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REVIEW ARTICLE

PTEN immunohistochemistry in endometrial hyperplasia: which are the optimal criteria for the diagnosis of precancer?

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Guidelines recommend protein phosphatase and tensin homolog (PTEN) immunohistochemistry for differentiating between benign endometrial hyperplasia (BEH) and atypical endometrial hyperplasia/endometrioid intraepithelial neoplasia (AEH/EIN). However, it is unclear when PTEN expression should be defined as 'lost' and thus suggestive of AEH/EIN. We aimed to determine the optimal immunohistochemical criteria to define PTEN loss in endometrial hyperplasia, through a systematic review and meta-analysis of diagnostic accuracy. Electronic databases were searched for studies assessing immunohistochemical expression of PTEN in both BEH and AEH/EIN specimens. PTEN status ('loss' or 'presence') was the index test; histological diagnosis ('AEH/EIN' or 'BEH') was the reference standard. Accuracy was quantified based on the area under the curve (AUC) on summary receiver operating characteristic (SROC) curves, for several different thresholds of PTEN expression. Eighteen studies with 1362 hyperplasias were included. Six different criteria to define PTEN loss were assessed. Low diagnostic accuracy was found for complete loss of expression (AUC = 0.71), presence of any null gland (AUC = 0.63), positive cells <10% (AUC = 0.64), positive cells <50% (AUC = 0.71) and moderate-to-null intensity (AUC = 0.64). Barely moderate diagnostic accuracy was only found for the subjective criterion 'weak-to-null intensity' (AUC = 0.78). Therefore, the clinical usefulness of PTEN immunohistochemistry in this field should be further investigated.

Key words: Atypical endometrial hyperplasia; endometrial intraepithelial neoplasia; endometrial hyperplasia without atypia; biomarker; cancer precursor; endometrioid adenocarcinoma.

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Endometrial hyperplasia includes two different conditions: benign endometrial hyperplasia (BEH), which is a proliferation reactive to unopposed action of estrogens, and atypical endometrial hyperplasia/endometrioid intraepithelial neoplasia (AEH/EIN), which is a precancerous lesion (1–3). It is crucial to differentiate between these two conditions in order to adopt an appropriate patient management. BEH may be managed with observation alone, or with progestins when symptomatic. On the other hand, AEH/EIN requires total hysterectomy; in selected cases, a conservative treatment can be chosen, using progestins alone or combined with hysteroscopic resection (4–7).

Histologic examination is considered as the gold standard for the differential diagnosis between AEH/EIN and BEH. However, histologic criteria appear to be poorly reproducible, and endometrial specimens may show ambiguous features; furthermore, the evaluation of little biopsies is often

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affected by tissue inadequacy and artifact changes (2, 8, 9).

Several immunohistochemical markers have been proposed in order to improve the differential diagnosis (2). In this field, the tumor suppressor protein phosphatase and tensin homolog (PTEN) has played a major role, since PTEN loss of expression is regarded as the crucial event in endometrial carcinogenesis and occurs in an early phase (10, 11). The 2017 guidelines of the European Society of Gynaecological Oncology (ESGO) recommend the use of immunohistochemistry for PTEN to differentiate AEH/EIN from BEH, not specifying the immunohistochemical criteria to define PTEN loss of expression (12).

However, in our previous study we found that the accuracy of PTEN loss as immunohistochemical diagnostic marker of AEH/EIN was low (13, 14). Anyway, immunohistochemical criteria to define loss of PTEN expression are not standardized, and different thresholds of expression have been adopted in the several studies. This might have affected the diagnostic accuracy evaluation.

In the current study, we aimed to determine: (i) the optimal criteria for interpreting PTEN immunostaining, by assessing how its diagnostic accuracy for AEH/EIN changes according to different thresholds of percentage of stained cells and intensity of staining; (ii) if PTEN immunohistochemistry is interpreted following the optimal criteria identified may be useful in the common practice.

