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SYSTEMATIC REVIEW

Loss of B-cell lymphoma 2 immunohistochemical expression in endometrial hyperplasia: A specific marker of precancer and novel indication for treatment

A systematic review and meta-analysis

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Abstract

Introduction: Endometrial hyperplasia is differentiated into benign or premalignant. Two histological classifications are used for this purpose: World Health Organization (WHO) classification, based on cytological atypia, disregarding glandular complexity, and endometrial intraepithelial neoplasia (EIN) classification, based on several different parameters. B-cell lymphoma 2 (Bcl-2) loss has been studied as immunohistochemical marker with the aim of improving the differential diagnosis between benign and premalignant hyperplasia. We aimed to evaluate: (A) Bcl-2 loss as marker of endometrial precancer, by assessing it in proliferative endometrium, benign hyperplasia, premalignant hyperplasia, and endometrial cancer; (B) the diagnostic accuracy of Bcl-2 in the differential diagnosis between benign and premalignant endometrial hyperplasia; (c) how the results change according to the histological classification and the thresholds of Bcl-2 expression used.

Material and methods: Electronic databases were searched from their inception to March 2018. All studies assessing Bcl-2 immunohistochemistry in endometrial specimens were included.

Results: In total, 20 observational studies assessing 1,278 specimens were included. Bcl-2 loss rates were not significantly different between proliferative endometrium and benign hyperplasia ($P = 0.12$) and between premalignant hyperplasia and endometrial cancer ($P = 0.53$). Among hyperplasias, Bcl-2 loss was significantly associated with premalignancy, according to both the WHO (OR = 4.39; $P < 0.00001$) and EIN classifications (OR = 6.07; $P = 0.01$), and also with architecture complexity (OR = 2.06; $P = 0.02$). Using the WHO classification, Bcl-2 loss showed low diagnostic accuracy in detecting premalignant hyperplasia (area under the curve [AUC] = 0.708), with a sensitivity of 0.41, a specificity of 0.81, a positive likelihood ratio of 3.22, and a negative likelihood ratio of 0.69. Using the EIN classification, accuracy was high (AUC = 0.938),

Abbreviations: AUC, area under the curve; EIN, endometrial intraepithelial neoplasia; LR–, negative likelihood ratio; LR+, positive likelihood ratio; OR, odds ratio; WHO, World Health Organization.

with a sensitivity of 0.18, a specificity of 0.97, a positive likelihood ratio of 5.16 and a negative likelihood ratio of 0.86. Thresholds of Bcl-2 expression not involving a complete loss showed lower diagnostic accuracy with a slight increase in sensitivity, but a severe decrease in specificity.

Conclusions: B-cell lymphoma 2 loss is a marker of endometrial precancer, with a high specificity and high diagnostic accuracy if the EIN classification is used. Thresholds of Bcl-2 expression not involving a complete loss should not be considered. Bcl-2 loss in endometrial hyperplasia may be a novel indication for treatment when precancerous features are ambiguous in a histological examination. Bcl-2 loss correlates better with EIN classification than with the WHO classification, suggesting that glandular complexity is an important precancerous feature.

KEYWORDS

apoptosis, biomarker, cancer, endometrial hyperplasia, neoplasia

1 | INTRODUCTION

Endometrial hyperplasia is defined as an irregular proliferation of endometrial glands, characterized by an increased gland : stroma ratio compared with proliferative endometrium.[1] Endometrial hyperplasia includes benign proliferations caused by the unopposed action of estrogens and premalignant lesions, which have a considerable risk of progression to endometrial cancer.[2,3]

While benign endometrial hyperplasia may be managed by follow-up, premalignant endometrial hyperplasia usually requires hysterectomy, or progestins and close follow-up in selected cases.[4] However, the distinction between these two conditions is often difficult.[2]

The gold standard for the differential diagnosis is the histological examination, which is based on two possible classification systems: the World Health Organization (WHO) system and the endometrial intraepithelial neoplasia (EIN) system.[2,3]

The 2014 WHO system is based on the presence of cytological atypia and distinguishes between premalignant atypical endometrial hyperplasia and benign endometrial hyperplasia without atypia. Nuclear atypia may include rounding, enlargement, pleomorphism, loss of polarity, and nucleoli.[1] The complexity of glandular architecture, included in the former classification (WHO, 1994), has been excluded, although it appears to be associated with higher risk of progression to endometrial cancer.[1-3]

The EIN system differentiates between premalignant EIN and benign endometrial hyperplasia based on the presence of three morphological parameters (glands exceeding stroma, lesion size > 1 mm and cytological differences with adjacent non-neoplastic endometrium) and a careful exclusion of benign mimics (e.g., polyps and secretory endometrium) and cancer (maze-like glands, solid or cribriform areas).[2,3,5,6] The EIN system classifies a considerable percentage of endometrial hyperplasia without atypia but with complex architecture as premalignant.[6] However, several concerns have been reported for the histological classification, which are related to the pathologist (low reproducibility) or to the specimen itself (tissue inadequacy, artifact changes and ambiguous features).[2,3,7]

Key message

Using endometrial intraepithelial neoplasia criteria, B-cell lymphoma 2 (Bcl-2) loss is a highly specific and accurate marker of endometrial precancer. Bcl-2 loss in endometrial hyperplasia may be a novel indication for treatment.

A great number of immunohistochemical markers have been studied to improve the reliability of the diagnosis. The anti-apoptotic protein B-cell lymphoma 2 (Bcl-2) is upregulated through the actions of estrogens and its expression has been observed to increase in proliferative endometrium and in benign endometrial hyperplasia. Several studies have found a loss of Bcl-2 expression in neoplastic endometrial samples (premalignant endometrial hyperplasia and endometrial cancer).[8,9] Therefore, Bcl-2 loss has been proposed as a marker to detect intraepithelial neoplasia in endometrial specimens.[10]However the role of Bcl-2 loss of expression in endometrial carcinogenesis has never been clarified. Furthermore, its possible clinical usefulness in the differential diagnosis of endometrial hyperplasia is still subject to debate.

Thus, the aims of this study were: (i) to assess Bcl-2 immunohistochemical expression in histological specimens of proliferative endometrium, benign endometrial hyperplasia, premalignant endometrial hyperplasia and endometrial cancer, to define whether or not Bcl-2 loss of expression may be considered a marker of neoplasia; (ii) to determine the clinical usefulness of immunohistochemistry for Bcl-2 in the differential diagnosis between benign and premalignant endometrial hyperplasia, through analyses of diagnostic accuracy; and (iii) to assess how the results are influenced by the histological criteria used to classify endometrial hyperplasia and by the thresholds of Bcl-2 expression considered.

2 | MATERIAL AND METHODS

The study protocol, including methods for electronic search, study selection, risk of bias assessment, data extraction, and data analysis, was designed previously. Two authors (AT, AR) independently conducted all stages of the study; disagreements were resolved by discussion with a third author (GS). The study was reported according to the Preferred Reporting Item for Systematic Reviews and Meta-analyses statement.

