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Xiaoyan Li
Wuxi Maternity and Child Health Care Hospital, Affiliated Women's Hospital of Jiangnan University
Meilikang Li
Women's Hospital of Nanjing Medical University Nanjing Women and Children's Healthcare Hospital
Yi Shen
Wuxi Maternity and Child Health Care Hospital, Affiliated Women's Hospital of Jiangnan University
Xiaopei Zhou
Wuxi Maternity and Child Health Care Hospital, Affiliated Women's Hospital of Jiangnan University
Boqun Xu
Women's Hospital of Nanjing Medical University Nanjing Women and Children's Healthcare Hospital
Xiaokang Deng
xiaokang@jiangnan.edu.cn

Wuxi Maternity and Child Health Care Hospital, Affiliated Women's Hospital of Jiangnan University

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Clinical, Ultrasonographic, and Laboratory Factors Associated With Endometrial Malignancy in Women With Postmenopausal Bleeding: A Retrospective Cohort Study

Xiaoyan Li[1#], Meilikang Li[2#], Yi Shen[1#], Xiaopei Zhou[1], Boqun Xu[2*] & Xiaokang Deng[1,3*]

[#]*These authors contributed equally to this work.*
[1] *Wuxi Maternity and Child Health Care Hospital, Affiliated Women's Hospital of Jiangnan University, Wuxi 214002, China*
[2] *Women's Hospital of Nanjing Medical University,Nanjing Women and Children's Healthcare Hospital, Nanjing 210000, China*
[3] *Key Laboratory of Reproductive Genetics (Zhejiang University), Ministry of Education, Hangzhou 310000, China*

Corresponding author:
Xiaokang Deng, E-mail: xiaokang@jiangnan.edu.cn;
Boqun Xu, E-mail: boqun_xu@163.com;

Abstract

Background: Postmenopausal bleeding (PMB) requires evaluation for malignant pathology, but the discriminatory relevance of metabolic biomarkers and serum tumor markers remains uncertain.

Objective: To examine clinical, ultrasonographic, and laboratory factors associated with malignant pathology among women undergoing histopathological evaluation for PMB.

Methods: We retrospectively analyzed 876 women with classifiable pathology (688 non-malignant and 188 malignant). A prespecified multivariable logistic regression model included clinical characteristics, endometrial thickness, D-dimer, and tumor markers. Complete-case analysis included 728 patients, with multiple imputation performed as a sensitivity analysis. Natural cubic splines assessed nonlinear associations, and nested models evaluated the incremental discrimination provided by CA125 and CA19-9.

Results: Women with malignant pathology were older and had greater endometrial thickness (both $P < 0.001$). In multivariable analysis, age (aOR 1.27 per 5 years, 95% CI 1.12–1.44), endometrial thickness (aOR 1.92 per 5 mm, 95% CI 1.60–2.31), CA125 (aOR 1.12 per 10 U/mL, 95% CI 1.04–1.21), and CA19-9 (aOR 1.16 per 10 U/mL, 95% CI 1.06–1.27) were associated with malignant pathology. Endometrial thickness showed evidence of nonlinearity ($P = 0.030$). Adding CA125 and CA19-9 to a model including age and endometrial thickness increased the apparent AUC from 0.744 to 0.781. BMI and conventional lipid-related measures showed limited discriminatory associations.

Conclusions: Age, endometrial thickness, and selected tumor markers were associated with malignant pathology in this pathology-selected PMB cohort. The incremental discrimination provided by tumor markers was modest, and the findings require external validation before clinical application.

Keywords: postmenopausal bleeding; malignant pathology; endometrial thickness; body mass index; lipid profile; logistic regression; restricted cubic splines

Introduction

Postmenopausal bleeding is a clinically important symptom because it may be the presenting manifestation of uterine malignancy, although many affected women have benign conditions(1). In a systematic review and meta-analysis, Clarke and colleagues reported that approximately 9% of women with PMB had endometrial cancer, with substantial variation across settings and study characteristics. Accordingly, the challenge in clinical practice is not merely identifying women with bleeding but distinguishing those with malignant from non-malignant underlying pathology.(2, 3)

Transvaginal ultrasonography and endometrial tissue assessment are central to the diagnostic evaluation of PMB. Endometrial thickness has long been used as a triage measure, but it is not a histologic diagnosis, and a thin endometrium cannot exclude every malignancy. The American College of Obstetricians and Gynecologists (ACOG) updated its guidance in 2026, emphasizing a more comprehensive initial evaluation in most patients with PMB (4). These developments support evaluating endometrial thickness alongside other patient-level information rather than treating a single thickness threshold as definitive.(5)

Obesity is a recognized determinant of endometrial cancer incidence in the general population.

A dose-response meta-analysis of prospective studies demonstrated a substantial increase in endometrial cancer risk with increasing BMI.(6) However, an association with cancer incidence in an unselected population does not necessarily imply that BMI can distinguish malignant from benign pathology within a selected group of women who already have PMB and undergo diagnostic assessment. Similarly, lipid-related markers may reflect metabolic disturbances, but their discriminatory relevance in this symptomatic clinical setting is uncertain(7-9).

We therefore examined demographic and reproductive characteristics, BMI, ultrasonographic measures, coagulation-related testing, lipid-related biomarkers, and serum tumor markers in women with PMB who had available pathology. Our primary objective was to identify factors associated with malignant rather than normal/non-malignant pathology. Secondary objectives were to explore potential nonlinear associations of continuous variables, assess lipid-related findings under strict complete-case selection, and evaluate the robustness of the primary multivariable findings to missing covariate data using multiple imputation. We hypothesized that age and endometrial thickness would be associated with malignant pathology, while associations of BMI and lipid markers were treated as exploratory.

Materials and Methods

Study design and setting

This retrospective cohort study included women evaluated for postmenopausal bleeding at Wuxi Maternity and Child Health Care Hospital, Affiliated Women's Hospital of Jiangnan University, between January 2023 and December 2025. Reporting was organized with reference to the STROBE statement.

Participants and cohort construction

The study population comprised women presenting with postmenopausal bleeding who underwent histopathological evaluation at our institution between January 2023 and December 2025. Tissue sampling was performed as part of routine clinical care according to individual clinical circumstances, including bleeding symptoms, ultrasonographic findings, relevant medical history, and the treating clinician's judgment. Diagnostic procedures included endometrial biopsy, hysteroscopy-guided sampling, and dilation and curettage. A uniform diagnostic pathway could not be retrospectively verified for all patients. Women managed without histopathological assessment were not represented in the source dataset.

