Ref: Ro Med J. 2021;68(Suppl6)
DOI: 10.37897/RMJ.2021.S6.10

Latest updates in the histopathological and molecular approach of endometrial carcinoma

Antonia Carmen Lisievici[1], Tiberiu-Augustin Georgescu[1,2], Nicolae Bacalbasa[3], Irina Balescu[4], Corina Grigoriu[3,5], Consuela-Madalina Gheorghe[6*], Ioana Teodora Vladareanu[7], Roxana Elena Bohiltea[3,8]

[1]Department of Pathology, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania
[2]Department of Pathology, "Alessandrescu-Rusescu" National Institute for Mother and Child Health, Bucharest, Romania
[3]Department of Obstetrics and Gynecology, "Carol Davila" University of Medicine and Pharmacy Bucharest, Romania
[4]Department of Visceral Surgery, Ponderas Academic Hospital, Bucharest, Romania
[5]Department of Obstetrics and Gynecology, University Emergency Hospital Bucharest, Bucharest, Romania
[6]Department of Marketing and Medical Technology,
"Carol Davila" University of Medicine and Pharmacy Bucharest, Romania
[7]Faculty of Medicine, "Carol Davila" University of Medicine and Pharmacy Bucharest, Romania
[8]Department of Obstetrics and Gynecology, Filantropia Clinical Hospital, Bucharest, Romania

ABSTRACT

Endometrial carcinomas are one of the most frequent carcinomas of the female genital tract. This article aims to highlight the particularities of both histopathological, immunohistochemical and molecular aspects, according to the new World Health Organization (WHO) Classification of endometrial tumors. These tumors can be divided into 4 groups, depending on the mutation that is present, which impacts the prognosis and consequently also the treatment of these patients. Tumors with the best prognosis are part of the low-risk category and are those harboring the POLE mutation, while on the opposite spectrum, TP53 mutated tumors are known to have an aggressive evolution. A thorough literature search has been performed in order to extract the up-to-date information that has been furtherly synthesized. The articles obtained have been reviewed, correlating the histological and molecular features with treatment and prognosis. Although no known reliable immunohistochemical stain can identify the presence of the POLE mutation, molecular analysis is necessary in order to further classify these tumors and to identify the cases that need to be put in the high-risk or high-intermediate risk categories. Considering the good prognosis of carcinomas with POLE mutations, the identification of these cases is recommended due to the possible descaling of treatment.

Keywords: endometrial carcinoma, molecular classification, POLE ultramutated

INTRODUCTION

During the last decade, extraordinary progresses have been made in the field of endometrial carcinoma, especially regarding the molecular classification. In comparison to the previous edition, the current World Health Organization classification of tumors of the female genital tract has introduced a new diagnostic algorithm by which a specific molecular type can be attributed to each carcinoma using an integrated histomolecular classification [1]. According to this algorithm, endometrial carcinomas can be divided into: Polymerase Epsilon (POLE) ultramutated, MMR deficient, p53 mutated, or without any specific molecular alterations [1-3]. This classification cannot be predicted by histopathological means, but it has great impact on the prognosis and overall survival rate, thus being relevant in the current practice for both pathologists and clinicians.

Corresponding author:
Consuela-Mădălina Gheorghe
E-mail: consuela.gheorghe@umfcd.ro

Article History:
Received: 27 November 2021
Accepted: 22 December 2021

HISTOLOGIC APPROACH OF ENDOMETRIAL CARCINOMA

Endometrial carcinoma has several tumoral subtypes, each with multiple histologic variants. For example, endometrioid carcinoma can have squamous differentiation, mucinous differentiation, tubal differentiation, secretory change, villoglandular pattern, small nonvillous papillae, sertoliform pattern or dedifferentiated areas. Other rare variants include: spindle cells, corded, hyalinized stroma, signet ring cells, trophoblastic differentiation, hepatoid differentiation or giant cells. Although extremely heterogeneous microscopically, the most important aspect in clinical practice is the distinction between endometrioid and non-endometrioid endometrial carcinomas.

