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REVIEW

PTEN expression in endometrial hyperplasia and risk of cancer: a systematic review and meta-analysis

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Abstract

Purpose Rates of progression of endometrial hyperplasia (EH) to endometrial cancer (EC) are highly variable. Among several prognostic markers, PTEN has been recommended by ESMO–ESGO–ESTRO to identify premalignant EH. However, its prognostic accuracy is unclear. Thus, we aimed to assess: (1) the association between PTEN loss in EH and risk of cancer, and (2) the prognostic accuracy of PTEN immunohistochemistry in EH.

Methods Electronic databases were searched from their inception to June 2018. All studies assessing PTEN immunohistochemistry in EH and the presence of EC on subsequent hysterectomy were included. Odds ratio (OR), sensitivity, specificity, positive and negative predictive value (PPV and NPV), positive and negative likelihood ratio (LR + and LR−) and area under the curve (AUC) on SROC curves were calculated with subgroup analysis (short/long-term; atypical/non-atypical EH).

Results Nine retrospective studies assessing 933 EH were included. PTEN loss in EH was significantly associated with increased risk of EC (OR = 3.32, $p = 0.001$). The association was significant only on the short term (< 1 year) (OR = 3.45, $p = 0.002$) and in atypical EH (OR = 1.89, $p = 0.01$). For overall analysis and short-term/atypical EH subgroup the prognostic accuracy was low, with sensitivity = 0.58 and 0.68, specificity = 0.60 and 0.48, VPp = 0.41 and 0.54, VPN = 0.75 and 0.63, LR + = 1.80 and 1.37, LR − = 0.62 and 0.56, AUC = 0.687 and 0.721, respectively.

Conclusion PTEN loss in EH is a risk factor for EC, but is not reliable in predicting the risk of EC. In atypical EH, PTEN loss is associated with a risk of concurrent EC of over 50%. This information might integrate the patients' informed consent for the choice of treatment (conservative/hysterectomy), especially in borderline cases. In conservative approach, PTEN loss might suggest closer follow-up.

Keywords EIN · Endometrial intraepithelial neoplasia · Endometrioid adenocarcinoma · Immunohistochemical · Prognosis · Tumor suppressor protein phosphatase · Tensin homolog

Introduction

Endometrial hyperplasia (EH) is an irregular proliferation of endometrial glands [1]. EH is involved in the development of endometrial cancer (EC) of endometrioid type [2], which is the most common gynecologic malignancy in the Western World [3]. Since EH can be a precancerous lesion as well as a polyclonal proliferation caused by unopposed action of estrogens [4, 5], its malignant potential is highly variable, with rates of progression to EC ranging from less than 1% to over 40% [6–9]. To choose an appropriate treatment a differential diagnosis between benign and premalignant EH is needed. In particular, benign EH requires only observation or progestins when symptomatic, while premalignant EH

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requires hysterectomy or progestins for conservative approach in selected cases [4, 10].

The World Health Organization (WHO) 2014 classification system differentiates atypical EH (premalignant) from EH without atypia (benign) based on the presence of cytologic atypia [1, 11]. Previous WHO systems had also considered the complexity of glandular architecture for EH classification, although its impact on the malignant potential was not well defined [5, 6, 11].

However, WHO system does not perfectly reflect the risk of progression to cancer. In fact, only a minority of atypical EH progress to EC (8.2–27.5%), while a little percentage of EH without atypia still progress (1.2–4.6%) [8]. Furthermore, WHO criteria have shown problems of reproducibility, with endometrial specimens often showing ambiguous features [5, 12]. The endometrial intraepithelial neoplasia (EIN) system is an alternative classification system based on nuclei diameters, glandular perimeter and gland to stroma ratio [5, 9]. These parameters can be assessed objectively through a computerized analysis calculating a prognostic score (D-score) and providing a reliable risk stratification [5, 9, 11]. Nonetheless, D-score was not widely applicable in the common practice due to the cost of a morphometry workstation [5, 13].

For these reasons, there has been great interest in the literature about cheaper prognostic markers in EH [11]. The tumor suppressor protein *phosphatase and tensin homolog* (PTEN) has probably been the most studied marker, since PTEN gene is the most commonly mutated in endometrial carcinogenesis [14].

In the 2016 ESMO-ESGO-ESTRO Consensus Conference on Endometrial Cancer, the immunohistochemical assessment of PTEN has been recommended to recognize premalignant EH, which often show a loss of expression of the protein [15]. Nonetheless, in our previous study we found that the accuracy of PTEN immunohistochemistry was low [16]. Such study was based on the histologic classification of EH into benign or premalignant. However, despite being the gold standard, histology might not reflect the actual rates of progression to EC, as discussed above.

Thus, the aim of this study was to analyse the prognostic value of immunohistochemical assessment of PTEN expression in EH, by assessing the actual risk of progression to cancer. In this regard, we analysed first the association between PTEN loss and risk of progression to EC, and then the prognostic accuracy of PTEN assessment in predicting progression to EC.

Materials and methods

Study protocol

This study was performed following a recommended protocol for systematic review and meta-analysis. The study protocol defining methods for collection, extraction and analysis of data, including subgroups analyses, was designed a priori. All review stages were conducted independently by two reviewers (AR, AT), who independently assessed electronic search, eligibility of the studies, inclusion criteria, risk of bias, data extraction and data analysis. Disagreements were resolved by discussing with a third reviewer (GS).

The study was reported following the Preferred Reporting Item for Systematic Reviews and Meta-analyses (PRISMA) statement [17].

Search strategy

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

Study selection

We included all peer-reviewed, retrospective or prospective studies assessing the association between immunohistochemical expression of PTEN in EH on endometrial biopsy and the presence of EC on a subsequent hysterectomy.

