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SYSTEMATIC REVIEW

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Loss of PTEN expression as diagnostic marker of endometrial precancer: A systematic review and meta-analysis

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Abstract

Introduction: Endometrial hyperplasia may be either a benign proliferation or a premalignant lesion. In order to differentiate these two conditions, two possible histologic classifications can be used: the World Health Organization (WHO) classification and the endometrial intraepithelial neoplasia (EIN) classification. The 2017 European Society of Gynaecological Oncology guidelines recommend the use of immunohistochemistry for tumor suppressor protein phosphatase and tensin homolog (PTEN) to improve the differential diagnosis. Nonetheless, its diagnostic accuracy has never been defined. We aimed to assess the diagnostic accuracy of immunohistochemistry for PTEN in the differential diagnosis between benign and premalignant endometrial hyperplasia.

Material and methods: Electronic databases were searched from their inception to May 2018 for studies assessing immunohistochemical expression of PTEN in endometrial hyperplasia specimens. PTEN status ("loss" or "presence") was the index test; histological diagnosis ("precancer" or "benign") was the reference standard. Sensitivity, specificity, positive and negative likelihood ratios (LR+, LR−), diagnostic odds ratio (DOR), and area under the curve (AUC) on summary receiver operating characteristic curves were calculated (95% CI), with a subgroup analysis based on the histologic classification adopted (WHO vs EIN).

Results: Twenty-seven observational studies with 1736 cases of endometrial hyperplasia were included. Pooled estimates showed low diagnostic accuracy: sensitivity 54% (95% CI 50%-59%), specificity 66% (63%-69%), LR+ 1.55 (1.29-1.87), LR− 0.72 (0.62-0.83), DOR 3.56 (2.02-6.28), AUC 0.657. When the WHO subgroup was compared with the EIN subgroup, higher accuracy (AUC 0.694 vs 0.621), and higher heterogeneity in all analyses, were observed.

Conclusions: Immunohistochemistry for PTEN showed low diagnostic usefulness in the differential diagnosis between benign and premalignant endometrial hyperplasia. In the absence of further evidence, the recommendation about its use should be reconsidered.

Abbreviations: AUC, area under the curve; D, D-score; DOR, diagnostic odds ratio; EH, endometrial hyperplasia; EIN, endometrial intraepithelial neoplasia; LR+, positive likelihood ratio; LR−, negative likelihood ratio; PTEN, tumor suppressor protein phosphatase and tensin homolog; SROC, summary receiver operating characteristic; WHO, World Health Organization.

KEYWORDS
atypical endometrial hyperplasia, biomarker, cancer precursor, endometrial hyperplasia without atypia, endometrial intraepithelial neoplasia, endometrioid adenocarcinoma, phosphatase and tensin homolog

1 | INTRODUCTION

Endometrial hyperplasia (EH) is an irregular proliferation of endometrial glands with increased gland to stroma ratio when compared with the normal proliferative endometrium.[1] It may be a benign condition caused by an unopposed action of estrogens or a precancerous process.[2,3]

It is necessary to distinguish between these two conditions. In fact, premalignant EH requires total hysterectomy, or a conservative progestin-based therapy with close follow up in selected women. On the other hand, benign EH may be managed with observation alone, or with progestins when symptomatic.[4,5]

Two different systems have been proposed to classify EH: the World Health Organization (WHO) system and the endometrial intraepithelial neoplasia (EIN) system.[2,3]

The WHO system distinguishes "EH without atypia" (benign) from "atypical EH" (premalignant) based on the presence of cytologic atypia.[1,2]

The EIN system distinguishes "benign EH" from "endometrial intraepithelial neoplasia" based on a combination of histologic criteria. The EIN system may also be applied objectively through a computerized morphometric analysis; such analysis allows calculation of the "morphometric D-score," which subdivides EH into "high/intermediate risk" (D-score [D] ≤ 1) or "low risk" (D > 1) of progression to cancer.[2,3]

In the revised 2014 WHO classification, the terms "atypical EH" and EIN are reported as synonyms, although EIN refers to "endometrioid intraepithelial neoplasia."[1]

The histologic evaluation is considered the gold standard in the differential diagnosis between benign and premalignant EH. The WHO system is recommended by the Royal College of Obstetricians and Gynaecologists, whereas the EIN system is recommended by the American College of Obstetricians and Gynecologists.[4,5] Nonetheless, histologic classifications may be affected by several problems, such as low reproducibility, tissue inadequacy, artefact changes, or ambiguous features.[3,6]

To improve the reliability of the differential diagnosis, several diagnostic markers have been proposed. Great emphasis has been given to the loss of expression of the tumor suppressor protein phosphatase and tensin homolog (PTEN),[2,7] because the mutation of PTEN is the most common molecular alteration found in endometrial carcinogenesis[8,9] and occurs in an early phase.[7,9] In the 2017 European Society of Gynaecological Oncology (ESGO) guidelines (based on the 2016 European Society for Medical Oncology-ESGO-European Society for Radiotherapy & Oncology Consensus Conference), the immunohistochemical assessment of PTEN expression is recommended to recognize endometrial precancerous lesions.[10] In spite of this, the several studies in the literature showed a highly variable degree of association between loss of PTEN expression and premalignant EH, missing an analysis of diagnostic accuracy. The actual usefulness of immunohistochemistry for PTEN has never been defined.

Key message

Immunohistochemistry for PTEN has low diagnostic usefulness in differentiating benign from premalignant endometrial hyperplasia. Hence, its recommended use should be reconsidered.

The aim of this study was to determine the diagnostic accuracy of immunohistochemical assessment of PTEN in differential diagnosis between benign and premalignant EH, by extracting data from the available literature.