The source dataset contained 931 records. After removing 10 records without valid hospitalization identifiers and five duplicate records, 916 unique patients remained. Forty patients were excluded from the strict binary analysis because of premalignant or indeterminate pathology (n = 27), missing or unfinished pathology (n = 10), or non-diagnostic findings (n = 3). The final analytical cohort comprised 876 patients, including 688 with non-malignant pathology and 188 with malignant pathology. The non-malignant group included benign pathological abnormalities and should not be interpreted as uniformly normal endometrium. Premalignant or indeterminate lesions were excluded to preserve a binary comparison between classifiable non-malignant and malignant findings.

Because the cohort was restricted to women who underwent histopathological assessment, the observed malignant proportion of 21.5% should not be interpreted as the prevalence of

malignancy among all women presenting with postmenopausal bleeding. The participant selection process is summarized in Figure 1

Variables and measurement

Extracted variables included age, reproductive history, anthropometric measurements, endometrial thickness, uterine dimensions, D-dimer, lipid-related markers, CA125, CA19-9, and CEA. Endometrial thickness was extracted retrospectively from routine clinical ultrasound reports. Because the examinations were not performed under a prospectively standardized research protocol, consistency in measurement technique could not be independently verified. Information regarding the handling of intracavitary abnormalities, including endometrial polyps, submucosal fibroids, and intracavitary fluid, was not consistently documented in the available records.

Laboratory results were obtained from routine clinical records. The analytical dataset did not consistently establish whether CA125 and CA19-9 were measured routinely or selectively, or their precise timing relative to histopathological diagnosis. Laboratory measurements were obtained from routine institutional clinical records, and the corresponding units are presented in Table 1. Assay-specific reference intervals, analytical platforms, and potential changes in laboratory methods during the study period could not be fully verified from the available retrospective dataset. Consequently, analytical variability cannot be completely excluded. When three uterine dimensions were available in millimeters, uterine volume was estimated using an ellipsoid approximation (length × width × height × 0.523/1000) in mL.

Ethical Approval

The study protocol was approved by the Ethics Committee of Wuxi Maternity and Child Health Care Hospital (Approval No. [YLSL2026-082]). Because of the retrospective nature of the study and the use of routinely collected clinical data, the requirement for individual informed consent was waived by the Ethics Committee. The study was conducted in accordance with the Declaration of Helsinki and applicable institutional requirements.

Data quality and missingness

Duplicate hospitalization records were reconciled, and obvious physiologically implausible entries were set to missing using predetermined ranges rather than excluding the entire patient from the descriptive cohort. The descriptive study cohort remained N=876; Table 1 provides variable-specific denominators. The prespecified multivariable logistic model used complete-case analysis because all selected covariates were required simultaneously, yielding 728 patients (578 normal/non-malignant; 150 malignant). This represents a subset of the full cohort and should not be confused with initial study eligibility. In the lipid sensitivity analysis, all eight prespecified coagulation and lipid-related markers were required, yielding 611 patients (470 normal/non-malignant; 141 malignant).

Missingness was not demonstrably completely at random: endometrial thickness was missing in 26/688 (3.8%) women with non-malignant pathology and 25/188 (13.3%) women with malignant pathology ($P<0.001$). Because complete-case estimates may therefore be susceptible to selection bias, we performed a multiple-imputation sensitivity analysis under a

missing-at-random working assumption, as described below.

Statistical Analysis

Normality was assessed separately by pathology group using the Shapiro–Wilk test. Normally distributed continuous variables were summarized as mean ± standard deviation and compared using independent-samples t tests after assessing variance homogeneity. Non-normally distributed continuous variables were summarized as median [interquartile range] and compared using two-sided Mann–Whitney U tests. Descriptive comparisons were performed using variable-specific available observations. The P values reported in Table 1 were considered descriptive and were not adjusted for multiple comparisons.

The primary outcome was binary pathological classification, coded as 1 for malignant pathology and 0 for non-malignant pathology. Univariable and prespecified multivariable logistic regression analyses were performed using the same complete-case population (N = 728). The multivariable model included ten prespecified predictors: age, body mass index (BMI), age at menarche, age at menopause, parity, endometrial thickness, D-dimer, CA125, CA19-9, and carcinoembryonic antigen (CEA). Predictors were selected based on clinical relevance rather than automated stepwise selection. Odds ratios (ORs) were expressed per 5 years for age, per 5 mm for endometrial thickness, per 0.1 mg/L for D-dimer, per 10 U/mL for CA125 and CA19-9, and per unit for the remaining variables. Adjusted odds ratios (aORs) with 95% confidence intervals (CIs) were reported. Multicollinearity was assessed using variance inflation factors.

To assess the robustness of the primary findings to missing covariate data, multiple imputation by chained equations (MICE) was performed as a sensitivity analysis using the full cohort (N = 876). Predictive mean matching with five donors was used to impute missing continuous covariate values. Twenty imputed datasets were generated using 20 burn-in cycles and five cycles between imputations. The observed pathological outcome and gravidity were included as predictors in the imputation procedure. Regression estimates were combined across imputed datasets using Rubin's rules. Multiple imputation was conducted under a conditional missing-at-random assumption. The complete-case analysis remained the primary analysis, and the possibility of missing-not-at-random mechanisms was considered when interpreting the findings.

Potential nonlinear associations were evaluated using natural cubic splines with four degrees of freedom. Each continuous predictor was examined separately by replacing its linear term with a spline term while retaining the remaining prespecified covariates. Likelihood-ratio tests were used to compare the nonlinear and corresponding linear models. The four-degree-of-freedom spline specification introduced three additional degrees of freedom relative to the linear term. These analyses were exploratory, and estimates at the sparsely populated extremes of the predictor distributions were interpreted cautiously. Endometrial thickness was additionally categorized as ≤4 mm, >4–10 mm, and >10 mm in a categorical sensitivity analysis.

To investigate whether CA125 and CA19-9 provided additional discriminatory information beyond established clinical factors, nested logistic regression models were compared within the same complete-case population. Model A included age and endometrial thickness, whereas Model B additionally included CA125 and CA19-9. Model C included the eight

prespecified predictors other than CA125 and CA19-9, and Model D included all ten predictors. Model discrimination was assessed using the area under the receiver operating characteristic curve (AUC). Absolute differences in AUC (ΔAUC) and their 95% confidence intervals were estimated using 3,000 paired stratified bootstrap resamples of the fitted predictions. Likelihood-ratio tests were used to compare nested models, and Brier scores were calculated to summarize overall prediction error. These performance measures were exploratory and represented apparent performance in the development dataset; they were not optimism-corrected or externally validated.

A separate strict complete-case sensitivity analysis examined coagulation- and lipid-related measurements. This analysis required non-missing values for D-dimer and seven lipid-related markers: total cholesterol, triglycerides, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, apolipoprotein A, apolipoprotein B, and lipoprotein(a). The resulting analytical subset comprised 611 patients. Between-group comparisons were performed using distribution-appropriate statistical tests.

