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SYSTEMATIC REVIEW

PAX2 in endometrial carcinogenesis and in differential diagnosis of endometrial hyperplasia: A systematic review and meta-analysis of diagnostic accuracy

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Abstract

Introduction: Benign and precancerous endometrial hyperplasias (EH) are differentiated according to two alternative histomorphologic classifications: World Health Organization (WHO) or endometrial intraepithelial neoplasia (EIN) system. The 2017 European Society of Gynaecological Oncology guidelines recommend paired box 2 protein (PAX2) immunohistochemistry to identify precancerous EH. However, methods for interpreting immunostaining and diagnostic accuracy are not defined, and the role of PAX2 in endometrial carcinogenesis is unclear. We aimed to assess: (a) PAX2 expression throughout endometrial carcinogenesis, from normal endometrium to benign EH, precancerous EH, and endometrial cancer (EC); (b) the diagnostic accuracy of PAX2 immunohistochemistry in diagnosing precancerous EH, defining criteria for its use.

Material and methods: Electronic databases were searched for from their inception to July 2018. All studies evaluating PAX2 immunohistochemistry in normal endometrium, EH, and EC were included. Univariate comparisons of PAX2 expression were performed with Fisher's exact test (significant $P < .05$). Sensitivity, specificity, positive and negative likelihood ratio, diagnostic odds ratio (DOR), and area under the curve on summary receiver operating characteristic curves were calculated. Subgroup analyses were based on expression thresholds (decrease vs. complete loss) and classifications used (WHO vs. EIN).

Results: Six studies with 266 normal endometrium, 586 EH, and 114 EC were included. Both decrease and complete loss of PAX2 expression were significantly more common in EC and precancerous EH than benign EH. Diagnostic accuracy was moderate for both PAX2 complete loss and decrease (areas under the curve 0.829 and 0.876, respectively). PAX2 complete loss with EIN system showed the best results (sensitivity = 0.72; specificity = 0.95; DOR = 43.13).

Conclusions: PAX2 seems to behave as a tumor suppressor in endometrial carcinogenesis. PAX2 is an accurate marker of precancerous EH; complete loss of PAX2 and EIN classification appear as the optimal diagnostic criteria.

Abbreviations: AUC, area under the curve; DOR, diagnostic odds ratio; EC, endometrial cancer; EH, endometrial hyperplasia; EIN, endometrial intraepithelial neoplasia; ESGO, European Society of Gynaecological Oncology; LR+, positive likelihood ratio; LR−, negative likelihood ratio; NE, normal endometrium; PAX2, paired box 2 protein; SROC, summary receiver operating characteristic; WHO, World Health Organization.

KEYWORDS
biomarker, cancer precursor, endometrial hyperplasia, endometrial intraepithelial neoplasia, endometrioid adenocarcinoma, paired box 2 protein

1 | INTRODUCTION

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

These two conditions are managed in different ways: benign EH with observation alone, or with progestins if symptomatic; premalignant EH with a total hysterectomy, or a conservative progestin-based treatment (with or without hysteroscopic resection) and close follow up in designated cases.[4-6] Thus, it is fundamental to differentiate these two processes.

In this regard two classification systems have been proposed: the World Health Organization (WHO) system and the endometrial intraepithelial neoplasia (EIN) system.[2,3]

The WHO system categorizes EH into "EH without atypia" (benign) and "EH with atypia" (premalignant) based on the presence of cytologic atypia.[1,2] The former WHO classification also classified EH based on architectural complexity in "simple EH" and "complex EH."[2,3] WHO classification is recommended by the Royal College of Obstetricians and Gynaecologists.[4]

The EIN system categorizes EH into "benign EH" and "endometrial intraepithelial neoplasia," based on a combination of histomorphologic criteria.[2,3] EIN classification is recommended by the American College of Obstetricians and Gynecologists.[5]

On the other hand, the revised 2014 WHO classification has used "atypical EH" and "endometrial intraepithelial neoplasia" as synonyms.[1]

Recently, a novel integration of both classification systems has been proposed in order to stratify the risk of coexistent cancer in premalignant EH. This novel classification categorized EH into three categories: benign EH, EIN without cytologic atypia (at lower risk), and EIN with cytologic atypia (at higher risk).[7]

To date, histologic examination is the reference standard in differential diagnosis between benign and premalignant EH. Nonetheless, this method is characterized by poor inter- and intra-observer reproducibility. Unclear features or tissue paucity—may cause additional problems in diagnostics.[3,8]

In this regard, several markers have been proposed to increase the reliability of the differential diagnosis.[9] In particular, 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 evaluation of paired box 2 protein (PAX2) has been recommended to distinguish premalignant EH from benign mimics.[10] Nevertheless, it is not specified if PAX2 nuclear expression should be assessed routinely or in selected cases, and in terms of complete loss or only decrease of expression.

Key message

PAX2 loss is an accurate diagnostic marker of endometrial precancer. A complete loss of PAX2 and the EIN classification appear as the optimal diagnostic criteria.

In the literature, the changes of PAX2 immunohistochemical expression from NE to simple and complex EH (with and without atypia), EIN, and endometrial cancer (EC) are not well defined. Furthermore, it is unclear if the *PAX2* gene acts as an oncogene or a tumor suppressor in endometrial carcinogenesis. No systematic review and/or meta-analysis analyzed the diagnostic accuracy of PAX2 immunohistochemical assessment in the differential diagnosis between benign and premalignant EH.

Objectives of our study were:

1. to determine the behavior of PAX2 in endometrial carcinogenesis, by assessing the differences in PAX2 expression among the above-mentioned histologic categories;
2. to determine the diagnostic accuracy of PAX2 immunohistochemistry in differential diagnosis between benign and precancerous EH, defining the optimal criteria for the interpretation of PAX2 immunostaining; for this purpose, we planned to assess how the accuracy changes according to the index test criteria (PAX2 complete loss or even only decrease of expression) and reference standard criteria (WHO or EIN system).

2 | MATERIAL AND METHODS

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

The study was reported according to the Preferred Reporting Item for Systematic reviews and Meta-Analyses (PRISMA) statement[11] and the Synthesizing Evidence from Diagnostic Accuracy TEsts (SEDATE) guideline.[12]

Several searches were conducted using EMBASE, OVID, MEDLINE, Scopus, Web of Sciences, Cochrane Library, ClinicalTrial.gov, and Google Scholar as electronic databases. The relevant articles were searched from the inception of each database to July 2018, by using a combination of the following text words and all their synonyms found in the Medical SubHeading (MeSH) vocabulary: "*PAX2*"; "*PAX-2*"; "Paired box gene 2"; "marker"; "biomarker"; "diagnosis"; "immunohistochemical"; "immunohistochemistry"; "endometrial hyperplasia"; "endometrial intraepithelial neoplasia"; "EIN"; "precancer"; "precancerous"; "premalignant"; "precursor." Review of articles also included the abstracts of all references retrieved from the search.

