APMIS JOURNAL OF PATHOLOGY, MICROBIOLOGY AND IMMUNOLOGY

APMIS 127: 699–709

DOI 10.1111/apm.12988

Nuclear expression of β-catenin in endometrial hyperplasia as marker of premalignancy

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Travaglino A, Raffone A, Saccone G, Mascolo M, D'Alessandro P, Arduino B, Mollo A, Insabato L, Zullo F. Nuclear expression of β-catenin in endometrial hyperplasia as marker of premalignancy. APMIS 2019; 127: 699–709.

We aimed to assess (1)-whether nuclear β-catenin is a marker of endometrial precancer, and (2)-the diagnostic accuracy of β-catenin immunohistochemistry in the differential diagnosis between benign and premalignant endometrial hyperplasia (EH), defining criteria for its use. Electronic databases were searched for studies evaluating β-catenin immunohistochemistry in normal endometrium (NE), benign and/or premalignant EH, and endometrioid carcinoma (EC). Odds ratio (OR; $p < 0.05$), sensitivity, specificity, diagnostic OR (DOR), positive and negative likelihood ratios (LR+, LR−) were calculated. Subgroup analyses were based on the classification system used (WHO or EIN) and criteria to define aberrant β-catenin expression (only nuclear or cytoplasmic/nuclear). Twelve studies with 1510 specimens were included. Nuclear β-catenin rate significantly increased from NE to benign EH (OR = 26.01; p = 0.0002, only in WHO subgroup), and from benign EH to premalignant EH (OR = 3.89; p = 0.0002; more markedly in EIN subgroup), but not from premalignant EH to EC (OR = 0.78; p = 0.29). Nuclear β-catenin accuracy was very low in WHO subgroup (sensitivity = 0.40, specificity = 0.76, LR+ = 1.85, LR− = 0.72; DOR = 2.89) and moderate in EIN subgroup (sensitivity = 0.19, specificity = 1.00, LR+ = 14.80, LR− = 0.83; DOR = 18.14). Cytoplasmic/nuclear β-catenin accuracy was absent in WHO subgroup (sensitivity = 0.45, specificity = 0.54, LR+ = 1.01, LR− = 1.01; DOR = 0.99) and low in EIN subgroup (sensitivity = 0.57, specificity = 0.86, LR+ = 3.63, LR− = 0.51; DOR = 8.30). Considering nuclear expression and using EIN system, β-catenin immunohistochemistry might be reliable as rule-in test for diagnosis of endometrial precancer, with perfect specificity and moderate overall accuracy.

Key words: Atypical endometrial hyperplasia; endometrial hyperplasia without atypia; endometrial intraepithelial neoplasia; endometrial precancer.

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β-catenin is a key protein in Wnt signaling pathway and is normally expressed on the cellular membrane, where it links cell-cell adherens junctions to the cytoskeleton. When Wnt pathway is activated, β-catenin accumulates in the cytoplasm and then migrates into the nucleus, where it binds to transcription factors of the LEF/TCF family. While Wnt-β-catenin pathway plays a physiological role in embryo development and cells proliferation, its pathologic activation can lead to cancerous transformation (1–3). In this respect, Wnt-β-catenin pathway is known to be involved in endometrial carcinogenesis, with specific regard to endometrioid adenocarcinoma and its precursor endometrial hyperplasia (EH) (2–4). In this field, the scientific interest in β-catenin has recently increased, since mutations in its codifying gene *CTNNB1* have shown an independent prognostic value in endometrial cancer, delineating a separate molecular subgroup (3, 5).

Great interest has also been given to β-catenin assessment in the precancerous phase (6–17). In

Received 14 March 2019. Accepted 23 July 2019

particular, β-catenin has been one of the most important markers studied to differentiate between premalignant EH and benign EH caused by unopposed action of estrogens (18). To date, the gold standard for such differential diagnosis is histologic examination, with two possible classification systems: the World Health Organization (WHO) system, based on the presence of cytologic atypia, and the endometrial intraepithelial neoplasia (EIN) system, based on several morphologic parameters (19–23). However, histologic examination has several issues such as low reproducibility and possibility of artifact changes, ambiguous features, or tissue inadequacy (24–28).

Although several studies assessed β-catenin expression by immunohistochemistry on EH specimens, the results are conflicting, and the activation of Wnt pathway also occurs in normal proliferative endometrium, consequently to the action of estrogens (2, 6). Therefore, the actual usefulness of β-catenin in this field was never determined.

Thus, aims of our study were:

1. to define whether nuclear expression of β-catenin is a marker of endometrial precancer, by assessing its expression in normal endometrium (NE), benign hyperplasia (EH), premalignant EH, and endometrioid carcinoma (EC), evaluating how the results change according to the histologic classification system used (WHO or EIN);
2. to define the diagnostic accuracy of β-catenin in differentiating premalignant EH from benign EH, assessing how the accuracy is influenced by the criteria used to define β-catenin pattern as aberrant (i.e., only nuclear or even cytoplasmic accumulation).