All statistical tests were two-sided. A P value < 0.05 was considered statistically significant for the prespecified regression analyses. Results from exploratory analyses, including spline comparisons, categorical sensitivity analyses, lipid-related comparisons, and model performance evaluations, were interpreted cautiously without adjustment for multiple testing. Statistical analyses were performed in Python using SciPy, statsmodels, and patsy.

Results

Participant selection and descriptive characteristics

The source dataset comprised 931 records from women with postmenopausal bleeding who had undergone histopathological evaluation. After removing 10 records without valid hospitalization identifiers and five duplicate records, 916 unique patients remained.

Forty patients were excluded from the primary binary comparison because their pathological findings were premalignant or indeterminate (n=27), missing or unfinished (n=10), or non-diagnostic (n=3). The final analytical cohort comprised 876 women, including 688 (78.5%) with non-malignant pathology and 188 (21.5%) with malignant pathology.

The observed malignant proportion of 21.5% was calculated among patients with classifiable pathological findings and should not be interpreted as the prevalence of endometrial malignancy in the general population of women presenting with postmenopausal bleeding.

Patients in the malignant group were older than those in the normal/non-malignant group (median 63.0 [57.0–71.0] vs 57.0 [53.0–64.0] years, P<0.001). Median endometrial thickness was 8.0 [3.0–15.0] mm versus 4.0 [3.0–7.0] mm (P<0.001). D-dimer, CA125, CA19-9, and CEA were higher in the malignant group (all P≤0.001 in the available-case comparisons). BMI did not differ significantly (24.03 [22.58–27.10] vs 24.03 [22.06–26.62] kg/m², P=0.605). No significant differences were found for uterine volume or most lipid-related measurements (Table 1).

The primary complete-case regression included 728 of 876 patients (83.1%). Endometrial thickness was missing in 25 of 188 patients (13.3%) with malignant pathology and 26 of 688 patients (3.8%) with non-malignant pathology. Multiple imputation was performed using the full cohort as a sensitivity analysis for missing covariate data.

Histopathological characteristics of the study cohort

Among the 188 patients assigned to the malignant group, linked postoperative histopathological records were available for 111 patients (59.0%), whereas 77 patients (41.0%) had no corresponding postoperative records in the institutional surgical dataset.

Among the 111 patients with available postoperative records, 85 (76.6%) were diagnosed with endometrioid carcinoma, 12 (10.8%) with serous carcinoma, and one each (0.9%) with clear cell carcinoma, carcinosarcoma, and mixed carcinoma. Histological subtype was unspecified in 11 patients (9.9%).

Regarding tumor grade, 56 patients had clearly documented single-grade classifications, including 26 with grade 1, 20 with grade 2, and 10 with grade 3 disease. An additional 31 patients had grade ranges or conflicting descriptions, while 24 lacked explicit grade documentation.

FIGO staging information was available for all 111 patients with linked postoperative records, with 80 patients (72.1%) classified as stage I, 16 (14.4%) as stage II, 11 (9.9%) as stage III, and four (3.6%) as stage IV.

Detailed histopathological characteristics are presented in Supplementary Table S3A. The incomplete availability of postoperative records should be considered when interpreting the distribution of histological subtypes, grades, and stages in the overall malignant group.

Among the 688 patients assigned to the non-malignant group, provisional classification of the original pathology descriptions identified endometrial polyps in 221 patients (32.1%), proliferative-pattern endometrium in 132 (19.2%), atrophic endometrium in 29 (4.2%), and endometrial hyperplasia described as simple or complex in 12 (1.7%). Other findings included leiomyoma or smooth-muscle lesions, cervical polyps, endometritis, and secretory-pattern endometrium. In 205 patients (29.8%), the available descriptions were benign or nonspecific without sufficient information for a more definitive principal histopathological classification. The distributions of malignant and non-malignant pathological findings are summarized in Supplementary Tables S3A and S3B, respectively.

Logistic regression

In the prespecified multivariable model, 728 women had complete data for all ten covariates, including 150 malignant cases. Older age was associated with malignant pathology (aOR 1.27 per 5 years, 95% CI 1.12–1.44, P<0.001). Greater endometrial thickness was associated with higher odds of malignancy (aOR 1.92 per 5 mm, 95% CI 1.60–2.31, P<0.001), as were CA125 (aOR 1.12 per 10 units, 95% CI 1.04–1.21, P=0.002) and CA19-9 (aOR 1.16 per 10 units, 95% CI 1.06–1.27, P=0.001). Later age at menarche was also associated with malignancy (aOR 1.25 per year, 95% CI 1.09–1.42, P=0.001). BMI was not statistically significant after adjustment (aOR 0.94 per kg/m², 95% CI 0.89–1.00, P=0.051). Neither D-dimer (aOR 1.00 per 0.1 unit, P=0.515) nor age at menopause, parity, or CEA reached the conventional significance threshold. The maximum variance inflation factor was 1.24, suggesting limited collinearity in the fitted model (Table 2; Figure 2).

Incremental discriminatory value of serum tumor markers

We further evaluated the incremental discriminatory value of CA125 and CA19-9 beyond age and endometrial thickness. The baseline model including age and endometrial thickness

yielded an apparent AUC of 0.744 (95% CI 0.695–0.791). Adding CA125 and CA19-9 increased the apparent AUC to 0.781 (95% CI 0.736–0.824), corresponding to an absolute improvement of 0.0366 (95% CI 0.0158–0.0610). The likelihood-ratio test indicated improved model fit ($\chi^2 = 40.98$, df = 2, $P < 0.001$). In the fully adjusted sensitivity analysis, the apparent AUC increased from 0.781 (95% CI 0.737–0.825) to 0.805 (95% CI 0.761–0.845) after inclusion of both markers, yielding an absolute improvement of 0.0232 (95% CI 0.0074–0.0414; likelihood-ratio test $P < 0.001$). These findings indicate a modest improvement in apparent discrimination after the addition of CA125 and CA19-9. However, the results were derived from the development dataset and do not establish clinical utility or externally validated predictive performance. Detailed model comparisons are presented in Supplementary Table S4.

Multiple-imputation sensitivity analysis

Multiple-imputation sensitivity analysis using the full cohort of 876 women yielded estimates broadly consistent with the complete-case model (Supplementary Table S2). Age (aOR 1.30 per 5 years, 95% CI 1.16–1.47; P<0.001), age at menarche (aOR 1.22 per year, 95% CI 1.09–1.38; P=0.001), endometrial thickness (aOR 1.88 per 5 mm, 95% CI 1.58–2.24; P<0.001), CA125 (aOR 1.12 per 10 units, 95% CI 1.05–1.20; P=0.001), and CA19-9 (aOR 1.14 per 10 units, 95% CI 1.02–1.26; P=0.018) remained associated with malignant pathology after multivariable adjustment. BMI was borderline significant after imputation (aOR 0.95 per kg/m², 95% CI 0.90–1.00; P=0.049), whereas it narrowly missed the conventional significance threshold in the complete-case analysis (P=0.051). Age at menopause, parity, D-dimer, and CEA remained non-significant. Overall, the direction and magnitude of the principal associations were similar across the complete-case and multiple-imputation analyses.