MEDLINE, EMBASE, Web of Sciences, Scopus, ClinicalTrial.gov, OVID, Cochrane Library and Google Scholar were searched for relevant articles from the inception of each database to March 2018, using a combination of the following text words and all their synonyms found on Medical SubHeading (MeSH): "endometrial hyperplasia"; "proliferative endometrium"; "endometrial cancer"; "endometrial intraepithelial neoplasia"; "EIN"; "precancer"; "premalignant"; "precursor"; "Bcl2"; "B-cl 2"; "B-cell lymphoma 2"; "apoptosis"; "apoptotic"; "anti-apoptotic"; "marker"; "biomarker"; "expression"; "immunohistochemistry"; "immunohistochemical". All relevant references were also assessed.

All peer-reviewed, prospective and retrospective studies assessing the immunohistochemical expression of Bcl-2 on histological specimens of proliferative endometrium, benign endometrial hyperplasias, premalignant endometrial hyperplasias and endometrial cancer were included in this systematic review. The exclusion criteria were: (i) data on Bcl-2 expression not available; (ii) endometrial hyperplasia unclassified; (iii) inclusion of only benign or only premalignant endometrial hyperplasia; (iv) sample size of < 5 endometrial hyperplasia; (v) case reports and reviews; (vi) not written in English; (vii) endometrial hyperplasia data overlapping with an already included study.

The risk of bias was assessed according to the revised Quality Assessment of Diagnostic Accuracy Studies.[11] Four domains related to the risk of bias were assessed in each study: (i) patient selection (ie, inclusion of consecutive patients); (ii) index test (ie, unbiased assessment of Bcl-2 immunohistochemical expression), (iii) reference standard (ie, unbiased histological classification), (iv) flow and timing (ie, all patients were assessed with the same index test and the same reference standard). Authors' judgments were categorized as "low risk," "high risk," or "unclear risk of bias." For the domains 1, 2, and 3, concerns about their applicability to the included studies were also assessed.

Data were extracted from the included studies without modification. Contingency 2 × 2 tables were prepared for each study, reporting two dichotomous qualitative variables: Bcl-2 expression and histological diagnosis. When discrepancies between values reported in the text and the tables were found, values from tables were used for the analysis.

The immunohistochemical expression of Bcl-2 was dichotomized into "presence" and "loss". For studies that did not dichotomize Bcl-2 expression, data were extracted using the following criteria for Bcl-2 loss:

- when Bcl-2 expression was graded according to the intensity of staining, the lowest grade, indicating absence of expression, indicated Bcl-2 loss;
- when Bcl-2 was graded according to the rate of stained cells, the lowest grade indicated Bcl-2 loss;
- when Bcl-2 expression was graded using a staining score (combining intensity and stained cells rate), the lowest grade indicated Bcl-2 loss.

The authors in one study were contacted to obtain the additional unpublished data regarding Bcl-2 expression in benign endometrial hyperplasia.[12]

The histological diagnosis was dichotomized as proliferative endometrium vs benign endometrial hyperplasia, benign endometrial hyperplasia vs premalignant endometrial hyperplasia, and premalignant endometrial hyperplasia vs endometrial cancer. Benign endometrial hyperplasia included non-atypical endometrial hyperplasia according to the WHO classification or benign endometrial hyperplasia according to the EIN classification; premalignant endometrial hyperplasia included atypical endometrial hyperplasia according to the WHO classification or EIN according to the EIN classification. Regarding benign and premalignant endometrial hyperplasia, two groups were composed according to the classification system used (WHO or EIN). Moreover, a third group was created according to the former WHO parameter of architecture complexity.

The association between the two variables (Bcl-2 expression and histological diagnosis) was assessed using odds ratios (OR), with 95% CI and a significant P value < 0.05. OR was calculated for each study and as pooled estimates, and reported graphically on forest plots.

The inconsistency index (I^2) was used to quantify the statistical heterogeneity among the studies: heterogeneity was considered insignificant for I^2 < 25%, low for I^2 < 50%, moderate for I^2 < 75%, and high for I^2 ≥ 75%. In case of I^2 ≥ 50%, a random effect model was adopted; otherwise, a fixed effect model was preferred.

Values of OR found in the three groups were compared by using chi-squared test, with a significant P value < 0.05.

In the analysis of diagnostic accuracy, the index test was Bcl-2 immunohistochemical expression (loss or presence), while the reference standard was endometrial hyperplasia histology (benign or premalignant). The following criteria were adopted:

- premalignant endometrial hyperplasia with Bcl-2 loss were considered true positives;
- premalignant endometrial hyperplasia with Bcl-2 presence were considered false negatives;
- benign endometrial hyperplasia with Bcl-2 loss were considered false positives;
- benign endometrial hyperplasia with Bcl-2 presence were considered true negatives.

Analyses of diagnostic accuracy were performed separately for the WHO and EIN classifications. The diagnostic accuracy was assessed as sensitivity, specificity, positive likelihood ratio (LR+), and negative likelihood ratio (LR–) with 95% CI. The area under the curve (AUC) was calculated on summary receiver operating characteristic curves. Diagnostic

accuracy was considered absent for AUC ≤ 0.5, low for 0.5 < AUC ≤ 0.75, moderate for 0.75 < AUC ≤ 0.9, high for 0.9 < AUC < 0.97, and very high for AUC ≥ 0.97. A random effect model was planned previously, since a significant heterogeneity is expected in meta-analyses of diagnostic accuracy.[13]

Where possible, we assessed whether the diagnostic accuracy could be improved using different thresholds of the expression of Bcl-2. Only studies considering both the intensity of staining and the rate of stained cells were suitable for such analysis.

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

3 | RESULTS

Altogether 20 observational studies[9,10,12,14-30] with a total of 1,278 specimens (137 proliferative endometrium, 676 endometrial hyperplasia, and 465 endometrial cancer), were included in the systematic review. The flow diagram reporting the process of study selection is shown in Figure 1.

A total of 17 studies adopted the WHO classification system and three used the the EIN system. Among the studies using the WHO system, eight studies differentiated endometrial hyperplasia based on cytological atypia, six did so based on the complexity of architecture, and three did so based on both parameters.

Six studies dichotomized Bcl-2 expression, five graded the intensity of staining, three assessed the rate of stained cells, while six used more than one parameter. Out of five studies using a combined score (intensity grade +stained cells rate), two allowed extracting data for the analysis of the thresholds of Bcl-2 expression; the two scoring systems were fully comparable.[12,30] All studies assessed the expression of Bcl-2 in endometrial glands. The characteristics of the included studies are shown in Table 1.