MATERIALS AND METHODS

Study protocol

This study followed the Preferred Reporting Item for Systematic Reviews and Meta-Analyses (PRISMA) statement (15) and the Synthesizing Evidence from Diagnostic Accuracy Tests (SEDATE) guideline (16). The protocol defining methods for collecting, extracting and analyzing data was designed *a priori*. All stages were conducted independently by two reviewers (AR, AT). Disagreements were resolved by discussion with a third reviewer (GS).

Search strategy and study selection

Methods of search strategy and study selection have been described previously (13). In brief, we conducted several researches using the following electronic databases: MEDLINE, EMBASE, Web of Sciences, Scopus, ClinicalTrial.gov, OVID, Cochrane Library and Google Scholar. A combination of the following text words was used: 'endometrial hyperplasia'; 'endometrial intraepithelial neoplasia'; 'EIN'; 'precancer'; 'premalignant'; 'precursor'; 'PTEN'; 'phosphatase and tensin homolog'; 'marker'; 'biomarker'; 'diagnosis' 'immunohistochemistry'; 'immunohistochemical'. In the current study, the research was updated to October 2018.

All peer-reviewed retrospective or prospective studies assessing immunohistochemical expression of PTEN on histological specimens of AEH/EIN and BEH were included. Exclusion criteria were: data on PTEN expression not extractable; no differentiation between AEH/EIN and BEH; assessment of only AEH/EIN or only BEH; case reports and reviews; overlapping patient data with a study already included.

Risk of bias assessment

Risk of bias within studies was assessed using the revised Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) (17). For each study, correctness criteria were applied to four different domains related to the risk of bias: (i) Patient selection: if the patients were consecutive; (ii) Index test: if criteria for interpreting PTEN immunostaining were clearly stated; (iii) Reference standard: if histologic classification was unbiased; (iv) Flow and Timing: if all patients were assessed with the same index test and the same reference standard. Authors' judgments were categorized as 'low risk' (the criterion was met), 'high risk' (the criterion was not met) or 'unclear risk of bias' (not clear whether or not the criterion was met).

Data extraction

Data extraction was based on methods from our previous study (13). For each study, we reported two dichotomous qualitative variables on 2 × 2 contingency tables:

1. PTEN expression ('loss' or 'normal'), which was the index test;
2. Histological diagnosis ('AEH/EIN' or 'BEH'), which was the reference standard.

Data regarding the index test were subdivided into groups based on the different criteria to define PTEN loss (positive index test):

1. Complete loss of PTEN expression in the whole lesion;
2. Presence of any PTEN-null gland;
3. Percentage of PTEN-positive cells below several thresholds;
4. Intensity of PTEN immunostaining lower than normal.

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

1. For the studies using the WHO classification (1–3), atypical endometrial hyperplasia (simple or complex) was considered as 'AEH/EIN', and endometrial hyperplasia without atypia (simple or complex) was considered as 'BEH';
2. For the studies using the endometrial intraepithelial neoplasia (EIN) classification (2, 3), endometrial intraepithelial neoplasia was considered as 'AEH/EIN', and benign endometrial hyperplasia was considered as 'BEH';
3. Hyperproliferative conditions caused by unopposed action of estrogens (e.g., 'disordered proliferative endometrium', 'persistent proliferative endometrium') were included in the 'BEH' group, as proposed in the literature (18), since they constitute a pathologic continuum with endometrial hyperplasia without atypia (1, 8).

AEH/EIN cases with PTEN loss were considered as true positive, BEH cases with PTEN presence as true negative, AEH/EIN cases with PTEN presence as false negative and BEH cases with PTEN loss as false positive.

