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CDO1 and CELF4 methylation assay as the dominant predictor of endometrial cancer: A cohort analysis across pre- and post-menopausal cohorts

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HIGHLIGHTS

- CDO1/CELF4 methylation assay shows high diagnostic accuracy for endometrial cancer.
- The methylation test is the strongest independent predictor of endometrial cancer.
- It identifies cancers missed by endometrial thickness measurement in premenopausal women.
- Combining methylation with ultrasound enhances sensitivity for cancer detection.

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ABSTRACT

Background. The rising global incidence of endometrial cancer (EC) necessitates the development of non-invasive, accurate detection methods to improve early diagnosis and optimize referrals for hysteroscopy. This study evaluated the diagnostic and triage value of a CDO1 and CELF4 methylation (CISENDO) assay using cervical scrapings for EC detection, and its independent association with EC across menopausal statuses.

Methods. In this prospective cohort study, 573 participants for diagnostic hysteroscopy were enrolled, including 524 non-EC, 41 EC, and 8 endometrial intraepithelial neoplasia (EIN) cases. The diagnostic performance of the CISENDO assay, alone and combined with endometrial thickness (ET) measured by transvaginal ultrasound (TVS), was assessed. Analyses were stratified by menopausal status. Univariate and multivariate logistic regression identified independent EC predictors.

Results. The CISENDO assay demonstrated high discriminatory performance. In premenopausal women with EC, sensitivity was 89.7%, specificity 93.0%, and AUC 0.91. In postmenopausal women with EC, sensitivity was 91.7%, specificity 91.8%, and AUC 0.92. Combining CISENDO with ET increased sensitivity (96.6% premenopausal; 100% postmenopausal) but reduced specificity. Notably, 55.2% of premenopausal EC cases had ET <11 mm but were CISENDO-positive, while 52.7% of non-EC postmenopausal women with ET ≥5 mm were CISENDO-negative. Multivariable analysis identified the CISENDO assay as the strongest independent predictor of EC (adjusted OR = 103.9 in premenopausal and 118.0 in postmenopausal women; $P < 0.001$).

Conclusions. The non-invasive CDO1 and CELF4 methylation assay demonstrates high diagnostic accuracy for EC and represents a promising tool for non-invasive diagnosis and triage, particularly in women at high risk for EC and those with early-stage disease.

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1. Introduction

Endometrial cancer (EC), namely uterine corpus carcinoma, is an increasingly important public health challenge. Global analyses show a sustained rise in age-standardized incidence rate (ASIR) from 1990 to 2021 (estimated annual percentage change [EAPC] = +0.54%), making it the only gynecologic malignancy with a growing incidence worldwide [1]. Projections for 2024 estimate 48,931 new uterine cancer cases and 8195 deaths in China alone, particularly in economically developed cities, reflecting both increasing incidence and continued clinical impact [2]. This not only highlights the increasing incidence rate but also underscores the ongoing clinical impact of this disease.

Epidemiologic patterns vary by region and socioeconomic development: high Socio-demographic Index (SDI) settings (notably North America) carry the highest incidence rates, attributable in part to the prevalence of obesity, declining parity and improved diagnostic ascertainment [3], whereas low- and middle-SDI regions shoulder a disproportionate share of mortality burden owing to constrained access to timely diagnosis and treatment [1,4]. Within East Asia, including China, EC incidence exceeds the global average, and the region accounts for 33% of global EC disability-adjusted life years (DALYs), reflecting a substantial burden of premature death and disability [1]. High body mass index (BMI) constitutes the predominant risk factor, contributing 34.3% of EC cases, with its attributable fraction exceeding 30% in middle and high SDI regions [5]. EC in Western series is predominantly type I with type II comprising roughly 10–20% [6], whereas Chinese cohorts report a broadly similar predominance but often present at a younger median age and the prevalence of type I and type II EC in Chinese postmenopausal women is lower than that of postmenopausal white women [7,8].

Although EC is diagnosed with the highest incidence among postmenopausal women [9], recent reports shift toward younger ages has been observed in multiple settings—an issue of particular relevance to studies that include both premenopausal and postmenopausal populations [10]. Rising obesity prevalence, earlier menarche, later childbearing, and other lifestyle changes have been implicated in this trend [10–12], and several national series report a lower mean age at diagnosis compared with Western cohorts [9,13].

Despite this increasing burden, there is currently no internationally endorsed, general population-level screening program for EC analogous to cervical cancer screening [14–16]. Expert summaries and guidelines conclude that routine screening, such as by transvaginal ultrasound (TVS) or endometrial biopsy, for asymptomatic, average-risk women is not supported by evidence of mortality benefit and is therefore not recommended [12,14,17]. Diagnostic efforts instead focus on stratified approaches targeting symptomatic individuals, particularly those experiencing vaginal bleeding in the postmenopausal phase, and those at high hereditary or clinical risk [11,12]. Current diagnostic pathways therefore rely principally on symptom-driven evaluation, most commonly the abnormal symptoms of metrorrhagia [18]. In China, it is advisable for women at increased risk—such as those with obesity and polycystic ovary syndrome (PCOS)—to undergo annual TVS examinations to evaluate endometrial thickness (ET) [19]. However, absence of a consistently established threshold for endometrial thickness, especially in premenopausal women, coupled with low specificity in asymptomatic postmenopausal women and inadequate discriminatory capacity between benign and malignant lesions [19–22]. Endometrial sampling, indicated for symptomatic women with thickened endometrium, faces acceptability barriers in asymptomatic populations due to procedural discomfort, bleeding, infection risks, potential need for anesthesia, and frequent inadequacy of specimens [23]. These limitations hinder scalable, acceptable screening strategies, particularly for community and resource-limited settings. Consequently, a critical need exists for non-invasive, highly accurate, and easily deployable biomarkers to triage women for definitive diagnostic evaluation.

Against this backdrop, tumor-specific molecular biomarkers are gaining attention as potential triage or screening tools that could non-invasively stratify risk and direct invasive diagnostics to those most likely to benefit [24,25]. Aberrant DNA methylation is a consistent and early feature across many neoplasms, including endometrial precancers and cancers, and methylation of specific gene promoters has been proposed as a robust marker of malignant transformation [26,27]. Recent prospective studies reported that Cysteine Dioxygenase Type 1 (*CDO1*) and CUGBP Elav-like family member 4 (*CELF4*) promoter hypermethylation assay using cervical scraping cells could identify women with endometrial intraepithelial neoplasia (EIN, also termed as atypical hyperplasia in this literature) or EC with promising accurate (84.9% sensitivity, 86.6% specificity), and that combining methylation testing with ET may further refine diagnostic performance [28]. Subsequent validation in northwest China confirmed high discriminatory power (87.5% sensitivity, 95.9% specificity) for distinguishing malignant from benign causes of postmenopausal bleeding (PMB) [29]. Notably, *CDO1*/*CELF4* methylation (*CDO1*^{m}/*CELF4*^{m}; CISENDO) assay also proved superior to other non-invasive indicators in premenopausal women with abnormal uterine bleeding (AUB) [30].

Accordingly, this study evaluated the diagnostic performance of *CDO1*/*CELF4* methylation assays—alone and in combination with ET—in a referred cohort undergoing hysteroscopic assessment, and examined the association between methylation status and established clinical risk factors for EC. The aim was to determine whether methylation testing can serve as a feasible, non-invasive triage method to improve detection and referral pathways for women with an increased risk of EC.

2. Method

2.1. Study population and enrollment

This prospective cross-sectional study systematically enrolled patients eligible for diagnostic hysteroscopy at Hainan General Hospital from January to June 2022. Following the application of inclusion and exclusion criteria, a total of 573 participants were ultimately included in the analysis from an initial cohort of 608 (Fig. 1).