Bokhman et al. have observed two distinct pathogenic types of endometrial carcinoma, which, to date, are still used in some pathology departments, especially in those where immunohistochemical tests or molecular investigations are not available [4]. Type I EC occurred in women with comorbidities (obesity, hyperlipidemia), or who have risk factors for hyperestrogenism. Upon histologic examination, these tumors are low-grade and have an endometrioid carcinoma histology [2]. Type II EC represents a broader category that incorporates non-endometrioid carcinomas, especially serous carcinoma, and clear cell carcinoma. These tumors have a more aggressive evolution and usually occur in women in the 6th decade of life [2,5].

A more practical approach of endometrial carcinoma, especially on curettage biopsies, is represented by the evaluation of the predominating pattern of the neoplastic proliferation. Carcinomas with a glandular pattern of growth are most often of endometrioid type, a diagnosis that can be supported by the low-grade morphology of the cells. The most important differential diagnosis is represented by a serous carcinoma with a pseudo-glandular pattern of growth. The most reliable histological feature that can help distinguish between the two entities is the high-grade nuclei that are normally encountered in the latter [6]. Additionally, the expression of a mutational p53 pattern of staining can support the diagnosis. The expression in serous carcinoma can be either: block diffuse, diffuse cytoplasmic or loss of expression [7].

A papillary pattern of growth is usually seen in both clear cell carcinoma and serous carcinoma. However, the character of the papillae is different between the two entities: serous carcinomas have larger papillae lined by multiple layers of cells, while clear cell carcinomas have smaller papillae with a hyalinized core [8]. A clear cell-rich pattern can be seen either in clear cell carcinoma, which is characterized by cells with a hobnail morphology, but also in endometrioid carcinoma with secretory changes, or with pale/ clear squamous differentiation [9,10]. Lastly, a squamous pattern can be observed in endometrioid carcinomas, endometrial hyperplasia but also in atypical polypoid adenomyoma. The latter entity can be differentiated by the SATB2 immunohistochemical expression of the squamous morules [11].

IMMUNOHISTOCHEMICAL APPROACH OF ENDOMETRIAL CARCINOMA

Immunohistochemical analysis can be used in two different scenarios: one in which there is a necessity to differentiate between an endometrial carcinoma and an endocervical carcinoma, while the other situation requires uncertainty regarding the histological subtype (e.g., high grade endometrial carcinoma versus serous carcinoma). The first scenario can be easily solved with the following classic panel: Vimentin, ER, monoclonal CEA, p16, the former two being positive in endometrial carcinoma, while the later favors an endocervical adenocarcinoma [12].

In order to differentiate between the subtypes of endometrial carcinoma, one should make use of both histological and immunohistochemical features. Endometrioid carcinoma shows diffuse reactivity for ER and PR, with only focal expression of p53 (wild type pattern of expression). The latter, together with the loss of PTEN, can help in the differential diagnosis with serous carcinoma, which shows a mutational pattern of staining for p53 (cytoplasmatic, strong diffuse or complete absence) and retained PTEN [12,13]. Serous carcinoma also features diffuse expression of p16, IMP3 and only focal, weak expression of ER and PR [1,12]. Clear cell carcinoma can show a similar immunohistochemical expression profile as serous carcinoma, because both can show diffuse or complete absence of p53 and loss of ER. However, HNF1beta and Napsin A are more commonly expressed in clear cell carcinoma [8].

MOLECULAR APPROACH OF ENDOMETRIAL CARCINOMA

According to the Cancer Genoma Atlas, endometrial carcinomas can be divided into four large groups. Group 1 is associated with a good prognosis and is characterized by mutations of the POLE gene [14]. Epidemiologically, studies have showed a lower incidence of this mutation in Chinese patients [15]. From a morphological point of view, these tumors are frequently grade 3 endometrioid carcinoma, with an ambiguous morphology and large front of invasion. Intratumoral or peritumoral lymphocytes, as well as giant cells, can also be observed [16]. This mutation is encountered in approximate-