All peer-reviewed prospective or retrospective studies evaluating immunohistochemical nuclear expression of PAX2 on histologic samples of NE, benign EH (EH without atypia/benign EH), precancerous EH (atypical EH/EIN) or EC were included in the systematic review. Exclusion criteria were: sample size <10 cases; language other than English; case reports.

For the meta-analysis, only the studies assessing both benign and precancerous EH were included. Exclusion criteria were: immunohistochemical assessment of PAX2 expression as a mean staining score; evaluation of only specific categories of EH (eg, EH with squamous morules, EH on polyps); overlapping patient data with a study already included.

Following the revised Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2),[13] four domains regarding risk of bias were evaluated in each study: (1) patient selection (ie, if the patients were consecutive); (2) index test (ie, if the assessment of PAX2 expression was unbiased, eg, index test blinded with reference standard, clearly specified method to interpret immunostaining); (3) reference standard (ie, if the histomorphologic examination was unbiased, eg, blinded re-evaluation of specimens); (4) flow and timing (ie, if all patients were assessed with both index and reference standard; if all patients were assessed with the same tests, if the latency time between index and reference standard did not affect the results). Review authors' judgments were categorized as "low risk," "unclear risk," or "high risk" of bias.

Concerns about applicability were also evaluated for the domains 1, 2, and 3 (ie, if the criteria used are correct but do not fit the objective of our review).

Data from each eligible study were extracted without modification of original data.

Two-by-two contingency tables were elaborated for each study, reporting two qualitative variables:

1. PAX2 nuclear expression (alternately dichotomized as "loss" or "presence" and "decrease" or "normal expression");
2. histologic category (NE, benign EH, precancerous EH and EC).

"PAX2 decrease" indicated an expression at least decreased, also including a complete loss of expression.

For meta-analysis of diagnostic accuracy, the index test was PAX2 nuclear expression, whereas the reference standard was histologic type of EH, dichotomized as "benign" or "precancerous".

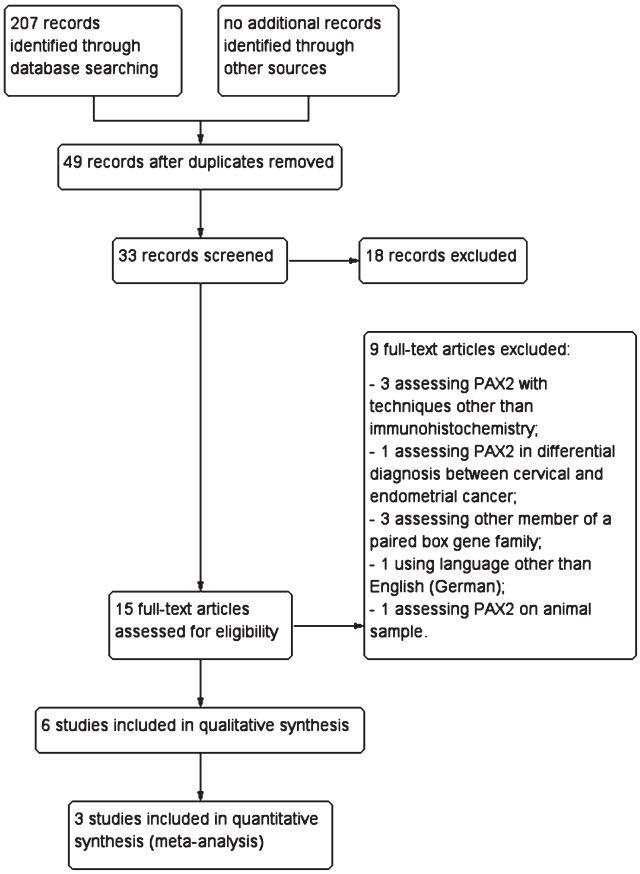

FIGURE 1 Flow diagram of studies identified in the systematic review (PRISMA template [Preferred Reporting Item for Systematic Reviews and Meta-analyses])

Precancerous cases with PAX2 loss or decrease were considered as true positives, benign cases with PAX2 presence or normal expression were considered as true negatives, precancerous cases with PAX2 presence or normal expression were considered as false negatives, and benign cases with PAX2 loss or decrease were considered as false positives.

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

1. for the study assessing the rate of PAX2-stained cells, PAX2 loss was considered as 0% cell staining, whereas PAX2 decrease was 1%-75% cell staining;
2. for the study adopting a qualitative staining score (normal, complete loss, noticeably decreased, or increased staining compared with background endometrium), PAX2 "complete loss" was considered as loss, while PAX2 "noticeably decreased" was considered as a decrease;
3. for the study adopting a quantitative staining score (0-6) composed by both intensity and proportional score, PAX2 loss was considered as a score of 0/6, whereas PAX2 decreased as a score of 2-3/6.

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

TABLE 1 Immunohistochemical assessment of PAX2 expression in the included studies

					Benign EH								
	Normal endometrium				Simple EH (WHO)				Complex EH (WHO)				Benign EH (EIN)
Study (ref)	n	L (%)	D (%)	MS	n	L (%)	D (%)	MS	n	L (%)	D (%)	MS	n
Monte 2010[18]	191	68 (35.6)	68 (35.6)	—	—	—	—	—	—	—	—	—	—
Allison 2012[19]	28	0 (0)	5 (17.6)	—	23	4 (17.4)	15 (65.2)	—	84	49 (59)	76 (90.5)	—	—
Kahraman 2012[15]	37	—	—	80.8 ± 18.5	—	—	—	—	18	—	—	88.6 ± 20.6	—
Upson 2012[16]	—	—	—	—	—	—	—	—	73	—	66 (90.4)	—	—
Joiner 2014[17]	10	4 (40)	4 (40)	—	7	0 (0)	1 (14.3)	—	25	13 (52)	15 (60)	—	26
Trabzonlu 2017[14]	—	—	—	—	—	—	—	—	—	—	—	—	34
Total	229 + 37	72 (31.4)	77 (33.6)	—	30	4 (13.3)	16 (53.3)		109 + 91	62 (56.9)	91 (83.5)+66	—	60
					n = 199 + 91 – L = 69 (34.7) – D = 120 (60.3)								

D, PAX2 expression at least decreased; EH, endometrial hyperplasia; EIN, endometrial intraepithelial neoplasia; L, complete loss of PAX2 expression; MS, PAX2 mean score; n, number of specimens; WHO, World Health Organization.