2 | MATERIAL AND METHODS

This study was performed according to a protocol recommended for systematic review and meta-analysis. The protocol defining methods for collecting, extracting, and analyzing data was designed a priori. All stages were conducted independently by two reviewers (AR, AT). The two authors independently assessed electronic search, eligibility of the studies, inclusion criteria, risk of bias, data extraction, and data analysis. Disagreements were resolved by discussion with a third reviewer (GS).

The study was reported following the Preferred Reporting Item for Systematic Reviews and Meta-analyses (PRISMA) statement[11] and the Synthesizing Evidence from Diagnostic Accuracy Tests (SEDATE) guideline.[12]

Searches were conducted using MEDLINE, EMBASE, Web of Sciences, Scopus, ClinicalTrial.gov, OVID, Cochrane Library, and Google Scholar as electronic databases. The relevant articles were searched from their inception to May 2018 using a combination of the following text words and all their synonyms found in the Medical SubHeading (MeSH) vocabulary: "endometrial hyperplasia"; "endometrial intraepithelial neoplasia"; "EIN"; "precancer"; "premalignant"; "precursor"; "PTEN"; "phosphatase and tensin homolog"; "marker"; "biomarker"; "diagnosis" "immunohistochemistry"; "immunohistochemical". Review of articles also included the abstracts of all references retrieved from the search.

All peer-reviewed retrospective or prospective studies assessing the immunohistochemical expression of PTEN on histological specimens of premalignant EH (atypical EH/endometrial intraepithelial neoplasia) or benign EH (EH without atypia/benign EH) were included in the systematic review.

Exclusion criteria were:

1. data on PTEN expression not extractable;
2. no distinction between premalignant and benign EH;
3. case reports and reviews;
4. patient data overlapping with a study already included.

Only the studies assessing both premalignant and benign EH were included in the meta-analysis.

According to the revised Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2),[13] four domains related to risk of bias were assessed in each study: (1) patient selection (low risk if the patients were selected as consecutive cohort); (2) index test (low risk if the assessment of PTEN expression was based on objective criteria); (3) reference standard (low risk if the histologic slides were reviewed to confirm the index diagnosis); (4) flow and timing (low risk if the latency time between index and reference standard did not affect the results, if all patients were assessed with the same tests, if all patients were assessed with both index and reference standards). Review authors' judgments were categorized as "low risk," "high risk," or "unclear risk" of bias.

Data from each eligible study were extracted without modification of the original data. Two-by-two contingency tables were prepared for each study, reporting two dichotomous qualitative variables:

1. PTEN expression ("loss" or "presence"), which was the index test;
2. histological diagnosis ("precancerous" or "benign"), which was the reference standard.

Precancerous cases with PTEN loss were considered as true positives, benign cases with PTEN presence were considered as true negatives, precancerous cases with PTEN presence were considered as false negatives, and benign cases with PTEN loss were considered as false positives.

Data regarding the index test were extracted by using the following criteria:

1. for the studies dichotomizing PTEN expression (positive vs negative) independently from distribution and intensity of expression, "negative" was considered as "PTEN loss";
2. for the studies using a semi-quantitative scale to grade the intensity of PTEN expression, independently from the distribution, the lowest grade (negative expression) was considered as "PTEN loss";
3. for the studies assessing the percentage of PTEN-positive glands, independently from the intensity of staining, the lowest percentage (negative expression) was considered as "PTEN loss."

Data regarding the reference standard were extracted by using the following criteria:

1. for the studies using the WHO classification, atypical EH (simple or complex) was considered as "precancer," while EH without atypia (simple or complex) was considered as "benign";
2. for the studies using the EIN classification, endometrial intraepithelial neoplasia or high/intermediate-risk EH (D ≤ 1) were considered as "precancer," while benign EH or low-risk EH (D < 1) were considered as "benign";
3. hyperproliferative conditions caused by unopposed action of estrogens (eg "disordered proliferative endometrium," "persistent proliferative endometrium") were included in the "benign" group, as proposed in the literature,[14] because they constitute a pathologic continuum with non-atypical EH.[6]

When discrepancies between values reported in the text and the tables were found, values from tables were used for the analysis. Data were also subdivided into two subgroups based on the classification system adopted (WHO vs EIN).

Sensitivity, specificity, positive likelihood ratio (LR+), negative likelihood ratio (LR−), and diagnostic odds ratio (DOR) were calculated for each study and as pooled estimate using the random effect model of DerSimonian and Laird and reported graphically on forest plots, with 95% CI. Statistical heterogeneity among studies was assessed using the Higgins I^2 statistic; heterogeneity was considered insignificant for $I^2 < 25\%$, low for $I^2 < 50\%$, moderate for $I^2 < 75\%$, and high for $I^2 \geq 75\%$.

Area under the curve (AUC) was calculated on summary receiver operating characteristic (SROC) curves. The diagnostic usefulness was considered absent for AUC ≤ 0.5, low for 0.5 < AUC ≤ 0.75, moderate for 0.75 < AUC ≤ 0.9, high for 0.9 < AUC < 0.97, and very high for AUC ≥ 0.97.

As additional analysis, we performed a subgroups analysis, assessing sensitivity, specificity, LR+, LR−, DOR, and AUC separately for the two subgroups.

The data analysis was performed using Review Manager 5.3 (Copenhagen: The Nordic Cochrane Center, Cochrane Collaboration, 2014) and Meta-DiSc version 1.4 (Clinical Biostatistics Unit, Ramon y Cajal Hospital, Madrid, Spain).

3 | RESULTS

We identified 635 articles through database searching and 13 through additional sources. After duplicate removal, 189 articles remained and 101 were screened. Forty-four articles were considered relevant and so were assessed for eligibility; 17 of them were excluded by applying our exclusion criteria. Finally, 27 studies were included in the systematic review, 18 of which were suitable for the meta-analysis. Details about the whole process of study selection are shown in Figure 1.