Data analysis

Sensitivity, specificity, positive likelihood ratio (LR+), negative likelihood ratio (LR−) and diagnostic odds ratio (DOR) were calculated for each study and as pooled estimate, and reported graphically on forest plots, with 95% confidence interval (CI). The random effect model of DerSimonian and Laird was used to pool values, as recommended by the SEDATE guidelines (16). Statistical heterogeneity among studies was quantified by using the inconsistency index (I^2) statistic: heterogeneity was considered null for $I^2 = 0$, minimal for $I^2 < 25\%$, low for $I^2 < 50\%$, moderate for $I^2 < 75\%$ and high for $I^2 \geq 75\%$.

The overall diagnostic accuracy was calculated as area under the curve (AUC) on summary receiver operating characteristic (SROC) curves. Diagnostic accuracy was considered absent for $AUC \leq 0.5$, low for $0.5 < AUC \leq 0.75$, moderate for $0.75 < AUC \leq 0.9$, high for $0.9 < AUC < 0.97$ and very high for $AUC \geq 0.97$.

Diagnostic accuracy assessment was performed for each group based on the different criteria used to define PTEN loss.

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

RESULTS

Study selection and characteristics

Eighteen observational studies (10, 19–35) were included in our review. The whole process of study selection was previously reported (13). The updated

Table 1. Characteristics of the included studies

Year	First author	Country	Period of enrollment	Sample size	Precancer	Benign	Method to assess PTEN staining	Thresholds
2000	Mutter (10)	USA	Not reported	28	21	7	Intensity of staining	Negative, weak, moderate
2001	Mutter (19)	USA	1998–2000	76	35	41	Dichotomous	Any null gland
2003	Orbo (20)	Norway	1980–1991	68	39	29	Dichotomous (percentage of stained cells)	10% positive cells
2005	Baak (21)	Norway	Not reported	103	21	82	Dichotomous	Any null gland
2007	Kapucoglu (22)	Turkey	Not reported	37	10	27	Dichotomous	Complete loss
	Norimatsu (23)	Japan	1998–2005	70	38	32	Dichotomous	Any null gland
2008	Lacey (24)	USA	1970–2002	308	73	235	Dichotomous	Any null gland
	Tantbirojn (25)	Thailand	2001–2004	45	20	25	Intensity of staining	Negative, weak, moderate
2009	Abd El-Masqoud (26)	Egypt	Not reported	20	8	12	Dichotomous	Complete loss
	Sarmadi (27)	Iran	Not reported	29	8	21	Intensity of staining Percentage of stained cells	Negative, weak 10%, 50%
2010	Pavlakis (28)	Greece	Not reported	83	58	25	Dichotomous	20%
	Xiong (29)	China	2001–2006	83	24	59	Percentage of stained cells	Complete loss, 50%
2011	Pieczynska (30)	Poland	1994–2001	132	16	116	Dichotomous	Complete loss
	Rao (31)	India	2005–2007	76	13	63	Intensity of staining Number of null glands Percentage of stained cells	Negative, weak, moderate Any, 2, 3, 4, 5 or more 10%, 50%
2012	Lee (32)	S. Korea	1991–2005	42	21	21	Percentage of stained cells	5%, 95%
	Upson (33)	USA	1985–2005	112	40	72	Dichotomous	75%
2013	Huang (34)	USA	Not reported	24	22	2	Dichotomous (percentage of stained cells)	10%
2014	Shawana (35)	Pakistan	2006–2010	26	6	20	Intensity of staining	Negative, weak, moderate
Total hyperplasias		–	–	1362	473	889	–	–

research (May 2018 to November 2018) did not reveal further eligible studies.

A total of 1362 endometrial hyperplasia specimens were assessed, out of which 473 were classified as AEH/EIN and 889 were classified as BEH. Eleven studies dichotomized PTEN expression, four studies graded PTEN expression based on the percentage of stained cells and five based on the intensity of immunostaining (Table 1).

Risk of bias assessment

Results of risk of bias assessment are shown in Fig. S1.

For the 'patient selection' domain, three studies were classified at unclear risk of bias, since they followed a case-control design; all the remaining studies were considered at low risk.