The risk of bias among the included studies is shown in Figure 2. For the patients' selection domain, three studies were considered to be at low risk of bias (since they included consecutive patients) and 17 were at unclear risk (because they did not report selection criteria); concerns about applicability were unclear for three studies (one assessed several different human neoplasms; one included only endometrial hyperplasia treated with progestins; one selected only non-atypical endometrial hyperplasia and reclassified them according to the EIN system). For the index test domain, 10 studies were considered at low risk of bias (since they considered both intensity and distribution of immunostaining) and 10 at unclear risk (because they considered only one parameter). For the reference standard domain, six studies were considered at low risk of bias (since all specimens were re-evaluated by several authors at the same time) and 14 at unclear risk (because they did not re-evaluate the specimens). For the flow and timing domain, only one study was at unclear risk and concern (because not all endometrial hyperplasia included were assessed by immunohistochemistry), while the other 19 were considered at low risk of bias. No study was at high risk of bias. No further concerns about applicability were found.

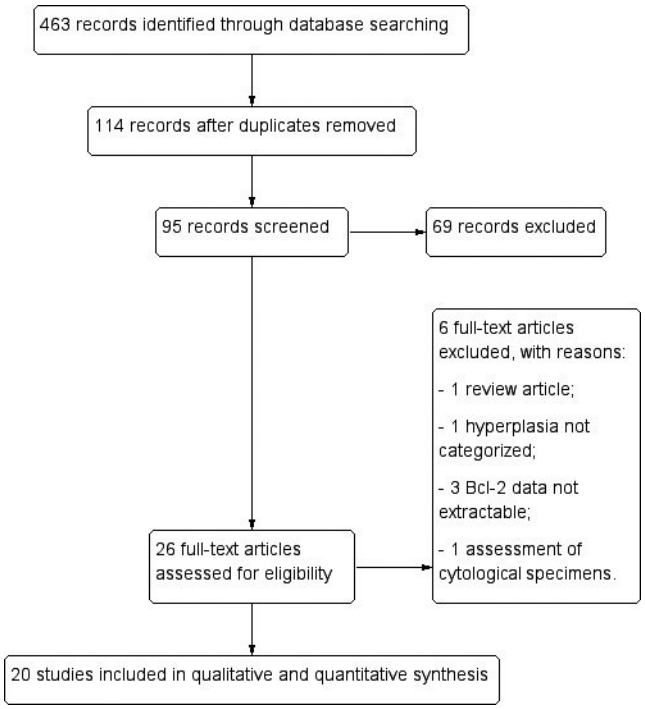

FIGURE 1 Flow diagram of studies identified in the systematic review using Preferred Reporting Item for Systematic Reviews and Meta-analyses] template. Bcl-2, B-cell lymphoma 2

3.1 | B-cell lymphoma 2 expression and histological diagnosis

B-cell lymphoma 2 loss was not significantly more common in benign endometrial hyperplasia than in proliferative endometrium (OR = 1.85, 95% CI, 0.86-4.02; $P = 0.12$), and without heterogeneity among studies ($I^2 = 0\%$) (Figure 3a).

Among endometrial hyperplasia, Bcl-2 loss was significantly associated with premalignant endometrial hyperplasia, according to both WHO and EIN systems:

- when the WHO system (cytological atypia) was adopted, the OR was 4.39 (95% CI, 2.56-7.53; $P < 0.00001$) with insignificant heterogeneity ($I^2 = 22\%$) (Figure 3b);
- when the EIN system was adopted, the OR was 6.07 (95% CI 1.50-24.56; $P = 0.01$), with no heterogeneity ($I^2 = 0\%$) (Figure 3c).

The difference between WHO and EIN groups was not significant (chi-squared = 0.18; P value = 0.67) (Figure 3c).

B-cell lymphoma 2 was also significantly associated with the complexity of glandular architecture, with an OR of 2.06 (95% CI, 1.13-3.74; P value of 0.02) and without heterogeneity ($I^2 = 0\%$). There was no significant difference with the group of cytological atypia (chi-squared = 3.39; P value = 0.07) (Figure 3d).

Among neoplastic samples, Bcl-2 loss was more common in endometrial cancer than in premalignant endometrial hyperplasia, without

TABLE 1 Characteristics of the included studies

Year	Study (ref)	Country	Period of enrollment	System adopted	Sample size	PE	WHO					EIN		endometrial cancer	Method to interpret immunostaining
							SH	CH	SH + CH	AH	CH + AH	Benign	EIN		
1995	Chan et al[14]	UK	nr	WHO	45	-	-	-	10	10	-	-	-	25	dichotomous
1996	Chhieng et al[15]	USA	1981-1992	WHO	99	17	8	5	-	12	-	-	-	57	intensity grade, cell rate
	Henderson et al[9]	USA	nr	WHO	71	11	-	-	18	11	-	-	-	31	intensity grade
	Niemann et al[16]	USA	nr	WHO	29	-	-	4	-	4	-	-	-	21	dichotomous
1997	Nakamura et al [17]	Japan	1993-1995	WHO	48	8	16	-	-	-	10	-	-	14	stained cell rate
1998	Ioffe et al[18]	USA	nr	WHO	64	10	18	-	-	-	18	-	-	18	intensity grade
	Miturski et al[19]	Poland	1995-1997	WHO	31	5	5	-	-	-	5	-	-	16	dichotomous
1999	Mora et al[20]	USA	nr	WHO	28	-	-	-	19	9	-	-	-	13	stained cell rate
2000	Morsi et al[21]	Germany	nr	WHO	67	18	12	-	-	-	8	-	-	29	stained cell rate
2001	Kokawa et al[22]	Japan	1988-2000	WHO	36	-	-	-	9	7	-	-	-	20	intensity grade
	Peiró et al[23]	Germany	1984-1994	WHO	88	-	10	-	-	-	22	-	-	56	score (intensity + cell rate)
2002	Cinel et al[24]	Turkey	1997-2000	WHO	49	13	11	-	-	-	8	-	-	17	dichotomous, score
	Sakuragi et al[25]	Japan	1996-1999	WHO	42	7	-	-	10	3	-	-	-	22	intensity grade
2003	Maia et al[10]	Brazil	nr	EIN	12	-	-	-	-	-	-	11	1	-	dichotomous
2004	Erkanli et al[26]	Turkey	1990-1999	WHO	50	9	-	-	5	5	-	-	-	31	intensity grade
2007	Kapucuoglu et al[27]	Turkey	nr	WHO	81	9	20	7	-	10	-	-	-	35	dichotomous, score
2009	Nunobiki et al[28]	Japan	1997-2003	WHO	180	30	30	30	-	60	-	-	-	30	dichotomous, cell rate
2012	Upson et al[29]	USA	1985-2005	WHO	111	-	-	71	-	40	-	-	-	-	dichotomous
2016	Bozkurt et al[30]	Turkey	2007-2015	EIN	85	-	-	-	-	-	-	32	23	30	intensity grade, cell rate
2017	Trabzonlu et al[12]	Turkey	2006-2011	EIN	49	-	-	-	-	-	-	34	15	-	intensity grade
Total hyperplasias					1278	137	560					116		465	

–, none; +, positive; - ; negative; AH, atypical hyperplasia; CH, complex hyperplasia; EC, endometrial cancer; EIN, endometrial intraepithelial neoplasia; nr, not reported; SH, simple hyperplasia; PE, proliferative endometrium; WHO, World Health Organization.