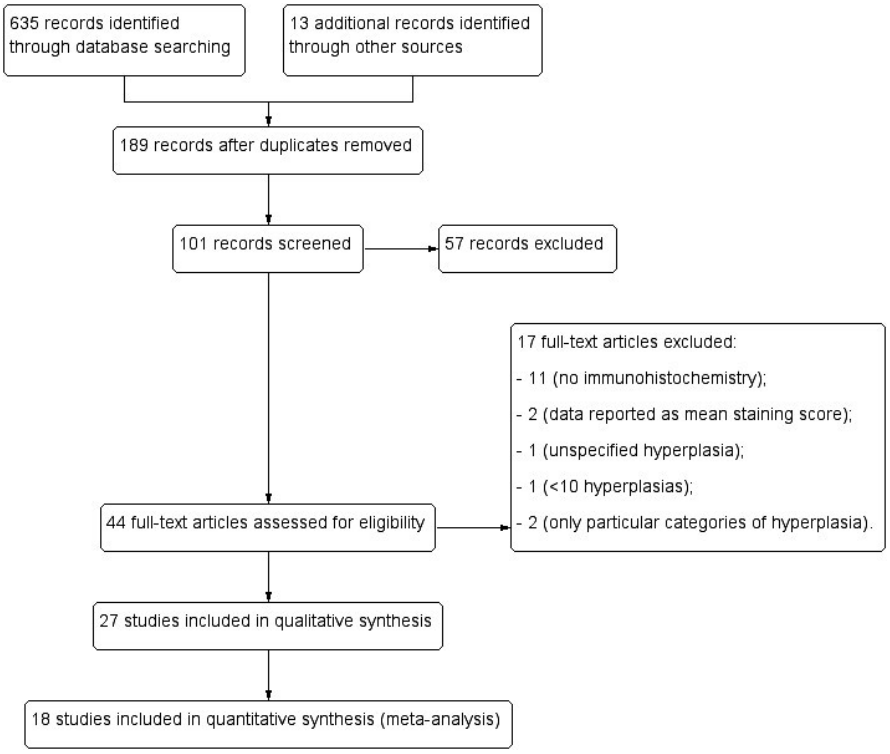

FIGURE 1 Flow diagram of studies identified in the systematic review (Prisma template [Preferred Reporting Item for Systematic Reviews and Meta-analyses])

Twenty-seven observational studies were included in the systematic review:[7,15-40] 19 adopted the WHO classification and eight adopted the EIN classification. A total of 1736 cases of EH were included; 847 (48.8%) EH were classified as "precancer" and 889 (51.2%) as "benign." PTEN loss was observed in 443 of 847 (52.3%) premalignant EH and 299 of 889 (33.6%) benign EH.

Among the 1232 EH classified according to the WHO system, PTEN loss was observed in 336 of 593 (56.7%) atypical EH and 221 of 639 (34.6%) non-atypical EH.

Among the 504 EH classified according to the EIN system, PTEN loss was observed in 107 (42.1%) of 254 premalignant EH and in 78 of 250 (31.2%) benign EH.

Fifteen studies dichotomized PTEN expression. Six studies used an intensity scale to grade PTEN expression, and four assessed the percentage of PTEN-positive cells. Three studies combined intensity of staining and percentage of stained cells to obtain a staining score.

Details about characteristics of the included studies and methods for immunohistochemistry are shown in Tables 1 and 2, respectively.

Results of the risk of bias assessment are shown in Figure 2. In particular, for the "patient selection" domain, three studies were classified as being at unclear risk of bias, because they followed a case–control design; nine studies were considered at high risk of bias because they selected only premalignant EH. All the remaining studies were considered at low risk.

For the "index test" domain, five studies were considered at unclear risk of bias, because they only used a qualitative scale to grade PTEN expression, regardless of the percentage of stained cells. The other studies were considered at low risk.

For the "reference standard" domain, 17 studies were classified as being at low risk of bias, because they specified that histological slides were reviewed to confirm the diagnosis of benign or premalignant. The other 10 studies were considered at unclear risk.

For the "flow and timing" domain, all the included studies were classified as being at low risk of bias, since both the index and the reference standard were performed on the same specimen and for all patients.

Eighteen studies assessing 1362 EH were included in the meta-analysis;[7,15-17,20,22,24-27,29-32,34,36-38] (34.7%) of total EH were premalignant and 889 (65.3%) were benign. The nine studies not assessing benign EH were excluded from the meta-analysis.

Pooled sensitivity and specificity of PTEN loss in detecting endometrial precancer were 54% (95% CI 50%-59%) and 66% (95% CI 63%-69%), respectively, with pooled LR+ and LR− of 1.55 (95% CI 1.29-1.87) and 0.72 (95% CI 0.62-0.83), respectively. Pooled DOR was 2.81 (95% CI 1.96-4.02).

Among the included studies, the heterogeneity was high in sensitivity (I^2 = 80%) and specificity (I^2 = 92.5%), low in LR+ (I^2 = 43.2%), moderate in LR− (I^2 = 56.9%), and insignificant in DOR (I^2 = 23.5%).

The SROC curves analysis demonstrated low overall accuracy with an AUC of 0.657.

Results are reported graphically in forest plots and SROC curves in Figure 3.

In the subgroup analysis, 12 studies assessing 934 EH by using the WHO system were included in first subgroup; 295 (31.6%) of total EH were premalignant and 639 (68.4%) were benign.