Nonlinearity and endometrial thickness categories

Spline analysis provided evidence of a nonlinear association between endometrial thickness and malignant pathology (P for nonlinearity = 0.030). The nonlinear association is shown in Figure 3. Among the 188 patients in the malignant group, endometrial thickness measurements were available for 163 patients (86.7%). Of these, 53 (32.5%) had an endometrial thickness of ≤4 mm, 46 (28.2%) had a thickness of >4–10 mm, and 64 (39.3%) had a thickness of >10 mm. Endometrial thickness was unavailable for the remaining 25 patients (13.3%). In the adjusted categorical analysis, thickness >10 mm was associated with substantially greater odds of malignant pathology compared with ≤4 mm (aOR 7.69, 95% CI 4.41–13.41; $P < 0.001$). However, confidence intervals widened at greater endometrial thicknesses, indicating increased uncertainty in the upper range of the distribution. These findings should not be interpreted as establishing a definitive diagnostic threshold. The histological composition of malignant cases with endometrial thickness ≤4 mm could not be reliably characterized from the available linked postoperative records for the entire malignant group.

Lipid-related complete-case sensitivity analysis

A strict eight-marker complete-case analysis included 611 patients (470

normal/non-malignant and 141 malignant). D-dimer was higher in the malignant group (0.27 [0.16–0.43] vs 0.21 [0.16–0.36], P=0.022). However, total cholesterol (P=0.658), triglycerides (P=0.526), HDL (P=0.629), LDL (P=0.547), apolipoprotein A (P=0.098), apolipoprotein B (P=0.619), and lipoprotein(a) (P=0.866) showed no statistically significant between-group differences (Supplementary Table S1).

Discussion

Principal findings

In this retrospective cohort of women with postmenopausal bleeding (PMB) undergoing histopathological evaluation, older age, greater endometrial thickness, and higher CA125 and CA19-9 levels were independently associated with malignant pathology. Endometrial thickness showed a nonlinear association, whereas BMI and conventional lipid-related markers demonstrated limited discriminatory associations. The unexpected positive association with later age at menarche requires cautious interpretation. The principal findings were broadly consistent in the multiple-imputation sensitivity analysis.

Endometrial thickness and clinical implications

Endometrial thickness was strongly associated with malignant pathology, particularly at values exceeding 10 mm. However, 53 of 163 malignant cases with available measurements (32.5%) had an endometrial thickness of ≤4 mm. This finding highlights that a thin endometrium does not completely exclude malignant pathology in a selected population undergoing histopathological evaluation.(10, 11) The observed proportion should not be interpreted as the malignancy risk among all women with PMB and a thin endometrium. Although spline analysis suggested nonlinearity, wider confidence intervals at greater thicknesses indicated reduced precision. Therefore, endometrial thickness should be interpreted alongside clinical findings rather than used as a standalone diagnostic criterion.(12,13)

BMI and lipid-related findings

Although obesity is an established risk factor for incident endometrial cancer, BMI showed an uncertain association with malignant pathology in our selected PMB cohort.(14,15) The adjusted estimates were similar in the complete-case and multiple-imputation analyses, but their P values were close to the conventional significance threshold, indicating sensitivity to the analytical approach rather than robust evidence of an independent association. Residual confounding by diabetes mellitus, hormone therapy, tamoxifen exposure, metabolic medications, and other unmeasured factors cannot be excluded. Conventional lipid-related measures also showed limited between-group differences, although incomplete measurements and unavailable information on fasting status and lipid-lowering therapy may have influenced these findings. Our results should therefore not be interpreted as evidence that adiposity or lipid metabolism is unrelated to endometrial cancer development.(16,17)

D-dimer and tumor markers

Although D-dimer differed between pathology groups in rank-based comparisons, it did not show an independent linear association in the adjusted logistic regression model. Its skewed

distribution and potential sensitivity to influential observations warrant cautious interpretation. CA125 and CA19-9 were independently associated with malignant pathology and provided modest additional apparent discrimination beyond age and endometrial thickness (Δ AUC = 0.0366) and in the fully adjusted model (Δ AUC = 0.0232). However, these markers are nonspecific, and retrospective selective testing may have introduced indication bias. Furthermore, the analyses were performed in the development dataset without external validation. Consequently, the findings do not establish sufficient clinical benefit to recommend routine measurement of these markers for diagnostic triage.(18,19)

Reproductive factors

The positive association between later age at menarche and malignant pathology was unexpected, as earlier menarche has generally been associated with greater endometrial cancer risk through prolonged lifetime estrogen exposure. Residual confounding by hormonal, metabolic, reproductive, or socioeconomic factors may have influenced this finding. Retrospective recall and documentation errors, selection of patients undergoing histopathological evaluation, and pathological heterogeneity may also have contributed. Therefore, this association should be considered exploratory rather than causal and requires independent confirmation.

Strengths and limitations

This study included a clinically relevant pathology-selected cohort and several sensitivity analyses. Nevertheless, its retrospective, single-center design and restriction to women undergoing histopathological evaluation introduce selection and verification bias, limiting generalizability to unselected women with postmenopausal bleeding. The malignant group was histologically heterogeneous, and detailed postoperative records were available for only 111 of 188 patients. Endometrial thickness was more frequently missing in malignant than non-malignant cases (13.3% vs. 3.8%). Such missingness may reflect technical difficulties, altered uterine anatomy, differences in clinical management, or documentation practices, although the specific reasons could not be verified. Multiple imputation was performed under a missing-at-random assumption, but missing-not-at-random mechanisms remain possible. Incomplete sonographic characterization, variation in ultrasound measurements, retrospectively documented reproductive histories, and unavailable metabolic or treatment-related covariates may have introduced additional bias. Furthermore, the potential influence of extreme biomarker values was not fully characterized, and model performance was assessed using apparent discrimination without formal calibration assessment or external validation.

Clinical and research implications

These findings support evaluating endometrial thickness and selected clinical factors together rather than relying on individual measurements to exclude malignant pathology. The incremental value of serum tumor markers was modest and requires further assessment of calibration and clinical utility. Future prospective multicenter studies should use standardized ultrasound and pathology protocols, comprehensive clinical data collection, and external validation to determine whether these associations can meaningfully improve the evaluation

of women with PMB.(20)

Conclusion

In this retrospective, pathology-selected cohort of women with postmenopausal bleeding, older age, greater endometrial thickness, and higher CA125 and CA19-9 levels were associated with malignant pathology. Although the addition of serum tumor markers modestly improved apparent discrimination, their clinical utility remains uncertain. Endometrial thickness should not be interpreted as a standalone criterion for excluding malignant pathology. These findings require prospective validation, standardized histopathological assessment, and further evaluation of calibration and clinical decision-making before clinical application.