FIGURE 2 (a) Assessment of risk of bias. Summary of risk of bias for each study; + low; – high; ? unclear risk of bias. (b) Risk of bias graph of each risk of bias item presented as percentages across all included studies [Color figure can be viewed at wileyonlinelibrary.com]

statistical significance (OR = 1.32, 95% CI, 0.55-3.15; $P = 0.53$), and with moderate heterogeneity ($I^2 = 57\%$) (Figure 3d).

3.2 | Diagnostic accuracy

3.2.1 | WHO group

In the WHO group the sensitivity and specificity of Bcl-2 loss in detecting premalignant endometrial hyperplasia were 0.41 (95% CI, 0.33-0.49) and 0.81 (95% CI, 0.75-0.86), respectively, with LR+ and LR− of 3.22 (95% CI, 1.45-7.14) and 0.69 (95% CI, 0.55-0.88), respectively. The diagnostic accuracy was low, with an AUC of 0.708. The heterogeneity was high for sensitivity ($I^2 = 82\%$) and specificity ($I^2 = 89.4\%$) and moderate for LR+ ($I^2 = 50.5\%$) and LR− ($I^2 = 61.4\%$) (Figure 4). Analyses of different thresholds of Bcl-2 expression were not available due to the lack of studies using a scoring system that combined the intensity of staining and the rate of stained cells.

3.2.2 | EIN group

In the EIN group we were able to perform analyses of diagnostic accuracy for a complete loss of expression and for three different thresholds of expression. For a complete loss of Bcl-2 expression, its sensitivity and specificity in detecting premalignant endometrial hyperplasia were 0.18 (95% CI, 0.08-0.34) and 0.97 (95% CI, 0.91-1.00), respectively, with LR+ and LR− of 5.16 (95%