Pooled sensitivity and specificity were 59% (95% CI 53%-65%) and 65% (95% CI 62%-69%), respectively, with pooled LR+ and

TABLE 1 Characteristics of the included studies

Year	First author (ref)	Country	Period of enrollment	Sample size		Precancer			Benign			Method to assess PTEN staining
				WHO	EIN	WHO	EIN	PTEN loss (%)	WHO	EIN	PTEN loss (%)	
2000	Mutter [7]	USA	Not reported	—	28	—	21	14 (67%)	—	7	2 (29%)	Intensity grade
2001	Mutter [15]	USA	1998-2000	—	76	—	35	22 (63%)	—	41	23 (56%)	Dichotomous
2003	Ørbo [16]	Norway	1980-1991	—	68	—	39	11 (28%)	—	29	3 (10%)	Dichotomous
2005	Baak [17]	Norway	Not reported	—	103	—	21	14 (67%)	—	82	29 (35%)	Dichotomous
2006	Cirpan [18]	Turkey	1998-2002	—	24	—	24	1 (4%)	—	—	—	Intensity grade
	McCampbell [19]	USA	Not reported	14	—	14	—	9 (64%)	—	—	—	Dichotomous
2007	Kapucuoglu [20]	Turkey	Not reported	37	—	10	—	2 (20%)	27	—	0 (0%)	Dichotomous + score
	Minaguchi [21]	Japan	1989-2003	12	—	12	—	3 (25%)	—	—	—	Intensity grade
	Norimatsu [22]	Japan	1998-2005	—	70	—	38	13 (34%)	—	32	4 (12%)	Dichotomous
2008	Chen [23]	China	2003-2005	34	—	34	—	20 (59%)	—	—	—	Dichotomous
	Lacey [24]	USA	1970-2002	308	—	73	—	41 (56%)	235	—	105 (47%)	Dichotomous
	Tantbirojn [25]	Thailand	2001-2004	45	—	20	—	12 (60%)	25	—	6 (24%)	Intensity grade
2009	Abd El-Masqoud [26]	Egypt	Not reported	20	—	8	—	2 (25%)	12	—	0 (0%)	Dichotomous + score
	Sarmadi [27]	Iran	Not reported	29	—	8	—	2 (25%)	21	—	0 (0%)	Intensity grade + percentage
2010	Monte [28]	USA	2006-2008	—	52	—	52	23 (44%)	—	—	—	Dichotomous
	Pavlakis [29]	Greece	Not reported	83	—	58	—	38 (66%)	25	—	15 (60%)	Dichotomous
	Xiong [30]	China	2001-2006	—	83	—	24	9 (37%)	—	59	17 (29%)	Percentage
2011	Pieczyńska [31]	Poland	1994-2001	132	—	16	—	1 (6%)	116	—	4 (3%)	Dichotomous + score
	Rao [32]	India	2005-2007	76	—	13	—	13 (100%)	63	—	46 (73%)	Intensity grade + percentage
2012	Feng [33]	China	2005-2009	28	—	28	—	12 (43%)	—	—	—	Dichotomous
	Lee [34]	South Korea	1991-2005	42	—	21	—	15 (71%)	21	—	5 (24%)	Percentage
	Robbe [35]	Belgium/Netherlands	1999-2006	39	—	39	—	15 (38%)	—	—	—	Dichotomous
	Upson [36]	USA	1985-2005	112	—	40	—	32 (80%)	72	—	39 (54%)	Dichotomous
2013	Huang [37]	USA	Not reported	24	—	22	—	12 (55%)	2	—	0 (0%)	Dichotomous
2014	Shawana [38]	Pakistan	2006-2010	26	—	6	—	4 (67%)	20	—	1 (5%)	Intensity grade
2015	Ayhan [39]	Japan/Taiwan	Not reported	114	—	114	—	80 (70%)	—	—	—	Dichotomous
	Berg [40]	Norway	2001-2013	57	—	57	—	23 (40%)	—	—	—	Intensity grade
Total WHO		—	—	1232	—	593	—	336 (57%)	639	—	221 (35%)	—
Total EIN		—	—	—	504	—	254	107 (42%)	—	250	78 (31%)	—
Total hyperplasias		—	—	1736		847		443 (52%)	889		299 (34%)	—

dichotomous, PTEN present or absent; EIN, endometrial intraepithelial neoplasia; intensity grade, grading of intensity of PTEN immunostaining; percentage, percentage of PTEN-null glands; score, combined immunostaining score; WHO, World Health Organization.