Authors' contributions

X.L., M.L., Y.S. contributed to study design, data collection and analysis, and preparation of the original manuscript. X.D. and B.X. contributed to study supervision, interpretation of the findings, and critical revision of the manuscript. All authors reviewed and approved the final manuscript.

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Competing interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Clinical trial number

Not applicable.

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Table 1. Baseline clinical, ultrasonographic, and laboratory characteristics

Variable	Non-malignant n	Non-malignant	Malignant n	Malignant	P value
Age, years	674	57.00 [53.00, 64.00]	184	63.00 [57.00, 71.00]	<0.001
Age at menarche, years	687	13.00 [13.00, 14.00]	187	14.00 [13.00, 15.00]	0.049
Age at menopause, years	682	51.00 [49.00, 53.00]	187	52.00 [50.00, 53.00]	0.062
Gravidity	688	2.50 [2.00, 3.00]	187	3.00 [2.00, 3.50]	0.443
Parity	687	1.00 [1.00, 2.00]	187	1.00 [1.00, 2.00]	0.005
BMI, kg/m²	686	24.03 [22.06, 26.62]	186	24.03 [22.58, 27.10]	0.605
Endometrial thickness, mm	662	4.00 [3.00, 7.00]	163	8.00 [3.00, 15.00]	<0.001
Uterine volume, mL	685	32.86 [17.12, 53.51]	184	31.15 [18.39, 56.45]	0.624
D-dimer, mg/L	680	0.21 [0.16, 0.34]	186	0.27 [0.16, 0.45]	0.001
Total cholesterol, mmol/L	489	4.94 [4.23, 5.59]	145	4.86 [4.39, 5.52]	0.782
Triglycerides, mmol/L	494	1.35 [0.99, 1.96]	145	1.42 [0.98, 1.95]	0.544
HDL, mmol/L	491	1.58 [1.37, 1.81]	143	1.58 [1.29, 1.84]	0.542
LDL, mmol/L	489	3.07 ± 0.86	144	3.12 ± 0.90	0.522
Apolipoprotein A, g/L	489	1.46 [1.29, 1.69]	144	1.41 [1.21, 1.65]	0.055
Apolipoprotein B, g/L	487	0.90 [0.75, 1.07]	143	0.91 [0.75, 1.06]	0.612
Lipoprotein(a), mg/L	487	127.30 [58.85, 250.40]	144	122.50 [58.27, 279.88]	0.853
CA125, U/mL	633	11.30 [8.00, 16.10]	178	16.25 [10.12, 35.92]	<0.001
CA19-9, U/mL	627	6.20 [3.50, 10.55]	177	10.20 [4.80, 21.00]	<0.001
CEA, ng/mL	628	1.29 [0.80, 1.91]	177	1.57 [1.07, 3.04]	<0.001

Data are presented as median [interquartile range] unless otherwise indicated; LDL is presented as mean ± standard deviation. P values were calculated using the Mann–Whitney U test for non-normally distributed variables and the independent-samples t test for LDL. Variable-specific denominators reflect available data. BMI, body mass index; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; CA125, carbohydrate antigen 125; CA19-9, carbohydrate antigen 19-9; CEA, carcinoembryonic antigen.

Table 2. Univariable and multivariable logistic regression analyses of factors associated with malignant pathology

Variable	Unit	Univariable OR (95% CI)	P value	Adjusted OR (95% CI)	P value
Age	per 5 years	1.32 (1.19-1.46)	<0.001	1.27 (1.12-1.44)	<0.001
BMI	per 1 kg/m²	1.01 (0.96-1.06)	0.738	0.94 (0.89-1.00)	0.051
Age at menarche	per 1 year	1.21 (1.08-1.37)	0.001	1.25 (1.09-1.42)	0.001
Age at menopause	per 1 year	1.05 (1.00-1.11)	0.064	1.03 (0.97-1.10)	0.299
Parity	per birth	1.26 (1.01-1.56)	0.041	0.99 (0.74-1.31)	0.929
Endometrial thickness	per 5 mm	2.02 (1.71-2.39)	<0.001	1.92 (1.60-2.31)	<0.001
D-dimer	per 0.1 mg/L	1.002 (0.995 – 1.010)	0.515	1.00 (0.99-1.01)	0.515
CA125	per 10 U/mL	1.22 (1.13-1.31)	<0.001	1.12 (1.04-1.21)	0.002
CA19-9	per 10 U/mL	1.23 (1.12-1.36)	<0.001	1.16 (1.06-1.27)	0.001
CEA	per 1 ng/mL	1.17 (1.07-1.28)	<0.001	1.08 (0.99-1.18)	0.071

Both columns use the same complete-case subset (N=728; 150 malignant events). The adjusted model includes all 10 listed covariates.