METHODS

Study protocol

The study protocol was designed *a priori*, and methods for electronic search, study selection, risk of bias assessment, data extraction, and data analysis were defined. All review stages were conducted independently by two authors (AT, AR), and all 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 (PRISMA) statement (29).

Search strategy

MEDLINE, EMBASE, Web of Sciences, Scopus, ClinicalTrial.gov, OVID, Cochrane Library, and Google Scholar were searched for relevant articles from the inception to January 2019, by using a combination of the following text words and all their synonyms found on Medical SubHeading (MeSH) vocabulary: 'endometrial hyperplasia'; 'endometrial cancer'; 'endometrial intraepithelial neoplasia'; 'EIN'; 'precancer'; 'premalignant'; 'precursor'; 'β-catenin'; 'beta-catenin'; 'nuclear'; 'Wnt pathway'; 'marker'; 'biomarker'; 'immunohistochemistry'; 'immunohistochemical'. Abstracts of all relevant references found were also reviewed.

Study selection

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

The following exclusion criteria, defined *a priori*, were adopted:

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

Risk of bias assessment

The revised Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) (30) was adapted to the included studies, and four domains related to risk of bias were assessed in each study: (i) patient selection (if the patients were selected consecutive, or at least inclusion criteria and period of enrollment were reported); (ii) index test (if criteria for assessment of β-catenin were specified), (iii) reference test (if histologic classification criteria were specified), (iv) flow and timing (if all patients were assessed with the same tests; if all patients were assessed with both index and reference tests). Authors' judgments were categorized as 'low risk', 'high risk', or 'unclear risk of bias'. Concerns about applicability (i.e., if study methods did not suit the aim of our meta-analysis) were also assessed for the domains 1, 2, and 3.

Data extraction and analysis

Statistical association of β-catenin with histologic categories

Data were extracted from the included studies without any modifications. For each study, 2×2 contingency tables were prepared, reporting two dichotomous qualitative variables:

1. β-catenin expression ('nuclear' vs 'non-nuclear');
2. histologic category (benign EH vs NE; premalignant EH vs benign EH; EC vs premalignant EH).

For the studies that did not dichotomize β-catenin expression, data were extracted by using the following criteria:

1. for the studies using a semi-quantitative scale (1–3) to grade the intensity β-catenin nuclear staining, the presence of an intensity at least moderate (grade 2) was considered as 'nuclear expression', since it has been suggested that a weak nuclear expression of β-catenin is non-specific and may be caused by hormonal action (3).
2. for the studies assessing the rate of cells showing β-catenin nuclear staining, the presence of at least a minimum percentage (1%) was considered as 'nuclear expression'.

Data regarding EH categories were extracted by using the following criteria:

1. for the studies using the WHO classification, atypical EH (simple or complex) was considered as 'premalignant EH', while EH without atypia (simple or complex) was considered as 'benign EH';
2. for the studies using the EIN classification, EH classified as 'EIN' was considered as 'premalignant EH', while EH classified as 'benign hyperplasia' was considered as 'benign EH'.

Statistical association was assessed by using odds ratio (OR), with 95% confidence interval (CI); a p-value < 0.05 was considered significant. OR was calculated for each study and as pooled estimate and reported graphically on a forest plot.

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 $I^2 \geq 50\%$, a random effect model was preferred; otherwise, a fixed-effect model was adopted.

A subgroup analysis was performed according to the classification system used (WHO vs EIN).

Diagnostic accuracy in differentiating between benign and premalignant EH

Beta-catenin expression was the index test, while EH morphology was the reference standard.

EH specimens with β-catenin nuclear expression were considered as 'true positive' when they showed premalignant morphology, and 'false positive' when they showed benign morphology. On the other hand, EH without β-catenin nuclear expression was considered as 'true negative' when they showed benign morphology and 'false negative' when they showed premalignant morphology.

Diagnostic accuracy was assessed as sensitivity, specificity, positive likelihood ratio (LR+), negative likelihood ratio (LR−), and diagnostic OR (DOR), with 95% CI. DOR was used to quantity the overall diagnostic accuracy, as follows: $DOR \leq 1$: no accuracy; $1 < DOR < 3$: very low accuracy; $3 \leq DOR < 10$: low accuracy; $10 \leq DOR < 25$: moderate accuracy; $25 \leq DOR < 100$: high accuracy; $DOR \geq 100$: very high accuracy. A random effect model was planned *a priori*, since a significant heterogeneity is expected in meta-analyses of diagnostic accuracy (31).

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

RESULTS

Selection and characteristics of the studies

Out of eighteen studies assessed for eligibility, 4 (32–35) were excluded because of non-extractable data and 2 (36, 37) because of the absence of EH specimens. Twelve observational studies (6–17) were finally included in the systematic review, with a total of 270 NE, 312 benign EH, 303 premalignant EH, and 625 EC. The flow diagram of the process of study selection is reported in Figure S1.