Exclusion criteria were:

- data regarding PTEN expression not extractable;
- case reports and reviews;
- overlapping patient data with a study already included;

Risk of bias assessment

The risk of bias was assessed according to the revised Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) [18]. Four domains related to risk of bias were assessed in each study: (1) patient selection (low risk if the

patients were consecutive or randomly selected from a consecutive series), (2) index test (low risk if the assessment of PTEN expression was made according to complete and clearly defined criteria), (3) reference test (low risk if the diagnosis of endometrial cancer was not affected by biases, such as cancer on biopsy not confirmed at hysterectomy), (4) flow and timing (low risk if the latency time between EH biopsy and cancer diagnosis did not introduce a bias, such as confusion between coexistent and subsequent cancer). Review authors' judgments were categorized as "low risk," "high risk" or "unclear risk of bias." Concerns about applicability of the domains 1, 2 and 3 (if methods of the included do not suit the aim of our review, regardless of their correctness) were also assessed and categorized as "low concerns," "high concerns" or "unclear concerns" about applicability.

Data extraction

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

- PTEN expression on the index endometrial biopsy ("loss" or "presence");
- subsequent diagnosis of EC at histologic examination of endometrial specimen ("cancer" or "no cancer").

If discrepancies between values reported in the text and the tables were found, values from tables were used for the analysis.

In the analysis of prognostic accuracy, PTEN expression on the index endometrial biopsy was considered the index test, and subsequent diagnosis of EC was considered the reference test. The combination "PTEN loss" with "cancer" was considered as true positive, "PTEN presence" with "no cancer" as true negative, "PTEN presence" with "cancer" as false negative, "PTEN loss" with "no cancer" as false positive.

The association of PTEN loss with cancer and the prognostic accuracy of PTEN immunohistochemistry for the risk of cancer were the two primary outcomes of our study.

Moreover, data were also subdivided according to two different criteria, defined a priori:

- on the basis of the latency time between index and reference test, the studies were subdivided into two "latency time subgroups": "short term subgroup" (mean latency time < 1 year) and "long term subgroup" (mean latency time ≥ 1 year); in fact, several authors highlighted that in case of a latency time < 1 year the cancer should be considered as "concurrent" rather than "subsequent", because of the typically slow growth of EC [9, 19];
- on the basis on the WHO classification, EH was subdivided into two "WHO subgroups": "EH without atypia subgroup" and "atypical EH subgroup".

Moreover, data were subdivided into four combined subgroups, based on the combination of latency time and WHO classification: (1) short term and EH without atypia; (2) short term and atypical EH; (3) long term and EH without atypia; (4) long term and atypical EH.

Data analysis

PTEN loss and risk of cancer

The association between PTEN loss and risk of cancer was assessed using odds ratio (OR); a p value < 0.05 indicated a significant impact on the risk. OR was calculated for each study and as pooled estimate and reported graphically on forest plot, with 95% confidence interval (CI). OR was also assessed separately in the different subgroups, and results were compared using χ^2 test with significant p value < 0.05.

Statistical heterogeneity among studies was assessed using the inconsistency index I^2: heterogeneity was considered insignificant for $I^2 < 25\%$, low for $I^2 < 50\%$, moderate for $I^2 < 75\%$ and high for $I^2 \geq 75\%$. In case of $I^2 < 50\%$, the fixed-effect model of Mantel–Haenszel was used; otherwise, the random effect model of DerSimonian–Laird was adopted.

Pre- and post-test probabilities of cancer were reported graphically using a Fagan's nomogram.

Review manager 5.3 (Copenhagen: The Nordic Cochrane Centre, Cochrane Collaboration, 2014) was used for the analysis.

Prognostic accuracy

The prognostic accuracy was assessed as sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPP), positive likelihood ratio (LR+) and negative likelihood ratio (LR−). The prognostic usefulness was assessed as "area under the curve" (AUC) on summary receiver operating characteristic (SROC) curves, and was considered absent for AUC ≤ 0.5, low for $0.5 < \text{AUC} \leq 0.75$, moderate for $0.75 < \text{AUC} \leq 0.9$, high for $0.9 < \text{AUC} < 0.97$, very high for AUC ≥ 0.97.

Sensitivity, specificity, PPV, NPV, LR+ and LR− were assessed for each study and as pooled estimate and reported graphically on forest plots, with 95% confidence interval (CI).

The random effect model of DerSimonian–Laird was planned a priori, since an actual heterogeneity is expected for meta-analyses of prognostic accuracy [20].

In the subgroup analysis, the prognostic accuracy was assessed only for combined subgroups, to contextualize the clinical usefulness of PTEN immunohistochemistry, and only in those showing a significant OR. For the subgroups in which OR was found non-significant, the possibility of a prognostic usefulness was excluded a priori.

Meta-DiSc version 1.4 (Clinical Biostatistics Unit, Ramon y Cajal Hospital, Madrid, Spain) was used for the analysis.

Results

Study selection

Nine retrospective studies [19, 21–28] with a total sample size of 933 EH were included. The process of study selection is reported in detail in Fig. 1.

Characteristics of the studies

Six studies had a retrospective cohort design, while three were case–control studies. Five studies adopted WHO classification system; two studies categorized EH specimens based on a computerized morphometric analysis (D-score); one study used the subjective EIN classification; the