Figure Legend

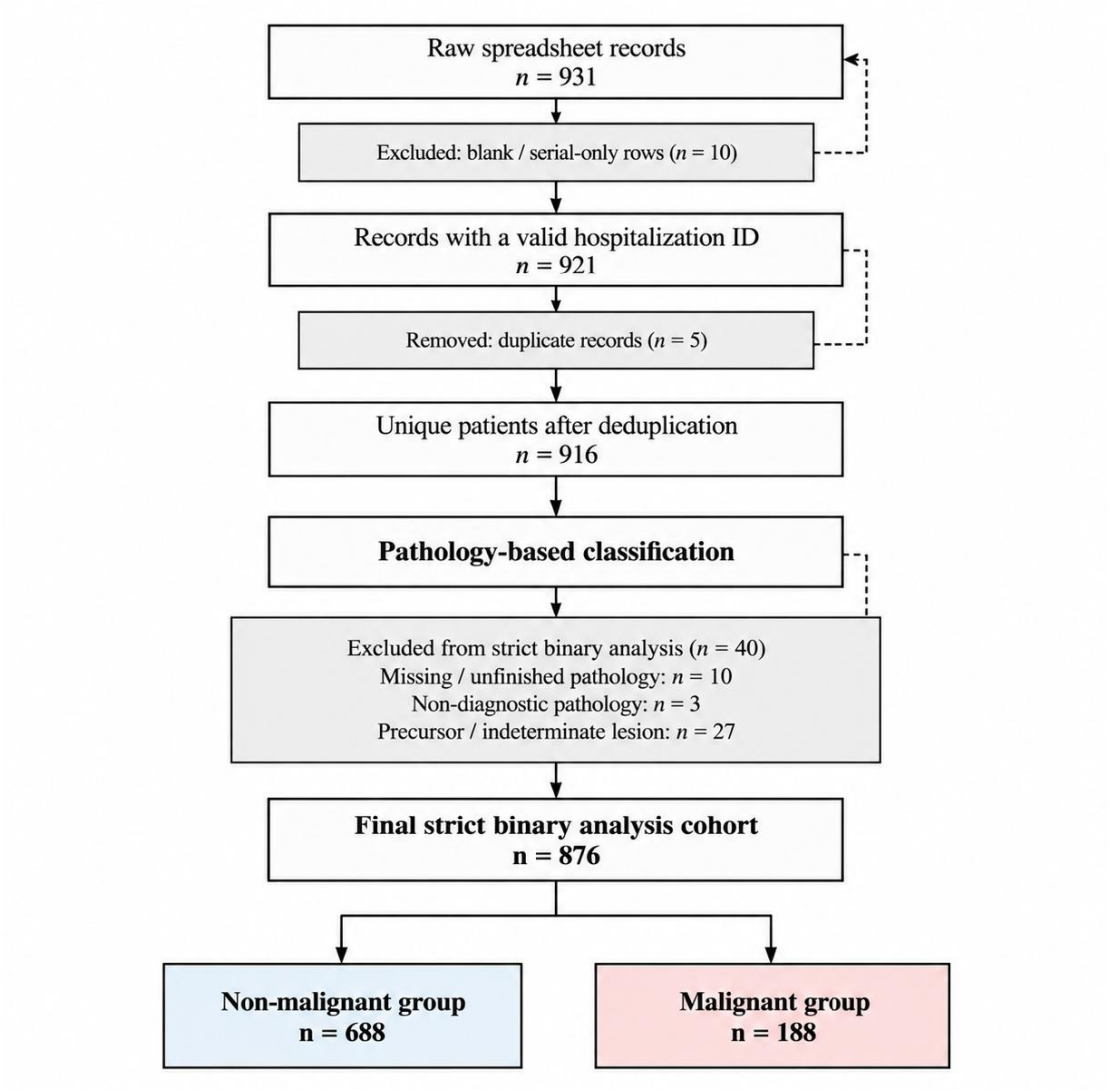


Figure 1. Participant selection and pathology-based classification.

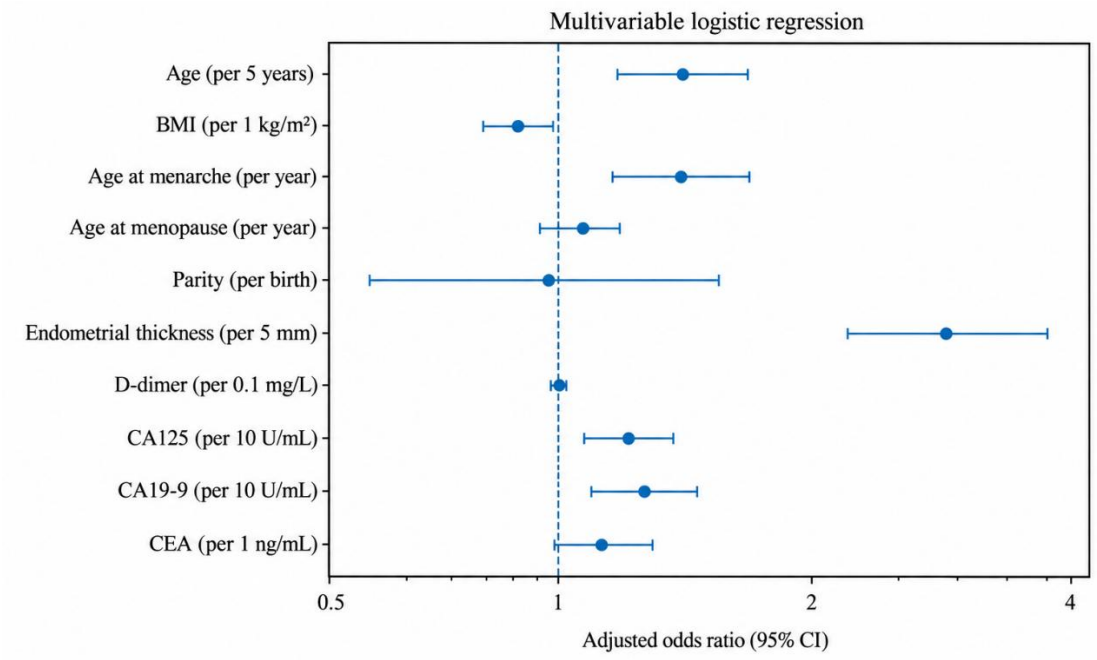


Figure 2. Adjusted odds ratios and 95% confidence intervals from the prespecified multivariable logistic regression model (N=728).

The analysis included 728 complete cases. Points represent adjusted odds ratios, and horizontal lines indicate 95% confidence intervals. The vertical reference line indicates an odds ratio of 1.

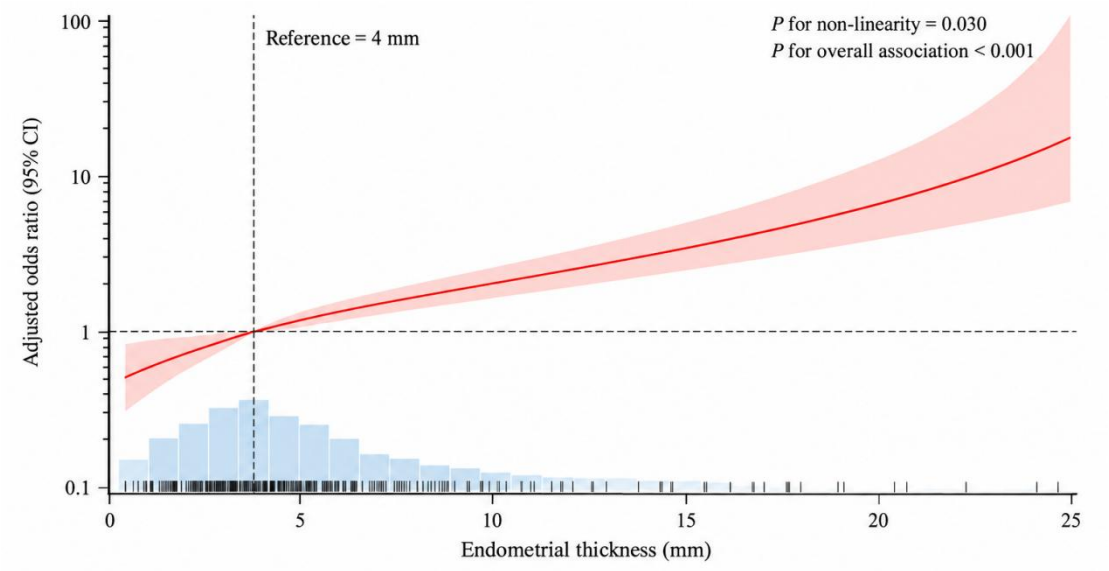


Figure 3. Natural cubic spline analysis of the association between endometrial thickness and malignant pathology.