ly 10% of all endometrioid tumors and in 16% of all clear cell carcinomas [17,18]. To date, there are no reliable immunohistochemical stains that can be used in order to predict the presence of the POLE mutation [19]. This group is also called "ultramutated" because the number of alterations is greater than 100 mutations/ mb [16,19]. If the tumors are classified as stage I or II, then the patient can be placed in the low-risk category [16]. However, POLE mutations have also been identified in patients who developed synchronous ovarian and endometrial carcinoma [20]. Leon-Castillo et al. have also identified cases with POLE exonuclease domain mutation and MMRd, which can be separated into two categories. Those cases with a pathogenic POLE exonuclease domain mutation (EDM) and MMRd can be considered as POLE ultramutated due to a 5-year disease free survival rate of 92.3. On the other hand, cases with non-pathogenic POLE mutation have a 5-year recurrence free survival rate of 76,2%, thus being more similar in their behavior to MMRd endometrial carcinoma [21]. Alexa et al. have observed that the patients with POLE mutation have received the second most aggressive adjuvant treatment after cases harboring TP53 mutations. This is most probably because POLE mutated carcinomas have a high grade endometrioid morphology [16].

Group 2 tumors present microsatellite instability, which can be easily identified by immunohistochemical staining for the following markers: MLH1, MSH2, PMS2 and MSH6 [22,23]. Raffone et al. have observed that even a panel made up of PMS2 and MSH6 can show similar results to using all four markers, but considerably reducing the costs of the laboratory [24]. From a morphological point of view, these tumors are frequently grade 3 endometrioid carcinoma, have multiple foci of lymphovascular invasion, low-uterine segment involvement and MELF-type invasion [16]. This mutation can be identified in about 40% of all endometrioid carcinomas [17,25]. Kaur et al. have reported the expression of INI1 and aberrant expression of the MMR proteins in a case of dedifferentiated endometrial carcinoma [26]. This group has a hypermutated state, meaning that there have between 10 and 100 mutations/ mb [16]. The hypermethylation of MLH1 has been known as a key factor in the tumorigenesis of endometrial carcinoma since 1999 [27,28]. The prognosis in the group 2 tumors is intermediate between group 1 and group 4 [2]. Stage IA low grade endometrioid carcinomas with no or only focal lymphovascular invasion can be placed in the low-risk category, while patients with low-grade endometrioid carcinoma - stage IB and high-grade endometrioid carcinoma stage IA need to be placed in the intermediate risk grade [16,29]. Cases of endometrioid carcinoma with diffuse lymphovascular invasion and microsatellite instability are automatically considered high-intermediate risk. High-grade stage IB and stage II endometrioid carcinomas are also placed in this risk category [16]. However, stage III or IV MMR deficient endometrioid carcinomas represent high-risk tumors [10,16]. However, Makker et al. have observed that a treatment with Lenvatinib and pembrolizumab in patients with advanced endometrial carcinoma can show promising results [30].

Group 3 has the lowest mutation burden, a moderate prognosis, and is not characterized by any of the mutations encountered in the other three groups (POLE, P53 or MSI-H) [2,19]. Clinically, these patients tend to have a higher BMI than the other tumor groups [16]. These tumors have less than 10 mutations/ mb [16]. They usually express ER and PR and from a histopathological point of view they represent an endometrioid-type carcinoma [2,16]. Although there are no specifical immunohistochemical stains that can be used to identify these cases, if none of the other available markers reveals a specific type of mutation, one can assume at least that these cases will probably have a better prognosis [19]. Most common mutations identified in this category are the following: PTEN, PIK3CA, PIK3R1, CTNNB1 and ARID1A [16].