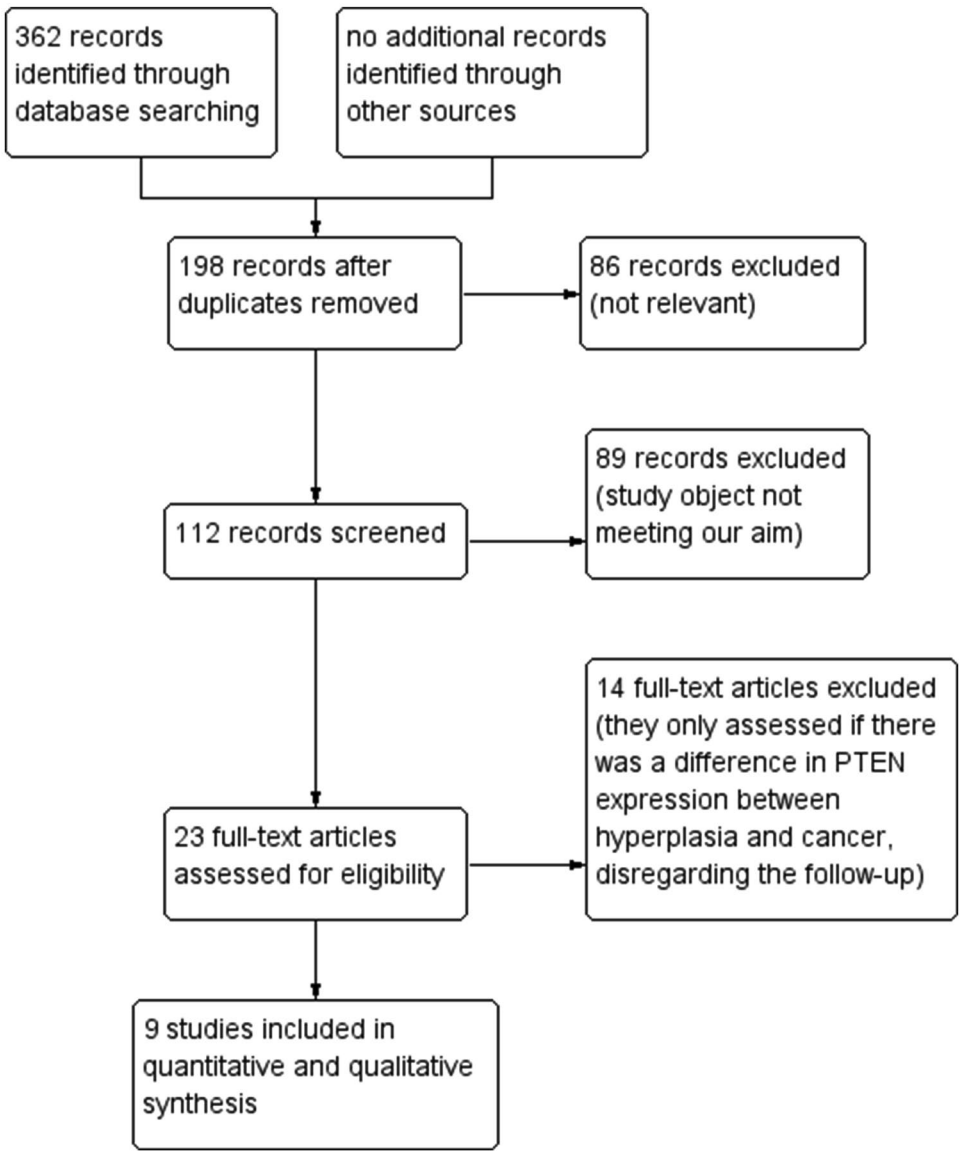

Fig. 1 Flow diagram of studies identified in the systematic review [Prisma template (preferred reporting item for systematic reviews and meta-analyses)]

remaining study adopted all three systems. Fifty-two point three percent of EH were classified as benign and 47.7% as precancerous.

Eight of nine studies dichotomized PTEN expression into positive and negative in the results; the remaining one graded PTEN expression as "low", "intermediate" and "high", where "low" denoted negative staining [27].

PTEN expression was assessed in EH glands, while the surrounding endometrial stroma was used as internal positive control.

PTEN loss was observed in 173 of 298 (58.1%) EH with progression to EC and in 252 of 635 (39.7%) EH without progression to EC.

Details are shown in Table 1.

Risk of bias assessment

Results of risk of bias assessment are shown in Fig. 2.

For the "patient selection" domain, three studies were categorized at low risk of bias, due to the inclusion of consecutive patients; the others were categorized at unclear risk. Concerns about the applicability of this domain were considered unclear for one study, since it assessed only atypical EH with metaplastic surface changes.

For the "index test" domain, three studies were categorized at low risk of bias, because they specified in detail the criteria to define PTEN loss; the others were considered at unclear risk.

For the "reference test" domain, one study was considered at unclear risk, since some diagnoses of EC were made at histologic examination of dilatation and curettage specimens, and it is not specified if the diagnosis was confirmed on hysterectomy specimen. For the other eight studies, no particular risks of bias were highlighted, thus all studies were considered at low risk.

For the "flow and timing" domain, three studies were categorized at unclear risk of bias: one of them did not clearly differentiate the results based on the latency times (< or > 1 year), the other two did not specify the latency time between index and reference test (Table 1). The remaining studies were considered at low risk.

No study was considered at high risk of bias. No further applicability concerns were found.

PTEN loss and risk of cancer

Overall results

PTEN loss in EH was significantly associated with increased risk of EC, with an overall OR of 3.32 (95% CI 1.59–6.97, $p=0.001$) and high heterogeneity among studies ($I^2=75\%$). Results of OR analysis are reported graphically in Fig. 3a.

Latency time subgroups

In the subgroup analysis based on latency time, five studies assessing 396 EH were included in the short-term subgroup, and four studies assessing 537 EH were included in the long-term subgroup. In the short-term subgroup, the association of PTEN loss with EC risk was stronger, with an OR of 3.45 (95% CI 1.59–7.50, $p=0.002$) and moderate heterogeneity ($I^2=60\%$). On the other hand, in the long latency subgroup the association was weaker and not statistically significant, with an OR of 3.37 (95% CI 0.70–16.33, $p=0.13$) and high heterogeneity ($I^2=78\%$) (Fig. 3a).

WHO subgroups

The subgroup analysis based on WHO classification included 260 EH without atypia and 199 atypical EH. The "EH without atypia subgroup" showed no significant association between PTEN loss and EC risk, with an OR of 0.75 (95% CI 0.44–1.29, $p=0.30$ and insignificant heterogeneity ($I^2=11\%$). For the "atypical EH subgroup", the association was significant, with an OR of 1.89 (95% CI 1.16–3.06, $p=0.01$) and low heterogeneity ($I^2=38\%$) (Fig. 3b).

Combined subgroups

Among the four combined subgroups, only the one considering "short term" and "atypical EH" (subgroup 2; $N=208$), showed significant association, with an OR of 2.51 (95% CI 1.36–4.61, $p=0.003$) and with low heterogeneity ($I^2=46\%$) (Fig. 3c).