Eight studies adopted the WHO classification system and 4 the EIN system. Nine studies considered only a nuclear expression of β-catenin as positive, two studies evaluated separately nuclear and cytoplasmic expression, and 3 studies lumped together nuclear expression and strong cytoplasmic expression.

Characteristics of the included studies are shown in Table 1.

Risk of bias assessment

Authors' judgements regarding risk of bias are summarized in Figure S2.

For the patient selection domain, 6 studies were considered at low risk of bias, since they included consecutive patients, or specified at least inclusion criteria and period of enrollment, while the other 6 were considered at unclear risk because the inclusion method was not fully specified. Concerns about applicability were raised for two studies [selection restricted to EH or EC conservatively treated (10); selection of EH with squamous morular metaplasia (11)].

For the index test domain, 11 studies were considered at low risk since they reported in detail the method to interpret immunostaining, while the

Table 1. Characteristics of the included studies

Year	First author	Country	Period of enrollment	System adopted	Sample size	Normal			Hyperplastic		
						TOT	↑	nucl	TOT	↑	nucl
1999	Nei	Japan	n.r.	WHO	80	30	0	0	14	8	8
2001	Saegusa	Japan	1988–1999	WHO	409	141	n.r.	0	37	n.r.	4
2002	Ashihara	Japan	n.r.	WHO	45	–	–	–	17	n.r.	7
2003	Moreno Bueno	Spain	n.r.	WHO	146	–	–	–	–	–	–
2003	Saegusa	Japan	1988–2000	WHO	24	–	–	–	–	–	–
2005	Brachtel	USA, Spain	n.r.	WHO	24	–	–	–	–	–	–
2007	Norimatsu	Japan	1998–2005	EIN	90	20	n.r.	0	32	n.r.	0
2009	Liao	China	n.r.	WHO	146	15	n.r.	0	14	n.r.	1
2010	Xiong	China	2001–2006	EIN	117	10	0	0	59	5	0
2013	Li	China	2008–2010	EIN	101	10	0	0	40	9	n.r.
2016	Senol	Turkey	2007–2014	WHO	279	30	n.r.	n.r.	99	44	n.r.
2018	Strickland	USA	n.r.	EIN	49	14	0	0	–	–	–

remaining study was considered at unclear risk because immunohistochemical data were reported approximatively. Concerns about applicability were raised for two studies, since they did not aim to specifically assess the expression of β-catenin in the nucleus.

For the reference test domain, all studies were considered at low risk because they referred to the two gold standard classification systems (WHO and EIN).

For the flow and timing domain, all studies were considered at low risk since all patients were assessed using the same index and reference test, and the histological specimens are not influenced by the timing.

No further concerns about applicability were found.

Meta-analysis

Association

Nuclear expression of β-catenin was significantly more common in benign EH than in NE (OR = 26.01, 95% CI 4.73–143.02; p = 0.0002) in the WHO subgroup; in the EIN subgroup, no case of nuclear expression of β-catenin was reported in benign EH or NE, not allowing a comparison (Fig. 1).

Nuclear β-catenin was significantly more common in premalignant EH than in benign EH in the overall analysis (OR = 3.89, 95% CI 1.90–7.98; p = 0.0002); as well as in the WHO subgroup (OR = 2.58, 95% CI 1.16–5.75; p = 0.02) and in the EIN subgroup (OR = 19.65, 95% CI 2.33–165.82; p = 0.0006) (Fig. 2).

No significant difference was found between premalignant EH and EC (OR = 0.78, 95% CI 0.50–1.23; p = 0.29); the EIN subgroup analysis was not feasible due to the presence of only one available study (Fig. 3).

Diagnostic accuracy

In the WHO subgroup, sensitivity and specificity of nuclear expression of β-catenin in diagnosing endometrial precancer were 0.40 (95% CI, 0.28–0.53) and 0.76 (95% CI, 0.65–0.84), respectively, with LR+ and LR− of 1.85 (95% CI, 0.79–4.31) and 0.72 (95% CI, 0.59–0.89), respectively; the overall accuracy was very low, with a DOR of 2.89 (95% CI, 1.00–8.39) (Fig. 4).

In the EIN subgroup, sensitivity and specificity of nuclear expression of β-catenin in diagnosing endometrial precancer were 0.19 (95% CI, 0.10–0.31) and 1.00 (95% CI, 0.96–1.00), respectively, with LR+ and LR− of 14.80 (95% CI, 1.91–114.60) and 0.83 (95% CI, 0.67–1.02), respectively, and a moderate diagnostic accuracy (DOR = 18.14, 95% CI 2.22–148.5) (Fig. 5).