TABLE 2 Methods for immunohistochemistry in each included study

		Antibody		Incubation		
Year	First author (ref)	Vendor	Clone	Time	Temperature	Dilution
2000	Mutter [7]	Not reported	6H2.1	1 h	Room temperature	1:100
2001	Mutter [15]	Cascade Biosciences	6H2.1	Overnight	4°C	1:300
2003	Ørbo [16]	Santa Cruz Biotechnology	A2B1	30 min	Room temperature	1:50
2005	Baak [17]	Cascade Biosciences	6H2.1	30 min	Not reported	1:300
2006	Cirpan [18]	Novocastra	28H6	30 min	Not reported	Not reported
	McCampbell [19]	Cascade Biosciences	6H2.1	Overnight	4°C	1:50
2007	Kapucuoglu [20]	LabVision	17.A	1 h	Not reported	1:50
	Minaguchi [21]	Santa Cruz Biotechnology	A2B1	Overnight	4°C	1:50
	Norimatsu [22]	Cascade Biosciences	6H2.1	Not reported	Not reported	1:100
2008	Chen [23]	Antibody Diagnostica	Not reported	1 h	Not reported	1:60
	Lacey [24]	Cascade Biosciences	6H2.1	Overnight	4°C	1:300
	Tantbirojn [25]	Cascade Biosciences	6H2.1	1 h	Room temperature	1:100
2009	Abd El-Masqoud [26]	LabVision	28H6	Not reported	Not reported	1:100
	Sarmadi [27]	Zymed Laboratories	Polyclonal	60 min	Not reported	1:100
2010	Monte [28]	Dako	6H2.1	Overnight	4°C	1:100
	Pavlakis [29]	Cascade Biosciences	6H2.1	Overnight	4°C	1:300
	Xiong [30]	Maixin Bio	Not reported	Not reported	Not reported	Not reported
2011	Pieczynska [31]	Novocastra	Not reported	1.5 h	Room temperature	1:800
	Rao [32]	Biogenex	28H6	Not reported	Not reported	Not reported
2012	Feng [33]	Antibody Diagnostica	Not reported	1 h	37°C	1:60
	Lee [34]	Cell Signaling Technology	138G6	Not reported	Not reported	1:100
	Robbe [35]	Not reported	Not reported	30 min	Room temperature	Not reported
	Upson [36]	Cascade Biosciences	6H2.1	40 min	Room temperature	1:100
2013	Huang [37]	Dako	6H2.1	Not reported	Not reported	1:100
2014	Shawana [38]	Millipore	6H2.1	1 h	Room temperature	1:50
2015	Ayhan [39]	Dako	6H2.1	Not reported	Not reported	1:100
	Berg [40]	Cell Signaling	#9188	Overnight	4°C	1:100

LR− of 1.56 (95% CI 1.22-1.98) and 0.67 (95% CI 0.52-0.85), respectively. Pooled DOR was 3.56 (95% CI 2.02-6.28).

The heterogeneity was high in sensitivity (I^2 = 81.4%) and specificity (I^2 = 94.5%), moderate in LR+ (I^2 = 50.7%) and LR− (I^2 = 71.8%), and low in DOR (I^2 = 30.1%).

The SROC curves analysis demonstrated low overall accuracy with an AUC of 0.694.

Six studies assessing 428 EH by using the EIN system were included in the second subgroup; 178 (41.6%) of total EH were premalignant and 250 (58.4%) were benign.

Pooled sensitivity and specificity were 47% (95% CI 39%-54%) and 69% (95% CI 63%-74%), respectively, with pooled LR+ and LR− of 1.59 (95% CI 1.17-2.17) and 0.77 (95% CI 0.67-0.89), respectively. Pooled DOR was 2.34 (95% CI 1.47-3.73).

The heterogeneity was moderate in sensitivity (I^2 = 74.1%), high in specificity (I^2 = 79.9%), low in LR+ (I^2 = 27%), and completely absent in LR− (I^2 = 0%) and DOR (I^2 = 0%).

The SROC curves analysis demonstrated low overall accuracy with an AUC of 0.621.

Results are reported graphically in forest plots and SROC curves for the WHO and EIN subgroups, respectively in Figures 4 and 5.

4 | DISCUSSION

Although a loss of PTEN function is involved in endometrial carcinogenesis, our study showed that immunohistochemical evaluation of PTEN expression has a low diagnostic usefulness in the differential diagnosis between benign and premalignant EH.

The PTEN gene is located at chromosome 10q23 and encodes a phosphatase that acts as a tumor suppressor. It has a lipid phosphatase activity, which induces cell cycle arrest, upregulates AKT-dependent pro-apoptotic mechanisms and downregulates Bcl-2-dependent anti-apoptotic mechanisms, acting in opposition

FIGURE 2 A, Assessment of risk of bias. Summary of risk of bias for each study. Plus sign: low risk of bias; minus sign: high risk of bias; question mark: unclear risk of bias. B, Risk of bias graph about each risk of bias item presented as percentages across all included studies [Color figure can be viewed at wileyonlinelibrary.com]

to phosphatidylinositol 3-kinase (PI3K). Moreover, PTEN has also a protein phosphatase activity, which is involved in the inhibition of focal adhesion formation, cell spread, and growth-factor-stimulated mitogen-activated protein kinase (MAPK) signaling.[41]

In the four categories of endometrial cancer identified by the Cancer Genome Atlas Research Network (ultramutated, hypermutated, copy number low, copy number high), PTEN mutations were found in 94%, 88%, 77%, and 15% of cases, respectively.[8]

According to our results, the immunohistochemical assessment of PTEN has a low diagnostic usefulness, as demonstrated by an AUC < 0.75 (0.657).

Such a test would determine which women should be treated to prevent cancer, so a high sensitivity appears crucial in order not to miss patients at risk. For this reason, a sensitivity of 59% appears to be not enough. On the other hand, as hysterectomy is the reference standard intervention for premalignant EH, a high specificity is also needed to avoid severe overtreatment. Hence, the specificity observed (66%) is too low. Given these findings, PTEN assessment appears inadequate as a stand-alone diagnostic test.

However, a suboptimal sensitivity might be expected, because not all endometrioid adenocarcinomas or their precursor lesions have underlying mutations of the PTEN gene.[8,9]

Concerning the low specificity, a possible cause may be that a loss of PTEN expression does not necessarily indicate a monoclonal lesion. In fact, Yilmaz et al observed PTEN loss using immunohistochemistry in 3/36 (8.3%) polyclonal endometrial specimens.[42] Furthermore, a loss of PTEN expression may be observed in morphologically normal clones of endometrial glands, which tend to spontaneously regress. Mutter et al showed that only a small proportion (6.7%) of these latent precancers actually progress to overt lesions.[43]

In the WHO subgroup, higher sensibility and DOR, and lower specificity, LR+, and LR−, were found when compared with the EIN subgroup. This resulted in a greater AUC for the WHO subgroup. The heterogeneity was higher in the WHO subgroup for all analyses, possibly because of the better reproducibility of the EIN system.[2,3] In a comparison study, the prognostic ability of the two classifications appeared to be superimposable.[14]