Group 4 tumors are characterized by the presence of p53 mutations and have a high copy-number alterations, although the mutational frequency is less than 10 mutations/ mb [2,16]. P53 mutations are encountered in up to 92% of all group 4 cases, and, from a histological point of view, they have a serous or clear cell carcinoma (grade 3) morphology, feature numerous tumoral emboli, with a destructive pattern of invasion [16,31]. Cytologically, there is high cytonuclear atypia, with large, irregular nuclei, relatively frequent giant cells, and occasional hobnailing of nuclei [16,18,32]. Travaglino et al. have observed that the TP53 mutation represents the most frequent mutation also in carcinosarcomas [33]. In order to identify this category, one can use the p53 immunohistochemical stain, which can function as a reliable surrogate marker for the presence of this mutation. Both overexpression and missense mutations are associated with an extremely poor prognosis [16,17,34]. Cases with p53 mutations of any stage, that do not show any residual disease, are automatically placed in the high-risk group, while those with residual disease are placed in the advanced risk group [16,35]. It is important to note that studies have revealed that grade 3 endometrial carcinomas, which harbor both PT53 and MMRd alterations, behave like other MMRd tumors [36]. However, the survival between TP53 mutated grade 3 endometrial carcinomas and serous carcinoma with the same mutation have a similar survival, jus-

tifying a similar therapy for both entities [36]. Akiyama et al. have also observed that cases with p53 overexpression, from patients aged 70 years or more, with advanced stage of disease or featuring a non-endometrioid subtype, are associated with a worse prognosis and a poor overall survival [37]. Additionally, these tumors appear not to respond as well to radiotherapy as other endometrial carcinomas that do not share this mutation [37].

CONCLUSIONS

Endometrial carcinoma encompasses a heterogenous group of tumors that can harbor different and even multiple mutations, including TP53, POLE and MSI-H. The morphological aspects do not give away the mutation associated, although a histopathologic aspect of serous carcinoma hints towards the presence of p53 mutation. Reliable immunohistochemical stains are available only for group 2 (MMRd carcinomas) and for group 4 (TP53 mutated carcinomas). However, the favorable outcome and better prognosis of cases with POLE EDM mutation require the identification of these cases either through immunohistochemical stains or through molecular analysis. Given the differences in the prognosis, placing the patients into one of the four categories should be a priority in their treatment and follow up. The favorable prognosis can impact the treatment and warrant its descaling for patients with POLE mutation, while patients with TP53 protein mutation need to be considered as high-grade and require a more aggressive treatment.