Inclusion Criteria: 1. Age ≥ 18 years; 2. Clinical indication for hysteroscopy examination; 3. Read and signed the informed consent form; 4. Presence of ≥1 clinical or sonographic indication including: Persistent or recurrent lower abdominal pain or distension; Vaginal discharge; AUB in premenopausal women, such as menorrhagia, cycle irregularity, or intermenstrual spotting; PMB; TVS exhibited heterogeneous endometrial echotexture, focal hypoechoic or hyperechoic intrauterine lesions, intrauterine space-occupying abnormalities suggestive of polyps or submucosal fibroids, endometrial thickening (≥5 mm in postmenopausal women or ≥ 11 mm in the proliferative phase of premenopausal women), intrauterine adhesions, and vascular proliferation or other suspicious masses; physician suspects possible uterine cavity lesions/diseases. Endometrial thickening thresholds are consistent with our institution's clinical protocol and guideline recommendations [11,19,31]. Menopausal status was determined by patient history (amenorrhea ≥12 months) the guidelines [32].

Exclusion Criteria: 1. Incomplete demographic or histopathological data; 2. Current or prior gynecologic malignancy (cervical, ovarian, tubal, vulvar), or any concurrent extra-uterine gynecologic tumor; 3. History of total hysterectomy or endometrial ablation; 4. Without pathological diagnosis through biopsy or surgery; 5. Pregnancy or lactation; 6. Autoimmune disorder (e.g., systemic lupus erythematosus, rheumatoid arthritis) under immunosuppressive or systemic corticosteroid therapy; 7. Severe cardiac, hepatic, or renal dysfunction substantially affecting patients' ability to safely undergo clinical procedures; 8. Within one month after hysteroscopy examination.

The protocol was approved by the Hainan General Hospital Institutional Review Board (Approval No. Med-Eth-Re[2025]627) in accordance with the Declaration of Helsinki.

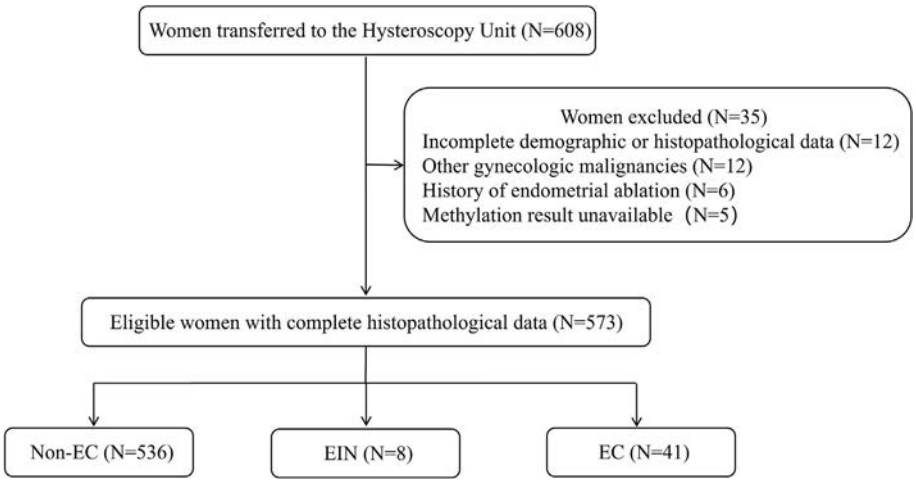

Fig. 1. Flowchart of recruitment for women at the hysteroscopy unit.
Abbreviation: EC: Endometrial cancer; EIN: Endometrial intraepithelial neoplasia; Non-EC: Non-endometrial carcinoma, including benign lesions including polyps, simple or complex hyperplasia, and normal endometrium; N: Number.

2.2. Data collection

Retrospective data extraction included demographics, TVS reports, and hysteroscopic images to evaluate BMI, ET, echotexture homogeneity, lesion dimensions, etc.

2.3. TVS measurement

TVS was performed by sonographers with over 5 years of experience per institutional practice before hysteroscopy, performing 3–7 days after the menstruation (proliferative phase). ET was measured in the midline sagittal plane at the thickest endometrium.

2.4. CDO1/CELF4 methylation detection

The methylation test was performed in a certified molecular laboratory (ISO15189), and all procedures followed the manufacturer's SOPs and the requirements [33]. Personnel conducting methylation analyses were blinded to clinical information, including participant demographics, imaging results, examinations, and clinical data, in the study.

Cervical specimens were obtained using a Rovers Cervex-Brush® (Rovers Medical Devices, Oss, The Netherlands) rotated clockwise 5–6 times within the cervix and endocervical canal. Exfoliated cells were immediately preserved in PreservCyt solution (Hologic, Bedford, MA, USA) at the point of collection. Cervical scraping for CISENDO testing was performed prior to hysteroscopy and any endometrial instrumentation.

Genomic DNA extraction employed the JH-DNA Isolation and Purifying kit (OriginPoly Bio-Tec., Beijing, China), with quantification via NanoDrop 2000c spectrophotometry (Thermo Fisher Scientific, Wilmington, DE, USA). Bisulfite conversion of 200 ng DNA was performed using the JH-DNA Methylation-Lightning MagPrep system (OriginPoly Bio-Tec.) following manufacturer specifications.

The methylation levels of *CDO1* and *CELF4* were detected using the CISENDO DNA Methylation Detection Kit (National Medical Products Administration, NMPA China-certified Class III medical device, No. 20243402610; OriginPoly Bio-Tec, Beijing, China), with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) serving as the endogenous control. This assay was conducted on the SLAN-96S real-time PCR platform (Hongshi Medical Technology, Shanghai). After mixing the sample, primer mix, master mix, and nuclease-free water (ddH_2O) to a final volume of 25 μL following the manufacturer's instructions, the PCR thermal cycling conditions were performed under the following protocol: initial denaturation at 96 °C for 10 min, followed by 45 cycles of 94 °C for 15 s (denaturation), 64 °C for 5 s (annealing), and 60 °C for 30 s (extension), with a cooling step at 25 °C for 1 min. Methylation quantification derived from ΔCp values: $\Delta Ct_{CDO1} = Ct_{CDO1} - Ct_{GAPDH}$, $\Delta Ct_{CELF4} = Ct_{CELF4} - Ct_{GAPDH}$. Thresholds were predefined per manufacturer's NMPA-certified criteria and prior validation as follows: *CDO1* hypermethylation ($\Delta Ct_{CDO1} \leq 8.4$) and *CELF4* hypermethylation ($\Delta Ct_{CELF_4} \leq 8.8$). The CISENDO (commercial name of *CDO1/CELF4* dual-gene methylation kit) (+) positive result is defined as either $\Delta Ct_{CDO1} \leq 8.4$ or $\Delta Ct_{CELF4} \leq 8.8$, as reported in previous studies [28–30]. Thresholds were predefined per manufacturer's NMPA-certified criteria and prior validation [28–30].

2.5. Histopathological diagnosis

During hysteroscopic evaluation, focal or diffuse endometrial hyperplasia and/or abnormal thickening area and/or suspicious area prompt immediate targeted biopsy or hysteroscopic curettage, adhering to clinical protocol for endometrial sampling. Where multiple specimens were available for a single participant, final diagnosis was assigned using the following hierarchy: surgical specimen > hysteroscopic excision/biopsy, as the diagnostic gold standard. Suspicious thickening / sonographic abnormalities triggered hysteroscopy. Specimens were processed underwent standardized hematoxylin and eosin (H&E) staining and histopathological evaluation. Histologic classification of endometrial lesions followed the WHO Classification of Tumours — Female Genital Tumours (5th edition, 2020) [34]. Tumor staging was recorded according to the International Federation of Gynecology and Obstetrics (FIGO) 2023 staging system [35]. Two certified gynecologic pathologists independently reviewed each case; discordant diagnoses were adjudicated by a third senior pathologist. The interval between recruitment and hysteroscopy was typically <2 week.