A possible cause of both the higher diagnostic accuracy and the heterogeneity for the WHO subgroup may be found in the "small-study effect." Nonetheless, a meta-epidemiological study published in 2014 showed that such an effect was not significant in meta-analyses of diagnostic test accuracy.[44]

To the best of our knowledge, this study is the first meta-analysis assessing the usefulness of the immunohistochemical evaluation of PTEN in the differential diagnosis between premalignant and benign EH. Most of the included studies only assessed the association between PTEN loss and premalignant features of EH, without

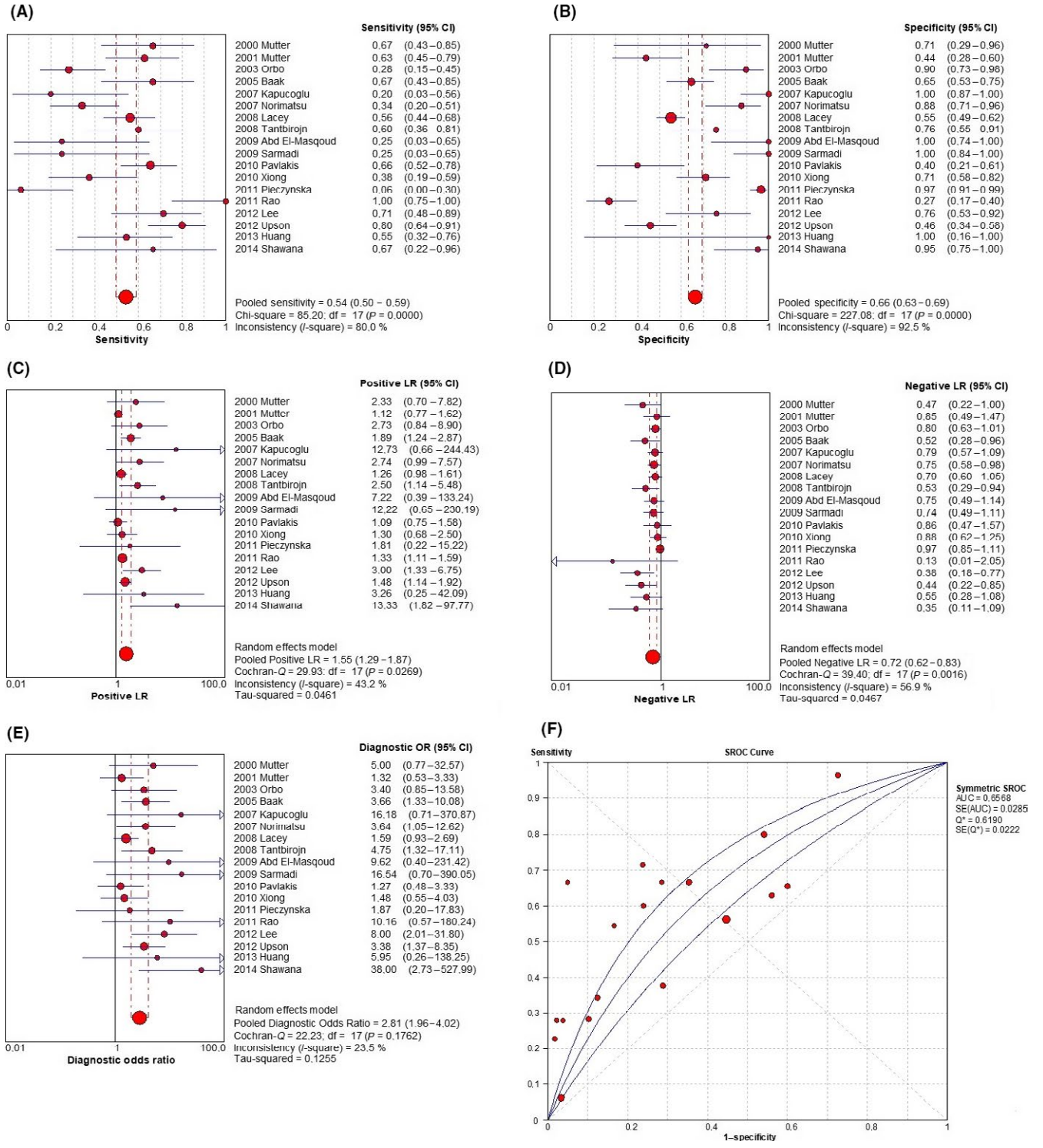

FIGURE 3 Forest plots of individual studies and pooled sensitivity (A), specificity (B), positive likelihood ratio (C), negative likelihood ratio (D), and diagnostic odds ratio (E) of PTEN immunohistochemical assessment in differential diagnosis between benign and premalignant endometrial hyperplasia, with summary receiver operating characteristic curves (F) [Color figure can be viewed at wileyonlinelibrary.com]

evaluating the diagnostic accuracy of PTEN. We aimed to define the actual diagnostic usefulness of PTEN assessment in EH. As current guidelines recommending evaluation of PTEN loss are based on level IV evidence,[10] a meta-analysis can provide a higher level of evidence for future recommendations. The current study provides both new and important insights into the field.

