Diagnostic Importance of PAX2, ARID1A, and FOXA1 Biomarkers in Atypical Endometrial Hyperplasia

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ABSTRACT

Objective: To evaluate the contribution of PAX2, ARID1A, and FOXA1 biomarkers to diagnosis in cases with atypical endometrial hyperplasia (AEH).

Study Design: Descriptive Study.

Place and Duration of the Study: Pathology Department of Umraniye Training and Research Hospital, from January 2018 to December 2020.

Methodology: Curettage materials of 100 patients diagnosed with AEH which stained PAX2, ARID1A, and FOXA1, were evaluated. The staining patterns in the atypical endometrial glandular areas were grouped as slight-no loss, moderate loss, and complete loss / severe loss for all three biomarkers. Complete or/severe loss in AEH was considered helpful in the diagnosis.

Results: Complete loss / severe loss rates in curettages were 84% for PAX2, 5% for ARID1A, and 15% for FOXA1, respectively. When used in combination, complete loss / severe loss rates were 85% in at least one of the three markers, 84% in PAX2 and/or ARID1A, 85% in PAX2 and/or FOXA1, and 17% in ARID1A and/or FOXA1.

Conclusion: Although all 3 biomarkers showed marked staining loss, PAX2 is the most sensitive biomarker for the diagnosis of AEH in curettage materials.

Key Words: *Endometrium, Atypical hyperplasia, PAX2, ARID1A, FOXA1.*

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INTRODUCTION

Endometrial carcinoma is the most common malign tumour of the female genital tract, accounting for 4% of all.[1] The term hyperplasia is used for precursor lesions in endometrial cancer. Accordingly, endometrial hyperplasias are divided into hyperplasia without atypia and hyperplasia with atypia/EIN (endometrial intraepithelial neoplasia).[2] Since 2017, with the recommendation of ESGO, PAX2, and ARID1A biomarkers have been used in the pathological diagnosis of atypical endometrial hyperplasia.[3] In addition, the loss of PTEN, PAX2, and MMR immunoreactivity is an auxiliary diagnostic tool in the 2020 WHO classification of female genital system tumours.[4] PAX2 is the member of the paired box gene family, that encodes transcription factors in embryonic development and organogenesis. It is also a tumour suppressor gene, mutates in endometrial carcinogenesis and shows loss of expression.[5,6]

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ARID1A is a nuclear protein involved in the SWI/SNF chromatin remodelling complex; provides cell differentiation, proliferation, and DNA damage repair; furthermore a tumour suppressor gene in endometrial carcinogenesis and shows loss of expression as a result of mutation.[7,8] FOXA1 is a member of the forkhead box transcription family, and regulates estrogenic activity in the gynaecological tract with the effect of ERα ligand-dependent transcription factor. It has a dual function in cancer development related to cancer type and subtype, that is, can act as a protooncogene or tumour suppressor.[9-11]

While evaluating AEHs microscopically, immunohistochemistry is used for definitive diagnosis in addition to histomorphologic and morphometric methods. The aim of this study was to evaluate 100 curettage cases diagnosed with AEH which had previously applied PAX2 immunostaining, to compare their expression at tissue level by applying ARID1A and FOXA1 immunostains.

METHODOLOGY

Between January 2018 and December 2020, curettage samples diagnosed as atypical endometrial hyperplasia and examined for PAX2 in the Pathology Department of Ümraniye Training and Research Hospital, were examined. One hundred and seventeen patient records were accessed through HIS, the hospital's electronic data system. H&E and PAX2 stained slides were collected from the archive and re-evaluated together with curet-

tage reports. Due to various technical problems (insufficient amount of tissue, inappropriate fixation, *etc.*), 17 cases were excluded from the study and a total of 100 cases were included. H&E slides (fixed in 10% formaldehyde, paraffinised with routine tissue application and standard H&E stained) were re-evaluated by a single pathologist under an Olympus CX31 brand light microscope, appropriate slide, and its paraffin block were selected. Care was taken to select samples with normal endometrium or benign changes next to the atypical area.

