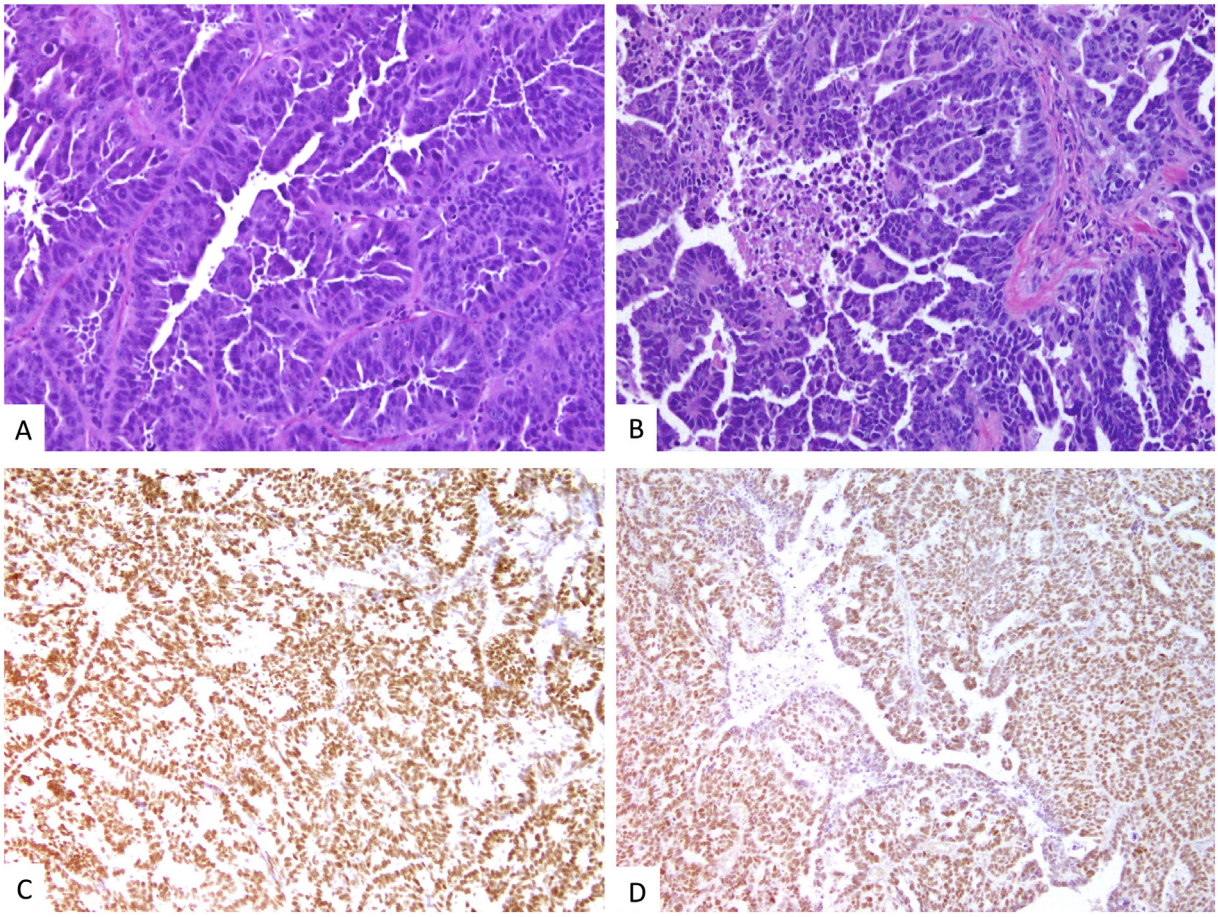

Fig. 3. A. and B. H&E images of serous carcinoma diagnosed on biopsy and hysterectomy specimens, respectively (200×). C. and D. IHC demonstrating aberrant p53 expression on both biopsy and hysterectomy specimens, respectively (200×).

patients and their clinical team with a reliable and objective classification that carries information to guide surgery, adjuvant treatments and subsequent cancer surveillance in a timely manner.

Author contribution

Eman Abdulfatah: analyzed the data and wrote the manuscript
Erin wakeling: performed the molecular testing
Sharif Sakr: collected the clinical and follow up information
Khaleel Al-Obaidy: collected the data and selected the appropriate tumor block for testing
Sudeshna Bandyopadhyay: revised the manuscript
Robert Morris: Provided clinical and follow up data
Gerald Feldman: analyzed the molecular results
Rouba Ali-Fehmi: mentor, supervised and revised the manuscript

Declaration of Competing Interest

All the contributing authors have nothing to disclose.

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