The solid line represents the estimated adjusted association from the natural cubic spline model, and the shaded area indicates the 95% confidence interval. Confidence intervals widen at greater endometrial thicknesses, reflecting increased uncertainty in the upper range of the observed distribution. The curve should not be interpreted as establishing a definitive diagnostic threshold.

Supplementary Table S1. Strict coagulation- and lipid-marker complete-case sensitivity analysis

Marker	Non-malignant (n=470)	Malignant (n=141)	P value
D-dimer, mg/L	0.21 [0.16, 0.36]	0.27 [0.16, 0.43]	0.022
Total cholesterol, mmol/L	4.96 [4.23, 5.60]	4.86 [4.34, 5.52]	0.658
Triglycerides, mmol/L	1.35 [0.98, 1.96]	1.42 [0.98, 1.95]	0.526
HDL cholesterol, mmol/L	1.57 [1.37, 1.81]	1.58 [1.29, 1.84]	0.629
LDL cholesterol, mmol/L	3.07 ± 0.85	3.12 ± 0.91	0.547
Apolipoprotein A, g/L	1.46 [1.29, 1.69]	1.42 [1.21, 1.65]	0.098
Apolipoprotein B, g/L	0.90 [0.75, 1.08]	0.92 [0.75, 1.07]	0.619
Lipoprotein(a), mg/L	128.90 [60.35, 248.75]	122.60 [59.70, 279.30]	0.866

The strict complete-case sensitivity analysis required eight non-missing measurements, comprising D-dimer as a coagulation-related marker and seven lipid-related markers: total cholesterol, triglycerides, HDL cholesterol, LDL cholesterol, apolipoprotein A, apolipoprotein B, and lipoprotein(a). The analytical subset included 611 patients (470 non-malignant and 141 malignant). LDL cholesterol was compared using an independent-samples t test; the remaining markers were compared using Mann–Whitney U tests.

Supplementary Table S2. Comparison of complete-case and multiple-imputation analyses for the prespecified multivariable logistic regression model

Variable	Complete-case aOR (95% CI)	P	Multiple-imputation aOR (95% CI)	P
Age, per 5 y	1.27 (1.12–1.44)	<0.001	1.30 (1.16–1.47)	<0.001
BMI	0.94 (0.89–1.00)	0.051	0.95 (0.90–1.00)	0.049
Age at menarche	1.25 (1.09–1.42)	0.001	1.22 (1.09–1.38)	0.001
Age at menopause	1.03 (0.97–1.10)	0.299	1.03 (0.98–1.09)	0.228
Parity	0.99 (0.74–1.31)	0.929	1.05 (0.82–1.36)	0.687
Endometrial thickness, per 5 mm	1.92 (1.60–2.31)	<0.001	1.88 (1.58–2.24)	<0.001
D-dimer, per 0.1 mg/L	1.00 (0.99–1.01)	0.515	1.00 (0.99–1.01)	0.564
CA125, per 10 U/mL	1.12 (1.04–1.21)	0.002	1.12 (1.05–1.20)	0.001
CA19-9, per 10 U/mL	1.16 (1.06–1.27)	0.001	1.14 (1.02–1.26)	0.018
CEA, per 1 ng/mL	1.08 (0.99–1.18)	0.071	1.07 (0.98–1.17)	0.153

The complete-case analysis included 728 participants (150 malignant events), whereas the multiple-imputation analysis included the full cohort of 876 participants. Twenty imputed datasets were generated using multivariate imputation by chained equations with predictive mean matching, and estimates were pooled according to Rubin's rules. aOR, adjusted odds ratio; CI, confidence interval; MICE, multivariate imputation by chained equations; PMM, predictive mean matching.

Supplementary Table S3A. Histopathological characteristics of patients assigned to the malignant pathology group (N = 188)

Histopathological characteristic	n	% of 188
Histological subtype		
Endometrioid carcinoma	85	45.2%
Serous carcinoma	12	6.4%
Clear cell carcinoma	1	0.5%
Carcinosarcoma	1	0.5%
Mixed carcinoma	1	0.5%
Unspecified subtype in linked postoperative records	11	5.9%
No linked local postoperative histology	77	41.0%
Tumor grade, as documented		
Grade 1, clearly documented	26	13.8%
Grade 2, clearly documented	20	10.6%
Grade 3, clearly documented	10	5.3%
Grade range or conflicting grade wording	31	16.5%
Grade not documented in linked records	24	12.8%
No linked local postoperative grade	77	41.0%
FIGO stage		
Stage I	80	42.6%
Stage II	16	8.5%
Stage III	11	5.9%
Stage IV	4	2.1%
No linked local postoperative stage	77	41.0%

Percentages were calculated using the total malignant group (N = 188). Linked postoperative histopathological records were available for 111 patients (59.0%), while 77 patients (41.0%) had no corresponding postoperative records in the available institutional surgical dataset. Histological subtype, tumor grade, and FIGO stage were summarized according to available documentation. Missing postoperative records should not be interpreted as evidence that these characteristics were unavailable at other institutions. Grade ranges and conflicting descriptions were retained without assigning a single grade.

Supplementary Table S3B. Histopathological characteristics of patients assigned to the non-malignant group (N = 688)

Principal histopathological category	n	% of 688
Endometrial polyp	221	32.1%
Endometrial hyperplasia, reported as simple/complex	12	1.7%
Atrophic endometrium	29	4.2%
Proliferative-pattern endometrium	132	19.2%
Secretory-pattern endometrium	6	0.9%
Endometritis	3	0.4%
Leiomyoma or smooth-muscle lesion	48	7.0%
Endocervical/cervical polyp	29	4.2%
Polyp, site unspecified	3	0.4%
Other benign or nonspecific tissue findings	205	29.8%
Total	688	100.0%*

The original non-malignant cohort included 688 patients. Principal histopathological categories were provisionally assigned from retrospective pathology descriptions, with each patient classified into one mutually exclusive category. When multiple benign findings were documented, a predefined hierarchy was applied to select the principal category. Polypoid proliferation was not automatically classified as a histologically confirmed endometrial polyp. Hyperplasia categories were based on the original diagnostic wording, and atypia status could not be established uniformly. The category of other benign or nonspecific tissue findings included limited or fragmented tissue descriptions without a more definitive principal diagnosis. Patients with premalignant or indeterminate lesions excluded from the primary binary analysis (n = 27) were not included. Histopathological categories were assigned according to the original pathology reports. Cases with insufficiently specific descriptions were retained in the other benign or nonspecific category rather than being assigned a more definitive diagnosis without supporting documentation.