ARID1A (BAF250a) Epitomics brand, clone EP303, concentrated rabbit monoclonal antibody, epitope retrieval 2 (EDTA), at 1/150 dilution and FOXA1 (HNF3a) Cell Marque brand, clone 2F83, mouse monoclonal antibody, epitope retrieval 1 (Citrate Buffer), at 1/50 dilution were used. Nonneoplastic colon tissue for ARID1A and malign carcinamatous breast tissue for FOXA1 were accepted as positive control. Fifty positively charged slides coated with poly-l-lysine were provided as two separate tissues on one slide. Sections of 3 microns prepared from paraffin blocks were taken on slides. The preparations were first deparaffinised by keeping them in an oven at 58°C for 1 hour. Afterwards, they were placed in a biotin-free, HRP polymer-based fully automatic immunohistochemistry staining device (Leica BOND-MAX Fully automated IHC&ISH) and all procedures were performed automatically. PAX2 applied to samples during standard reporting, was Cell Marque brand, clone EP235, rabbit monoclonal antibody, at 1/50 dilution (Table I).

Table I: Details of antibodies.

Antibody	Brand	Clone	Concentration
PAX2	Cell Marque	EP235	1/50
ARID1A	Epitomics	EP303	1/150
FOXA1	Cell Marque	2F83	1/50

The prepared sections were evaluated by two pathologists with the Olympus BX51 light microscope at x4, x10, x20, and x40 magnifications so that all areas on the slide were scanned. Atypical areas on H&E sections were matched on immunohistochemistry stained sections, and the staining pattern of antibodies was examined. Nuclear staining in epithelial cells lining the endometrial glands was considered positive for ARID1A and FOXA1. Since the surrounding normal endometrium was accepted as internal control in each case, the staining patterns of atypical endometrial glands were compared with the surrounding normal endometrium. In atypical endometrial glands, the staining intensity was graded as; no staining/negative (score: 0), mild-strength staining (score: 1), moderate-strength staining (score: 2), and severe staining (score: 3). The staining percentage was graded as no staining/negative (score:0), 1-25% staining (score: 1), 26-75% staining (score:2), and 76-100% staining (score: 3). Then the scores obtained from the staining intensity and staining percentage were added to get a total score between 0-6 (without 1); 0-2 as complete loss/-severe loss; 3 as moderate loss; and 4-6 as slight loss/no loss were accepted. PAX2 staining pattern was graded by the same scoring system for ARID1A and FOXA1. Nuclear staining in epithelial cells lining the endometrial glands was considered positive while evaluating PAX2.

NCSS (Number Cruncher Statistical System) program was used for statistical analysis. Descriptive statistical methods (mean, standard deviation, median, frequency, percentage, minimum, maximum) were used while examining the study data. In the comparison of qualitative data, the one-eyed layout Chi-Square test was used. Statistical significance was accepted as $p<0.01$.

RESULTS

The patients, aged between 18 and 74 years, with an average of 49.39±10.87 years, were diagnosed with AEH in their curettages. Seventy-nine cases were operated, 16 were not operated, and 5 of them could not reach the information. Diagnoses of the operated patients were 22% endometrioid carcinoma, 47% AEH, and 31% other diagnoses (hyperplasia without atypia, proliferative pattern, secretory pattern, and atrophy) in late pathology reports.

Four (4%) of the PAX2 staining intensity was mild, 3% (n=3) was moderate,11% (n=11) was severe and 3% (n=3) of the PAX2 staining percentage was between 1-25%, 1% (n=1) was between 26-75%, and 14% (n=14) was above 75%. Also in 82% (n=82) of the cases, PAX2 was negative, 0%. PAX2 total staining scores; 84% showed complete loss/severe loss, 2% moderate loss, 14% slight loss/no loss (Figure 1 a-c and 4-a). A statistically significant difference was found between the PAX2 staining groups ($p=0.001$ and $p<0.01$, respectively). PAX2 was 84% sensitive test in AEH.

Eight (8%) of the ARID1A staining intensity was mild, 9% (n=9) was moderate, 82% (n=82) was severe and 6% (n=6) of the ARID1A staining percentage was between 1-25%, 7% (n=7) was between 26-75%, 86% (n=86) was above 75%. It was negative, 0% in only 1% (n=1) of the cases. ARID1A total staining scores; 5% showed complete loss/severe loss, 3% moderate loss, and 92% slight loss/no loss (Figures 2a-c and 4-a, respectively). A statistically significant difference was found between the ARID1A staining groups ($p=0.001$ and $p<0.01$). ARID1A was 5% sensitive test in showing complete loss/severe loss.