Considering the criterion 'cytoplasmic and/or nuclear expression', β-catenin showed sensitivity = 0.45 (95% CI, 0.32–0.59), specificity = 0.54 (95% CI, 0.44–0.64), LR+=1.01 (95% CI, 0.71–1.42), LR- = 1.01 (95% CI, 0.76–1.36) and no diagnostic accuracy (DOR = 0.99, 95% CI, 0.51–1.89) in the WHO subgroup (Fig. 4), and sensitivity = 0.57 (95% CI 0.42–0.71), specificity = 0.86 (95% CI, 0.77–0.92), LR+=3.63 (95% CI, 2.15–6.14), LR− = 0.51 (95% CI, 0.29–0.91), and low diagnostic accuracy (DOR = 8.30, 95% CI, 3.60–19.1) in the EIN subgroup (Fig. 5).

DISCUSSION

Main findings and interpretation

Our analysis showed that nuclear expression of β-catenin was significantly associated with both WHO and EIN criteria of premalignancy. No

Premalignant			Malignant			Immunostaining interpretation	Antibody manufacturer
TOT	↑	nucl	TOT	↑	nucl		
6	4	4	30	9	9	Intensity grade	Transduction Lab. (USA)
32	n.r.	10	199	n.r.	65	Stained cells rate	Transduction Lab. (USA)
8	n.r.	3	20	n.r.	8	Intensity grade	Transduction Lab. (USA)
20	n.r.	4	126	n.r.	31	Combined score	Transduction Lab. (USA)
5	n.r.	2	19	n.r.	12	Stained cells rate	Transduction Lab. (USA)
24	n.r.	15	–	–	–	Intensity grade	Transduction Lab. (USA)
38	n.r.	10	–	–	–	Dichotomous	Novocastra (UK)
37	n.r.	12	80	n.r.	18	Stained cells rate	Transduction Lab. (USA)
24	10	2	24	14	0	Stained cells rate	Maixin-Biotech. (CHN)
25	18	n.r.	26	25	n.r.	Dichotomous	Neomarkers Inc. (USA)
49	21	n.r.	101	76.	n.r.	Stained cells rate	Cell Marque (USA)
35	6	n.r.	–	–	–	Comparison with background	Dako (DK)

difference was found between premalignant EH and EC, while significant difference between NE and benign EH was found only for the WHO criteria. The criterion 'only nuclear expression' of β-catenin showed very low accuracy using WHO criteria, and moderate accuracy using EIN criteria. On the other hand, 'cytoplasmic and/or nuclear expression' showed no accuracy using WHO criteria and low accuracy using EIN criteria.

These findings support that nuclear β-catenin is a marker of endometrial neoplasia. This was not a foregone conclusion. In fact, since Wnt-β-catenin signaling is stimulated by estradiol, an unopposed estrogen action leads to a constitutive activation of this pathway (2); therefore, a nuclear expression of β-catenin might have been expected in benign EH. However, as we discussed in our previous study, it seems that hormonal action may only lead to a weak nuclear expression of β-catenin, which appears as a light brown nuance. On the other hand, a moderate-to-strong nuclear expression correlates with mutations in the exon 3 of *CTNNB1* (β-catenin gene), particularly in endometrial specimens (3, 6, 38). Our current study tried to eliminate