TABLE 2 Characteristics of the included studies

						Patients age	
Study (ref)	Country	Period of recruitment	Study design	Specimen type	Patients selection	Normal/benign EH	Malignant/ premalignant
Monte 2010[18]	USA	2006-2008	n.r.	Biopsies, curettages	Consecutive	41.8 ± 6.1	50.3 ± 10.1
Allison 2012[19]	USA	1985-2009	Cohort	n.r.	Randomized	<39 to >70	<39 to >70
Kahraman 2012[15]	Turkey	n.r.	n.r.	Biopsies, curettages	n.r.	58 ± 9.8 AE 46.0 ± 3.8 PE 48.9 ± 5.3 CH	54.7 ± 9.8 CAH 61.9 ± 11.5 EC
Upson 2012[16]	USA	1995-2005	Nested case control	n.r.	Consecutive	<39 to >70	<39- >70
Joiner 2014[17]	USA	2005-2013	n.r.	Biopsies, curettages	Consecutive	n.r.	n.r.
Trabzonlu 2017[14]	Turkey	2006-2011	n.r.	n.r.	Randomized	22-72 (45.4)	36-57 (47.6)

AE, atrophic endometrium; CAH, complex atypical hyperplasia; CH, complex hyperplasia; EC, endometrial cancer; EH, endometrial hyperplasia; n.r., not reported; PE, proliferative endometrium.

1. for the studies adopting the WHO system, atypical EH (simple or complex) was considered as "precancerous," and EH without atypia (simple or complex) was considered as "benign";
2. for the studies using the EIN system, EIN was considered as "precancerous," and benign EH was considered as "benign."

We contacted doctor Levent Trabzonlu to obtain additional unpublished data from his study,[14] regarding PAX2 expression in benign EH, as recommended by the SEDATE guideline.[12]

Data were also subdivided into four subgroups based on the classification system adopted (WHO or EIN) and the immunohistochemical expression of PAX2 (loss or decrease).

Univariate comparisons of PAX2 expression were performed with Fisher's exact test for two-tailed *P* value with $\alpha = 0.05$ being the significance level for each histologic category. Two included studies were excluded from this analysis, because one assessed PAX2 expression as a mean staining score[15] and the other had overlapping patient data with a study already included.[16]

			Precancerous EH											
			Atypical EH (WHO)				Premalignant EH (EIN)				Endometrial cancer			
L (%)	D (%)	MS	n	L (%)	D (%)	MS	n	L (%)	D (%)	MS	n	L (%)	D (%)	MS
–	–	–	–	–	–	–	52	37 (71.2)	37 (71.2)	–	62	48 (77.4)	48 (77.4)	–
–	–	–	56	40 (74.1)	52 (92.9)	–	-	–	–	–	15	3 (20)	14 (90.3)	–
–	–	–	19	–	–	92.7 ± 11.6	–	–	–	–	47	–	–	99.2 ± 1.2
–	–	–	41	–	38 (92)	–	–	–	–	–	–	–	–	–
3 (11.5)	6 (23.1)	–	25	20 (80)	22 (88)	–	39	33 (84.6)	35 (89.7)	–	–	–	–	–
0 (0)	7 (20.6)	4.32 ± 1.06	–	–	–	–	15	6 (40)	11 (73.3)	2.19 ± 2.34	–	–	–	–
3 (5)	13 (21.7)	–	81 + 60	60 (74.1)	74 (91.4)+38	–	106	76 (71.7)	83 (78.3)	–	77 + 47	51 (66.2)	62 (80.5)	–
			n = 187 + 60 – L = 136 (72.7) – D = 157 (84)+38											

	Immunohistochemistry				
Confounding factors evaluated	Antibody manufacturer	Clone	Diluition	Antigen retrieval	Method to assess PAX2 staining
Clinical indication, sampling device, age	Invitrogen	Z-RX2	1:300	Microwave antigen retrieval incubated at 4°	At least one null gland, proportional score
Age, BMI	Zymed	Z-RX2	1:100	EDTA 15 min	Semi-quantitative proportional score
None	LifeSpan BioSciences	Polyclonal (pSer393)	1:100	CC2 solution	Mean combined staining score for each category
Age, BMI	Zymed	Z-RX2	1:100	EDTA 15 min 40 min at room temperature	Semi-quantitative proportional score
None	Cell Marque	n.r.	7 mL pre-diluited	EDTA Incubated 16-32 min at 37°	Comparison with adjacent endometrium
None	Zeta	EP235	1/20	EDTA	Intensity score, proportional score, combined score

Sensitivity, specificity, positive and negative likelihood ratio (LR+ and LR–), and diagnostic odds ratio (DOR) of both loss and decrease of PAX2 expression were calculated for each study and as a pooled estimate adopting the random effect model of DerSimonian and Laird and reported graphically on forest plots, with 95% CI.

Statistical heterogeneity among the included studies was evaluated using the Higgins I^2 statistic; heterogeneity was categorized as null for $I^2 = 0\%$, insignificant for $0\% < I^2 \leq 25\%$, low for $25\% < I^2 \leq 50\%$, moderate for $50\% < I^2 \leq 75\%$ and high for $I^2 > 75\%$.

Area under the curve (AUC) was calculated on summary receiver operating characteristic (SROC) curves. The diagnostic usefulness was categorized as 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, very high for AUC ≥ 0.97.

We performed additional analysis as a subgroups analysis, calculating sensitivity, specificity, LR+, LR–, and DOR separately for the four subgroups. Given that only two studies were suitable for inclusion in each subgroup, AUC was not calculated.

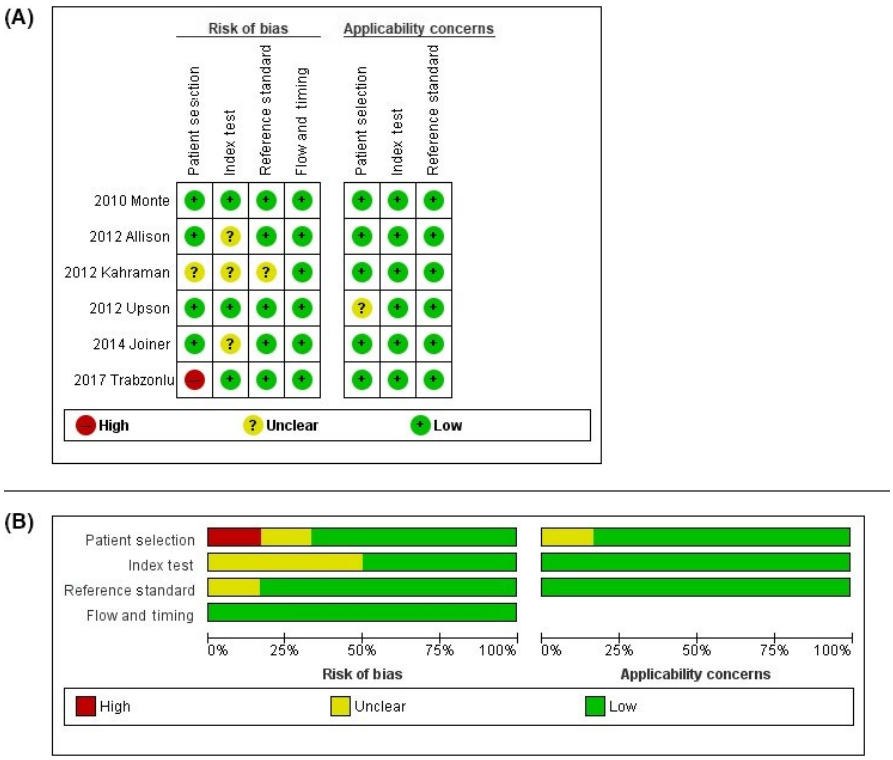

FIGURE 2 A, Assessment of risk of bias. Summary of risk of bias for each study. Plus sign: low risk of bias; minus sign: high risk of bias; question mark: unclear risk of bias. B, Risk of bias graph about each risk of bias item presented as percentages across all included studies [Colour figure can be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