Nineteen (19%) of the FOXA1 staining intensity was mild, 17% (n=17) was moderate, 64% (n=64) was severe and 17% (n=17) of the FOXA1 staining percentage was between 1-25%, 15% (n=15) was between 26-75%, 68% (n=68) was above 75%. In none of the cases (n=0) FOXA1 was negative, 0%. FOXA1 total staining scores; 15% showed complete loss/severe loss, 6% moderate loss, 79% slight loss/no loss (Figures 3a-c and 4-a, respectively). In only one case, increased expression of FOXA1 was observed in the area of atypia compared to the surrounding normal endometrium, which was admitted as slight loss/no loss. A statistically significant difference was found between the FOXA1 staining groups ($p=0.001$ and $p<0.01$, respectively). FOXA1 was 15% sensitive test in AEH.

In combinations; at least one of the three markers was complete loss/severe loss in 85% of the cases, and all three

markers were complete loss/severe loss in only 3% of the cases. In dual combinations complete loss/severe loss rates were; PAX2 and/or ARID1A 84%, PAX2 and/or FOXA1 85%, ARID1A and/or FOXA1 17% (and/or means at least one marker shows complete loss/severe loss, Figure 4-b).

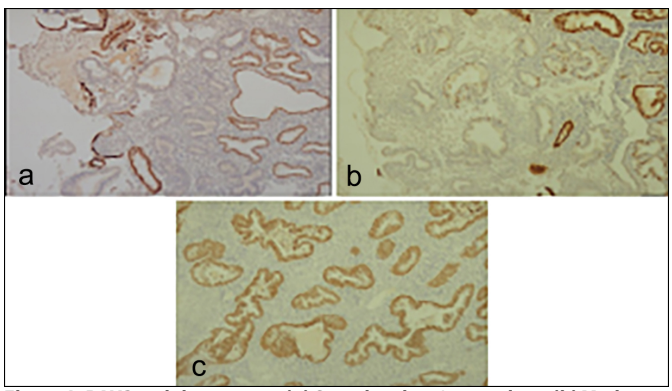

Figure 1: PAX2 staining groups. (a) Complete loss/severe loss. (b) Moderate loss. (c) Slight loss/no loss.

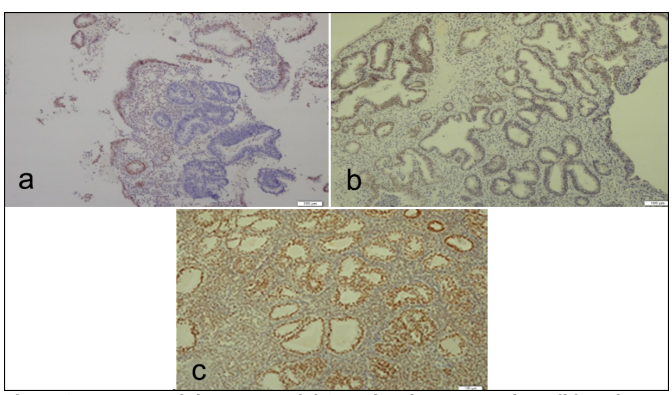

Figure 2: ARID1A staining groups. (a) Complete loss/severe loss. (b) Moderate loss. (c) Slight loss/no loss.

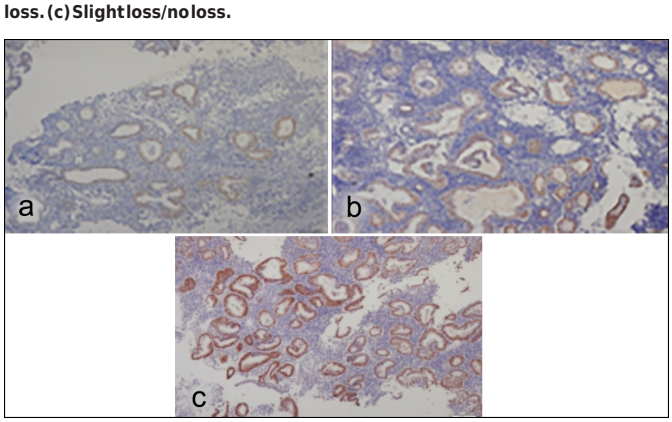

Figure 3: FOXA1 staining groups. (a) Complete loss/severe loss (b) Moderate loss. (c) Slight loss/no loss.