For the 'index test' domain, all studies were considered at low risk of bias, since criteria used to define PTEN loss were detailed enough to be suitable for the meta-analysis.

For the 'reference standard' domain, five studies were classified at unclear risk of bias, since they did not specify if histological slides were reviewed to confirm the diagnosis of benignity or premalignancy.

For the 'flow and timing' domain, all studies were considered at low risk of bias, since in each study every specimen underwent both the same index test and the same reference standard.

Diagnostic accuracy assessment

Complete loss of PTEN expression

A complete loss of PTEN expression was used to define PTEN loss in eight studies. Pooled sensitivity and specificity were 0.41 (95% CI, 0.32–0.50) and 0.90 (95%CI, 0.86–0.93) with moderate and high heterogeneity (I^2 = 71.1% and 83.9%), respectively. Pooled LR+ and LR− were 2.62 (95% CI, 1.46–4.72) and 0.74 (95% CI, 0.59–0.94), with low and moderate heterogeneity (I^2 = 26.8% and 65.4%), respectively. Pooled DOR was 4.34 (95%CI, 1.99–9.47), with low heterogeneity (I^2 = 22.4%). The overall diagnostic accuracy was low, with an AUC of 0.71 (Fig. S2).

Presence of any PTEN-null gland

The presence of any PTEN-null gland was used to define PTEN loss in five studies. Pooled sensitivity and specificity were 0.57 (95% CI, 0.50–0.65) and 0.55 (95%CI, 0.50–0.59) with high heterogeneity (I^2 = 83.3% and 89.9%), respectively. Pooled LR+ and LR− were1.38 (95% CI, 1.18–1.60) and 0.74 (95% CI, 0.62–0.87), with minimal and moderate heterogeneity (I^2 = 18.7% and 65.4%), respectively. Pooled DOR was 2.10 (95% CI, 1.40–3.17) with minimal heterogeneity (I^2 = 7.1%). The overall diagnostic accuracy was low, with an AUC of 0.63 (Fig. S3).

Percentage of stained cells

Several thresholds of PTEN expression in terms of percentage of stained cells were used to define PTEN loss. Unfortunately, thresholds of 5%, 20%, 75% and 95% PTEN-positive cells were assessed only in one study each, not allowing a meta-analysis.

On the other hand, thresholds of expression to define PTEN loss suitable for meta-analysis were <10% (considered in four studies) and <50% (considered in three studies) of cells positive for PTEN.

Pooled sensitivity and specificity of the 10% threshold were 0.37 (95% CI, 0.26–0.48) and 0.91 (95% CI, 0.85–0.96) with low heterogeneity (I^2 = 35.7% and 36.3%). Pooled LR+ and LR− were 3.40 (95% CI, 1.69–6.88) and 0.75 (95% CI, 0.63–0.90) with null heterogeneity (I^2 = 0%), respectively. Pooled DOR was 4.75 (95% CI, 1.95–11.59) with null heterogeneity (I^2 = 0%). The

Table 2. Summary of diagnostic accuracy metrics for all criteria assessed

	Complete loss of expression	Any null gland	10% positive cells	50% positive cells	Weak-to-null expression	Moderate-to-null expression
Sensitivity	0.41 (0.32–0.50)	0.57 (0.50–0.65)	0.37 (0.26–0.48)	0.69 (0.53–0.82)	0.69 (0.57–0.80)	0.92 (0.82–0.97)
Specificity	0.90 (0.86–0.93)	0.55 (0.50–0.59)	0.91 (0.85–0.96)	0.59 (0.51–0.68)	0.76 (0.68–0.83)	0.39 (0.30–0.49)
Positive likelihood ratio	2.62 (1.46–4.72)	1.38 (1.18–1.60)	3.40 (1.69–6.88)	1.75 (1.35–2.27)	1.90 (1.39–2.59)	1.21 (0.95–1.53)
Negative likelihood ratio	0.74 (0.59–0.94)	0.74 (0.62–0.87)	0.75 (0.63–0.90)	0.53 (0.34–0.83)	0.40 (0.12–1.38)	0.38 (0.11–1.29)
Diagnostic odds ratio	4.34 (1.99–9.74)	2.10 (1.40–3.17)	4.75 (1.95–11.59)	3.71 (1.74–7.90)	6.66 (2.47–17.97)	3.57 (1.09–11.69)
Area under the curve	0.7055 (low)	0.6289 (low)	0.6427 (low)	0.7143 (low)	0.7823 (moderate)	0.6400 (low)