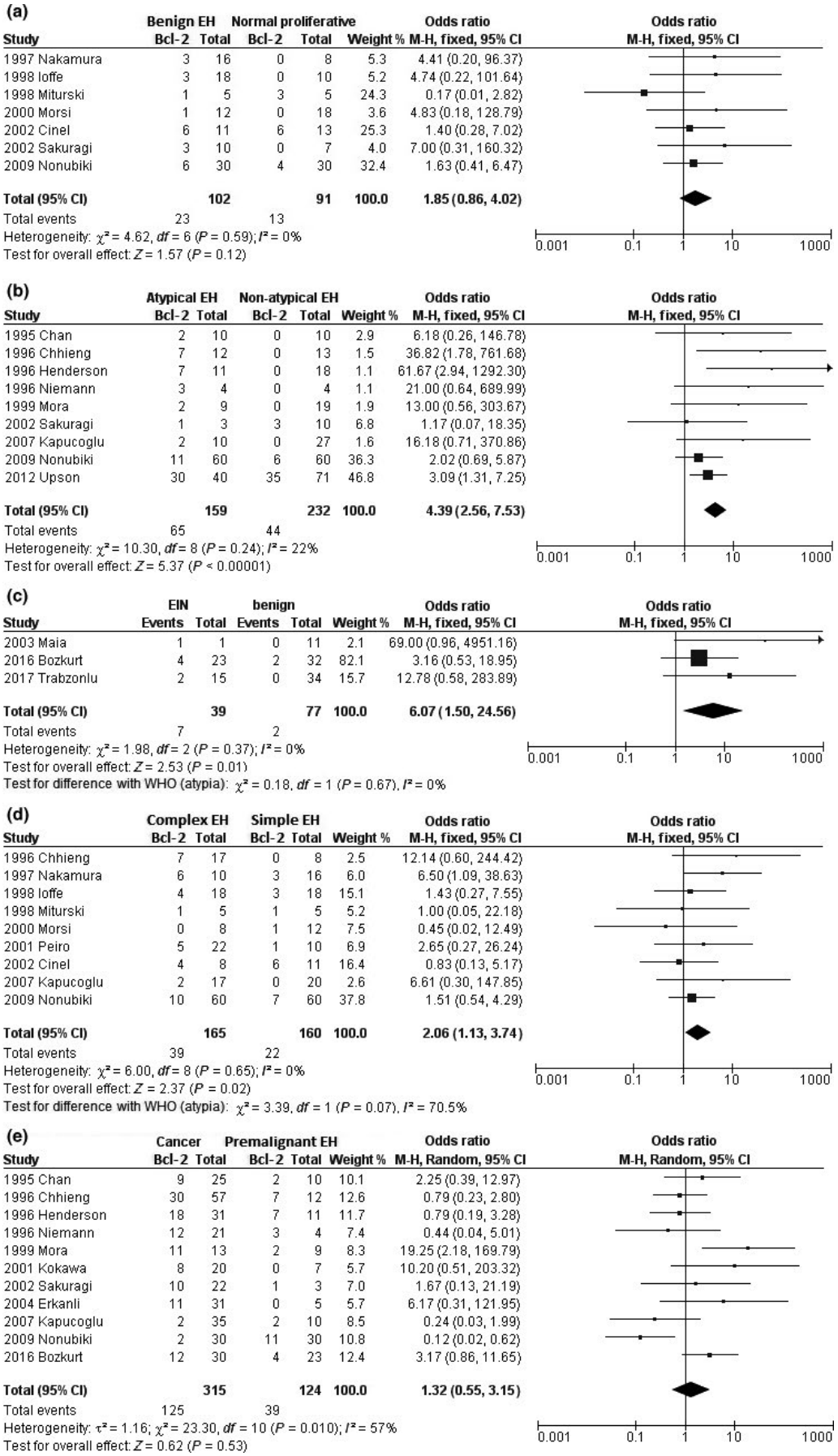
(a)
Benign EH
Normal proliferative
Odds ratio
Odds ratio
Study
Bcl-2 Total
Bcl-2 Total
Weight %
M-H, fixed, 95% CI
M-H, fixed, 95% CI
1997 Nakamura 3 16 0 8 5.3 4.41 (0.20, 96.37)
1998 Ioffe 3 18 0 10 5.2 4.74 (0.22, 101.64)
1998 Miturski 1 5 3 5 24.3 0.17 (0.01, 2.82)
2000 Morsi 1 12 0 18 3.6 4.83 (0.18, 128.79)
2002 Cinel 6 11 6 13 25.3 1.40 (0.28, 7.02)
2002 Sakuragi 3 10 0 7 4.0 7.00 (0.31, 160.32)
2009 Nonubiki 6 30 4 30 32.4 1.63 (0.41, 6.47)
Total (95% CI) 102 91 100.0 1.85 (0.86, 4.02)
Total events 23 13
Heterogeneity: χ² = 4.62, df = 6 (P = 0.59); I² = 0%
Test for overall effect: Z = 1.57 (P = 0.12)
0.001 0.1 1 10 1000
(b)
Atypical EH
Non-atypical EH
Odds ratio
Odds ratio
Study
Bcl-2 Total
Bcl-2 Total
Weight %
M-H, fixed, 95% CI
M-H, fixed, 95% CI
1995 Chan 2 10 0 10 2.9 6.18 (0.26, 146.78)
1996 Chhieng 7 12 0 13 1.5 36.82 (1.78, 761.68)
1996 Henderson 7 11 0 18 1.1 61.67 (2.94, 1292.30)
1996 Niemann 3 4 0 4 1.1 21.00 (0.64, 689.99)
1999 Mora 2 9 0 19 1.9 13.00 (0.56, 303.67)
2002 Sakuragi 1 3 3 10 6.8 1.17 (0.07, 18.35)
2007 Kapucoglu 2 10 0 27 1.6 16.18 (0.71, 370.86)
2009 Nonubiki 11 60 6 60 36.3 2.02 (0.69, 5.87)
2012 Upson 30 40 35 71 46.8 3.09 (1.31, 7.25)
Total (95% CI) 159 232 100.0 4.39 (2.56, 7.53)
Total events 65 44
Heterogeneity: χ² = 10.30, df = 8 (P = 0.24); I² = 22%
Test for overall effect: Z = 5.37 (P < 0.00001)
0.001 0.1 1 10 1000
(c)
EIN
benign
Odds ratio
Odds ratio
Study
Events Total
Events Total
Weight %
M-H, fixed, 95% CI
M-H, fixed, 95% CI
2003 Maia 1 1 0 11 2.1 69.00 (0.96, 4951.16)
2016 Bozkurt 4 23 2 32 82.1 3.16 (0.53, 18.95)
2017 Trabzonlu 2 15 0 34 15.7 12.78 (0.58, 283.89)
Total (95% CI) 39 77 100.0 6.07 (1.50, 24.56)
Total events 7 2
Heterogeneity: χ² = 1.98, df = 2 (P = 0.37); I² = 0%
Test for overall effect: Z = 2.53 (P = 0.01)
Test for difference with WHO (atypia): χ² = 0.18, df = 1 (P = 0.67), I² = 0%
0.001 0.1 1 10 1000
(d)
Complex EH
Simple EH
Odds ratio
Odds ratio
Study
Bcl-2 Total
Bcl-2 Total
Weight %
M-H, fixed, 95% CI
M-H, fixed, 95% CI
1996 Chhieng 7 17 0 8 2.5 12.14 (0.60, 244.42)
1997 Nakamura 6 10 3 16 6.0 6.50 (1.09, 38.63)
1998 Ioffe 4 18 3 18 15.1 1.43 (0.27, 7.55)
1998 Miturski 1 5 1 5 5.2 1.00 (0.05, 22.18)
2000 Morsi 0 8 1 12 7.5 0.45 (0.02, 12.49)
2001 Peiro 5 22 1 10 6.9 2.65 (0.27, 26.24)
2002 Cinel 4 8 6 11 16.4 0.83 (0.13, 5.17)
2007 Kapucoglu 2 17 0 20 2.6 6.61 (0.30, 147.85)
2009 Nonubiki 10 60 7 60 37.8 1.51 (0.54, 4.29)
Total (95% CI) 165 160 100.0 2.06 (1.13, 3.74)
Total events 39 22
Heterogeneity: χ² = 6.00, df = 8 (P = 0.65); I² = 0%
Test for overall effect: Z = 2.37 (P = 0.02)
Test for difference with WHO (atypia): χ² = 3.39, df = 1 (P = 0.07), I² = 70.5%
0.001 0.1 1 10 1000
(e)
Cancer
Premalignant EH
Odds ratio
Odds ratio
Study
Bcl-2 Total
Bcl-2 Total
Weight %
M-H, Random, 95% CI
M-H, Random, 95% CI
1995 Chan 9 25 2 10 10.1 2.25 (0.39, 12.97)
1996 Chhieng 30 57 7 12 12.6 0.79 (0.23, 2.80)
1996 Henderson 18 31 7 11 11.7 0.79 (0.19, 3.28)
1996 Niemann 12 21 3 4 7.4 0.44 (0.04, 5.01)
1999 Mora 11 13 2 9 8.3 19.25 (2.18, 169.79)
2001 Kokawa 8 20 0 7 5.7 10.20 (0.51, 203.32)
2002 Sakuragi 10 22 1 3 7.0 1.67 (0.13, 21.19)
2004 Erkanli 11 31 0 5 5.7 6.17 (0.31, 121.95)
2007 Kapucoglu 2 35 2 10 8.5 0.24 (0.03, 1.99)
2009 Nonubiki 2 30 11 30 10.8 0.12 (0.02, 0.62)
2016 Bozkurt 12 30 4 23 12.4 3.17 (0.86, 11.65)
Total (95% CI) 315 124 100.0 1.32 (0.55, 3.15)
Total events 125 39
Heterogeneity: τ² = 1.16; χ² = 23.30, df = 10 (P = 0.010); I² = 57%
Test for overall effect: Z = 0.62 (P = 0.53)
0.001 0.1 1 10 1000

FIGURE 3 Forest plots reporting graphically odds ratio for B-cell lymphoma 2 loss of expression in: (a) benign hyperplasia vs normal proliferative endometrium; (b) atypical vs non-atypical hyperplasia; (c) Endometrial intraepithelial neoplasia vs benign hyperplasia; (d) simple vs complex hyperplasia; (e) premalignant hyperplasia vs cancer. Odds ratios were calculated for each study and as pooled estimates with 95% CI. Bcl-2, B-cell lymphoma 2; EH, endometrial hyperplasia; LR, likelihood ratio; MH, Mantel-Haenszel method