Major limitations to our results might be the intra- and inter-observer variability for both index test and reference standard. Such

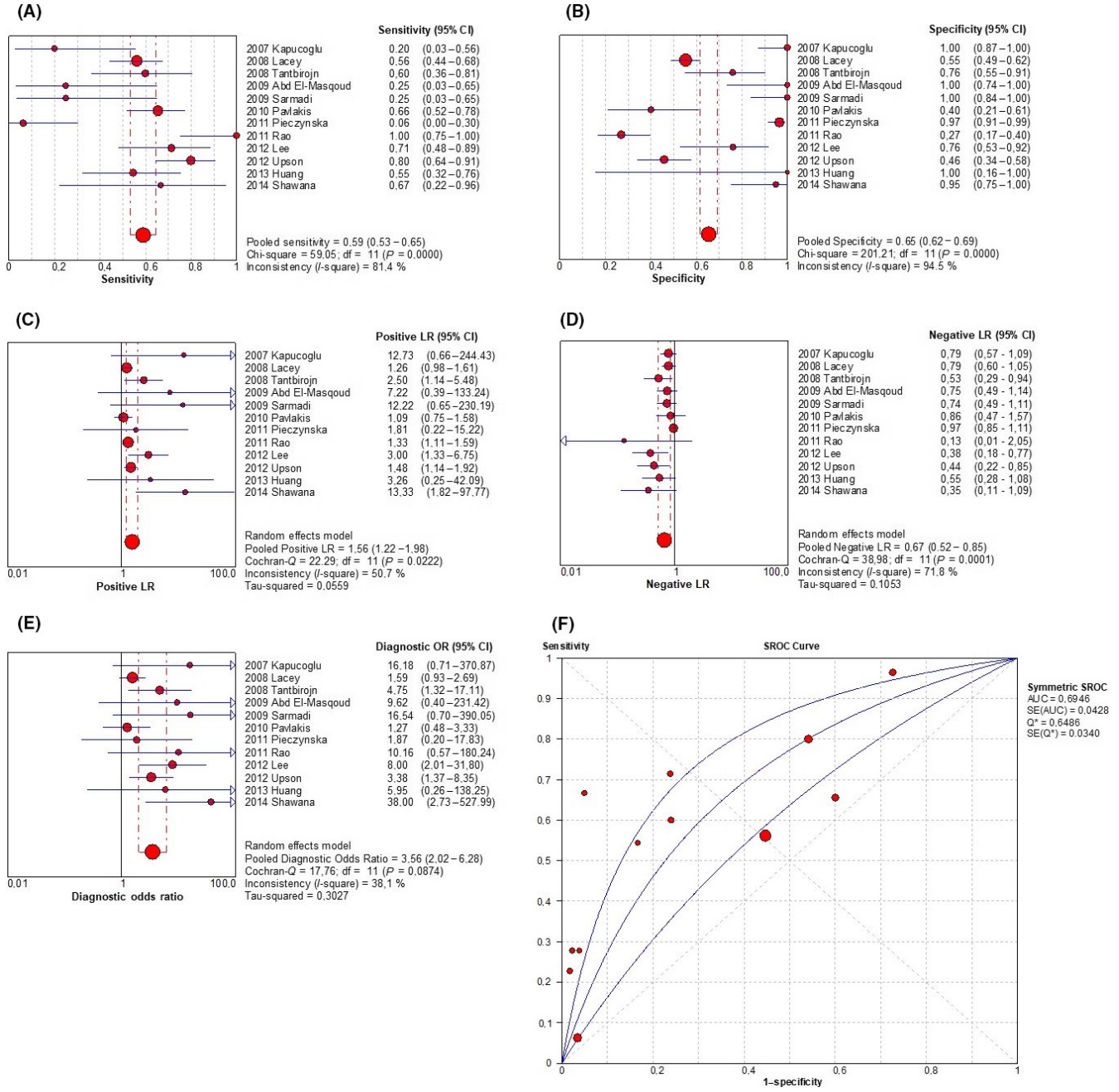

FIGURE 4 Forest plots of individual studies and pooled sensitivity (A), specificity (B), positive likelihood ratio (C), negative likelihood ratio (D), and diagnostic odds ratio (E) of PTEN immunohistochemical assessment in differential diagnosis between benign and premalignant endometrial hyperplasia, with summary receiver operating characteristic curves (F), for the World Health Organization subgroup [Color figure can be viewed at wileyonlinelibrary.com]

variability may explain the high heterogeneity observed for sensitivity and specificity analysis.

Concerns about the index text regard the lack of standardized and objective criteria for interpreting PTEN immunostaining. However, it was shown that a subjective categorization of PTEN immunostaining as "normal," "heterogeneous," or "loss," was highly reproducible.[45]

Regarding the reference standard, well-described concerns relate to the variability in the histologic criteria. In particular, the diagnosis of cytologic atypia has shown poor reproducibility.[2,46] Furthermore, the characteristics of atypia specific for endometrial epithelium are not included in the WHO classification system, and metaplastic and regenerative changes may mimic true atypia.[1,6,46] Other concerns refer to the fact that premalignant hyperplasia is a focal change,[1-3,47] and the amount of tissue/cells harboring the atypical features may be scant, particularly in aspiration biopsies or curettage samples.[2,6] In addition, the degree of atypia is often variable, further complicating the determination of diagnostic atypia.[46] As discussed, the 2014