overall diagnostic accuracy was low, with an AUC of 0.64 (Fig. S4).

For the 50% threshold, pooled sensitivity and specificity were 0.69 (95% CI, 0.53–0.82) and 0.59 (95%CI) with minimal heterogeneity (I^2 = 12.9% and 36.3%), respectively. Pooled LR+ and LR− were 1.75 (95% CI, 1.35–2.27) and 0.53 (95% CI, 0.34–0.83) with null heterogeneity (I^2 = 0), respectively. Pooled DOR was 3.71 (95%CI, 1.74–7.90) with null heterogeneity (I^2 = 0). The overall diagnostic accuracy was low, with an AUC of 0.71 (Fig. S5).

Intensity of immunostaining

Based on the intensity of PTEN immunostaining, criteria to define PTEN loss suitable for meta-analysis were 'weak-to-null expression' (in five studies) and 'moderate-to-null expression' (in four studies).

For weak-to-null expression, pooled sensitivity and specificity were 0.69 (95% CI, 0.57–0.80) and 0.76 (95% CI, 0.68–0.83) with high heterogeneity (I^2 = 89.5% and 92.3%), respectively. LR+ and LR− were 1.90 (95% CI, 1.39–2.59) and 0.40 (95% CI, 0.12–1.38) with minimal and high heterogeneity (I^2 = 5.3% and 88.5%), respectively. Pooled DOR was 6.66 (95% CI, 2.47–17.97) with null heterogeneity (I^2 = 0%). The overall diagnostic accuracy was moderate, with an AUC of 0.78 (Fig. S6).

For moderate-to-null expression, pooled sensitivity and specificity were 0.92 (95% CI, 0.82–0.97) and 0.39 (95% CI, 0.30–0.49) with high heterogeneity (I^2 = 82.5% and 89.7%), respectively. LR+ and LR− were 1.21 (95% CI, 0.95–1.53) and 0.38 (95% CI, 0.11–1.29) with moderate and low heterogeneity (I^2 = 52.6% and 29.9%), respectively. Pooled DOR was 3.57 (95% CI, 1.09–11.69) with low heterogeneity (I^2 = 11.8%). The overall diagnostic accuracy was low, with an AUC of 0.64 (Fig. S7).

Diagnostic accuracy metrics for all immunohistochemical criteria evaluated are summarized in Table 2.

DISCUSSION

Main findings and interpretation

Among the different criteria used to define PTEN loss in AEH/EIN, only the criterion of weak-to-null expression based on the intensity of PTEN immunostaining showed moderate accuracy in the differential diagnosis between BEH and EH/EIN. A complete loss of PTEN expression, the presence of any PTEN-null gland and thresholds based on the percentage of stained cells showed low accuracy instead.

The diagnosis of endometrial hyperplasia is a well-described problem (2). Although several histologic classifications have been proposed, the reproducibility still appears suboptimal (9). The main problem is in differentiating between BEH and AEH/EIN (1, 3, 36); furthermore, some benign conditions (endometrial polyps, secretory phase, metaplastic changes) and artifactual changes may mimic AEH/EIN (3, 8, 9). For this purpose, the 2016 ESMO-ESGO-ESTRO consensus conference has recommended the use of immunohistochemistry for PTEN to recognize AEH/EIN (37); such recommendation has been confirmed in the 2017 ESGO guidelines (12). However, information about its accuracy was lacking, as well as criteria to use for its interpretation.