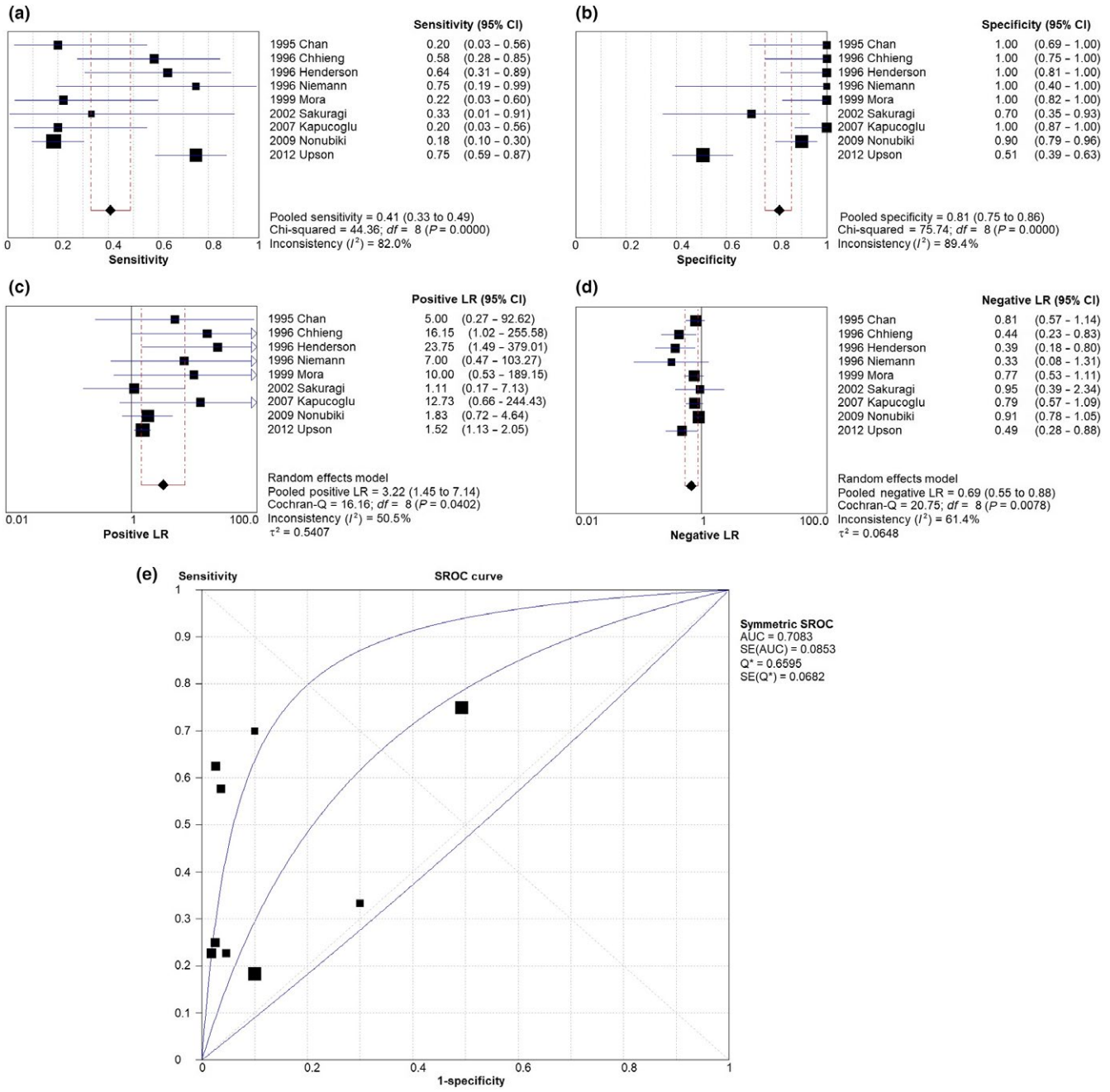

FIGURE 4 Forest plots reporting graphically diagnostic accuracy of immunohistochemical loss of B-cell lymphoma 2 expression in differentiating between benign and premalignant hyperplasia as defined by 2014 WHO criteria: (a) sensitivity; (b) specificity; (c) positive likelihood ratio; (d) negative likelihood ratio; (e) area under the curve (AUC) on summary receiver operating characteristic curves (SROC). LR, likelihood ratio [Color figure can be viewed at wileyonlinelibrary.com]

CI, 1.46-18.40) and 0.86 (95% CI, 0.75-1.00), respectively. The diagnostic accuracy was high, with an AUC of 0.938 calculated on summary receiver operating characteristic curves. The heterogeneity was insignificant for sensitivity (I^2 = 45.6) and specificity (I^2 = 44.3), and absent for LR+ (I^2 = 0%) and LR– (I^2=0%) (Figure 5).

For threshold I (weak intensity in ≤ 33% cells), sensitivity and specificity were 0.18 (95% CI, 0.08-0.34) and 0.91 (95% CI, 0.81-0.97), respectively, with LR+ and LR– of 1.88 (95% CI, 0.69-5.16) and 0.91 (95% CI, 0.77-1.07), respectively. Heterogeneity was absent for all analyses (I^2 = 0%).

For threshold II (weak intensity in 33%-67% cells or moderate intensity in ≤ 33% cells), sensitivity and specificity were 0.26 (95% CI, 0.13-0.43) and 0.76 (95% CI, 0.64-0.85), respectively, with LR+ and LR− of 1.11 (95% CI, 0.48-2.60) and 0.98 (95% CI, 0.74-1.29), respectively. Heterogeneity was absent for sensitivity (I^2 = 0%), insignificant for specificity (I^2 = 2.9%) and LR+ (I^2 = 24.7%), low for LR− (I^2 = 30.3%).

For threshold III (weak intensity in > 67% cells, moderate intensity in 33%-67% cells, or strong intensity in ≤ 33% cells), sensitivity and specificity were 0.47 (95% CI, 0.21-2.82) and 0.44 (95% CI 0.32-0.57), respectively, with LR+ and LR− of 0.78 (95% CI, 0.48-2.60) and 1.26 (95% CI, 0.39-4.05), respectively. Heterogeneity was high for sensitivity (I^2 = 77.2%), LR+ (I^2 = 84.8%) and LR− (I^2 = 87.5%), and moderate for specificity (I^2 = 74.1%).

The AUC calculation was not feasible in the analyses of expression thresholds.

4 | DISCUSSION

Our study showed that Bcl-2 loss was significantly associated with premalignant features of endometrial hyperplasia, while no significant differences were found between proliferative endometrium and benign endometrial hyperplasia, and between premalignant