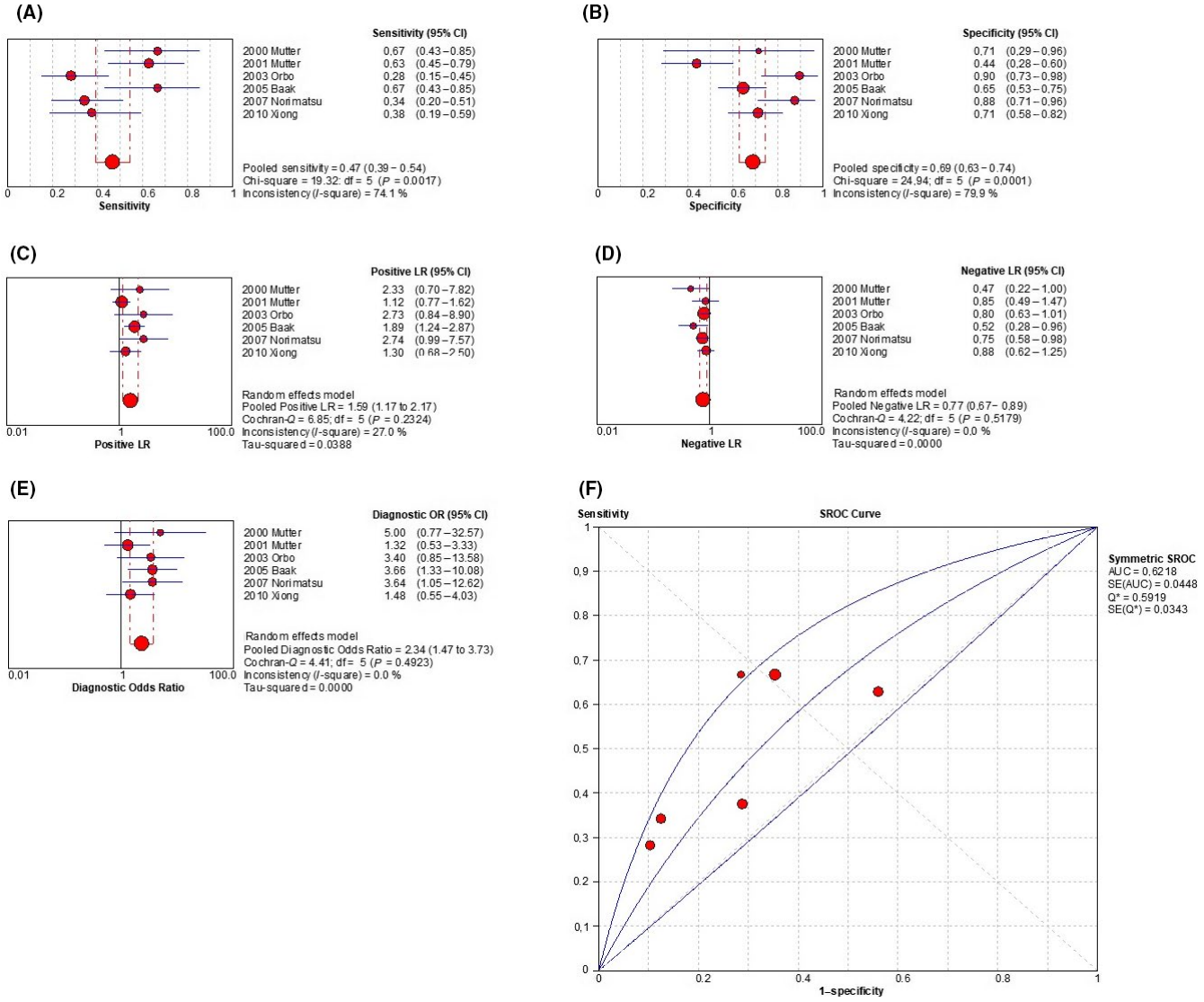

FIGURE 5 Forest plots of individual studies and pooled sensitivity (A), specificity (B), positive likelihood ratio (C), negative likelihood ratio (D), and diagnostic odds ratio (E) of PTEN immunohistochemical assessment in differential diagnosis between benign and premalignant endometrial hyperplasia, with summary receiver operating characteristic curves (F), for the endometrial intraepithelial neoplasia subgroup [Color figure can be viewed at wileyonlinelibrary.com]

revision of the WHO classification has tried to improve the diagnostic criteria by referring to the EIN system. Such revised classification uses "EIN" as a synonym of atypical EH.[1] However, WHO 2014 terms might be confounding because the acronym "EIN" refers to "endometrioid intraepithelial neoplasia." It is therefore unclear if it actually relates to the EIN (endometrial intraepithelial neoplasia) classification. In spite of such revision, the WHO classification still appears to be based on the presence of cytologic atypia.[1] Unfortunately, we were not able to consider the 2014 WHO classification separately from the former versions, because of the lack of studies published after 2014 in the WHO subgroup.

In our opinion, further studies in this field should improve the reliability of both the index test and the reference standard.

The reliability of the index test might be improved by standardizing criteria for interpreting PTEN immunostaining, in terms of intensity of staining and percentage of stained cells. The antibody to be used should also be standardized. Among the available anti-PTEN antibodies, it is unclear which one should be used. In fact, the clone 6H21 was used in the study showing the highest DOR,[38] as well as in the one showing the lowest DOR.[29] A study published in 2011 suggested the clone 138G6 to be the most reliable.[48] In our systematic review, the only study using such a clone was not included in the meta-analysis because of the lack of a control group of benign EH.[34]

On the other hand, the reliability of the reference standard might be partially improved through consensus among several pathologists in the evaluation of histologic slides. A combination of several markers more specific than PTEN (such as Bcl-2[49]) might considerably reduce the variability of the reference standard.

However, given the limitations inherent to PTEN and discussed above, it is probable that the diagnostic usefulness of PTEN alone

might be fair at best, even with optimized criteria. Further studies appear necessary to clarify the prognostic value of PTEN immunohistochemistry in EH, with specific regard to the progression to cancer.

5 | CONCLUSION

Although a loss of PTEN expression was associated with endometrial precancer, immunohistochemistry for PTEN showed a low diagnostic usefulness in the differential diagnosis between benign and premalignant EH, independently from the histologic classification used (WHO or EIN). In the absence of further evidence, the recommendation about the use of PTEN for this purpose should be reconsidered.

CONFLICT OF INTEREST

The authors report no conflict of interest.

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