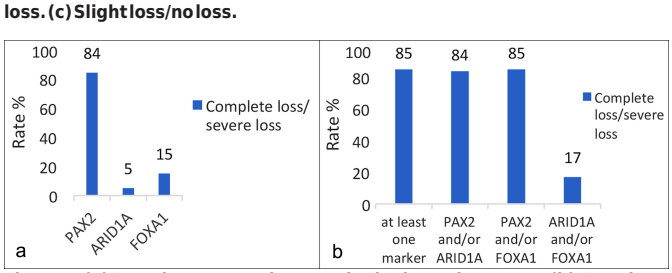

Figure 4: (a) Complete / severe loss rate in single marker usage. (b) Complete/ severe loss rate in combined marker usage.

DISCUSSION

AEHs have been described as precursors of endometrioid carcinomas; it may progress to or co-exist with them. Although clinical factors are considered in the treatment method, the preference is mostly surgery. Therefore, both the risk of carcinoma and the surgical treatment method make diagnosis critical. In general, histomorphological evaluation and immunohistochemical methods are used together.

PAX2 is a transcription factor identified in endometrial carcinogenesis and AEHs. In the study by Zhai *et al*., PAX2 was evaluated in normal, reactive, tumoural and metastatic tissues. It was shown that PAX2 is synthesised in mature endometrial glands and provides homeostasis.[12] Allison *et al*. evaluated normal endometrium, hyperplasia and endometrioid carcinoma according to PAX2 expression; 74% of atypical hyperplasias had complete loss of PAX2. It was emphasised that loss of PAX2 is an early finding in endometrial carcinogenesis and that it can only be used to differentiate hyperplasia with atypia from normal endometrium.[13] In the study by Quick *et al*., the contribution of PAX2 to the diagnosis of EIN was defined (helpful, unnecessary, confusing) and PAX2 loss was found helpful for diagnosis by 53%, especially in subdiagnostic cases.[14] Meng *et al*. assessed the staining pattern of PAX2 in benign hyperplasia, EIN, and endometrioid carcinomas and found that it showed staining loss in 40%, 73%, 79%, respectively. As a result, PAX2 could be used as a reliable marker in the diagnosis of EIN, since there was a statistically significant difference between the included groups.[15] Although different threshold values are used in the literature, loss of PAX2 expression occurs early in endometrial carcinogenesis and is a significant finding for the diagnosis of AEH.[6]

Mao *et al*. classified ARID1A expression as no loss, clonal loss and complete loss; clonal loss was observed in 16% of cases which were diagnosed as hyperplasia with atypia. Different numbers of low-grade and high-grade endometrioid carcinoma groups were also included, ARID1A loss increased proportionally from clonal to complete as the severity of malignancy increased. Thus, it has been suggested that ARID1A loss plays an important role in endometrial tumour progression.[16] In the study conducted by Yen *et al*. loss of ARID1A expression was observed in 16 of 52 (31%) patients diagnosed with atypical hyperplasia in their endometrial samplings, and endometrioid carcinoma was found in 15 of these 16 patients in their subsequent hysterectomies. ARID1A loss was classified as complete and heterogeneous, but both were included in the same group. In this study, ARID1A loss has been proposed to be a good predictive marker for concomitant carcinoma.[17] Vierkoetter *et al*. researched expression loss of six biomarkers, including ARID1A, in patients who were diagnosed with EIN in endometrial samplings and underwent hysterectomy in the follow-up. While no ARID1A expression loss was observed in EIN, 5 of 40 (13%) endometrioid carcinoma cases which were diagnosed in the postoperative report showed ARID1A expression loss. With this result, it was emphasised that ARID1A loss has high specificity

but low sensitivity in the diagnosis of endometrioid carcinoma.[18] Similarly, there are studies in which ARID1A loss was not observed in AEHs.[19,20] So, the lack of standardisation regarding the assessment of ARID1A loss and the different antibody clones used in the studies may explain the inconsistency of the literature data.