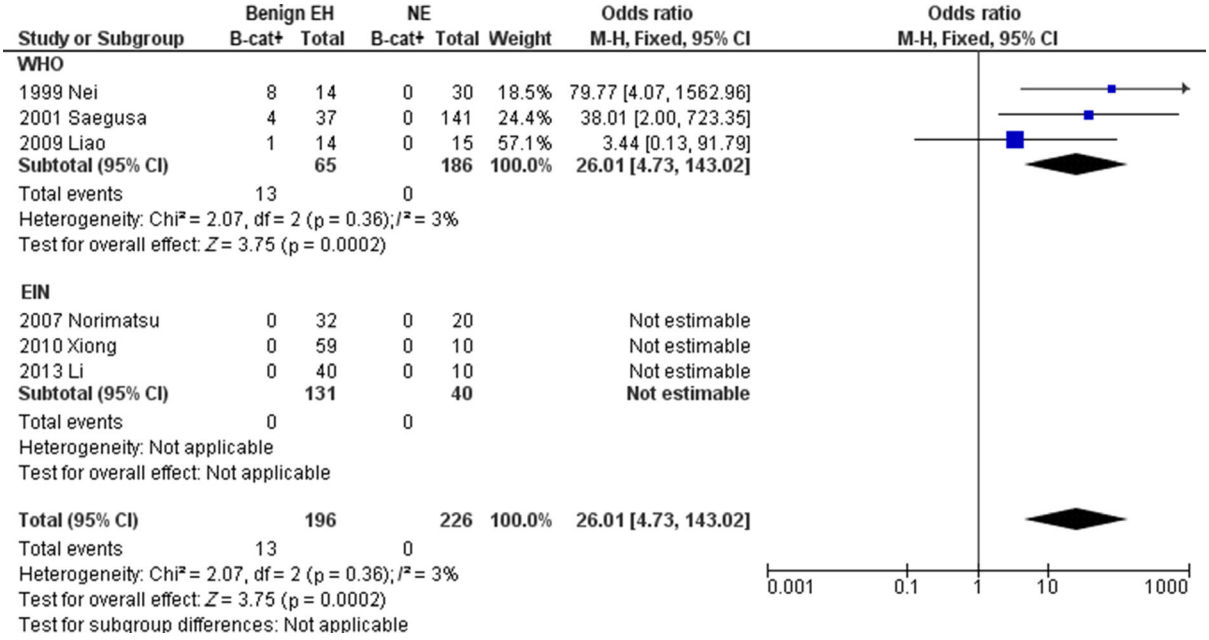

Fig. 1. Forest plot reporting graphically odds ratio for β-catenin nuclear expression in benign endometrial hyperplasia (EH) vs normal endometrium (NE).

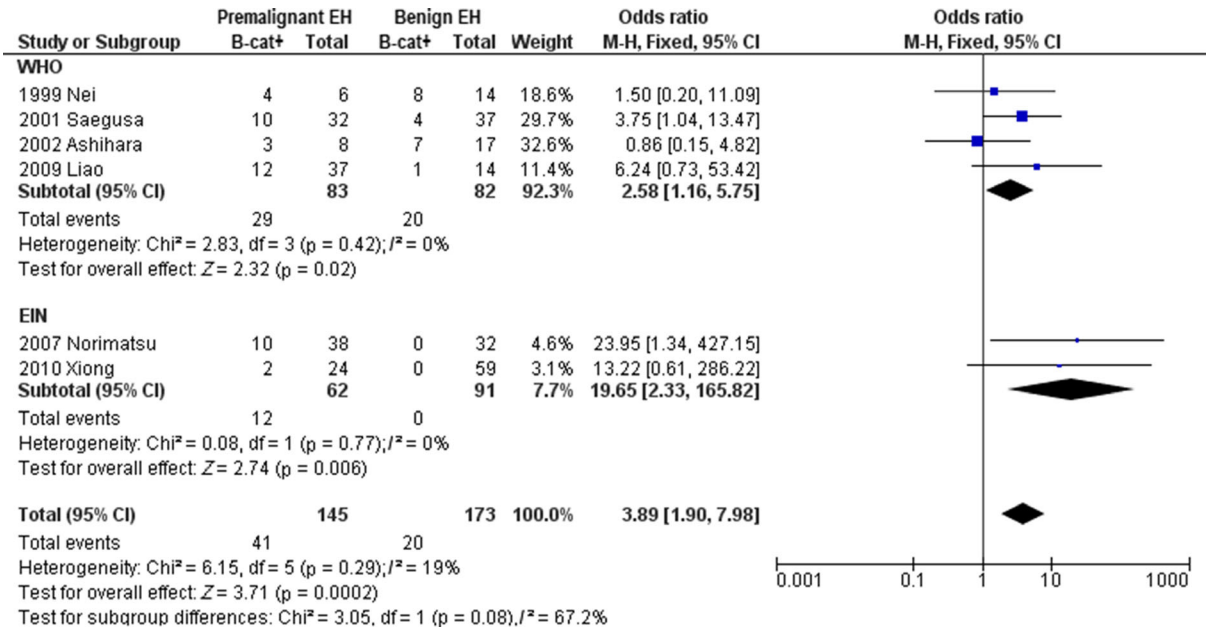

Fig. 2. Forest plot reporting graphically odds ratio for β-catenin nuclear expression in premalignant endometrial hyperplasia (EH) vs benign EH.

such confounding factor by considering only a nuclear expression of β-catenin at least moderate.

In the second place, our results might support the better reliability of EIN criteria in diagnosing precancer. We found that, in the EIN subgroup, no cases of β-catenin nuclear expression were found among benign EH, suggesting that EIN criteria had correctly classified them as benign; by contrast,