One study assessed EH according to both classification systems.[17] Hence, it was included in both subgroups, whereas data according to only the EIN system were used for total diagnostic accuracy.

The data analysis was performed using META-DISC version 1.4 (Clinical Biostatistics Unit, Ramon y Cajal Hospital, Madrid, Spain) and REVIEW MANAGER 5.3 (Copenhagen: The Nordic Cochrane Centre, Cochrane Collaboration, 2014).

3 | RESULTS

A total of 207 articles were identified through database searching. Forty-nine articles remained after duplicate removal. Thirty-three articles remained after title screening. Fifteen articles were evaluated for eligibility after abstract screening. Lastly, six studies were included in the systematic review, three of which were appropriate for the meta-analysis. Details about the whole process of study selection are shown in Figure 1.

Among the six observational studies included in the systematic review,[14-19] three were classified EH according to the WHO system, three according to the EIN system, and one according to both systems. A total of 266 NE, 587 EH, and 114 EC were included. Of 537 EH, 247 (46%) were categorized as "precancerous" (141 atypical EH and 106 EIN) and 290 (54%) as "benign" (30 simple EH, 200 complex EH and 60 benign EH according to the EIN system).

Details about PAX2 immunohistochemical assessment are shown in Table 1.

Characteristics of the included studies are summarized in Table 2.

Regarding the assessment of risk of bias among the studies, for the "patient selection" domain, four studies were categorized as being at low risk of bias, because they included consecutive patients, whereas one was classified at unclear risk because it did not report this information,[15] and one was high risk because it only selected cases previously diagnosed as non-atypical hyperplasia.[14] Concerns about the applicability of this domain were considered high for one study, because it assessed only EH of women treated with progestin.[16]

For the "index test" domain, three studies were categorized at low risk of bias, because they clearly specified criteria to define loss or decrease of PAX2 expression, whereas three studies were classified at unclear risk, because only one used a qualitative staining score to assess PAX2 expression,[17] and two did not report if the index test was blinded with reference standard.[15,19]

For the "reference standard" domain, five studies were categorized at low risk of bias since they reported a blinded re-evaluation of specimens, whereas one was classified at unclear risk because this information was not clearly reported.[15]

For the "flow and timing" domain, all the included studies were categorized at low risk of bias, given that both the index and the reference standard were performed on the same sample and for all patients; moreover, the latency time between index and reference standard did not affect the results.

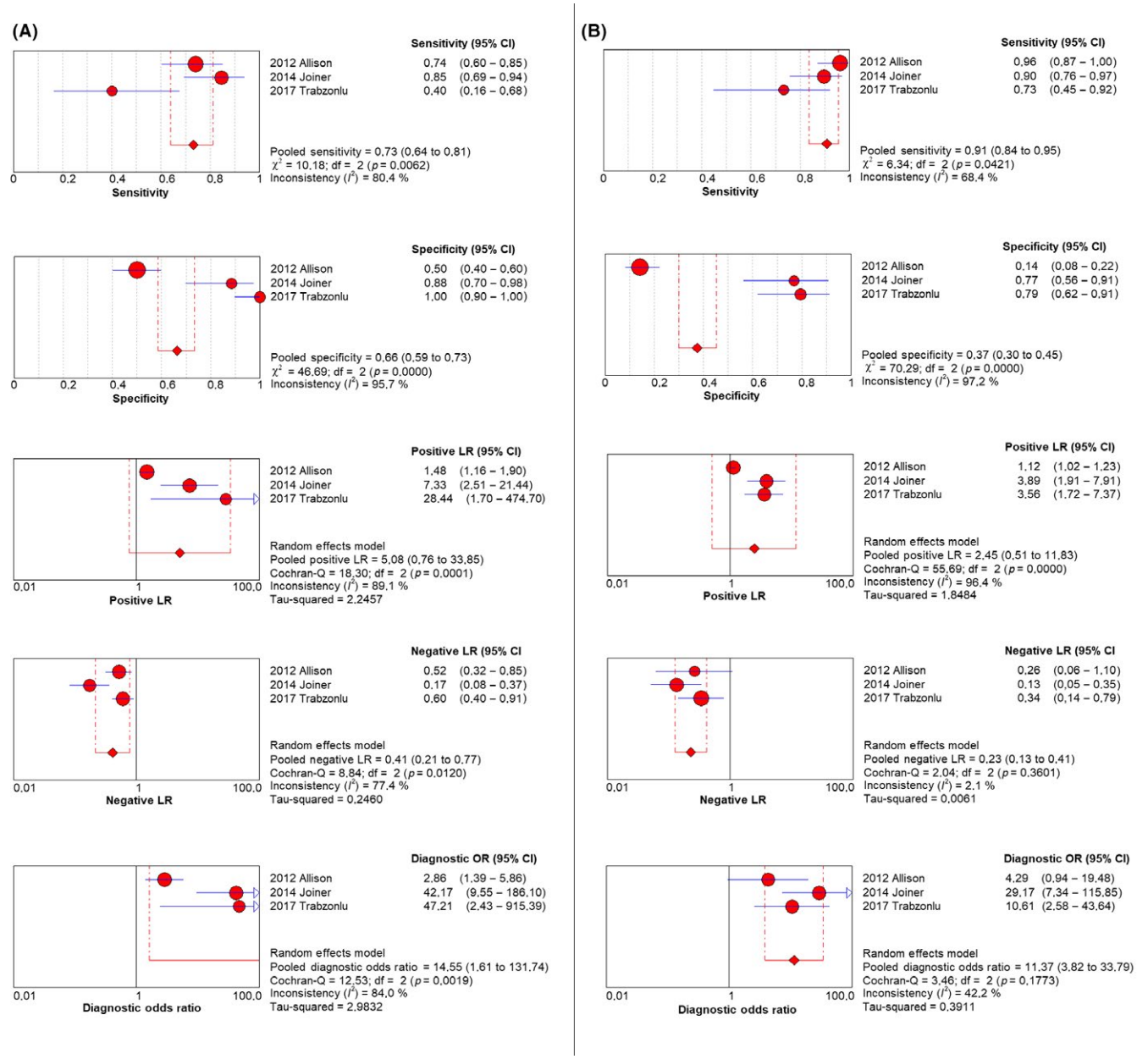

FIGURE 3 Forest plots of individual studies and pooled sensitivity, specificity, positive likelihood ratio (LR+), negative likelihood ratio (LR–), diagnostic odds ratio of immunohistochemistry for loss (A) and decrease (B) of paired box 2 protein expression in differential diagnosis between benign and premalignant endometrial hyperplasia, with summary receiver operating characteristic curves [Colour figure can be viewed at wileyonlinelibrary.com]

No further applicability concerns were found.