Conflict of interest: none declared
Financial support: none declared

REFERENCES

1. Cree IA, Cheung AN-Y, Lax SF, Oliva E. WHO Classification of Tumours-Female Genital Tumours. Vol. 4, 5th ed. Lyon (France): International Agency for Research on Cancer, 2020.
2. Cree IA, White VA, Indave BI, Lokuhetty D. Revising the WHO classification: female genital tract tumours. *Histopathology.* 2020 Jan;76(1):151-156.
3. Santoro A, Angelico G, Travaglino A, Inzani F, Arciuolo D, et al. New Pathological and Clinical Insights in Endometrial Cancer in View of the Updated ESGO/ESTRO/ESP Guidelines. *Cancers* (Basel). 2021 May 26;13(11):2623.
4. Bokhman JV. Two pathogenetic types of endometrial carcinoma. *Gynecol Oncol.* 1983 Feb;15(1):10-7.
5. Suarez AA, Felix AS, Cohn DE. Bokhman Redux: Endometrial cancer "types" in the 21st century. *Gynecol Oncol.* 2017 Feb;144(2):243-249.
6. Gatius S, Matias-Guiu X. Practical issues in the diagnosis of serous carcinoma of the endometrium. *Mod Pathol.* 2016 Jan;29 Suppl 1:S45-58.
7. Köbel M, Ronnett BM, Singh N, Soslow RA, Gilks CB, McCluggage WG. Interpretation of P53 Immunohistochemistry in Endometrial Carcinomas: Toward Increased Reproducibility. *Int J Gynecol Pathol.* 2019 Jan;38 Suppl 1(Iss 1 Suppl 1):S123-S131.
8. Vang R, Yemelyanova A, Seidman J, Epstein J. Differential Diagnoses in Surgical Pathology: Gynecologic Tract. Philadelphia: Lippincott Williams & Wilkins. 2017.
9. Nucci MR, Oliva E. Diagnostic Pathology: Gynecological. 2nd edition. Salt Lake City UT: Elsevier, 2018.
10. Kurman RJ, Hedrick Ellenson L, Ronnett BM. Blaustein's Pathology of the Female Genital Tract. Seventh ed. Switzerland AG: Springer, 2019.
11. McCluggage WG, Van de Vijver K. SATB2 is Consistently Expressed in Squamous Morules Associated With Endometrioid Proliferative Lesions and in the Stroma of Atypical Polypoid Adenomyoma. *Int J Gynecol Pathol.* 2019 Sep;38(5):397-403.
12. Djordjevic B, Westin S, Broaddus RR. Application of Immunohistochemistry and Molecular Diagnostics to Clinically Relevant Problems in Endometrial Cancer Bojana Djordjevic, Shannon Westin, Russell R. Broaddus. *Surg Pathol Clin*. 2012 Dec 1;5(4):859-878.
13. Köbel M, Ronnett BM, Singh N, Soslow RA, Gilks CB, McCluggage WG. Interpretation of P53 Immunohistochemistry in Endometrial Carcinomas: Toward Increased Reproducibility. *Int J Gynecol Pathol.* 2019 Jan;38 Suppl 1(Iss 1 Suppl 1):S123-S131.
14. Travaglino A, Raffone A, Mascolo M, Guida M, Insabato L, Zannoni GF, Zullo F. TCGA Molecular Subgroups in Endometrial Undifferentiated/Dedifferentiated Carcinoma. *Pathol Oncol Res.* 2020 Jul;26(3):1411-1416.
15. Li Y, He Q, Li S, Wen X, Ye L, Wang K, Wan X. *POLE* Mutation Characteristics in a Chinese Cohort with Endometrial Carcinoma. *Onco Targets Ther.* 2020 Jul 28;13:7305-7316.
16. Alexa M, Hasenburg A, Battista MJ. The TCGA Molecular Classification of Endometrial Cancer and Its Possible Impact on Adjuvant Treatment Decisions. *Cancers* (Basel). 2021 Mar 23;13(6):1478.
17. Cancer Genome Atlas Research Network, Kandoth C, Schultz N, et al. Integrated genomic characterization of endometrial carcinoma. *Nature.* 2013 May 2;497(7447):67-73.
18. Baniak N, Fadare O, Köbel M, DeCoteau J, Parkash V, et al. Targeted Molecular and Immunohistochemical Analyses of Endometrial Clear Cell Carcinoma Show that POLE Mutations and DNA Mismatch Repair Protein Deficiencies Are Uncommon. *Am J Surg Pathol.* 2019 Apr;43(4):531-537.
19. Soslow RA, Tornos C, Park KJ, Malpica A, Matias-Guiu X, Oliva E, et al. Endometrial Carcinoma Diagnosis: Use of FIGO Grading and Genomic Subcategories in Clinical Practice: Recommendations of the International Society of Gynecological Pathologists. *Int J Gynecol Pathol.* 2019 Jan;38 Suppl 1(Iss 1 Suppl 1):S64-S74.
20. Ishikawa M, Nakayama K, Nakamura K, Ono R, Yamashita H, et al. High frequency of POLE mutations in synchronous endometrial and ovarian carcinoma. *Hum Pathol.* 2019 Mar;85:92-100.
21. León-Castillo A, Britton H, McConechy MK, McAlpine JN, Nout R, Kommoss S, Brucker SY, Carlson JW, Epstein E, Rau TT, Bosse T, Church DN, Gilks CB. Interpretation of somatic POLE mutations in endometrial carcinoma. *J Pathol.* 2020 Mar;250(3):323-335.
22. Kurnit KC, Westin SN, Coleman RL. Microsatellite instability in endometrial cancer: New purpose for an old test. *Cancer.* 2019 Jul 1;125(13):2154-2163.
23. Sakr S, Abdulfatah E, Munkarah AR, Morris RT, Elshaikh MA, Pardeshi V, Ali-Fehmi R. Prognostic value of loss of MMR protein expression in endometrial carcinoma in African American and White women. *Gynecologic Oncology.* 2016; 141(1):112.
24. Raffone A, Travaglino A, Cerbone M, Gencarelli A, Mollo A, Insabato L, Zullo F. Diagnostic Accuracy of Immunohistochemistry for Mismatch Repair Proteins as Surrogate of Microsatellite Instability Molecular Testing in Endometrial Cancer. *Pathol Oncol Res.* 2020 Jul;26(3):1417-1427.
25. Johnson C, Bradley WH, Mackinnon C. Assessment of the clinical significance of the variable microsatellite instability phenotype observed in MMR-defective endometrial carcinoma. *Gynecologic Oncology.* 2020; 159.
26. Kaur R, Mehta J, Borges AM. Role of SMARCA4 (BRG1) and SMARCB1 (INI1) in Dedifferentiated Endometrial Carcinoma With Paradoxical