The role of FOXA1 in endometrial carcinogenesis is still controversial and there is limited information about its effect in AEHs. Yildirim *et al*. observed that FOXA1 was more expressed in endometrioid carcinoma compared to normal endometrium and EIN, so thought to be poor prognostic factor in endometrioid carcinomas.[9]

In this study, it was aimed to experimentally analyse the staining pattern of FOXA1 and evaluate the accuracy of ARID1A in AEHs, so contributing to current literature. In addition, the use of panels containing more than one immunostain in the diagnosis of AEH was analysed. PAX2 alone shows 84% loss, PAX2 and/or ARID1A 84%, PAX2 and/or FOXA1 85%, and ARID1A and/or FOXA1 17% in AEHs, respectively. In other words, using a combination of PAX2 and ARID1A instead of PAX2 alone in the AEH diagnosis does not change the sensitivity. Aguilar *et al*. created a biomarker panel for the diagnosis of AEH; PAX2, PTEN, beta-catenin, ARID1A, MLH1, and p53 markers alone and in combination were evaluated. While PAX2 showed the highest sensitivity with 81% alone in 111 AEH cases, this rate reached 93% in triple combination use of PAX2, PTEN, beta-catenin in which at least one was abnormal. However, the addition of ARID1A, MLH1 and p53 to the first combination did not change the rate.[21] This result was similar with this study, as the single most sensitive marker is PAX2 and the addition of ARID1A to the panel did not change the sensitivity.

In this study, PAX2, ARID1A and FOXA1 biomarkers were evaluated in 100 curettage cases diagnosed with AEH. All three biomarkers show significant staining loss in AEH ($p=0.001$ $p<0.01$), although PAX2 alone has the highest sensitivity. Other markers recommended for the diagnosis of atypical endometrial hyperplasia, such as PTEN or MMR, were not preferred. Because the staining pattern of PTEN is nucleocytoplasmic in both endometrial glands and stroma so it is difficult to evaluate and compare with normal endometrium; while MMR is not financially sustainable that it contains 4 different markers.

CONCLUSION

PAX2 alone could be preferred over ARID1A, FOXA1 and dual combinations because of its higher sensitivity in diagnosing atypical endometrial hyperplasia.

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ETHICAL APPROVAL:
This study was approved before its starting by the Health Science University, Umraniye Training and Research Hospital, Clinical Research Ethics Committee (Date: 08.04.2021 Session No: B.10.1.TKH.4.34.H.GP.0.01/105).

PATIENTS' CONSENT:
Informed consent were obtained from patients to publish the data.

COMPETING INTEREST:
The authors declare no competing interest.

AUTHORS' CONTRIBUTION:
ONY: Concept and study design, data collection, drafting of discussion, literature review, analysis and interpretation, and manuscript writing.
CST: Idea, statistical analysis of data, review, and proofreading.
IEZ: Statistical analysis of data and review.
All the authors have approved the final version of the manuscript to be published.

REFERENCES

1. Robert J. Kurman, Maria Luisa Carcangiu, C. Simon Herrington RHY. WHO classification tumours of female reproductive organs. 4th ed. Lyon: IARC; 2014. 124-154 p.
2. Zaino RJ, Carinelli S, Ellenson LH. Tumours of the uterine corpus: Epithelial tumours and precursors. In: Kurman RJ, Carcangiu M, Herrington C, Young R, eds. WHO classification of tumours of female reproductive organs. 4th ed. Lyon: WHO Press; 2014:125-26 p.
3. ESGO - European society of gynaecological oncology. Pocket guidelines - endometrial cancer; 2017. www.esgo.org/media/2015/12/Endometrial_broz_A6_b.pdf.
4. Mutter GL, Lax SF. WHO classification of tumours editorial board FGT. Endometrial atypical hyperplasia/endometrioid intraepithelial neoplasia. WHO classification of tumours series, 5th ed. Lyon, France: International agency for research on cancer; 2020:250-251 p.
5. Bedi D, Kataria AS, Sharma S *et al*. Evaluation of Loss of Paired Box 2 Gene Expression in Endometrial Hyperplasia for Detecting Endometrial Intraepithelial Neoplasia National. *J Labor Med* 2018; **7(3)**:23-8. doi: 10.7860/NJLM/2018/35743:2302.
6. Raffone A, Travaglino A, Saccone G. PAX2 in endometrial carcinogenesis and in differential diagnosis of endometrial hyperplasia: A systematic review and meta-analysis of diagnostic accuracy. *Acta Obstet Gynecol Scand* 2019; **98(3)**:287-99. doi: 10.1111/aogs.13512.
7. Raffone A,Travaglino A, Saccone G. Diagnostic and prognostic value of ARID1A in endometrial hyperplasia: A novel marker of occult cancer. *J Pathol Microbiol Immonol* 2019; **127(9)**:597-606. doi: 10.1111/apm.12977.
8. Werner H, Berg A, Wik E, Birkeland E. ARID1A loss is prevalent in endometrial hyperplasia with atypia and low-grade endometrioid carcinomas. *Modern Pathol* 2013; **26**:428-34. doi: 10.1038/modpathol.2012.174.
9. Yıldırım H, Nergiz D, Sadullahoğlu C. FOXA1 is associated with high tumour grade, myometrial invasion and lymph node invasion in endometrial endometrioid carcinoma *Ginekologia Polska* 2021; **92(1)**. doi: 10.5603/GP.a2021.0016.