Prognostic accuracy

Overall results

Pooled sensitivity and specificity of PTEN loss in predicting EC were 0.58 (95% CI 0.52–0.64) and 0.60 (95% CI 0.56–0.64), respectively. Pooled PPV and NPV were 0.41 (95% CI 0.36–0.46) and 0.75 (95% CI 0.71–0.79), respectively. Pooled LR + and LR− were 1.80 (95% CI 1.26–2.56) and 0.62 (95% CI 0.45–0.86), respectively. The heterogeneity was moderate for sensitivity ($I^2=67.8\%$), high for specificity ($I^2=89.3\%$), PPV ($I^2=82.8\%$), NPV ($I^2=91.1$) and LR + ($I^2=83\%$), and moderate for LR− ($I^2=66.3\%$) (Fig. 4a). The SROC curves analysis demonstrated low overall prognostic accuracy with an AUC of 0.687 (Fig. 4b).

Short term/atypical EH subgroup

In the combined subgroup, pooled estimates showed sensitivity of 0.68 (95% CI 0.58–0.77), specificity of 0.48 (95% CI 0.39–0.59), PPV of 0.54 (95% CI 0.45–0.63), NPV of

Table 1 Characteristics of the included studies

Year	Study	Country	Study design	Period of enrollment	Sample size	Age (mean)	Sampling method	System adopted	Diagnosed as		Latency time (mean)	Cancer		No cancer	
									Benign	Precancer		Total	PTEN loss	Total	PTEN loss
2003	Orbo [21]	Norway	Case–control	1980–1991	68	28–77 (48.2)	Curettage	D-score	42.6%	57.4%	3–39 months (6.6)	18	10 (55.6%)	50	4 (8%)
2005	Baak [22]	Norway	Retrospective cohort	n.r.	103	29–71 (50.3)	Curettage, aspiration, Pipelle	D-score	79.6%	20.4%	12–154 months (50)	7	7 (100%)	96	36 (37.5%)
2008	Lacey [19]	USA	Case–control	1970–2002	308	(51.7)	n.r.	WHO	76.3%	23.7%	≥ 12 months (78)	115	51 (44.3%)	193	95 (49.2%)
2009	Quddus [23]	USA	Retrospective cohort	n.r.	18	n.r.	Hysteroscopy, curettage	WHO	0%	100%	21–84 months (54)	9	6 (66,7%)	9	4 (44.4%)
2010	Pavlakis [25]	Greece	Retrospective cohort	n.r.	83	35–67	Curettage	WHO	30.1%	69.9%	≤ 12 weeks	33	25 (75.8%)	50	28 (56%)
2011	Steinbakk [24]	Norway	Retrospective cohort	1980–204	106	21–88 (53)	Curettage	WHO, EIN** D-score	92.1%*	7.9%*	12–283 months (57)	9	6 (66.7%)	99	20 (20.2%)
2012	Robbe [26]	Belgium, Netherlands	Retrospective cohort	1999–2006	39	34–90	Pipelle, curettage	WHO	0%	100%	0.5–9 months (1.4)	25	14 (56%)	14	1 (7.1%)
2015	Berg [27]	Norway	Case–control	2001–2013	111	n.r.	n.r.	WHO	0%	100%	n.r.***	42	30 (71.4%)	69	41 (59.4%)
2018	Vierkoetter [28]	USA	Retrospective cohort	2009–2014	95	n.r.	Hysteroscopy, curettage	EIN**	0%	100%	n.r.****	40	24 (60%)	55	23 (41.8%)
Total					933	–	–	–	52.3%	47.7%	–	298	173 (58.1%)	635	252 (39.7%)

Latency time: time from index EH biopsy to cancer on hysterectomy

n.r. not reported

*Rates of precancer and benign refer to WHO classification

**'EIN' refers to the subjective EIN system, while D-score is the objective EIN system [5]

***In the paper the authors stated that index biopsies were performed in the preoperative phase

****In the paper the diagnosis of cancer was made alternately on hysterectomy specimen (for women undergone surgery as primary treatment). or on dilatation and curettage specimen after the first cycle of progestin treatment