Patients were then stratified into: EC; EIN; Non-endometrial cancer (non-EC) including benign lesions including polyps, simple or complex hyperplasia, and normal endometrium.

2.6. Data analysis

All statistical analyses were conducted using R software (version 4.4.2). Continuous variables were presented as median with interquartile range (IQR), while categorical variables were summarized

as frequencies and percentages. Comparisons of continuous variables between groups were conducted using the Wilcoxon rank-sum test or Kruskal–Wallis test, as appropriate; categorical variables were compared with Pearson's Chi-squared (χ^2) test or Fisher's exact test as appropriate. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and an area under the receiver operating characteristic (ROC) curve (AUC) with their 95% confidence intervals (CIs) were computed with the pROC package. Multivariable analyses adjusted for multiple comparisons using false discovery rate (FDR) correction. In univariate screening, variables with $p < 0.10$ were selected for inclusion in the multivariable model. To guard against multicollinearity, variables with variance inflation factor (VIF) > 10 were sequentially removed. Both univariate and multivariable models report odds ratios (ORs) with 95% CIs and two-sided p values. For binary predictors with sparse counts, Firth's penalized likelihood logistic regression was used. A $P < 0.05$ was considered statistically significant.

3. Result

3.1. Cohort characteristics and Indicator distribution

Of the 608 participants referred to the Hysteroscopy clinic per Chinese Endometrial Cancer Guidelines (2022) [11], 35 were excluded for incomplete demographic or histopathological data, concurrent gynecologic malignancies, prior endometrial ablation, or unavailable methylation results (Fig. 1). The 573 eligible participants comprised 524 non-EC, 8 EIN, and 41 EC cases. Stratified by menopausal status, 462 premenopausal women (median age 42 years, IQR 36–47) and 111 postmenopausal women (median age 58 years, IQR 52–63) were enrolled (Table 1).

Premenopausal EC patients showed a trend toward higher BMI (median 24.7 (22.5, 27.0) vs. 22.6 (20.7, 25.0) kg/m^2; $P = 0.067$) and ET (median 10.0 vs. 8.0 mm; $P = 0.069$) than their benign counterparts, though these differences did not reach statistical

Table 1
Baseline information.

Characteristic	Premenopausal women					Postmenopausal women				
	Overall $N = 462$	Non-EC $N = 426$	EIN N = 7	EC $N = 29$	*p*-value	Overall $N = 111$	Non-EC $N = 98$	EIN N = 1	EC $N = 12$	p-value
Age (years)	42 (36, 47)	42 (35, 46)	35 (30, 42)	45 (40, 51)	0.007	58 (52, 63)	58 (52, 63)	64 (64, 64)	57 (55, 59)	0.530
BMI	22.7 (20.7, 25.0)	22.6 (20.7, 25.0)	21.2 (19.1, 24.9)	24.7 (22.5, 27.0)	0.067	23.4 (21.7, 26.8)	23.2 (21.5, 26.7)	32.9 (32.9, 32.9)	25.7 (25.0, 26.9)	0.016
Endometrial thickness (mm)	8.0 (6.0, 11.0)	8.0 (6.0, 11.0)	6.0 (4.0, 13.0)	10.0 (8.0, 14.0)	0.069	6.0 (4.0, 8.5)	5.0 (3.4, 8.0)	7.0 (7.0, 7.0)	12.0 (5.5, 16.0)	0.039
Hypertension					0.019					>0.999
No	453 (98%)	420 (99%)	6 (86%)	27 (93%)		106 (95%)	93 (95%)	1 (100%)	12 (100%)	
Yes	9 (1.9%)	6 (1.4%)	1 (14%)	2 (6.9%)		5 (4.5%)	5 (5.1%)	0 (0%)	0 (0%)	
Diabetes					0.034					>0.999
No	460 (100%)	425 (100%)	6 (86%)	29 (100%)		110 (99%)	97 (99%)	1 (100%)	12 (100%)	
Yes	2 (0.4%)	1 (0.2%)	1 (14%)	0 (0%)		1 (0.9%)	1 (1.0%)	0 (0%)	0 (0%)	
PCOS					0.056					
No	459 (99%)	424 (100%)	6 (86%)	29 (100%)		111 (100%)	98 (100%)	1 (100%)	12 (100%)	
Yes	3 (0.6%)	2 (0.5%)	1 (14%)	0 (0%)						
Intrauterine lesions					0.875					0.737
No	363 (79%)	333 (78%)	6 (86%)	24 (83%)		93 (84%)	81 (83%)	1 (100%)	11 (92%)	
Yes	99 (21%)	93 (22%)	1 (14%)	5 (17%)		18 (16%)	17 (17%)	0 (0%)	1 (8.3%)	
Heterogeneous endometrium					0.025					>0.999
No	435 (94%)	404 (95%)	5 (71%)	26 (90%)		103 (93%)	91 (93%)	1 (100%)	11 (92%)	
Yes	27 (5.8%)	22 (5.2%)	2 (29%)	3 (10%)		8 (7.2%)	7 (7.1%)	0 (0%)	1 (8.3%)	
Endometrial polyp					<0.001					0.003
No	288 (62%)	255 (60%)	5 (71%)	28 (97%)		68 (61%)	55 (56%)	1 (100%)	12 (100%)	
Yes	174 (38%)	171 (40%)	2 (29%)	1 (3.4%)		43 (39%)	43 (44%)	0 (0%)	0 (0%)	
Uterine fibroid					0.032					0.626
No	375 (81%)	340 (80%)	7 (100%)	28 (97%)		102 (92%)	89 (91%)	1 (100%)	12 (100%)	
Yes	87 (19%)	86 (20%)	0 (0%)	1 (3.4%)		9 (8.1%)	9 (9.2%)	0 (0%)	0 (0%)	
Adenomyosis					0.571					
No	439 (95%)	403 (95%)	7 (100%)	29 (100%)		111 (100%)	98 (100%)	1 (100%)	12 (100%)	
Yes	23 (5.0%)	23 (5.4%)	0 (0%)	0 (0%)						
AUB (Premenopausal only)					0.037					
No	121 (26%)	118 (28%)	0 (0%)	3 (10%)		0 (NA%)	0 (NA%)	0 (NA%)	0 (NA%)	
Yes	341 (74%)	308 (72%)	7 (100%)	26 (90%)		0 (NA%)	0 (NA%)	0 (NA%)	0 (NA%)	
PMB (Postmenopausal only)										0.037
No	0 (NA%)	0 (NA%)	0 (NA%)	0 (NA%)		42 (38%)	41 (42%)	0 (0%)	1 (8.3%)	
Yes	0 (NA%)	0 (NA%)	0 (NA%)	0 (NA%)		69 (62%)	57 (58%)	1 (100%)	11 (92%)	
CDO1m					<0.001					<0.001
Negative	423 (92%)	410 (96%)	5 (71%)	8 (28%)		96 (86%)	92 (94%)	1 (100%)	3 (25%)	
Positive	39 (8.4%)	16 (3.8%)	2 (29%)	21 (72%)		15 (14%)	6 (6.1%)	0 (0%)	9 (75%)	
CELF4m					<0.001					<0.001
Negative	416 (90%)	402 (94%)	6 (86%)	8 (28%)		97 (87%)	92 (94%)	1 (100%)	4 (33%)	
Positive	46 (10.0%)	24 (5.6%)	1 (14%)	21 (72%)		14 (13%)	6 (6.1%)	0 (0%)	8 (67%)	
CISENDO					<0.001					<0.001
Negative	404 (87%)	396 (93%)	5 (71%)	3 (10%)		92 (83%)	90 (92%)	1 (100%)	1 (8.3%)	
Positive	58 (13%)	30 (7.0%)	2 (29%)	26 (90%)		19 (17%)	8 (8.2%)	0 (0%)	11 (92%)	