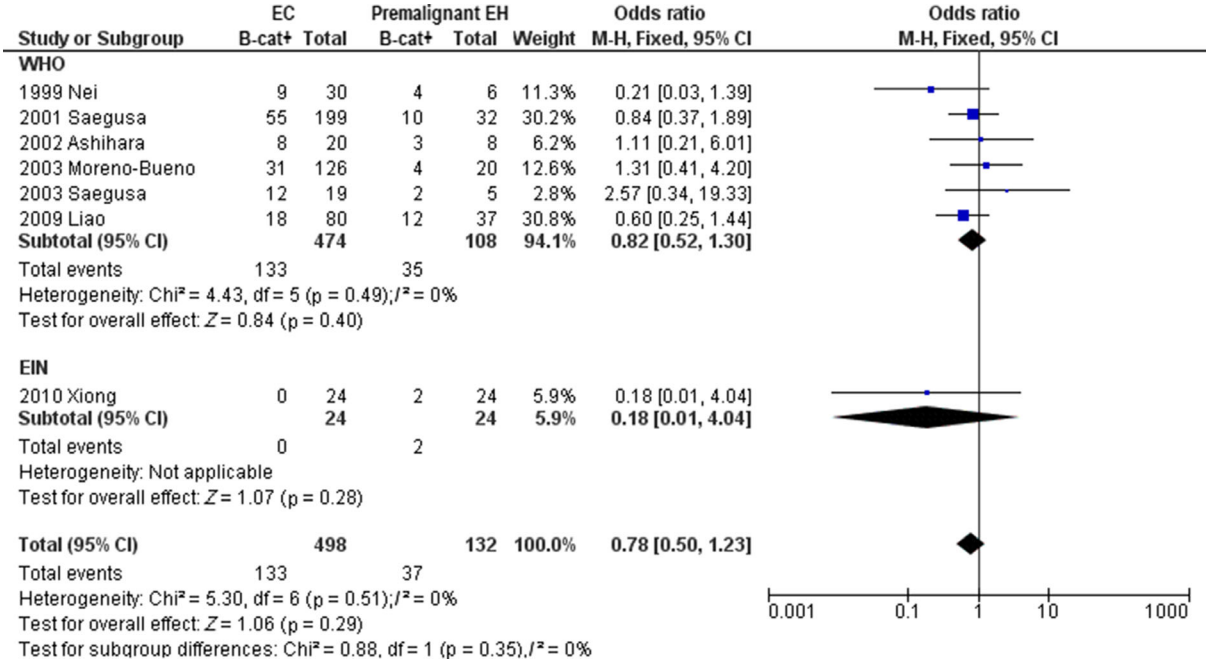

Fig. 3. Forest plot reporting graphically odds ratio for β-catenin nuclear expression in endometrioid carcinoma (EC) vs premalignant endometrial hyperplasia (EH).