In our previous study, we assessed the diagnostic accuracy of immunohistochemistry for PTEN in the differential diagnosis between AEH/EIN and BEH. We found that the diagnostic accuracy was low, regardless of the histologic classification system used (13). However, we pointed out the lack of standardized criteria to interpret PTEN immunohistochemistry. In fact, different criteria to define 'PTEN loss' were proposed by several authors, ranging from a single PTEN-null gland to a wide and complete loss of expression. Furthermore, there are two crucial parameters that should be considered: the percentage of stained cells and the intensity of immunostaining.

In the current study, we aimed to define which are the optimal criteria for the interpretation of PTEN immunohistochemistry in endometrial hyperplasia. We found that six different criteria to define PTEN loss were suitable for our meta-analysis: a complete loss of expression, the presence of any null gland, two thresholds based on the percentage of stained cells (<10% and <50%) and two criteria based on the intensity of staining ('weak-to-null expression' and 'moderate-to-null expression').

Considering a complete loss of PTEN expression, we found good specificity (0.90), but very low sensitivity (0.40), with a low overall accuracy (0.71). This finding suggests that a complete loss of PTEN expression occurs in only 40% of AEH/EIN. On the other hand, a complete loss of PTEN expression might be observed in 10% of BEH, and BEH is much more common than AEH/EIN. Therefore, such criterion might often be misleading. Consistently with our results, a study by Ylmaz et al. found a loss of PTEN expression in several polyclonal endometrial specimens (38).

The presence of any null gland appeared as the least accurate criterion, with low sensitivity (0.57), low specificity (0.55) and low overall accuracy (0.63). Such results are consistent with the possibility of PTEN-null gland with benign appearance, which tend to spontaneously regress (39). Therefore, the presence of only one PTEN-null gland

should not be considered as a marker of endometrial precancer.

With regard to the percentage of stained cells, we assessed two thresholds to define PTEN loss: <10% and <50% of PTEN-positive cells. The values found for the <10% threshold were similar to those found for a complete loss (sensitivity = 0.37 vs 0.40; specificity = 0.91 vs 0.90) with low accuracy (AUC = 0.64), suggesting that the two criteria are basically superimposable. Using the <50% threshold, the sensitivity was higher and the specificity was lower, as it could be expected from a less stringent criterion; however, the overall accuracy was still low (AUC = 0.71).

The only one criterion that achieved moderate diagnostic accuracy (AUC = 0.78) was 'weak-to-null expression of PTEN', based on the intensity of immunostaining, with a sensitivity of 0.69 and a specificity of 0.76. In fact, even when most cells are PTEN-positive, the immunostaining might be much lighter than the normal strong staining, indicating an important decrease of PTEN expression. In order to establish if PTEN expression is actually deficient, such criterion would require comparison with normal endometrium as internal positive control. In fact, it has been shown than PTEN immunostaining might appear slightly even in benign endometrium (40). In that case, correlation with glandular morphology is indispensable.

Instead, a moderate intensity of PTEN immunostaining, which could be interpreted as a slight decrease of PTEN expression, did not appear as a reliable criterion to define PTEN loss, based on the low accuracy found.

Nonetheless, also the highest AUC value found slightly exceeded the cut-off to consider AUC as moderate. Several other markers have shown an accuracy higher than that of PTEN in differentiating between AEH/EIN and BEH (40, 41). Moreover, contrary to other markers, PTEN seems not to be informative about the responsiveness of endometrial hyperplasia to progestin therapy (41–45), and appears only weakly associated with the status of its encoding gene (38, 46). In this regard, the actual clinical usefulness of PTEN immunohistochemistry in endometrial hyperplasia appears limited.