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

Analyzing PAX2 expression changes among histologic categories according to the WHO system, PAX2 loss was significantly more common in complex EH than simple EH and NE ($P < 0.0001$), and in atypical EH than complex EH ($P = 0.0152$). A decrease of PAX2 expression was significantly more common in simple EH than NE ($P = 0.0429$), in complex EH than simple EH ($P = 0.0011$), and in EC than simple EH ($P = 0.0073$).

Adopting EIN criteria, PAX2 loss was significantly more common in NE than benign EH ($P < 0.0001$), in EIN than benign EH and NE ($P < 0.0001$), and in EC than NE ($P < 0.0001$). PAX2 decrease was significantly more common in EIN than benign EH ($P < 0.0001$) and NE ($P = 0.0011$).

Considering both WHO and EIN system, PAX2 loss was significantly more common in precancerous EH than benign EH ($P < 0.0001$), and in EC than benign EH ($P < 0.0001$). PAX2 decrease was significantly more common in benign EH than NE ($P < 0.0001$), in precancerous EH than benign EH, and in EC than benign EH ($P = 0.0017$).

Details about PAX2 immunohistochemical expression are shown for each included study in Table 1. Univariate comparisons of PAX2

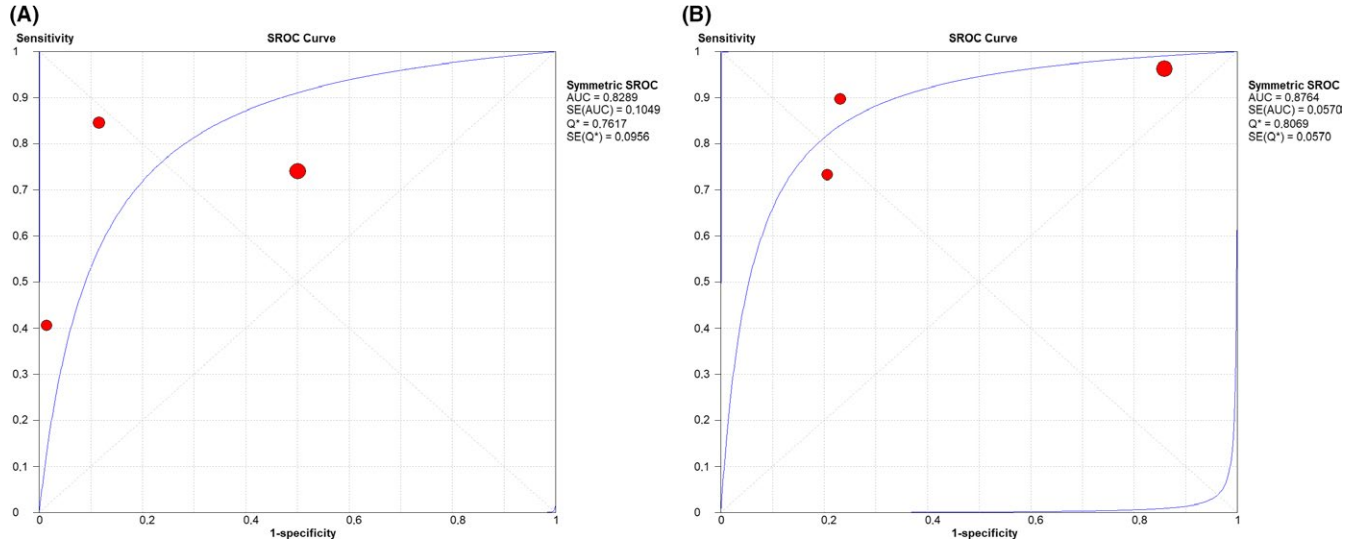

FIGURE 4 Summary receiver operating characteristic curves related to loss (A) and decrease (B) of paired box 2 protein expression in differential diagnosis between benign and premalignant endometrial hyperplasia [Colour figure can be viewed at wileyonlinelibrary.com]

expression in each histologic category with related *P* values were reported in detail for WHO, EIN, and both systems together in the Supplementary material (Tables S1-S3, respectively).

Regarding diagnostic accuracy assessment, three studies evaluating 274 EH were included in the meta-analysis.[14,17,19] In all, 166 (60.6%) of the total EH were benign and 108 (39.4%) were precancerous. Of the total, 160 EH were categorized according to the WHO system and 49 according to the EIN system; moreover, the study adopting both classification systems[17] assessed 65 EH according to the EIN system and 57 according to the WHO system.

Pooled sensitivity and specificity of PAX2 loss in diagnosing endometrial precancer with both WHO and EIN systems were 73% (95% CI 64%-81%) and 66% (95% CI 59%-73%), respectively, with pooled LR+ and LR− of 5.08 (95% CI 0.76-33.85) and 0.41 (95% CI 0.21-0.77), respectively. Pooled DOR was 14.55 (95% CI 1.61-131.74). Among the included studies, the heterogeneity was high with I^2 = 80% for sensitivity, I^2 = 95.7% for specificity, I^2 = 89.1% for LR+, I^2 = 77.4% for LR−, and I^2 = 84% for DOR. The SROC curves analysis showed moderate overall accuracy with an AUC of 0.8289.

Pooled sensitivity and specificity of PAX2 decreases in diagnosing endometrial precancer with both classification systems were 91% (95% CI 84%-95%) and 37% (95% CI 30%-45%), respectively, with pooled LR+ and LR− of 2.45 (95% CI 0.51-11.83) and 0.23 (95% CI 0.13-0.41), respectively. Pooled DOR was 11.37 (95% CI 3.82-33.79). Among the included studies, the heterogeneity was moderate in sensitivity (I^2 = 68.4%), high in specificity (I^2 = 97.2%) and LR+ (I^2 = 96.4%), insignificant in LR− (I^2 = 2.1%) and low in DOR (I^2 = 42.2%). The SROC curves analysis showed moderate overall accuracy with an AUC of 0.8764.