Supplementary Table S4. Incremental discriminatory value of CA125 and CA19-9 for malignant pathology

Model	Predictors	Apparent AUC (95% CI)	ΔAUC (95% CI)	LRT χ^2	P value	Brier score
Model A	Age + endometrial thickness	0.744 (0.695–0.791)	Reference	—	—	0.1370
Model B	Model A + CA125 + CA19-9	0.781 (0.736–0.824)	0.0366 (0.0158–0.0610)	40.98	<0.001	0.1257
Model C	Prespecified multivariable model excluding CA125 and CA19-9	0.781 (0.737–0.825)	Reference	—	—	0.1299
Model D	Full prespecified multivariable model including CA125 and CA19-9	0.805 (0.761–0.845)	0.0232 (0.0074–0.0414)	31.71	<0.001	0.1211

Models were compared within the same reconstructed complete-case dataset (N = 728; 150 malignant and 578 non-malignant cases). Model A included age and endometrial thickness; Model B additionally included CA125 and CA19-9. Model C included age, BMI, age at menarche, age at menopause, parity, endometrial thickness, D-dimer, and CEA; Model D additionally included CA125 and CA19-9. ΔAUC represents the absolute increase in AUC relative to the corresponding reference model (Model A for Model B; Model C for Model D). Confidence intervals were estimated using 3,000 paired stratified bootstrap resamples of the fitted predictions. Likelihood-ratio tests compared nested models with two degrees of freedom. Lower Brier scores indicate better overall prediction accuracy. AUC estimates were apparent and were not optimism-corrected or externally validated.

AUC, area under the receiver operating characteristic curve; CI, confidence interval; LRT, likelihood-ratio test;

BMI, body mass index; ET, endometrial thickness.

References

1. Makker V, MacKay H, Ray-Coquard I, Levine DA, Westin SN, Aoki D, et al. Endometrial cancer. Nat Rev Dis Primers. 2021;7(1):88.
2. Clarke MA, Long BJ, Del Mar Morillo A, Arbyn M, Bakkum-Gamez JN, Wentzensen N. Association of Endometrial Cancer Risk With Postmenopausal Bleeding in Women: A Systematic Review and Meta-analysis. JAMA Intern Med. 2018;178(9):1210–22.
3. Matteson KA, Robison K, Jacoby VL. Opportunities for Early Detection of Endometrial Cancer in Women With Postmenopausal Bleeding. JAMA Intern Med. 2018;178(9):1222–3.
4. Updated Guidance Regarding The Role of Transvaginal Ultrasonography in Evaluating the Endometrium of Individuals With Postmenopausal Bleeding. Obstet Gynecol. 2026;148(1):e87–e91.
5. Naftalin J, Nunes N, Hoo W, Arora R, Jurkovic D. Endometrial cancer and ultrasound: why measuring endometrial thickness is sometimes not enough. Ultrasound Obstet Gynecol. 2012;39(1):106–9.
6. Aune D, Navarro Rosenblatt DA, Chan DS, Vingeliene S, Abar L, Vieira AR, et al. Anthropometric factors and endometrial cancer risk: a systematic review and dose-response meta-analysis of prospective studies. Ann Oncol. 2015;26(8):1635–48.
7. Clim IA, Faur IF, Prodan-Barbulescu C, Neamtu AA, Pasca P, Burta C, et al. Fats and Facts: A Meta-Analysis of Lipid Biomarkers in Endometrial Cancer. Life (Basel). 2026;16(2).
8. Kho PF, Amant F, Annibali D, Ashton K, Attia J, Auer PL, et al. Mendelian randomization analyses suggest a role for cholesterol in the development of endometrial cancer. Int J Cancer. 2021;148(2):307–19.
9. Li W, Xu Y, Zeng X, Tan J, Wang Y, Wu H, et al. Etiological relationship between lipid metabolism and endometrial carcinoma. Lipids Health Dis. 2023;22(1):116.
10. Epstein E, Van Holsbeke C, Mascilini F, Måsbäck A, Kannisto P, Ameye L, et al. Gray-scale and color Doppler ultrasound characteristics of endometrial cancer in relation to stage, grade and tumor size. Ultrasound Obstet Gynecol. 2011;38(5):586–93.
11. Chen B, Wang P, He W, Yang P, Kong Z, Wang D, et al. Standardized IETA criteria enhance accuracy of junior and intermediate ultrasound radiologists in diagnosing malignant endometrial and intrauterine lesions. Ultrasound Obstet Gynecol. 2024;64(4):528–37.
12. Hurtado S, Shetty MK. Post-Menopausal Bleeding: Role of Imaging. Semin Ultrasound CT MR. 2023;44(6):519–27.
13. ACOG Committee Opinion No. 734: The Role of Transvaginal Ultrasonography in Evaluating the Endometrium of Women With Postmenopausal Bleeding. Obstet Gynecol. 2018;131(5):e124–e9.
14. Byun D, Hong S, Ryu S, Nam Y, Jang H, Cho Y, et al. Early-life body mass index and risks of breast, endometrial, and ovarian cancers: a dose-response meta-analysis of prospective studies. Br J Cancer. 2022;126(4):664–72.
15. Kundu I, John D, Ansari I, Pavithran K, Idicula-Thomas S. Risk of gynaecologic cancers in women with metabolic syndrome: A systematic review & meta-analysis. Indian J Med Res. 2025;162(2):197–210.
16. Liu F, Cheung ECW, Lao TT. Obesity increases endometrial cancer risk in Chinese women with postmenopausal bleeding. Menopause. 2021;28(10):1093–8.

17. Kang SY, Kim YJ, Kim S, Park HS. Different Patterns of the Relationship Between Gynecological Malignancy and Obesity Index: A Nationwide Retrospective Cohort Study in Korea. J Korean Med Sci. 2025;40(11):e31.
18. Li H, Liao H, Jing B, Wang Y. Effects of coagulation function indicators and tumor markers on diagnosis and clinicopathological characteristics of endometrial cancer. Int J Biol Markers. 2023;38(3-4):214–22.
19. Yu Z, Sun Y, Guo C. Evaluating pretreatment serum CA-125 levels as prognostic biomarkers in endometrial cancer: a comprehensive meta-analysis. Front Oncol. 2024;14:1442814.
20. Peremiquel-Trillas P, Gómez D, Martínez JM, Fernández-González S, Frias-Gomez J, Paytubi S, et al. Cost-effectiveness analysis of molecular testing in minimally invasive samples to detect endometrial cancer in women with postmenopausal bleeding. Br J Cancer. 2023;129(2):325–34.

Supplementary Files

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