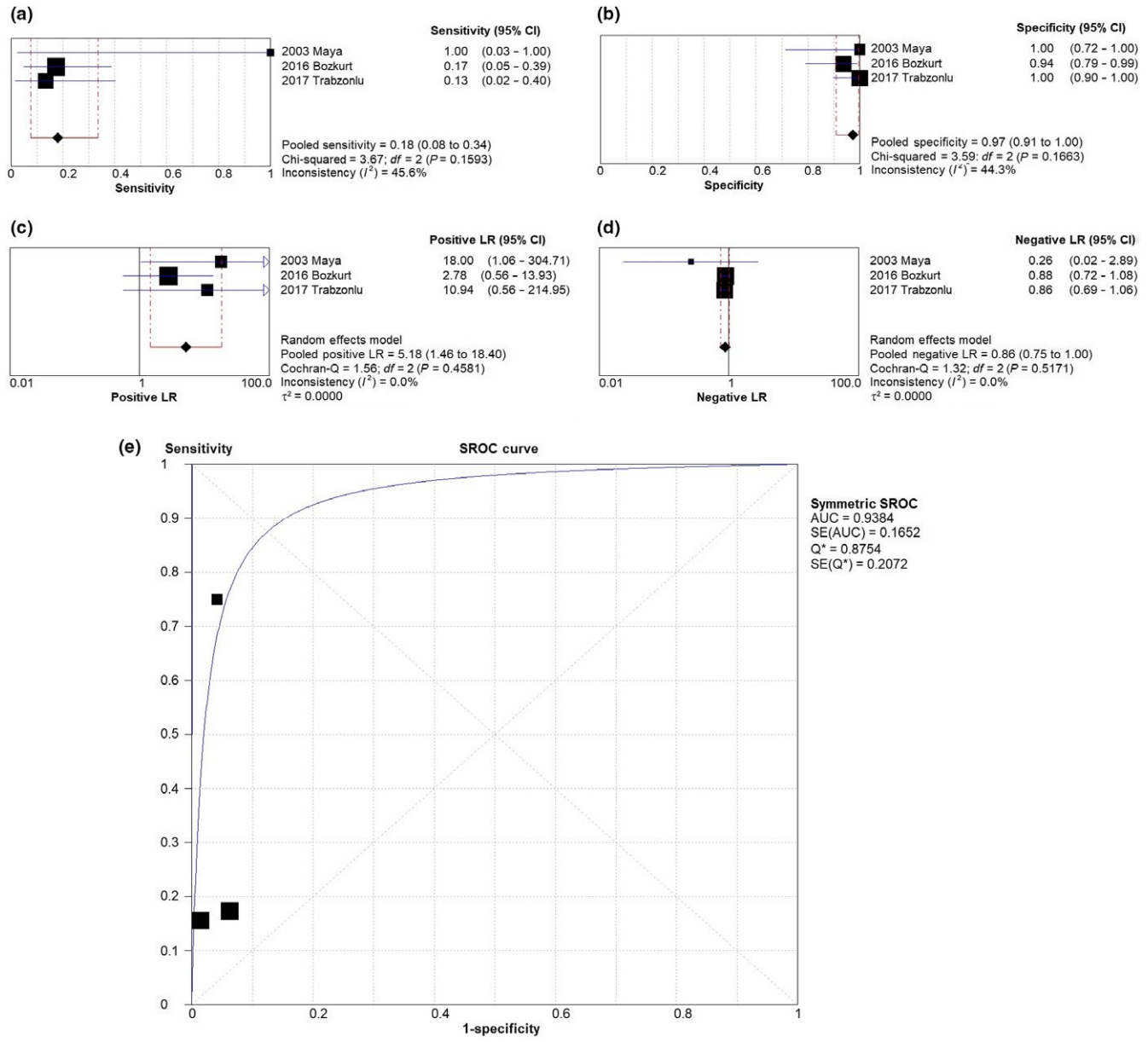

FIGURE 5 Forest plots reporting graphically diagnostic accuracy of immunohistochemical loss of B-cell lymphoma 2 expression in differentiating between benign and premalignant hyperplasia as defined by endometrial intraepithelial neoplasia criteria: (a) sensitivity; (b) specificity; (c) positive likelihood ratio; (d) negative likelihood ratio; (e) area under the curve (AUC) on summary receiver operating characteristic curves. LR, likelihood ratio [Color figure can be viewed at wileyonlinelibrary.com]

endometrial hyperplasia and endometrial cancer. These findings strongly suggest that Bcl-2 loss may be a marker of endometrial neoplasia, and they are compatible with scientific evidence on Bcl-2 physiology. In fact, as Bcl-2 is under hormonal control, its expression is expected to be high in benign proliferative conditions caused by the action of estrogens such as proliferative endometrium and benign endometrial hyperplasia.[8,9,31]

Our results showed there was a stronger association between Bcl-2 loss and the premalignant morphology of endometrial hyperplasia when the EIN system was adopted. Once it is assumed that Bcl-2 loss is a marker of precancer, such results suggest that the EIN criteria are more reliable than the WHO criteria. This observation is in agreement with several findings reported in the literature, supporting the view that the EIN system is more accurate than the WHO system in predicting progression to endometrial cancer.[2,3,6] However, the difference between the OR found in the two groups was not significant ($P = 0.67$). EIN criteria of premalignancy include glandular crowding, lesion size > 1 mm, different cytology from the adjacent endometrium, exclusion of mimics and cancer.[3] On the other hand, the WHO system is based on cytological atypia alone, and the complexity of glandular architecture has been disregarded.[1,2] However, Kurman et al. observed a higher rate of progression to cancer in simple atypical endometrial hyperplasia compared with complex non-atypical endometrial hyperplasia, thus considering cytological atypia as the crucial feature in the risk stratification.[32] Although Bcl-2 loss showed a stronger association with atypia than complexity (4.39 vs 2.06), the difference between the two OR was not significant ($P = 0.07$). In fact, it has been shown that complexity is associated with a higher risk of malignant progression. The study by Kurman et al showed that, among non-atypical endometrial hyperplasia, complex endometrial hyperplasia progresses to cancer more frequently than simple endometrial hyperplasia.[32] Furthermore, Baak et al. reported similar rates of progression between complex and simple atypical endometrial hyperplasia, showing that the EIN system classifies as premalignant a considerable percentage of complex non-atypical endometrial hyperplasias.[6]

As a diagnostic marker in the differential diagnosis between benign and premalignant endometrial hyperplasia the accuracy of Bcl-2 appears to vary according to the classification system adopted. In fact, while in the WHO group the diagnostic accuracy was low (AUC = 0.708), in the EIN group the accuracy become high (AUC = 0.938) due to a considerably higher specificity compared with the WHO group (0.97 vs 0.81). This finding means that Bcl-2 loss may frequently be found in non-atypical endometrial hyperplasia, which is considered benign in the WHO system. Under the assumption that Bcl-2 loss is a marker of premalignancy, such endometrial hyperplasias would actually be precancer misdiagnosed by the WHO system. As discussed above, complex non-atypical endometrial hyperplasia may account for most of these cases.