1. The CISENDO, the combination of CDO1/CELF4 methylation testing, positive result is defined as either marker's positive result(s).
2. Abbreviations: BMI: body mass index; ET: endometrial thickness; AUB: abnormal uterine bleeding; PMB: postmenopausal bleeding; EC: endometrial carcinoma; EIN: endometrial intraepithelial neoplasia; Non-EC: non–endometrial cancer; PCOS: polycystic ovary syndrome.

significance. This association became statistically significant in postmenopausal women, among whom EC cases had higher BMI (median 25.7 (25.0, 26.9) vs. 23.2 (21.5, 26.7) kg/m^2; $P = 0.016$) and ET (median 12.0 vs. 5.0 mm; $P = 0.039$). Hypertension was threefold more prevalent in premenopausal EC patients (6.9% vs. 1.4%; $P = 0.019$), yet both pre- and postmenopausal EC groups had no cases of diabetes or PCOS. Sonographically, only premenopausal EC patients exhibited a higher frequency of heterogeneous endometrium ($P = 0.025$), whereas intrauterine lesions did not differ in pre- and postmenopausal women ($P > 0.5$). As for metrorrhagia, AUB in premenopausal women and PMB were both more common in EC (both $P < 0.05$). Benign lesions were frequent across the cohort: endometrial polyps occurred in 38% (174/462) of premenopausal and 39% (43/111) of postmenopausal women, uterine fibroids in 19% (87/462) and 8.1% (9/111), respectively (Table 1). *CDO1* and *CELF4* methylation was negative in over 94% of non-EC cases, and the CISENDO (*CDO1*/*CELF4* methylation) assay positivity reached 90% in premenopausal and 92% in postmenopausal EC patients ($p < 0.001$).

Histologic characteristics and stage for the 41 EC cases were summarized in Supplementary Table S1. The majority were endometrioid histology (38/41; 92.7%), with the remainder comprising serous/other high-grade subtypes. Most tumours were FIGO stage I (29/41; 70.7%), reflecting detection in early disease; however, numbers for non-endometrioid subtypes and higher stages were small, limiting subtype-specific performance analyses. Due to the small number of EIN cases ($n = 8$; premenopausal $n = 7$; postmenopausal $n = 1$), EIN cases were also excluded from subsequent analyses.

3.2. Differential methylation signatures and diagnostic stratification

For methylation levels in four groups (Fig. 2A, B), *CDO1* and *CELF4* methylation levels robustly discriminated EC from non-EC cases in both premenopausal and postmenopausal strata ($P < 0.001$). Median methylation values among EC patients fell below the predefined ΔCt thresholds (*CDO1* ≤ 8.4; *CELF4* ≤ 8.8). Within the non-EC cohort, postmenopausal women exhibited lower *CDO1* ($P = 0.006$) and *CELF4* ($P = 0.033$) methylation levels compared to premenopausal women. By contrast, methylation levels within the EC cohort showed no significant difference by menopausal status (*CDO1*: $P = 0.720$; *CELF4*: $P = 0.875$).

Categorical methylation negativity was highly prevalent among non-EC subjects: 396 of 426 (93.0%) premenopausal non-EC participants and 101 of 110 (91.8%) postmenopausal non-EC participants were methylation-negative for both markers (Fig. 2C). A small number of non-EC participants exhibited isolated (premenopausal: 4.7%; postmenopausal: 4.1%) or concurrent (premenopausal: 2.3%; postmenopausal: 4.1%) hypermethylation, whereas EC cohorts exhibited 89.7% (premenopausal) and 91.7% (postmenopausal) combined methylation positivity (≥1 marker)) (Fig. 2C).

3.3. Diagnostic performance of methylation biomarkers and endometrial thickness

CISENDO assay demonstrated consistently high diagnostic accuracy across both menopausal strata (Table 2). In premenopausal women, the assay yielded a sensitivity of 89.7% (95%CI: 72.6–97.8%) and specificity of 93.0% (95%CI: 90.1–95.2%) with an AUC of 0.91 (95%CI: 0.86–0.97).

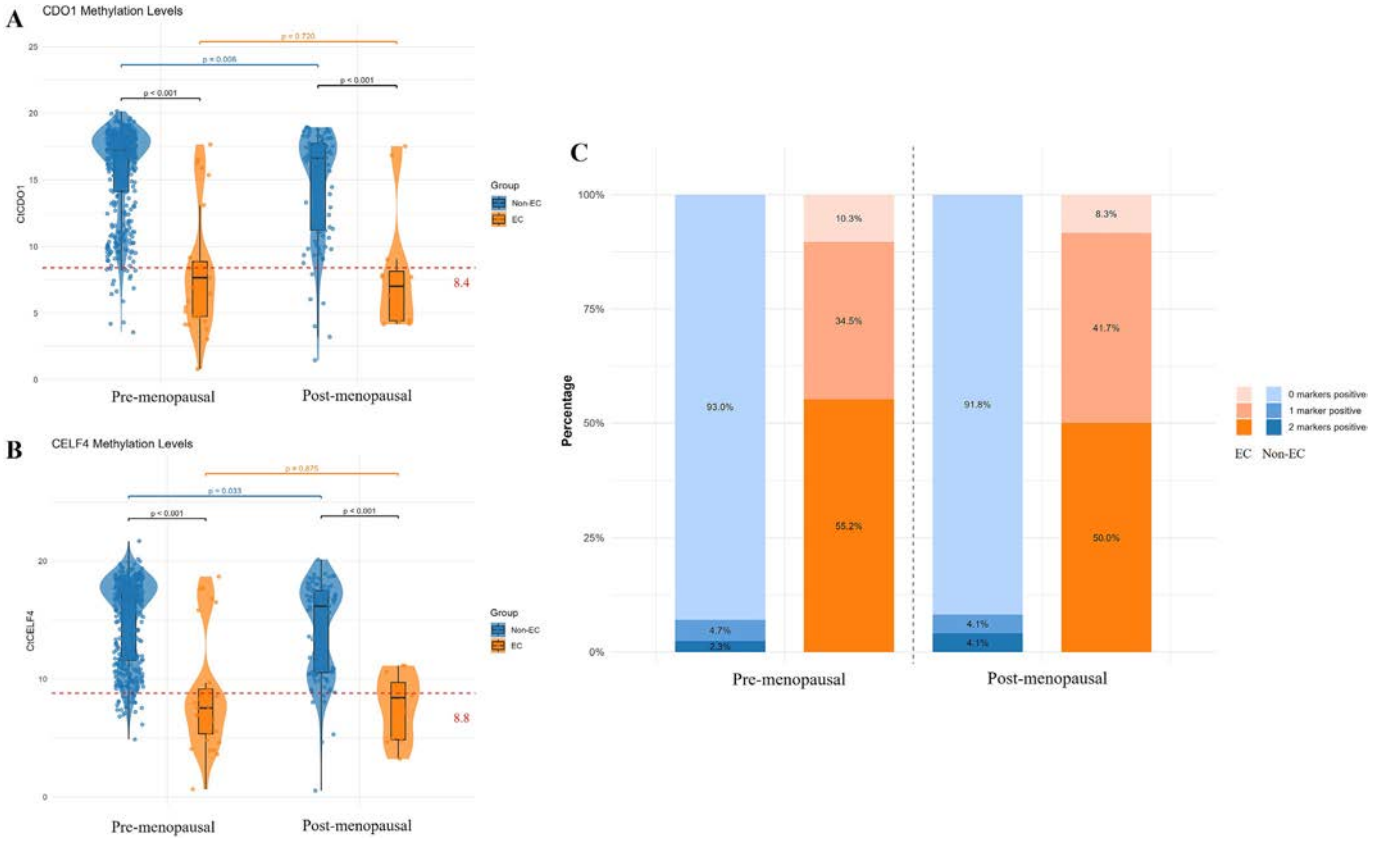

Fig. 2. CDO1 and CELF4 methylation profiles stratified by endometrial cancer and menopause status.
(A) CDO1 methylation levels; (B) CELF4 methylation levels; (C) Frequency of positive methylation markers.
Dashed line means $\Delta Ct_{CDO1} = 8.4$, $\Delta Ct_{CELF4} = 8.8$.