Results are reported graphically on forest plots in Figure 3 and on SROC curves in Figure 4.

With respect to subgroup analysis, two studies evaluating both loss and decrease of PAX2 expression in 217 EH according to the WHO system were included, respectively in the first and second subgroups.[17,19] One hundred and thirty-eight (63.6%) of the total EH were benign and 79 (36.4%) were premalignant.

On the other hand, two studies evaluating both loss and decrease of PAX2 expression in 114 EH according to the EIN system were included, respectively in the third and fourth subgroups.[14,17] Sixty (52.6%) of the total EH were benign and 54 (47.4%) were premalignant.

In subgroup 1 (PAX2 loss and WHO system), pooled sensitivity and specificity of PAX2 loss in diagnosing atypical hyperplasia were 76% (95% CI 65%-85%) and 52% (95% CI 44%-61%), respectively, with pooled LR+ and LR− of 1.59 (95% CI 1.25-2.04) and 0.46 (95% CI 0.3-0.71), respectively. Pooled DOR was 3.44 (95% CI 1.86-6.38). The heterogeneity was null in sensitivity (I^2 = 0%), specificity (I^2 = 0%), and LR− (I^2 = 0%), insignificant in LR+ (I^2 = 12.7%), and low in DOR (I^2 = 30.1%).

In subgroup 2 (PAX2 decrease and WHO system), pooled sensitivity and specificity of PAX2 decrease in diagnosing atypical hyperplasia were 94% (95% CI 86%-98%) and 22% (95% CI 16%-30%), respectively, with pooled LR+ and LR− of 0.25 (95% CI 0.1-0.6) and 0.67 (95% CI 0.52-0.85), respectively. Pooled DOR was 5.73 (95% CI 2.06-15.9). The heterogeneity was low in sensitivity (I^2 = 45.1%), high in specificity (I^2 = 93.8%), and LR+ (I^2 = 87.2%), and null in LR− (I^2 = 0%) and DOR (I^2 = 0%).

In subgroup 3 (PAX2 loss and EIN system), pooled sensitivity and specificity of PAX2 loss in diagnosing EIN were 72% (95% CI 58%-84%) and 95% (95% CI 56%-99%), respectively, with pooled LR+ and LR− of 8.71 (95% CI 3.2-23.73) and 0.34 (95% CI 0.0.9-1.26), respectively. Pooled DOR was 43.13 (95% CI 11.44-162.6). The heterogeneity was high in sensitivity (I^2 = 90.1%), specificity (I^2 = 80.9%), and LR− (I^2 = 89.6%), and null in LR+ (I^2 = 0%) and DOR (I^2 = 0%).

In subgroup 4 (PAX2 decrease and EIN system), pooled sensitivity and specificity of PAX2 decreases in diagnosing EIN were 85%

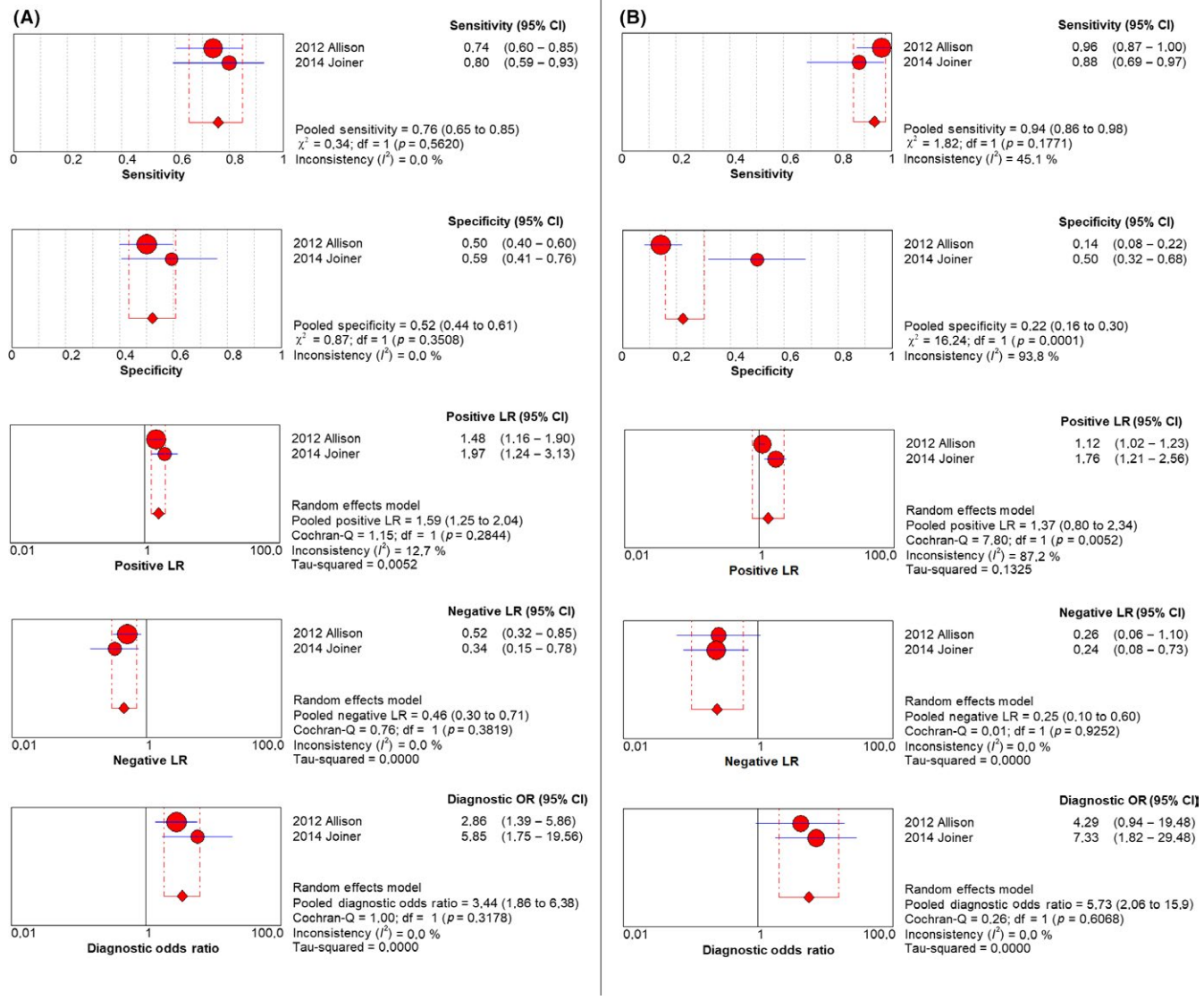

FIGURE 5 Forest plots of individual studies and pooled sensitivity, specificity, positive likelihood ratio (LR+), negative likelihood ratio (LR–), diagnostic odds ratio of immunohistochemistry for loss (A) and decrease (B) of paired box 2 protein expression in differential diagnosis between benign and premalignant endometrial hyperplasia, with summary receiver operating characteristic curves, for World Health Organization subgroup [Colour figure can be viewed at wileyonlinelibrary.com]

(95% CI 73%-93%) and 78% (95% CI 66%-88%), respectively, with pooled LR+ and LR– of 3.73 (95% CI 2.24-6.19) and 0.22 (95% CI 0.09-0.54), respectively. Pooled DOR was 17.81 (95% CI 6.61-47.9). The heterogeneity was moderate in sensitivity (I^2 = 52.7%) and LR– (I^2 = 50.2%), null in specificity (I^2 = 0%) and LR+ (I^2 = 0%), and insignificant in DOR (I^2 = 0.7%).