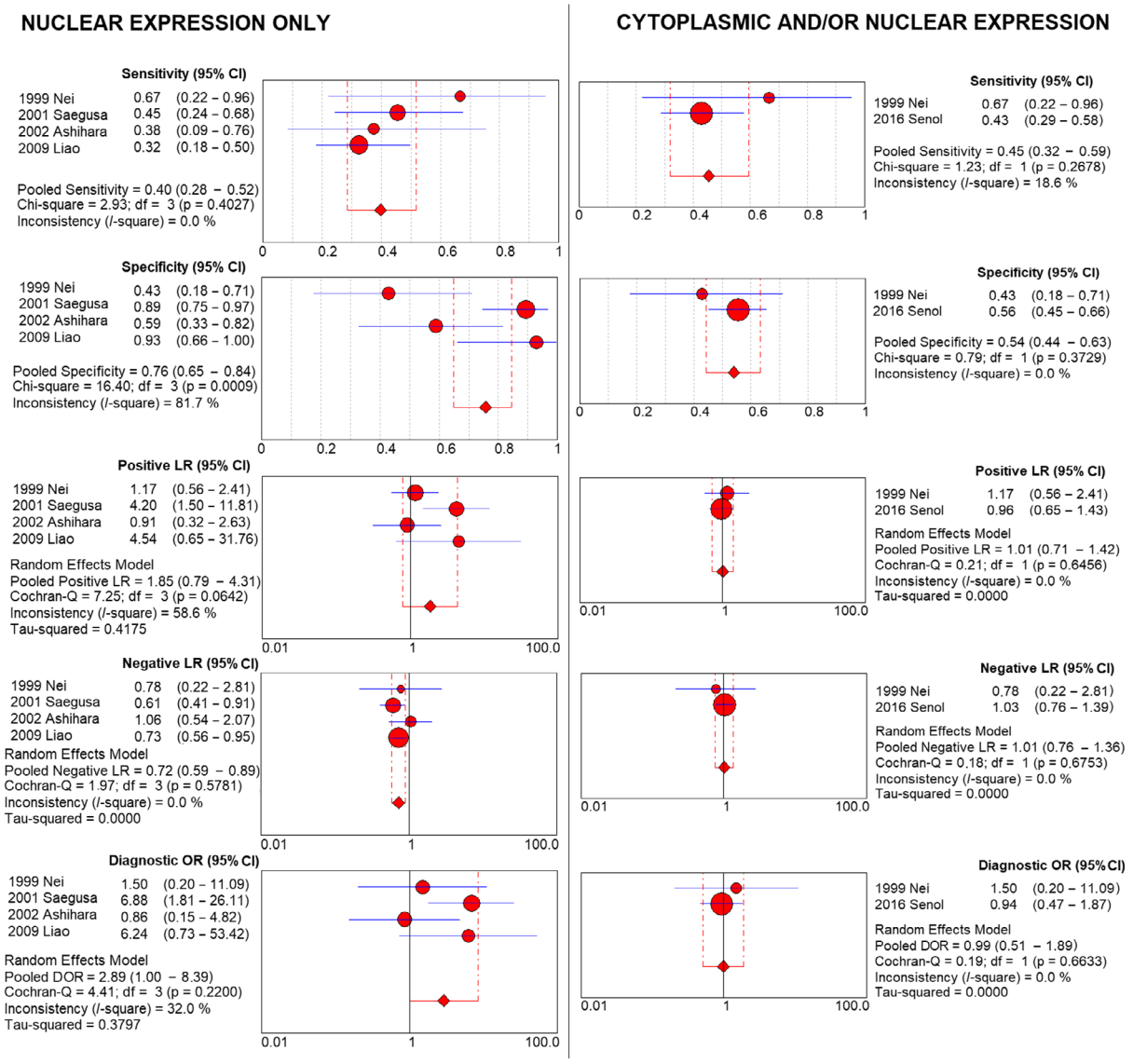

Fig. 4. Forest plots reporting graphically diagnostic accuracy metrics of immunohistochemistry for β-catenin in the differential diagnosis between benign and premalignant hyperplasia according to WHO criteria.

nuclear β-catenin was observed in a significant proportion of EH categorized as benign by the WHO criteria. Furthermore, the association of nuclear β-catenin with premalignant EH was stronger in the EIN subgroup than in the WHO subgroup. In our previous studies, we found that also the expression of PAX2 and Bcl2 correlated better with EIN criteria than WHO criteria (26, 27); on the other hand, no difference was found for PTEN, although it appeared overall non-specific (28, 39, 40). We hypothesized that the main cause of the discrepancy between WHO and EIN system might lie in the former WHO category of 'complex non-atypical EH'. Such category would fall in the benign category according to the current WHO system, while about half of them meet EIN criteria for precancer (41–43).

The relevance of nuclear β-catenin as a marker of endometrial precancer calls into question whether β-catenin may be clinically applicable in the differential diagnosis of EH. The analysis of diagnostic accuracy was conceived to address this issue. Consistently with the previously presented results, in the WHO subgroup the accuracy was very low. On the other hand, moderate accuracy was found using EIN criteria, with a low sensitivity but a perfect specificity.