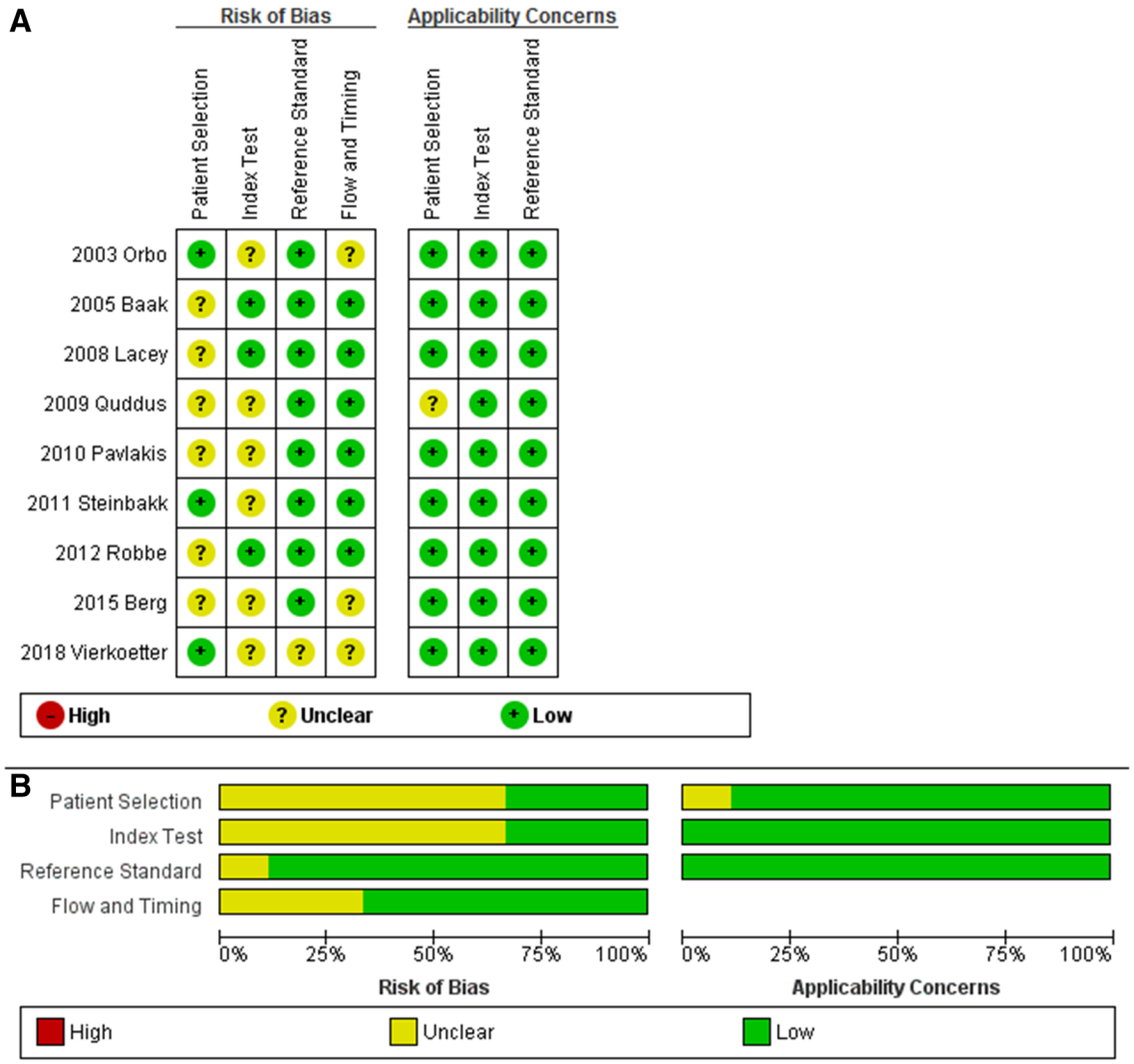

Fig. 2 **a** Assessment of risk of bias. Summary of risk of bias for each study; Plus sign: low risk of bias; minus sign: high risk of bias; question mark: unclear risk of bias. **b** Risk of bias graph about each risk of bias item presented as percentages across all included studies

0.63 (95% CI 0.52–0.73), LR + of 1.37 (95% CI 0.89–2.12) and LR− of 0.56 (95% CI 0.41–0.77). The heterogeneity was high for specificity ($I^2 = 86.5$) and PPV ($I^2 = 87.5\%$), moderate for LR + ($I^2 = 56.4\%$), insignificant for sensitivity ($I^2 = 15.4\%$), and absent for NPV and LR− ($I^2 = 0\%$) (Fig. 5a). The SROC curves analysis demonstrated low overall prognostic accuracy with an AUC of 0.721 (Fig. 5b).

Discussion

Main findings and interpretations

Overall results

According to our results, the loss of PTEN expression in EH is significantly associated with increased risk of EC. These findings might be expected, since PTEN mutation is known to be involved in endometrial carcinogenesis. Among the four molecular categories of EC identified by the Cancer Genome Atlas Research Network (*'ultramutated', 'hypermutated' and 'copy number low'*, which are predominantly endometrioid, and *'copy number high',* most of which are serous), PTEN mutations were found in 94%, 88%, 77% and 15%, respectively [14]. Furthermore, several studies

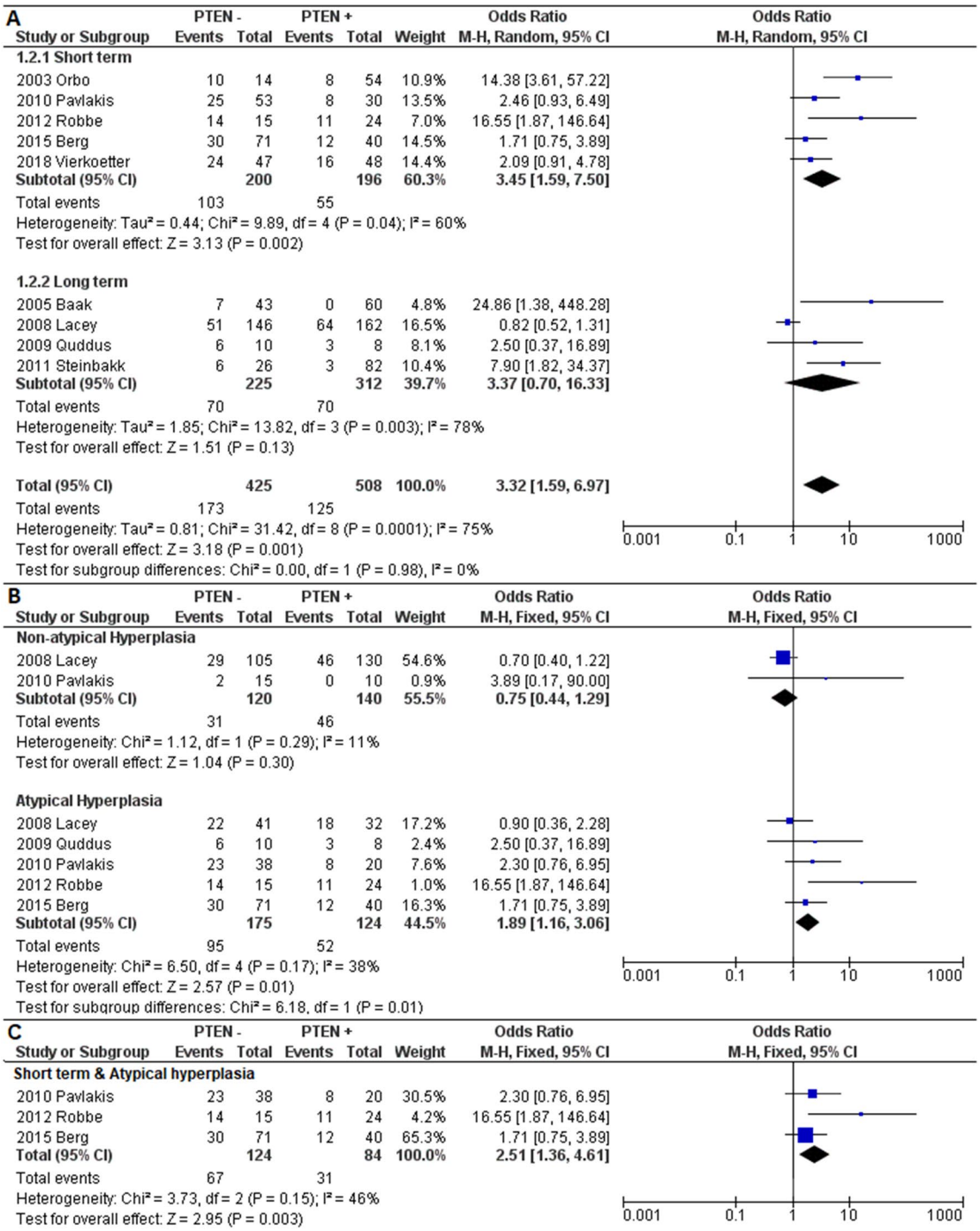

Fig. 3 Forest plot of individual studies and pooled odds ratio for risk of cancer, with subgroups analyses based on the latency time between index biopsy and cancer diagnosis (**a**) and the presence of cytologic atypia in the index biopsy (**b**). In (**c**) the analysis of the selected combined subgroup of 'short term' and 'atypical hyperplasia' is reported