As PTEN loss is a crucial and early event in endometrioid carcinogenesis (10), a possible usefulness of PTEN as a support marker and combined with other markers might be considered. However, in order to avoid misinterpretations, its immunohistochemical pattern needs to be strictly correlated to the whole histomorphologic setting, with particular regard to glandular cyto-architecture and comparison with normal endometrium.

Further studies are necessary to define whether a more elaborate scoring system of PTEN immunohistochemistry (combining together percentage of stained cells and intensity of expression) may be clinically useful, and whether PTEN may have a role in a panel of immunohistochemical markers.

Strengths and limitations

To the best of our knowledge, our study may be the first meta-analysis assessing the best criteria to define PTEN loss and their actual usefulness in differentiating BEH and AEH/EIN. We followed a thorough methodology to assess the accuracy of the different thresholds of PTEN expression.

However, some limitation to our results should be taken into account. A limitation to our index test may lie in the subjectivity of the evaluation of intensity of staining, which may be reliable only if compared to an internal positive control. Nonetheless, it has been suggested that subjective scoring of PTEN immunohistochemistry is highly reproducible (47).

On the other hand, the reliability of the reference standard may be affected by the low reproducibility of histologic criteria for differentiating between BEH and AEH/EIN (9).

Moreover, we were unable to assess the accuracy of immunohistochemical scores that combine percentage of stained cells and intensity of staining. In this regard, a marked decrease of PTEN expression in >10% of cancer cells has been used as a reliable scoring system in prostate cancer (48).

CONCLUSION

Among different criteria used to define PTEN loss in differentiating BEH and AEH/EIN., the only one that showed moderate diagnostic accuracy was 'weak-to-null expression', based on the subjective assessment of the intensity of immunostaining. Other criteria, including complete loss of expression, presence of any null gland and percentage of stained cells, showed low accuracy instead. However, even with optimized criteria, PTEN loss appears barely a moderately accurate marker of premalignant hyperplasia. Therefore, its usefulness in the common practice should be further investigated.

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CONFLICT OF INTEREST

The authors report no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Fig. S1. 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.
Fig. S2. Forest plots of individual studies and pooled sensitivity, specificity, positive and negative likelihood ratio and diagnostic odds ratio of PTEN immunohistochemical assessment in differential diagnosis between benign and premalignant endometrial hyperplasia, with SROC curve, using a complete loss of PTEN expression as positive index test.
Fig. S3. Forest plots of individual studies and pooled sensitivity, specificity, positive and negative likelihood ratio and diagnostic odds ratio of PTEN

immunohistochemical assessment in differential diagnosis between benign and premalignant endometrial hyperplasia, with SROC curve, using the presence of any PTEN-null glands as positive index test.

Fig. S4. Forest plots of individual studies and pooled sensitivity, specificity, positive and negative likelihood ratio and diagnostic odds ratio of PTEN immunohistochemical assessment in differential diagnosis between benign and premalignant endometrial hyperplasia, with SROC curve, using a percentage of PTEN-positive glands <10% as positive index test.

Fig. S5 Forest plots of individual studies and pooled sensitivity, specificity, positive and negative likelihood ratio and diagnostic odds ratio of PTEN immunohistochemical assessment in differential diagnosis between benign and premalignant endometrial hyperplasia, with SROC curve, using a percentage of PTEN-positive glands <50% as positive index test.

Fig. S6 Forest plots of individual studies and pooled sensitivity, specificity, positive and negative likelihood ratio and diagnostic odds ratio of PTEN immunohistochemical assessment in differential diagnosis between benign and premalignant endometrial hyperplasia, with SROC curve, using weak-to-null expression of PTEN as positive index test.

Fig. S7 Forest plots of individual studies and pooled sensitivity, specificity, positive and negative likelihood ratio and diagnostic odds ratio of PTEN immunohistochemical assessment in differential diagnosis between benign and premalignant endometrial hyperplasia, with SROC curve, using moderate-to-null expression of PTEN as positive index test.