Aberrant Expression of MMR in the Well-Differentiated Component: A Case Report and Review of the Literature. *Int J Surg Pathol.* 2021 Aug;29(5):571-577.
27. Fleisher AS, Esteller M, Wang S, Tamura G, Suzuki H, Yin J, Zou TT, et al. Hypermethylation of the hMLH1 gene promoter in human gastric cancers with microsatellite instability. *Cancer Res.* 1999 Mar 1;59(5):1090-5.
28. Esteller M, Catasus L, Matias-Guiu X, Mutter GL, Prat J, Baylin SB, Herman JG. hMLH1 promoter hypermethylation is an early event in human endometrial tumorigenesis. *Am J Pathol.* 1999 Nov;155(5):1767-72.
29. Timmerman S, Van Rompuy AS, Van Gorp T, Vanden Bempt I, et al. Analysis of 108 patients with endometrial carcinoma using the PROMISE classification and additional genetic analyses for MMR-D. *Gynecol Oncol.* 2020 Apr;157(1):245-251.
30. 30Makker V, Taylor MH, Aghajanian C, Oaknin A, Mier J, Cohn AL, et al. Lenvatinib Plus Pembrolizumab in Patients With Advanced Endometrial Cancer. *J Clin Oncol.* 2020 Sep 10;38(26):2981-2992.
31. Jones NL, Wu S, Xiu J, Craig A, Mantia-Smaldone G, Hernandez E, et al. Immune-response markers and actual response to immune-oncology therapy in uterine serous carcinoma. *Journal of Clinical Oncology.* 2021; 39(15_suppl).
32. Kosmas K, Stamoulas M, Marouga A, Kavantzas N, Patsouris E, Athanassiadou P. Expression of p53 in imprint smears of endometrial carcinoma. *Diagn Cytopathol.* 2014 May;42(5):416-22.
33. Travaglino A, Raffone A, Gencarelli A, Mollo A, Guida M, Insabato L, Santoro A, Zannoni GF, Zullo F. TCGA Classification of Endometrial Cancer: the Place of Carcinosarcoma. *Pathol Oncol Res.* 2020 Oct;26(4):2067-2073.
34. Saegusa M, Hashimura M, Suzuki E, Yoshida T, Kuwata T. Transcriptional up-regulation of Sox9 by NF-κB in endometrial carcinoma cells, modulating cell proliferation through alteration in the p14(ARF)/p53/p21(WAF1) pathway. *Am J Pathol.* 2012 Aug;181(2):684-92.
35. Co NN, Iglesias D, Celestino J, Kwan SY, Mok SC, Schmandt R, Lu KH. Loss of LKB1 in high-grade endometrial carcinoma: LKB1 is a novel transcriptional target of p53. *Cancer.* 2014 Nov 15;120(22):3457-68.
36. Brett MA, Atenafu EG, Singh N, Ghatage P, Clarke BA, Nelson GS, Bernardini MQ, Köbel M. Equivalent Survival of p53 Mutated Endometrial Endometrioid Carcinoma Grade 3 and Endometrial Serous Carcinoma. *Int J Gynecol Pathol.* 2021 Mar 1;40(2):116-123.
37. Akiyama A, Minaguchi T, Fujieda K, Hosokawa Y, Nishida K, et al. Abnormal accumulation of p53 predicts radioresistance leading to poor survival in patients with endometrial carcinoma. *Oncol Lett.* 2019 Dec;18(6):5952-5958.