Table 2
Diagnostic performance.

Indicator	Sensitivity	Specificity	PPV	NPV	AUC
Postmenopausal					
CISENDO	91.7% (61.5–99.8%)	91.8% (84.5–96.4%)	57.9% (33.5–79.7%)	98.9% (94.0–100.0%)	0.92 (0.83–1.00)
CDO1	75.0% (42.8–94.5%)	93.9% (87.1–97.7%)	60.0% (32.3–83.7%)	96.8% (91.0–99.3%)	0.84 (0.71–0.97)
CELF4	66.7% (34.9–90.1%)	93.9% (87.1–97.7%)	57.1% (28.9–82.3%)	95.8% (89.7–98.9%)	0.80 (0.66–0.94)
ET ≥5 mm	83.3% (51.6–97.9%)	39.8% (30.0–50.2%)	14.5% (7.2–25.0%)	95.1% (83.5–99.4%)	0.62 (0.50–0.74)
CISENDO+ET ≥5 mm	41.7% (15.2–72.3%)	98.0% (92.8–99.8%)	71.4% (29.0–96.3%)	93.2% (86.5–97.2%)	0.70 (0.55–0.84)
CISENDO/ET ≥5 mm	100.0% (73.5–100.0%)	38.8% (29.1–49.2%)	16.7% (8.9–27.3%)	100.0% (90.7–100.0%)	0.69 (0.65–0.74)
Premenopausal					
CISENDO	89.7% (72.6–97.8%)	93.0% (90.1–95.2%)	46.4% (33.0–60.3%)	99.2% (97.8–99.8%)	0.91 (0.86–0.97)
CDO1	72.4% (52.8–87.3%)	96.2% (94.0–97.8%)	56.8% (39.5–72.9%)	98.1% (96.3–99.2%)	0.84 (0.76–0.93)
CELF4	72.4% (52.8–87.3%)	94.4% (91.7–96.4%)	46.7% (31.7–62.1%)	98.0% (96.2–99.2%)	0.83 (0.75–0.92)
ET ≥11 mm	41.4% (23.5–61.1%)	73.2% (68.8–77.4%)	9.5% (5.0–16.0%)	94.8% (91.9–97.0%)	0.57 (0.48–0.67)
CISENDO+ET ≥11 mm	34.5% (17.9–54.3%)	97.2% (95.1–98.5%)	45.5% (24.4–67.8%)	95.6% (93.2–97.3%)	0.66 (0.57–0.75)
CISENDO/ET ≥11 mm	96.6% (82.2–99.9%)	69.0% (64.4–73.4%)	17.5% (12.0–24.3%)	99.7% (98.1–100.0%)	0.83 (0.79–0.87)

1. Abbreviations: AUC: area under the curve; ET: Endometrial thickness; CI: confidence interval; NPV: negative predictive value; PPV: positive predictive value.
2. Combination rules: "+" indicates AND rule (both tests positive); "/" indicates OR rule (either test positive).

Postmenopausal performance showed sensitivity of 91.7% (95%CI: 61.5–99.8%) and specificity of 91.8% (95%CI: 84.5–96.4%) (AUC 0.92, 95%CI: 0.83–1.00).

ET thresholds displayed divergent operating characteristics by menopausal status: ET ≥5 mm in postmenopausal participants produced a sensitivity of 83.3% (95%CI: 51.6–97.9%) but a low specificity of 39.8% (95%CI: 30.0–50.2%). In contrast, ET ≥11 mm in premenopausal subjects showed limited sensitivity of 41.4% (95%CI: 23.5–61.1%) and a specificity of 73.2% (95%CI: 68.8–77.4%), with an AUC of merely 0.57—indicating limited discriminative power. Thus, CISENDO represented the highest discriminative capacity among evaluated indices, substantially outperforming ET alone.

When methylation testing was combined with ET, performance metrics changed in a directionally consistent manner across groups. Using an OR rule (either CISENDO or ET positive) increased sensitivity but reduced specificity. In premenopausal women CISENDO combined ET ≥11 mm increased sensitivity to 96.6% (95%CI: 82.2–99.9%) with specificity 69.0% (95%CI: 64.4–73.4%) and AUC 0.83 (0.79–0.87), while in the postmenopausal cohort sensitivity rose to 100% (95% CI 73.5–100%) with specificity falling to 38.8% (95% CI 29.1–49.2%) and AUC 0.69 (0.65–0.74). By contrast, an AND rule (both CISENDO and ET positive) produced high specificity (pre: 97.2%; post: 98.0%) at the expense of lower sensitivity (pre: 34.5%; post: 41.7%) and AUC (pre: 0.66; post: 0.70). These results illustrate the trade-off between sensitivity and specificity depending on the combination rule chosen; importantly, NPV remained high across most combinations (>90%).

3.4. Integrated methylation-TVS profiling distribution

The combination of *CDO1* and *CELF4* methylation testing exhibits enhanced diagnostic accuracy for the detection of EC, demonstrating consistent performance across various menopausal statuses (Table 2). Particularly, the AUC for premenopausal individuals increased from 0.57 (ET alone) to 0.83 (co-test). Analysis of methylation-TVS