reported a higher rate of PTEN loss in EC compared to EH [29–31], suggesting a prognostic significance of PTEN. In spite of this, we found a low prognostic usefulness of PTEN assessment in predicting EC, with sensitivity and specificity of 0.58 and 0.60, respectively, considerably lower than the values reported by Baak et al. for WHO system (0.67 and 0.76, respectively) [9]. Our results confirm those of our previous study [16], indicating that PTEN evaluation is not a

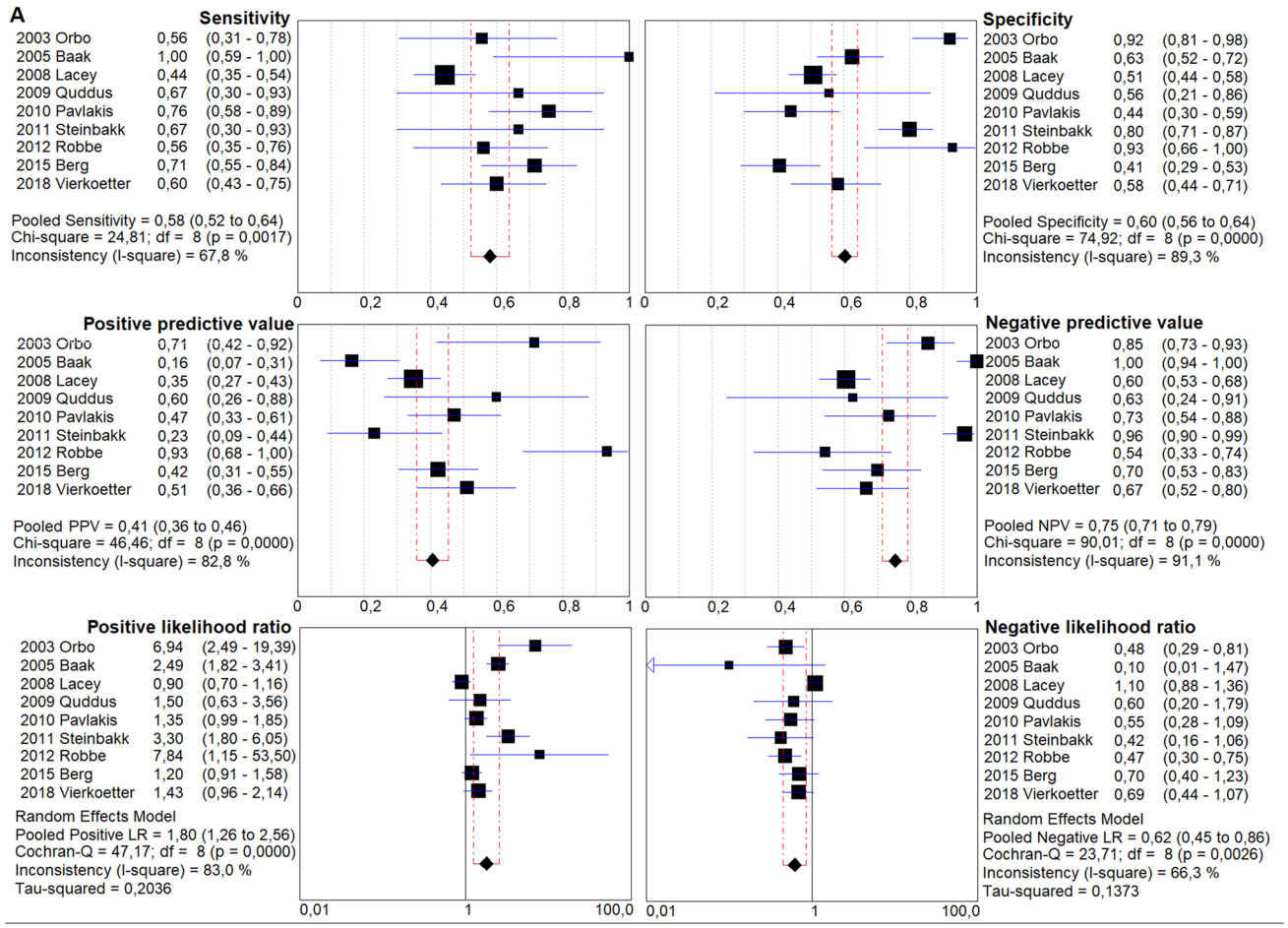

Fig. 4 Overall analysis for prognostic accuracy of PTEN immunohistochemical status. Forest plots of individual studies and pooled sensitivity, specificity, positive and negative predictive value, positive and negative likelihood ratio (**a**), with SROC curves (**b**)