Results are reported graphically in forest plots for subgroups 1 and 2 in Figure 5 and for subgroups 3 and 4 in Figure 6.

4 | DISCUSSION

Both complete loss and decrease of PAX2 expression were significantly more common in EC and precancerous EH than in benign EH, demonstrating that PAX2 expression decreases in endometrial carcinogenesis.

In the overall analysis, both PAX2 complete loss and decrease showed moderate diagnostic accuracy in identifying precancerous EH. Excellent accuracy was achieved by combining PAX2 complete loss (as index test) with EIN classification (as reference standard).

The *PAX2* gene is a member of a paired box gene family consisting of nine components (*PAX1* to *PAX9*), especially expressed during the embryonic development and organogenesis.[20] However, PAX proteins are also involved in several malignancies, acting as proto-oncogenes by transactivating promoters of target genes that regulate cell growth, self-sufficiency, apoptosis, and cellular transformation.[21-23] In particular, PAX2 protein has anti-apoptotic effects binding to the regulatory region at the 5′ end of P53 gene and inhibiting its protein

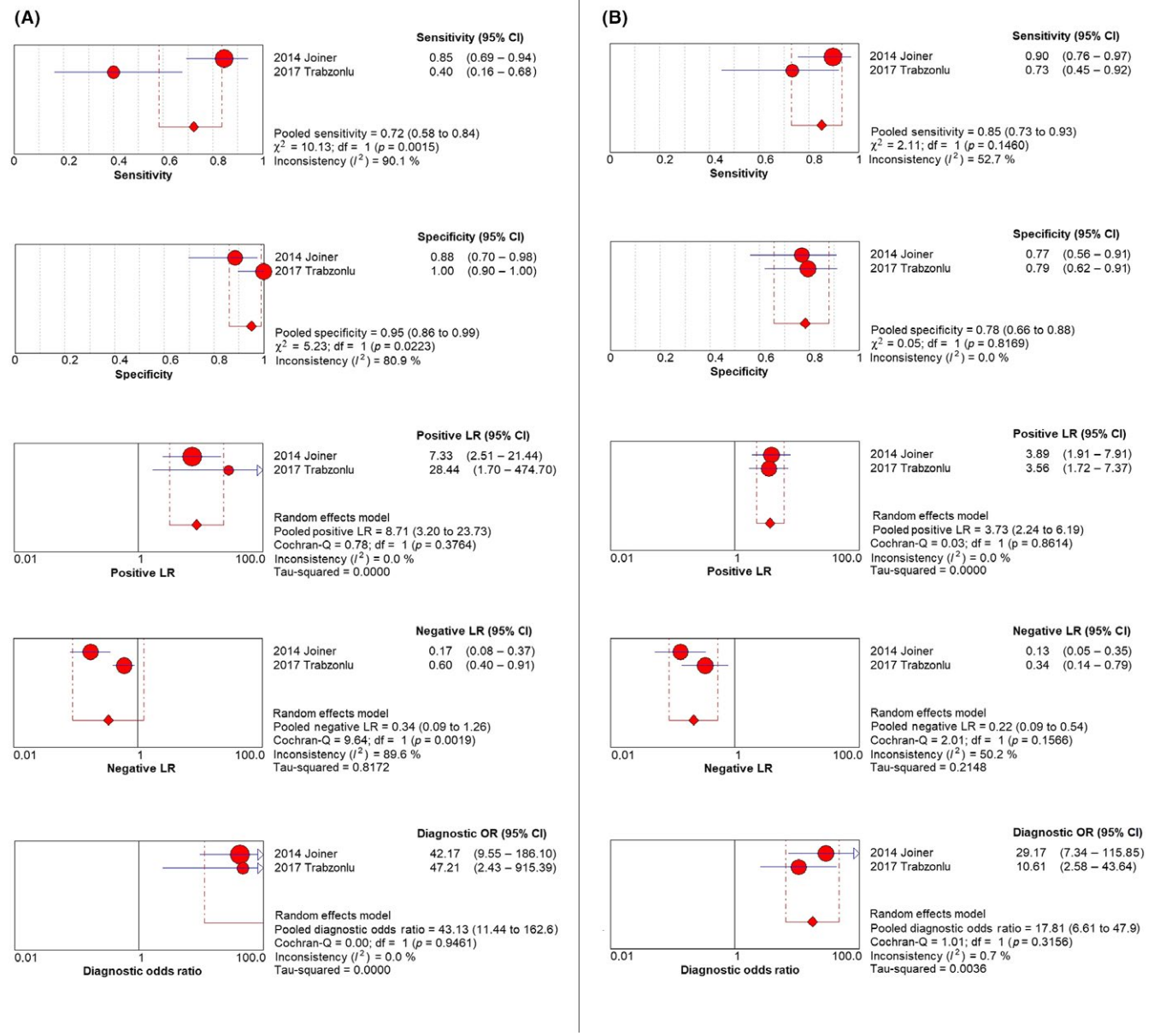

FIGURE 6 Forest plots of individual studies and pooled sensitivity, specificity, positive likelihood ratio (LR+), negative likelihood ratio (LR−), diagnostic odds ratio of immunohistochemistry for loss (A) and decrease (B) of paired box 2 protein expression in differential diagnosis between benign and premalignant endometrial hyperplasia for endometrial intraepithelial neoplasia subgroup [Colour figure can be viewed at wileyonlinelibrary.com]

production at the transcriptional level.[24,25] The expression of PAX2 is upregulated indirectly by the estrogen receptor α pathway.[26] Nevertheless, to date, the role of PAX2 and its changes of expression in endometrial carcinogenesis are still unclear, with the common suggestion that PAX2 expression decreases in EC and precancer and a few studies advocating an opposing viewpoint.[15,26-28]