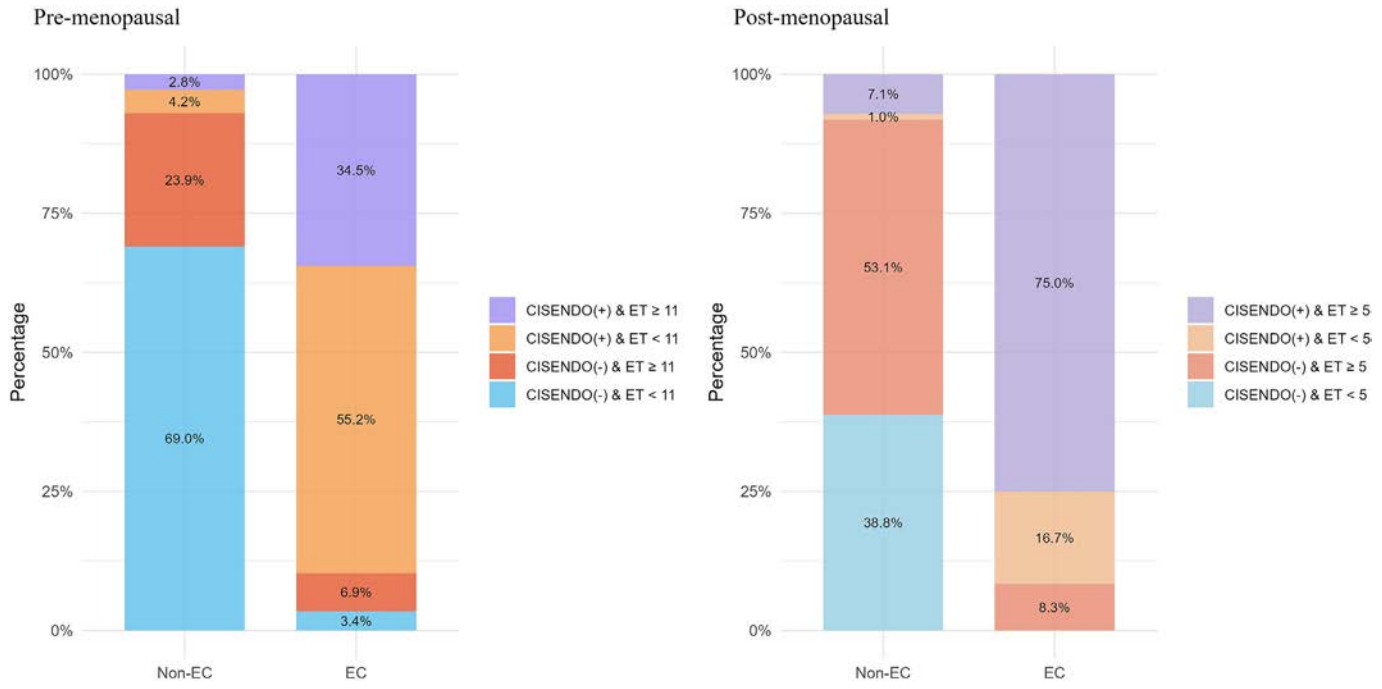

Fig. 3. Proportion distribution of combined status of CISENDO and endometrial thickness in EC and non-EC groups.
CISENDO (+): CISENDO positive; CISENDO (−): CISENDO negative; CISENDO positive criteria: $\Delta Ct_{CDO1} \leq 8.4$ and (or) $\Delta Ct_{CELF4} \leq 8.8$; CISENDO negative criteria: $\Delta Ct_{CDO1} > 8.4$ and $\Delta Ct_{CELF4} > 8.8$.

concordance revealed critical risk-stratification patterns (Fig. 3). Among premenopausal non-EC participants, 97.2% (414/426) exhibited either CISENDO(−) negative or ET <11 mm. Notably, 23.9% (102/426) of non-EC women with ET ≥11 mm maintained CISENDO(−), while only 4.2% (18/426) showed isolated methylation positivity with ET <11 mm. In non-EC postmenopausal subjects with ET ≥5 mm, a striking 53.1% (52/98) were CISENDO(−), reducing false-positive concerns. Complementing this, 92.9% of all non-EC postmenopausal women exhibited either CISENDO(−) or ET <5 mm, with minimal isolated methylation positivity (1.0% in low-ET group). These results indicated that methylation testing effectively reduces the misclassification of non-EC women as being at risk of EC due to unreliable ET measurements.

In premenopausal EC, 96.4% displayed either CISENDO(+) positivity or ET ≥11 mm. Crucially, methylation detection identified 55.2% (16/29) of EC cases despite ET <11 mm. Postmenopausal EC universally tested for CISENDO(+) or ET ≥5 mm, though 16.7% manifested CISENDO(+) with ET <5 mm. Similarly, methylation testing demonstrated the ability to detect EC cases that would have been missed by ET criteria alone, particularly among premenopausal women.

3.5. Univariable and multivariable analysis of endometrial cancer risk factors

Table 3 summarized univariate and multivariate logistic regression analyses stratified by menopausal status. In the premenopausal cohort, univariate analysis showed trends toward increased odds of EC for Age ≥ 45 years (OR 2.012, 95% CI 0.935–4.306, $P = 0.070$) and BMI ≥25 kg/m^2 (OR 1.868, 95% CI 0.830–4.031, $P = 0.117$), although neither reached statistical significance. Hypertension yielded an elevated univariate OR (5.185, 95% CI 0.736–23.765, $P = 0.050$). Diabetes and PCOS had no events in the case group, thus ORs and CIs were not estimable. TVS findings—including intrauterine lesions, heterogeneous endometrium, and ET ≥11 mm—did not show strong associations in univariate testing. AUB demonstrated significant association (OR 2.908, 95% CI 1.058–10.964, $P = 0.037$). Methylation markers exhibited strong univariate associations: *CDO1* methylation (OR 67.266, 95% CI 26.907–184.770, $P < 0.001$), *CELF4* methylation (OR 43.969, 95% CI 18.306–115.633, $P < 0.001$), and the combined CISENDO assay (OR 114.400, 95% CI 37.620–499.654, $P < 0.001$).

After applying the univariate screening threshold ($P < 0.10$) and addressing multicollinearity by removing variables with VIF > 10, the multivariable model in premenopausal women included CISENDO, Age, Hypertension, ET ≥11 mm, and AUB. In that adjusted model, CISENDO assay was the only variable that showed a strong and statistically significant independent association (adjusted OR 103.939, 95% CI 33.683–458.321, $P < 0.001$), whereas Age, Hypertension, ET ≥11 mm and AUB were not statistically significant.

In the postmenopausal cohort, univariate analysis identified BMI >25 kg/m^2 as a strongly risk factor (OR 5.167, 95% CI 1.437–24.424, $P = 0.019$). Hypertension and diabetes had no events among cases and thus yielded non-estimable ORs/CIs. Intrauterine lesions, heterogeneous endometrium and ET ≥5 mm were not associated with case status in univariate testing, and PMB was also significantly associated with EC (OR 5.533, 95% CI 1.242–52.420, $P = 0.022$). Methylation markers again demonstrated strong univariate associations: *CDO1*methylation test (OR 46.000, 95% CI 9.954–255.553, $P < 0.001$), CELF4 methylation test (OR 30.667, 95% CI 6.328–147.211, $P < 0.001$), and CISENDO (OR 123.750, 95% CI 20.434–2417.348, $P < 0.001$). The postmenopausal multivariable model, included CISENDO assay, BMI and PMB. In the adjusted analysis, CISENDO assay retained a highly independent association with EC (adjusted OR 117.981, 95% CI 16.961–2578.872, $P < 0.001$). The other covariates in the model (BMI and PMB) did not achieve statistical significance after adjustment.

Table 3
Univariate and multivariate logistic regressions for risk factors of endometrial cancer.

	Univariate logistic regression			Multivariate logistic regression		
Group / Variable	OR	95% CI	P	OR	95% CI	P
Premenopausal						
Age	2.012	0.935–4.306	0.070	1.413	0.503–3.963	0.508
BMI	1.868	0.830–4.031	0.117			
Hypertension	5.185	0.736–23.765	0.050	2.102	0.181–24.260	0.544
Diabetes	/	/	0.989			
PCOS	/	/	0.990			
Intrauterine lesions	0.746	0.246–1.859	0.562			
Heterogeneous endometrium	2.119	0.480–6.642	0.246			
ET ≥ 11 mm	1.932	0.875–4.142	0.093	1.193	0.421–3.320	0.736
AUB	2.908	1.058–10.964	0.037	2.781	0.725–14.178	0.165
CDO1m	67.266	26.907–184.770	<0.001			
CELF4m	43.969	18.306–115.633	<0.001			
CISENDO	114.400	37.620–499.654	<0.001	103.939	33.683–458.321	<0.001
Postmenopausal						
BMI	5.167	1.437–24.424	0.019	5.612	0.848–54.513	0.093
Hypertension	/	/	0.993			
Diabetes	/	/	0.993			
Intrauterine lesions	0.433	0.019–1.949	0.438			
Heterogeneous endometrium	1.182	0.060–7.585	0.881			
ET ≥ 5 mm	3.305	0.815–22.262	0.136			
PMB	5.533	1.242–52.420	0.022	11.146	1.089–298.417	0.073
CDO1m	46.000	9.954–255.553	<0.001			
CELF4m	30.667	6.328–147.211	<0.001			
CISENDO	123.750	20.434–2417.348	<0.001	117.981	16.961–2578.872	<0.001

1. Variable coding: Age: ≥45 vs <45 (≥45 coded as high risk). BMI: ≥25 vs <25 (≥25 coded as high risk).
2. "/" indicates the 95% CI and OR could not be estimated or is not available.
3. Univariate screening rule: Variables with $p < 0.10$ in univariate analysis were entered into the multivariable model.
4. Multicollinearity handling: To guard against multicollinearity, variables with variance inflation factor (VIF) > 10 were sequentially removed.
5. *P* values and significance: All tests are two-sided; $P < 0.05$ is considered statistically significant.
6. Abbreviations: OR: odds ratio; CI: confidence interval; ET: endometrial thickness; AUB: abnormal uterine bleeding; PMB: postmenopausal bleeding; CDO1m: *CDO1* methylation test; CELF4m: *CELF4* methylation test.