However, independent of the classification adopted, the sensitivity was low, indicating that only a minor percentage of premalignant endometrial hyperplasia shows the loss of Bcl-2 expression. Such limits cannot be overcome, even by increasing the threshold of Bcl-2 expression to be considered. As the threshold increases, sensitivity improves slightly (complete loss: 0.18→threshold I: 0.18→threshold II: 0.26→threshold III: 0.44), but specificity dramatically worsens (0.97→0.91 → 0.76→0.44), as well as the LR+ (5.16→1.88→1.11→0.78) and the LR− (0.86→0.91→0.98→1.26). This makes immunohistochemistry for Bcl-2 inadequate as a stand-alone test in the routine diagnosis of endometrial hyperplasia, since many patients at risk of progression would be missed. On the other hand, the specificity of Bcl-2 loss for the EIN criteria of premalignancy appeared excellent. Thus, Bcl-2 loss in endometrial

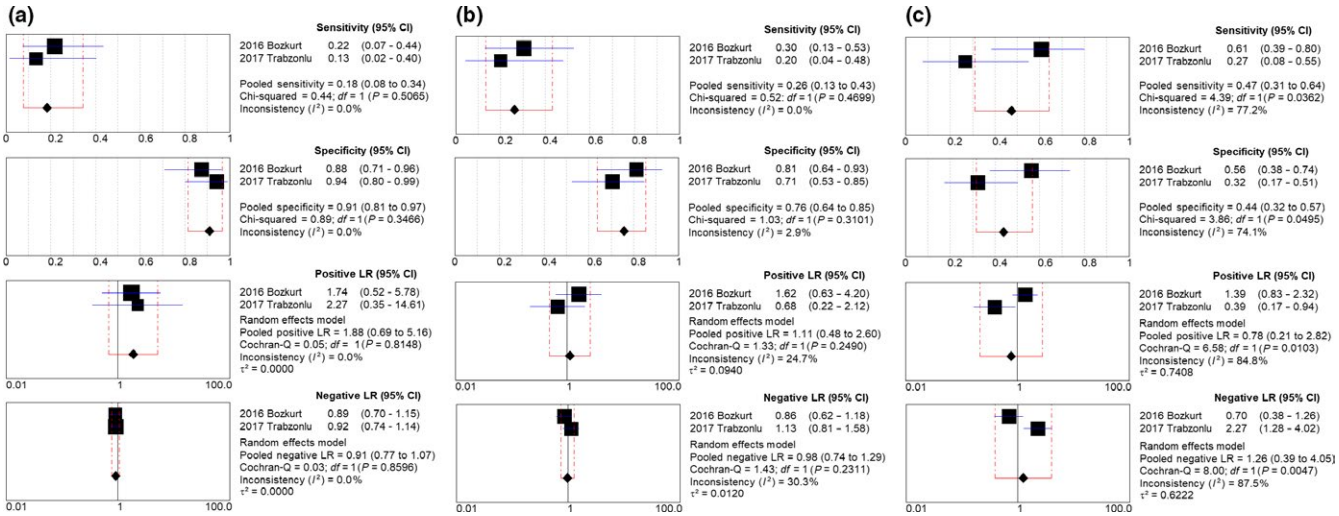

FIGURE 6 Forest plots reporting graphically sensitivity, specificity, positive, and negative likelihood ratio of immunohistochemical loss of B-cell lymphoma 2 (Bcl-2) expression in differentiating between benign and premalignant hyperplasia as defined by endometrial intraepithelial neoplasia criteria, based on three thresholds of Bcl-2 expression: (a) I (weak expression in < 33% cells); (b) II (weak intensity in 33%-67% cells or moderate intensity in < 33% cells); (c) III (weak intensity in > 67% cells or moderate intensity in 33%-67% cells or strong intensity in < 33% cells) [Color figure can be viewed at wileyonlinelibrary.com]

hyperplasia should be considered to be strongly indicative of the neoplastic process. When the premalignant features in endometrial hyperplasia are ambiguous the finding of Bcl-2 loss may still indicate treatment, especially in presence of a complex glandular architecture. Bcl-2 loss may be a novel indication for treatment and follow up in women with endometrial hyperplasia.

The value of Bcl-2 may be confirmed by its evaluation as a prognostic marker of progression to cancer. In fact, we found only one study that assessed Bcl-2 as a marker of the progression of endometrial hyperplasia to endometrial cancer, showing a significant association between a decreased expression (rate of stained cells ≤ 80%) and the subsequent development of endometrial cancer.[33]

As Bcl-2 expression may not decrease simultaneously in glands and stroma[34] it would be interesting to study the possible relevance of Bcl-2 stromal expression in the differential diagnosis of endometrial hyperplasia. Nevertheless, given the recent discoveries on the genetics of endometrial cancer, the need for a molecular definition of endometrial neoplastic specimens has been growing, as well as the search for cheaper immunohistochemical surrogates of genetic prognostic markers.[35] Thus, the importance of Bcl-2 and other immunohistochemical markers may be set to increase in the near future.

To the best of our knowledge, this study may be the first meta-analysis assessing the expression of Bcl-2 in endometrial hyperplasia. We defined the association of Bcl-2 loss with the neoplastic nature of endometrial hyperplasia, and the accuracy of immunohistochemistry for Bcl-2 in a differential diagnosis between benign and premalignant endometrial hyperplasia. Furthermore, we assessed the influence of the histological criteria adopted on the results, and we interpreted the results based on the scientific evidence reported in the literature. On the other hand, one limitation of our study may be found in the low uniformity of the methods among the included studies. In fact, several differences were found with regard to the baseline characteristics of the patients (such as age and body mass index) and the type of sample (such as hysteroscopic biopsy, curettage, or surgical specimen). The selection criteria for participants differed among the studies, and in most cases they were not specified (Figure 2). Further studies in this field should include consecutive patients to avoid spectrum bias.

Histological slides were reviewed simultaneously by at least two pathologists in fewer than 50% of the included studies, creating a possible bias in the reference standard (Figure 2). However, statistical heterogeneity among studies was low or absent in most of our analyses, giving solidity to our results. High heterogeneity was observed only for the diagnostic accuracy analysis, where it is expected.[13]

Another limitation may consist in the absence of a validated method to interpret Bcl-2 immunostaining in terms of the intensity of the staining and the rate of stained cells. Nonetheless, we considered only a complete loss of Bcl-2 expression in the main analysis, limiting the risk of bias caused by the low reproducibility of a qualitative scoring. Moreover, for the assessment of the different thresholds of Bcl-2 expression, we limited the analysis only to studies adopting a complete and clearly defined scoring system, which considered both the intensity of the staining and the rate of the stained cells.

5 | CONCLUSION

A loss of immunohistochemical expression of Bcl-2 in endometrial hyperplasia is significantly associated with premalignancy, regardless of the classification system adopted. When assessed with the EIN criteria, Bcl-2 loss appeared as a highly specific marker of endometrial precancer, with high diagnostic accuracy. Thus, the finding of Bcl-2 loss in endometrial hyperplasia might be a novel indication for treatment and follow-up, especially when precancerous features are ambiguous at histological examination. Thresholds of Bcl-2 expression that differ from a complete loss showed a lower diagnostic accuracy and their use should not be considered. Our results also suggest that EIN classification system is more reliable than the WHO system, suggesting that the complexity of glandular architecture as a premalignant feature is an important criterion. However, further studies examining Bcl-2 loss as independent prognostic factor are needed to use this certain marker for treatment in the absence of overt precancerous features in histological examination.

CONFLICT OF INTEREST

The authors have stated explicitly that there are no conflicts of interest in connection with this article.

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