Although one study among those included reported that PAX2 expression increases as endometrial carcinogenesis progresses,[15] we found that both complete loss and decrease of PAX2 expression were significantly more common in EC and precancerous EH than in benign EH. This behavior seems to indicate a tumor suppressor action of the *PAX2* gene in endometrial carcinogenesis. Similar results were reported in studies assessing PAX2 by techniques other than immunohistochemistry.[29,30] To date, data regarding the molecular mechanisms of PAX2 loss with specific regard to endometrial carcinogenesis are lacking. However, pathway models for the possible tumor suppressor activity of PAX2 in endometrial carcinogenesis might be suggested by studies about carcinogenesis in other tissues. In particular, several mechanisms have been proposed for tumor suppressor activity of PAX2 in ovarian carcinogenesis. PAX2 knockdown in fallopian tube epithelial cell lines increased expression of the stem cell markers CD44 and SCA1 and reduced the capability of these cells to form differentiated epithelial luminal structures.[31] It has been shown in murine oviductal epithelial cells that wild-type

p53 improves PAX2 transcription, while mutant p53 decreases.[32] In a fallopian tube model of ovarian cancer with PAX2 and PTEN (phosphatase and tensin homolog) loss, re-expression of PAX2 repressed the oncogenic properties of these cells and extended survival.[32] On the other hand, PAX2 expression in a spontaneous ovarian surface epithelium derived model of high-grade serous ovarian carcinoma reduced proliferation and metastasis by increasing cyclo-oxygenase subunit 2 and reducing HTRA1 (HtrA serine peptidase 1) expression.[33] Altogether, these results suggest that PAX2 loss may be an early molecular event in ovarian cancer progression that predisposes cells to further mutations that can drive tumorigenesis, regardless of the cell of origin.[34] Such mechanisms might underlie endometrial carcinogenesis.

However, a *PAX2* oncogenic action cannot be excluded in endometrial carcinogenesis, as suggested by Kahraman et al[15] and other EC cell-culture studies.[26,27] These findings might indicate a *PAX2* gene double action (tumor suppressor and oncogene) in endometrial carcinogenesis, as well as in ovarian carcinogenesis, as suggested.[33,34] Regarding the increase of PAX2 expression, further studies are needed to define its significance and its possible usefulness in differential diagnosis between benign and precancerous EH.

It is interesting to note that when adopting the WHO system, both complete loss and decrease of PAX2 expression were significantly more common in complex EH without atypia than in NE and simple EH without atypia. This finding supports that the WHO category of complex EH without atypia might also include premalignant lesions, as suggested by comparison with EIN classification.[3] Therefore, immunohistochemical assessment of PAX2 might assist histomorphologic examination to diagnose precancerous EH even before the appearance of overt cytologic atypia, as discussed below.

According to our results, immunohistochemical evaluation of both complete loss and decrease of PAX2 expression have a moderate diagnostic accuracy in differential diagnosis between benign and precancerous EH, with AUC, respectively, of 0.829 and 0.876.

In the face of a similar diagnostic accuracy, PAX2 complete loss showed higher specificity and DOR, but lower sensitivity than PAX2 decrease. This finding suggests that a decrease of PAX2 may occur in an early stage in endometrial carcinogenesis, but also exists in a major percentage of benign EH without malignant potential. Instead, a complete loss of PAX2 may be a later and more specific event of precancerous EH, with a major diagnostic value in the challenging cases on histologic examination. On the other hand, PAX2 normal expression may be strongly indicative of benign EH.

Through subgroup analysis, we found that the diagnostic accuracy of both PAX2 complete loss and decrease was higher when the EIN system was used as reference standard. In fact, using EIN criteria, sensitivity, specificity, and DOR greatly increased. The highest diagnostic accuracy was found for the combination of PAX2 complete loss (as index test) and EIN system (as reference standard), with a DOR of 43.13. This excellent value supports the diagnostic usefulness of PAX2 immunohistochemistry, suggesting its introduction as routine diagnostic test in support of histologic examination. Given its high specificity (95%), it might be extremely important in reducing the rate of a serious overtreatment—hysterectomy being the reference standard therapy for precancerous EH. On the other hand, the sensibility observed (72%) appeared to be more than enough for a support test. In fact, since PAX2 loss may rarely be found in NE and benign EH, it is fundamental to identify a lesional focus of interest by hematoxylin & eosin staining before performing PAX2 immunohistochemistry. As pointed out by Joiner et al, it is always necessary to correlate histomorphologic and immunohistochemical findings.[17]

Our results also suggest the better accuracy of the EIN system in identifying early precancerous lesions, when overt cytologic atypia is still absent. Such a finding is in accordance with the results of our previous review regarding Bcl-2 expression in EH.[35] The EIN system is based on three main histomorphologic features (glandular crowding, lesion diameter >1 mm, cytology different from adjacent endometrium) and a careful exclusion of benign mimics (eg, polyps, secretory changes) and cancer.[2,3] PAX2 loss may be used as an adjuvant finding for the EIN diagnosis when comparison with NE is not possible, or there is a secretory pattern as background endometrium, as suggested by Quick et al.[36]

Further studies would be necessary to validate these excellent results. Moreover, it would also be interesting to study the possible role of PAX2 as predictive markers of response to conservative treatment in EH, as for other markers.[37] Raffone A. Unpublished data.

To the best of our knowledge, this study is the first systematic review specifically assessing the immunohistochemical expression changes of PAX2 in NE, benign and precancerous EH, and EC, evaluating its behavior in endometrial carcinogenesis.

To date, this study is also the first meta-analysis calculating the diagnostic accuracy of PAX2 immunohistochemical evaluation in differential diagnosis of benign and precancerous EH.

We analyzed PAX2 diagnostic accuracy in terms of both loss and decrease of expression and in terms of both classification systems of EH (WHO and EIN), in order to clarify the method to interpret the immunostaining, not specified in the 2017 ESGO guidelines.[7]

Limitations of our results may arise from some characteristics of the included studies, which showed high or unclear risk of bias in several domains (Figure 2).

Methods in performing the primary studies were uneven. Details about the study protocol, baseline characteristics of the patients and thorough analysis of confounding factors were lacking in several studies. Moreover, the method to grade the expression of PAX2 needs to be standardized in terms of intensity of staining and percentage of stained glands. However, we found the best results for a complete loss of PAX2, which may be easily read even without a validated method.

5 | CONCLUSION

Both complete loss and decrease of PAX2 expression were significantly more common in EC and precancerous EH than benign EH, suggesting a tumor suppressor action of the *PAX2* gene in

endometrial carcinogenesis. However, an oncogenic role cannot be excluded.

Immunohistochemistry for PAX2 may be an accurate routine test to aid the histomorphologic differential diagnosis of EH; in particular a complete loss of PAX2 expression with EIN criteria showed an excellent diagnostic accuracy, with high specificity. Moreover, PAX2 loss may identify precancerous EH even in the absence of evident cytologic atypia.

Further studies are necessary to confirm and validate these results.

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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.

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