4. Discussion

In this prospective cohort of 573 participants referred for hysteroscopy, hypermethylation of *CDO1* and *CELF4* genes (CISENDO assay) demonstrated robust diagnostic performance across menopausal strata and emerged as the strongest independent predictor of EC in multivariable models. CISENDO assay achieved high sensitivity and specificity in both premenopausal and postmenopausal groups and retained statistical significance after adjustment for clinical covariates, whereas conventional TVS indicator demonstrated limited discrimination and variable trade-offs between sensitivity and specificity. Importantly, CISENDO assay identified a substantial fraction of cancers with subthreshold ET. Furthermore, many non-EC tested negative for CISENDO assay even when their ET surpassed the threshold recommended by the guidelines [11,19]. This observation suggests that integrating methylation status with TVS can improve case triage by increasing detection accurate and reducing unnecessary invasive assessment of many benign cases.

EC has exhibited rising incidence and mortality over recent 4 decades and remains among the few malignancies with an upward trend in fatal outcomes [36]. Aberrant DNA methylation is a pervasive and functionally important feature of EC as studies have identified hypermethylated tumor-suppressor loci and hypomethylated oncogenic regions in EC [37]. Aberrant DNA methylation patterns, notably promoter hypermethylation of tumor suppressor genes, silence critical regulators of cellular adhesion, proliferation, and apoptosis, thereby driving neoplastic transformation and metastatic dissemination [38]. The detection of such EC specific hypermethylation in noninvasive specimens like cervical scraping cells provides a biologically plausible and clinically actionable basis for molecular testing strategies [24,39]. The *CDO1* gene, encoding the cysteine dioxygenase enzyme critical for cysteine metabolism and lipid homeostasis, is frequently silenced by promoter hypermethylation—a common epigenetic alteration in cancers that enhances tumor survival by suppressing ROS-mediated apoptosis and promoting oxidative stress adaptation [40]. Consistent with this, *CDO1* hypermethylation has been demonstrated in both type I and II EC and serves as a diagnostic marker distinguishing malignant from normal endometrium [41]. Similarly, *CELF4* gene, an RNA-binding protein involved in post-transcriptional regulation, has been reported to display aberrant promoter methylation in EC [42,43].

In our cohort, the non-EC group showed lower *CDO1* and *CELF4* methylation in postmenopausal versus premenopausal women, consistent with an accumulation of focal hypermethylation at certain genes with advancing age [44]. However, methylation levels among histologically confirmed EC cases did not differ by menopausal status, and thus the absence of menopausal-status differences within the EC group suggests that tumor-specific epigenetic reprogramming of *CDO1* and *CELF4* predominates over background age-related methylation variation, supporting the utility of these markers for cancer discrimination across age strata.

Notably, in multivariable analyses CISENDO assay emerged as the dominant independent predictor of histologically confirmed EC after adjustment for age, BMI, ET, and bleeding status (adjusted ORs: premenopausal =103.9; postmenopausal =117.981). This strengthens the argument that methylation status provides prognostic information beyond conventional clinical parameters. Furthermore, neither AUB nor PMB remained independent predictors in adjusted models. These findings underscore the superior discriminative capacity of the molecular markers over symptomatic and anthropometric variables in EC risk stratification. However, the extreme magnitude and breadth of some adjusted effect estimates warrant cautious interpretation: wide confidence intervals reflect limited event numbers and potential model instability. We therefore recommend interpreting the adjusted ORs as evidence of strong association rather than precise effect-size estimates.

And what's interesting was that the seven of eight EIN cases in this study were relatively young (median age 35 years, IQR 30–42), a finding that is concordant with reports that EIN may present in younger women, particularly those with obesity, anovulatory disorders, or infertility, underscoring the relevance of metabolic and reproductive factors in disease pathogenesis [45,46]. Given that EIN is a recognized premalignant lesion with a substantial risk of concurrent or subsequent EC [47,48], accurate triage methods are crucial for this younger, high-risk and at risk increased women to ensure early detection and careful evaluation before adopting conservative management. However, in the primary diagnostic-performance analysis we restricted the case cohort to pathologically confirmed invasive EC. Cases diagnosed as EIN (also reported as atypical hyperplasia) were treated as premalignant lesions and were therefore excluded from the main EC group. First, the management, prognosis and immediate clinical implications of EIN and low-grade EC are different, so combining them would increase heterogeneity when evaluating tests aimed at detecting invasive disease [49]. Second, the histomorphologic diagnosis of EIN on endometrial sampling is challenging and subject to interobserver variation, including sampling fragmentation, variable hormonal effects, prior hormonal therapy, and polyps can obscure gland architecture and cytologic assessment, reducing diagnostic certainty [50]. Third, the number of EIN cases in our cohort was small ($n = 8$), which does not provide sufficient power to estimate subgroup diagnostic performance reliably.

AUB and PMB are the most frequent presenting complaints that prompt evaluation for endometrial pathology. Importantly, they often lead patients to seek care at an early, potentially treatable stage [18]. However, these symptoms are non-specific and are commonly attributed to benign conditions such as polyps, fibroids, adenomyosis and endometrial atrophy [51,52]. In postmenopausal populations only a minority (approximately 5–10%) of bleeding events are due to EC [53,54]. In addition, determining menopausal status can be clinically nebulous due to variable bleeding patterns during the menopausal transition, and this diagnostic uncertainty may affect the choice and performance of ET cutoffs in practice Consequently, misattribution of bleeding to benign causes contributes to diagnostic delays, and symptom-driven pathways generate substantial false positives, underscoring the need for improved triage strategies. A UK study (2006–2010) revealed age-related disparities in fast-track referral rates, with younger women (ages 35–44) exhibiting lower prompt referral likelihoods compared to older patients (ages 65–74) [55]. In contrast to prior studies focusing exclusively on symptomatic cohorts or failing to stratify by bleeding status [28–30], our sample explicitly included both premenopausal and postmenopausal women and patients presenting with both typical and atypical symptoms. These data suggest that CISENDO assay's high specificity prioritizes high-risk patients for immediate intervention, complementing anatomical imaging in a risk-stratified triage model. While the combination's high NPV allows safe, conservative follow-up for those with concordant negative molecular and imaging findings, meaning CISENDO assay could serve as a triage tool to refine referral pathways.