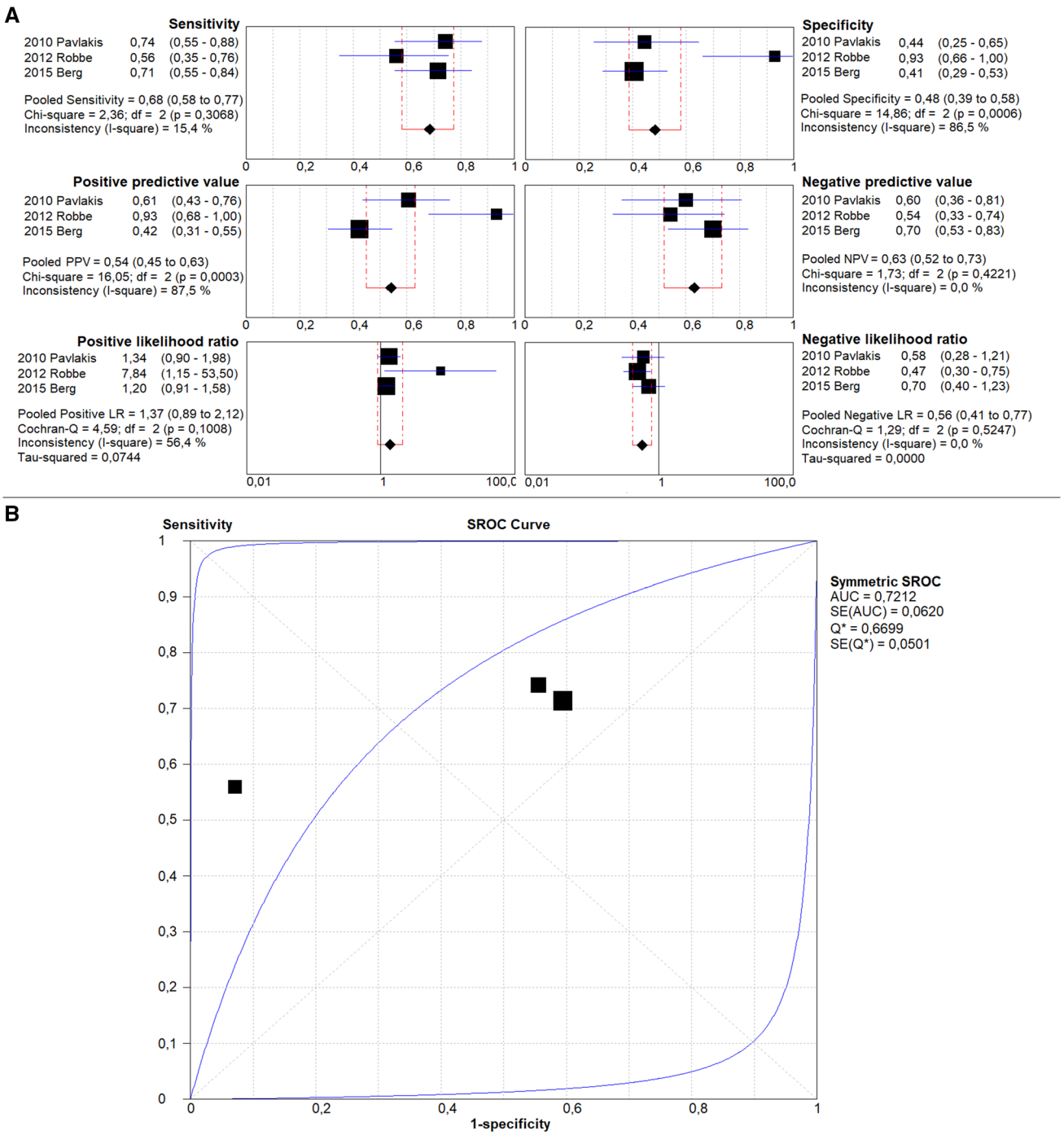

Fig. 5 Analysis of prognostic accuracy of PTEN immunohistochemical status in the selected subgroup of atypical hyperplasia with a short follow-up (< 1 year). Forest plots of individual studies and pooled sensitivity, specificity, positive and negative predictive value, positive and negative likelihood ratio (**a**), with SROC curves (**b**)

reliable tool in differentiating benign and premalignant EH. Thus, it appears inadequate to replace WHO histomorphologic criteria in selecting patients who need to be treated. For this reason, we do not recommend the indiscriminate use of PTEN immunohistochemistry in the diagnostic algorithm of EH. However, it should be remarked that PTEN is not the only molecule involved in endometrial carcinogenesis. In two included articles, mismatch repair proteins appeared associated with the risk of EC more strongly than PTEN [21, 28]. One of these papers also suggested to use a combination of several markers to better stratify the risk [28]. In this regard, many other proteins, such as PAX2, Bcl-2, β-catenin

and ARID1A [32–36], might have a role in improving the risk stratification in EH. Further studies are necessary in this field.

Latency time and WHO criteria-weighted results

We found that the association of PTEN loss with risk of EC was significant only when a short follow-up (< 1 year) was considered. Several authors pointed out that an EC diagnosed within 1 year after EH diagnosis should be considered as already present at the time of EH biopsy, due to the typically slow growth of EC [9, 19]. Thus, our results suggest that PTEN loss in EH predicts the presence of a coexistent EC rather than the progression of EH to EC. The reason could be that PTEN status does not affect the responsiveness of EH and EC to progestins [37], resulting in similar outcomes between PTEN-null and PTEN-positive specimens on the long term in treated patients.

In the analysis of WHO subgroups, the association of PTEN loss with cancer risk was significant only in atypical EH, while it was absent in EH without atypia. A possible explanation might be that the loss of PTEN expression alone is not enough to activate malignant transformation. In fact, Mutter et al. showed that endometrial glands with loss of PTEN at immunohistochemistry, but normal histomorphology, tended to spontaneously regress in most cases [37]. On the other hand, atypical EH have several genetic alterations [38], which might determine the progression to EC when combined with PTEN loss. Moreover, our results are also in accordance with Pavlakis et al., who reported that the combination of cytologic atypia and PTEN loss predicted coexistent cancer better than cytologic atypia alone [24].

Given these observations, the question is how PTEN evaluation may influence the management of patients with atypical EH, with regard to the risk of concurrent cancer. Even in this subset of patients (short term and atypical EH), the prognostic accuracy was low (AUC = 0.721), with suboptimal sensitivity (0.68) and very low specificity (0.48). Instead, the identification of women at risk of cancer would require a good sensitivity, to avoid progression to cancer in untreated patients. At the same time, a high specificity would also be needed to avoid a severe overtreatment such as hysterectomy. Thus, even for atypical EH, PTEN evaluation can be indiscriminately used in the diagnostic-therapeutic algorithm.