10. Wang J, Bao W, Qiu M. Forkhead-box A1 suppresses the progression of endometrial cancer via crosstalk with estrogen receptor α. *Oncol Rep* 2014; **31**:1225-34, doi: 10.3892/or.2014.2982.

11. Qiu M, Bao W, Wang J. FOXA1 promotes tumour cell proliferation through AR involving the Notch pathway in endometrial cancer. *BMC Cancer* 2014; **14**:78. doi: 10. 1186/1471-2407-14-78.

12. Zhai QJ, Ozcan A, Hamilton C. PAX-2 expression in non-neoplastic, primary neoplastic, and metastatic neoplastic tissue: A comprehensive immunohistochemical study. Appl Immunohistochem *Mol Morphol* 2010; **18(4)**:323-32. doi: 10.1097/PAI.0b013e3181d712ef.

13. Allison KH, Upson K, Reed SD. PAX2 loss by immuno-histochemistry occurs early and often in endometrial hyperplasia. *Int J Gynecol Pathol* 2012; **31(2)**:159-67. doi: 10.1097/PGP.0b013e318226b376.

14. Quick CM, Laury AR, Monte NM, Mutter GL. Utility of PAX2 as a marker for diagnosis of endometrial intraepithelial neoplasia. *Am J Clin Pathol* 2012; **138(5)**:678-84. doi: 10.1309/AJCP8OMLT7KDWLMF.

15. Yiting MENG, Donglin MA, Li LI. Expressions of PAX-2 and PTEN in endometrial lesions and their correlation with endometrial intraepithelial neoplasia. *Cancer Res Clinic* 2017; **6**:27-31. doi: 10.3760/cma.j.issn.1006-9801.2017. 01.007.

16. Mao TL, Ardighieri L, Ayhan A. Loss of ARID1A expression correlates with stages of tumour progression in uterine endometrioid carcinoma. *Am J Surg Pathol* 2013; **37**: 1342–8. doi: 10.1097/PAS.0b013e3182889dc3.

17. Yen TT, Miyamoto T, Asaka S. Loss of ARID1A expression in endometrial samplings is associated with the risk of endometrial carcinoma. *Gynecol Oncol* 2018; **150**:426-31. doi: 10.1016/j.ygyno.2018.06.025.

18. Vierkoetter K, Wong J, Ahn HJ. Using gene expression in patients with endometrial intraepithelial neoplasia to assess the risk of cancer. *Gynecol Oncol Rep* 2018; **24**:24-6. doi: 10.1016/j.gore.2018.02.006.

19. Wiegand KC, Lee AF, Al-Agha OM. Loss of BAF250a (ARID1A) is frequent in high-grade endometrial carcinomas. *J Pathol* 2011; **224**:328333. doi: 10.1002/path. 2911.

20. Zhang ZM, Xiao S, Sun GY, LiuYP, Zhang. The clinico-pathologic significance of the loss of BAF250a (ARID1A) expression in endometrial carcinoma. *Int J Gynecol Cancer* 2014; **24**:534-40. doi: 10.1097/igc.0000000000000092.

21. Aguilar M, Chen H, Glorimar RC, Niu S, Carrick K, Gwin K, *et al*. Reliable ıdentification of endometrial precancers through combined Pax2, β-Catenin, and Pten immuno-histochemistry. *Am J Surg Pathol* 2022; **43(3)**:404-14. doi: 10.1097/pas.0000000000001810.