TVS remains the first-line modality for women with PMB, with established ET thresholds (typically 3–5 mm) guiding selection for histologic assessment for postmenopausal women, as supported by substantial evidence and professional guidelines [22,56,57]. Clinical models based on imaging have identified that an ET ≥11 mm is a high-risk factor for EC in premenopausal women [58]. However, in premenopausal and peri-menopausal women, ET exhibits considerable variability across the menstrual cycle, compromising the reliability of isolated measurements and precluding the application of a universally validated cutoff. Within these populations, TVS often lacks the specificity to reliably differentiate benign structural anomalies from premalignant or malignant endometrial pathology [20–22,59]. While an ET threshold of ≤4 mm is an accepted rule-out for EC in women with PMB, in our study, the choice of 11 mm (proliferative phase) for premenopausal women just follows our institutional protocol and Chinese guideline recommendations [11,19] as a pragmatic choice. However, menstrual phase variability and a lack of universal consensus mean such a threshold has limited external validity and may not generalize

across all clinical settings. Moreover, obesity and diabetes can degrade ultrasound performance [60], and definitive diagnosis still requires invasive sampling—procedures that carry pain, anxiety and occasional sampling failure [61]. Although TVS demonstrates high sensitivity for excluding malignancy in postmenopausal bleeding when using appropriate cutoffs [56,57], meta-analytic evidence further indicates that studies with partial disease verification, where not all participants undergo biopsy reported lower EC risks compared to those with fully verified examinations implying potential under-ascertainment among TVS-negative women [53]. These uncertainties regarding symptom interpretation and imaging cutoffs often lead to diagnostic delays, especially in this high-risk referral population, underscoring the urgent need for objective, high-accuracy molecular biomarkers to triage patients effectively.

In our cohort, the CISENDO assay alone demonstrated high diagnostic accuracy across both menopausal strata (Sensitivity and Specificity ~90%; AUC >0.90), aligning with recent studies [28–30]. Moreover, integrating methylation with TVS using an 'OR' rule maximized NPV, providing a robust safety margin for excluding malignancy in double-negative patients, potentially serving as a triage tool during the EC screening process.

Notably, many women without EC who had ET exceeding conventional thresholds were CISENDO(−), and meanwhile a substantial proportion of EC cases—particularly among premenopausal women—exhibited CISENDO(+) despite subthreshold ET. These observations, as illustrated in Fig. 3 and summarized in Table 2, suggest a potential triage approach in referred or symptomatic populations: women who are both CISENDO(−) and have normal endometrial thickness could reasonably be considered for conservative management with appropriate clinical follow-up, whereas women who are CISENDO(+) and/or have abnormal ET might be prioritized for clinician-led risk assessment and selective further testing, such as hysteroscopy with dilation and curettage following a comprehensive assessment by the physician. Although this proposal requires further validation, based on our results, CISENDO's clinical utility does lie in: (1) identifying patients at high-risk who require timely invasive assessment or surgical intervention among those with ambiguous ultrasound findings; and (2) potentially reducing the urgency of invasive procedures for methylation-negative women with borderline endometrial thickness. Given their relatively low cost, feasibility for self-collection, and capacity to minimize unnecessary referrals, methylation assays such as CISENDO represent promising scalable tools for risk stratification and primary triage—especially in high-risk symptomatic cohorts or resource-constrained settings.

Unlike single-gene assays, our dual-gene approach demonstrates comparable accurate to emerging multi-marker panels (e.g., WID-qEC [20]), while utilizing cervical scrapings for minimal invasiveness. A range of non-invasive sampling strategies (vaginal fluid, tampon collections, urine, and cervical scrapings) have been explored. Vaginal fluid (tampon) approaches are generally well tolerated and acceptable to patients [62], but have shown lower discriminatory power [63] and may fail to provide enough endometrial cells in samples [64]. Urine specimens are attractive for convenience but cannot reliably capture endometrial cells directly, limiting their current diagnostic role [63]. In contrast, some studies report superior methylation test performance on cervical Pap-brush/scrape samples compared with endometrial brushes or tampon pools. For example, PCDHGB7 methylation had better performance in Pap brush vs Tao-brush (AUC 0.86 vs 0.83, sensitivity 80.7% vs 61.3%) [65], and methylated RASSF1A AUCs reported higher for cervical scrapes than for tampon samples(AUC 0.93 vs 0.75) [66,67]. The biological rationale for cervical scrapings lies in the anatomical continuity of the uterine cavity, allowing endometrial tumor cells to shed into the endocervical canal and be captured by cervical sampling [68,69]. And the ability of sensitive molecular assays to detect tumor-derived DNA among abundant background cells supports this approach as a rational, operationally practical specimen for methylation-based triage and diagnostic testing [69,70]. The WID-qEC assay (targeting methylated GYPC and ZSCAN12) demonstrated 90–100% sensitivity and 75–89% specificity in symptomatic patients [20]. In addition, triage with WID-qEC testing in abnormal uterine bleeding may reduce histological referrals and false positives [71] These findings, together with our and previous results using CDO1/CELF4 methylation test from cervical scrapings (sensitivity and specificity 0.85–0.9, AUC 0.85–0.9) [28–30], support the operational and diagnostic advantages of cervical scraping methylation assays as feasible triage/diagnostic tools in symptomatic or referred populations.

This study has several limitations. First, its single-center, prospective study design and use of a hysteroscopy referred cohort may introduce selection bias, notably an elevated disease prevalence that affects the generalizability of positive and negative predictive values. Second, the small number of EIN cases led to their exclusion from analysis, precluding meaningful conclusions regarding precancerous lesions. Furthermore, the sample size was insufficient to evaluate histological subtypes of EC (e.g., type I vs. type II) or to reliably estimate ORs for covariates with rare or absent events in certain subgroups (e.g., diabetes and PCOS). Although the diagnostic accuracy of methylation testing was established, the study did not assess its impact on healthcare resource utilization, or long-term survival outcomes. Future multicenter studies should prioritize: (1) Validation in type II EC subtypes; (2) Clinical endpoint assessment (e.g., diagnostic delay reduction, mortality); (3) Cost-effectiveness analysis via long-term follow-up.

5. Conclusion

The CISENDO assay, detecting cervical *CDO1/CELF4* methylation in cervical scraping samples, provides a highly accurate and reliable molecular method for EC diagnosis across menopausal status. It emerged as the strongest independent predictor of EC in multivariable analysis, outperforming ultrasound and clinical parameters. This assay offers robust objective triage by identifying malignancies in women with normal ET while reducing unnecessary invasive procedures in benign cases. Integration of this methylation assay into clinical pathways could optimize patient stratification, guide referrals for hysteroscopy, and improve early detection rates.

Authors' contribution

Xiuzhen Wang and Lang Zheng contributed equally to this work. Xiuzhen Wang, Lang Zheng, and Genhai Zhu were involved in study design, data collection, and manuscript drafting. Shengtan Wang, Wei Li, Jun Liu, and Haocheng Gao participated in data acquisition and statistical analysis. Xiaohang Liu and Guifei Li contributed to the laboratory assays and interpretation of molecular data. Lan Hong and Lei Li supervised the study, provided critical revisions, and are co-corresponding authors. All authors reviewed and approved the final manuscript.

CRediT authorship contribution statement

Xiuzhen Wang: Writing – review & editing, Writing – original draft, Funding acquisition, Formal analysis, Data curation. **Lang Zheng:** Writing – review & editing, Writing – original draft, Formal analysis, Data curation. **Genhai Zhu:** Formal analysis, Data curation. **Shengtan Wang:** Methodology, Investigation. **Wei Li:** Methodology, Investigation. **Jun Liu:** Methodology, Investigation. **Haocheng Gao:** Methodology, Investigation. **Xiaohang Liu:** Methodology, Investigation. **Guifei Li:** Methodology, Investigation. **Lei Li:** Writing – review & editing, Supervision, Project administration, Data curation, Conceptualization. **Lan Hong:**

Writing – review & editing, Supervision, Project administration, Funding acquisition, Data curation, Conceptualization.

Consent for publication

All participants provided written informed consent for the publication of the study results, including any anonymized data and findings presented in this manuscript.

Ethics statement

This study strictly followed institutional and National Research Council ethics guidelines for research involving human subjects, and was approved by the Ethics Committee of Hainan General Hospital (Approval No. Med-Eth-Re[2025]627). All patients participating in this study provided informed consent.

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Declaration of competing interest

The authors declare that they have no competing interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ygyno.2026.01.776.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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