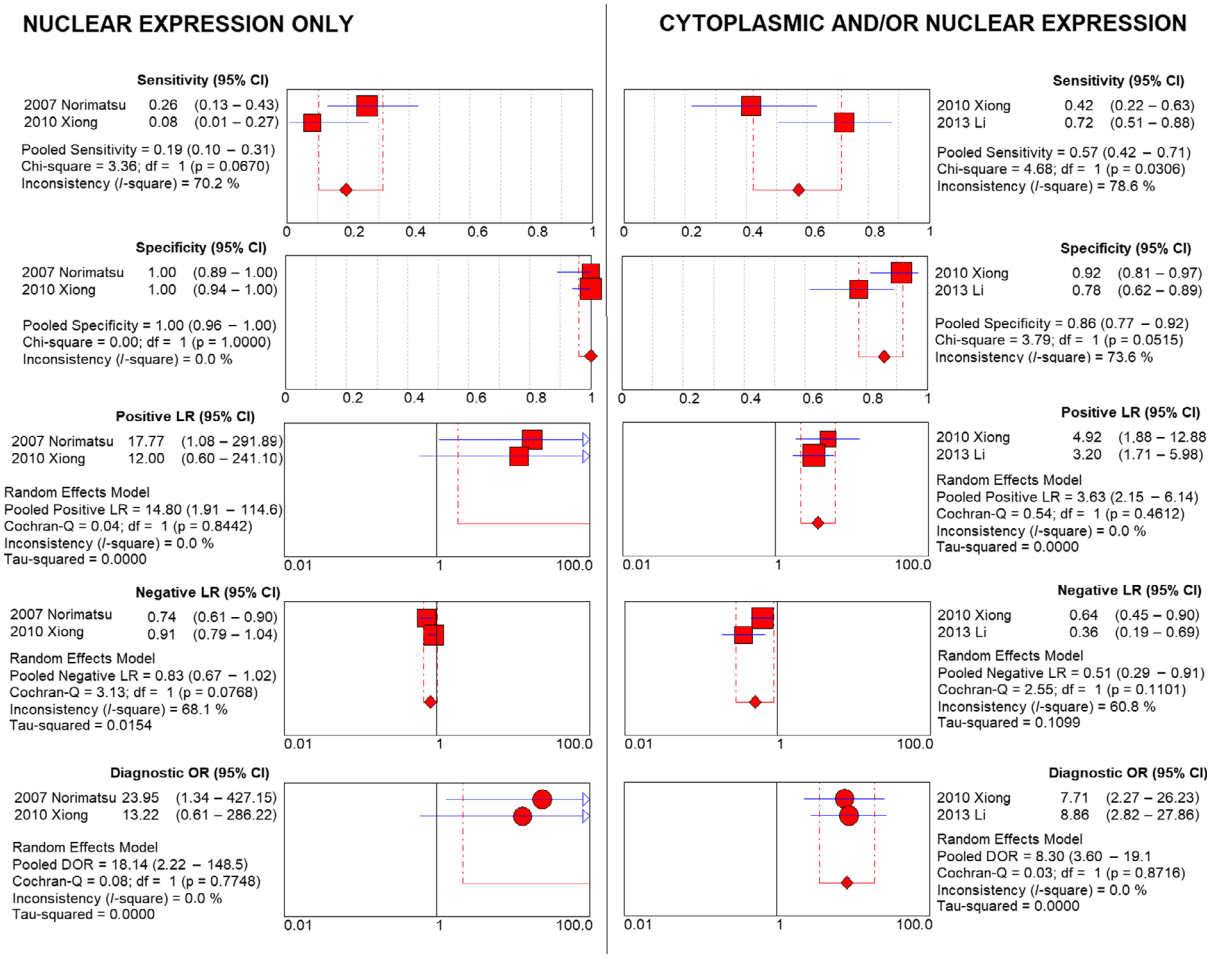

Fig. 5. Forest plots reporting graphically diagnostic accuracy metrics of immunohistochemistry for β-catenin in the differential diagnosis between benign and premalignant hyperplasia according to EIN criteria.

In spite of the moderate accuracy, such a low sensitivity makes β-catenin immunohistochemistry inadequate as a stand-alone diagnostic test. According to guidelines, EH diagnosed as benign do not always need a treatment and may be managed with observation alone (44). A little sensitive diagnostic test would miss many patients at risk of cancer. We tried to improve sensitivity by considering also a cytoplasmic accumulation as positive test; unfortunately, despite the increase in sensitivity, specificity decreased, resulting in an overall low accuracy. The low sensitivity appears as an intrinsic problem, since several different molecular pathways are involved in endometrial carcinogenesis; therefore, it is unlikely that only one marker may perfectly differentiate between benign and premalignant (4, 18, 27, 28).

On the other hand, given its perfect specificity, β-catenin immunohistochemistry might be highly reliable as a rule-in test for diagnosis of endometrial precancer. In fact, as hysterectomy is the standard treatment for premalignant EH (21, 22, 44), a highly specific test may avoid the risk of a severe overtreatment.

In our previous study, we found that also Bcl-2 loss of expression was a highly specific but little sensitive marker of endometrial precancer (26). The issue of the low sensitivity might be resolved by integrating several specific immunohistochemical markers into a diagnostic panel. Given the recent developments regarding the impact of genetics on the prognosis of endometrial cancer, the need for a molecular definition of endometrial neoplastic samples has been growing (4, 5, 45). In the near future, prognostic immunohistochemical markers could allow a customized approach based on the malignant potential of EH, choosing not only between progestin and hysterectomy, but also among different conservative approaches [e.g., progestin alone or combined with hysteroscopic resection (46, 47)]. Thus, the costs for additional immunohistochemistry would

be justified in order to reduce both overtreatment and undertreatment. In this regard, since mutations in the exon 3 of *CTNNB1* were found to bear prognostic value in endometrial cancer (29), a prognostic role of β-catenin may also be hypothesized in EH. Remarkably, compared to other molecules involved in endometrial carcinogenesis, little is known about the impact of β-catenin on the conservative management of EH (48–50). Further studies are necessary in this field.

Strength and limitations

To the best of our knowledge, this study is the first meta-analysis assessing the relevance of β-catenin in EH. We defined the association between nuclear expression of β-catenin and premalignant features in EH and how this association is influenced by the classification system adopted. Furthermore, we defined the diagnostic accuracy of β-catenin immunohistochemistry in the differential diagnosis between benign and premalignant EH, assessing different criteria to interpret β-catenin staining.

The major limitation for our results may lie in the low number of studies included in the subgroup analysis. Minor limitation might be the low reproducibility of histologic criteria for EH and differences in the interpretation of immunohistochemistry.

CONCLUSION

A moderate-to-strong nuclear expression of β-catenin appears as a little sensitive, but perfectly specific marker of endometrial precancer. Considering even cytoplasmic expression might improve sensitivity, although with lower specificity and lower overall accuracy. Moreover, the expression pattern of β-catenin supports the higher reliability of the EIN system compared to the WHO system.

Despite being inadequate as a stand-alone diagnostic test, β-catenin immunohistochemistry may be a highly reliable rule-in test for diagnosis of endometrial precancer. Furthermore, β-catenin might be included in a panel of specific immunohistochemical markers, overcoming the low sensitivity. Given its recently found prognostic value in endometrial cancer, the possibility of a prognostic and/or predictive role of β-catenin in EH should be considered. Further studies are necessary in this field.

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SUPPORTING INFORMATION

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

Figure S1. Flow diagram of studies identified in the systematic review (Prisma template [Preferred Reporting Item for Systematic Reviews and Meta-analyses]).
Figure S2. 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.