Despite the low prognostic accuracy, we found a PPV of 0.54 (Fig. 5), indicating a risk of concurrent EC of over 50% (Fig. 6). Since EC conservatively treated has shown lower regression and higher recurrence rates compared to atypical EH [39], it may be crucial to obtain this information in the decision-making between conservative treatment or hysterectomy, especially in borderline cases, such as age older than 40 years, pluriparity, wish to get pregnant not in the short term, low couple fertility potential. In these conditions, a finding of PTEN loss may integrate the informed consent, making the patient aware that it is more likely that a cancer is already present than not. However, if a conservative approach is still chosen, PTEN loss may indicate the need for a closer and more careful follow-up.

Strength and limitations

To the best of our knowledge, this is the first meta-analysis assessing PTEN as a prognostic marker of cancer progression in EH. Since data in this field are based only on retrospective studies with unclear results, this meta-analysis may considerably increase the strength of evidence.

However, the retrospective design of the studies may itself limit the significance of the results found.

The first limitation for our findings may be the high heterogeneity among the included studies, especially about the sample size. In fact, the greatest study included 308 EH, while the smaller one only 18. Since the number of included studies is not large ($N = 9$), such a limitation might affect in particular the subgroups analysis. Differences in the patient management may also be a limitation, as different treatments may lead to different outcomes [40–43]. Power calculation was not performed given the study design of our review.

The inter-observer variability in the interpretation of PTEN immunostaining might constitute a minor limitation for our study. In fact, Allison et al. pointed out that the several authors adopted different criteria to define PTEN loss [44]. However, even in the absence of a validated method for grading PTEN expression, Garg et al. showed that a solely qualitative scoring of PTEN immunohistochemistry in EC into positive, negative and heterogeneous, was highly reproducible [45]. In our previous study, we showed that a subjective interpretation of PTEN immunohistochemistry in EH was even more accurate than an objective, quantitative scoring [46].

Conclusion

The loss of PTEN immunohistochemical expression in EH is significantly associated with increased overall risk of EC. However, its prognostic accuracy is too low to replace histomorphologic criteria.

In the subset of patients with atypical EH, PTEN loss is associated with a risk of coexistent cancer of over 50%. This information might integrate the patients' informed consent for the choice of treatment (conservative or hysterectomy), and might be crucial in borderline cases, such as age older than 40 years, pluriparity, wish to get pregnant not in the short term, low couple fertility potential. Finally,

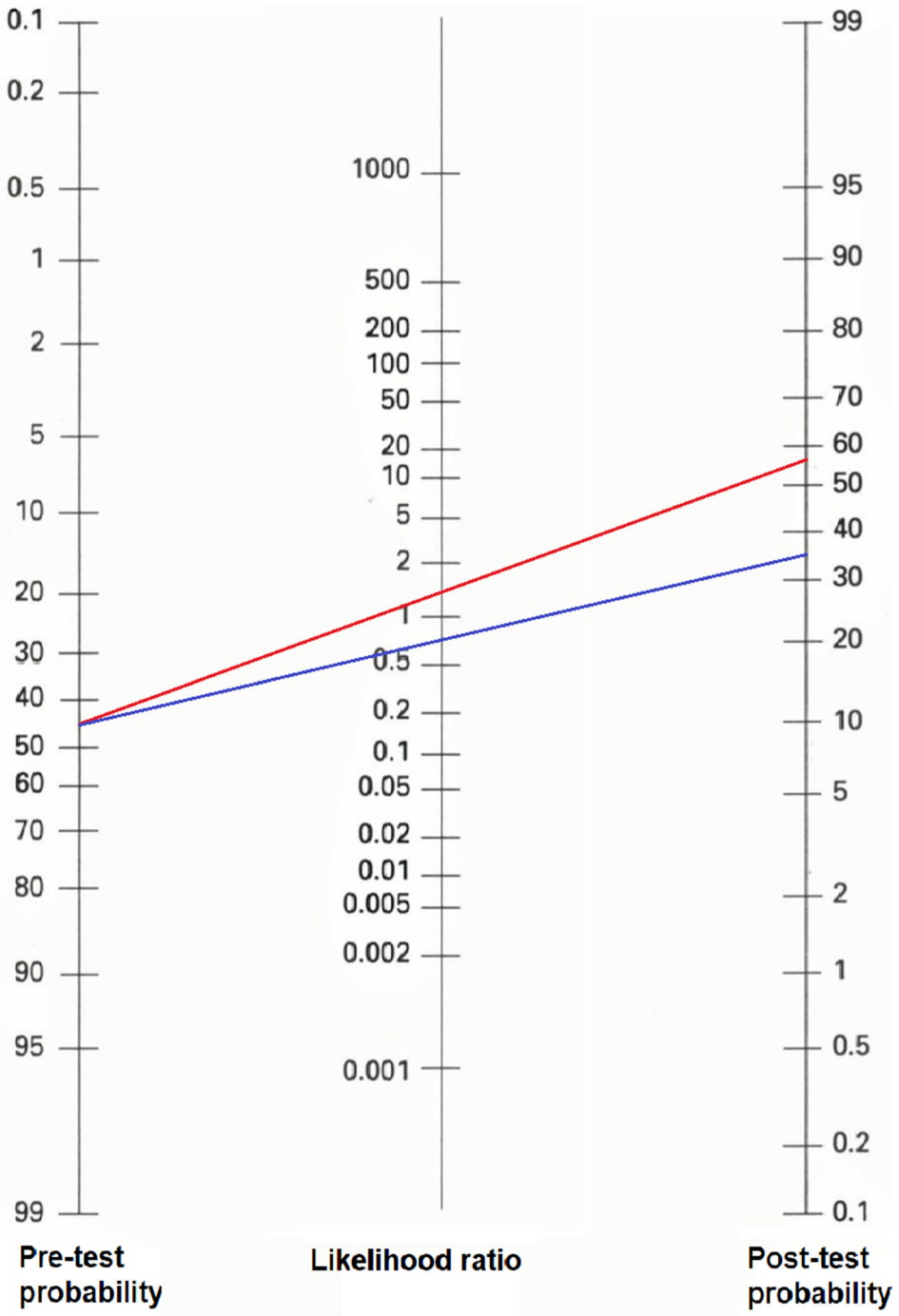

Fig. 6 Fagan's nomogram comparing pre- and post-test probabilities of cancer

if a conservative approach is still chosen, PTEN loss might indicate the need for a closer and more careful follow-up.

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Compliance with ethical standards

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