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Original Article

Development and validation of a prediction model for lymph node metastasis based on molecular typing in clinically early-stage endometrial carcinoma

Qiuyue Han [1,*] **Quanhong Jiang** [1,*] **Jiaqi Xu** [1,*] **Yuan Zhang** [2,*] **Zhuang Li** [1] **Yong Zhao** [1] **Zhaoyang Zhang** [1,3] **Ziyuan Yang** [4] **Helgi B. Schiöth** [5] **Yawen Zhang** [1] **Lingliya Tang** [1] **Shuaixin Wang** [1] **Beihua Kong** [1,3] **Ruifen Dong** [1]

[1]Department of Obstetrics and Gynecology, Qilu Hospital of Shandong University, Jinan, China
[2]Clinical Epidemiology Unit, Qilu Hospital of Shandong University, Jinan, China
[3]Key Laboratory of Gynecology Oncology, Qilu Hospital of Shandong University, Jinan, China
[4]Advanced Medical Research Institute, Cheeloo College of Medicine, Shandong University, Jinan, China
[5]Department of Surgical Sciences, Functional Pharmacology and Neuroscience, Uppsala University, Uppsala, Sweden

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Correspondence to
Ruifen Dong
Department of Obstetrics and Gynecology, Qilu Hospital of Shandong University, 107 Wenhua Xi Road, Jinan, Shandong Province 250012, China.
Email: ruifen.dong@sdu.edu.cn

*Qiuyue Han, Quanhong Jiang, Jiaqi Xu, and Yuan Zhang contributed equally to this work.

ORCID iDs
Qiuyue Han
https://orcid.org/0000-0002-5419-3966
Quanhong Jiang
https://orcid.org/0000-0002-6261-0408
Jiaqi Xu
https://orcid.org/0009-0008-8532-7358
Yuan Zhang
https://orcid.org/0000-0003-0527-6017

ABSTRACT

Objective: To develop and externally validate a machine learning-based preoperative model integrating molecular typing and clinical features to predict lymph node metastasis (LNM) in patients with early-stage endometrial carcinoma (EC).
Methods: This retrospective study included 465 patients with clinically early-stage EC treated at Qilu Hospital of Shandong University. Tumors were classified into molecular subtypes using The Cancer Genome Atlas-based methods. Least Absolute Shrinkage and Selection Operator regression identified five preoperative predictors: molecular typing (CN-H vs. non–CN-H), histological subtype, depth of myometrial invasion, neutrophil-to-lymphocyte ratio, and CA125 levels. Multiple machine learning algorithms were evaluated, and logistic regression (LR) was selected based on optimal discrimination and clinical applicability. Model performance was assessed using area under the curve (AUC), calibration plots, and decision curve analysis (DCA). A web-based nomogram was developed for clinical use.
Results: The LR model demonstrated excellent discrimination, with AUCs of 0.843 in the training cohort and 0.809 in the testing cohort. The CN-H subtype was significantly associated with increased LNM risk. The model enabled effective risk stratification and calibration curves and DCA confirmed the model's accuracy and clinical utility.
Conclusion: By integrating molecular and preoperative clinical features, this model offers accurate LNM risk stratification for early-stage EC. It supports clinical decision-making and has been implemented as a user-friendly online tool. Further prospective multicenter validation is warranted.

Keywords: Endometrial Carcinoma; Lymph Node Metastasis; Molecular Typing; Machine Learning; Preoperative Period; Nomogram

Zhuang Li
https://orcid.org/0000-0001-6855-2803
Yong Zhao
https://orcid.org/0000-0003-4264-4149
Zhaoyang Zhang
https://orcid.org/0000-0003-1280-0905
Ziyuan Yang
https://orcid.org/0009-0009-2771-4226
Helgi B. Schiöth
https://orcid.org/0000-0001-7112-0921
Yawen Zhang
https://orcid.org/0009-0000-9328-0301
Lingliya Tang
https://orcid.org/0000-0003-1940-9546
Shuaixin Wang
https://orcid.org/0000-0003-1781-3959
Beihua Kong
https://orcid.org/0000-0002-1627-1150
Ruifen Dong
https://orcid.org/0009-0001-5829-8327

Conflict of Interest
No potential conflict of interest relevant to this article was reported.

Data Availability
The data is available from the corresponding author upon reasonable request.

Author Contributions
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Synopsis
This study developed a machine learning model incorporating molecular typing to predict lymph node metastasis in early-stage endometrial cancer. It utilized key clinicopathological and molecular typing predictors, achieving high accuracy in risk stratification to guide surgical decisions.

INTRODUCTION

Endometrial carcinoma (EC) is the second most prevalent gynecologic malignancy globally, with the incidence steadily increasing [1,2]. Lymph node metastasis (LNM) is a critical prognostic indicator, significantly associated with decreased survival. Patients with pelvic or para-aortic LNM face worse outcomes compared to those without nodal involvement [3].

Sentinel lymph node biopsy (SLNB) has emerged as the preferred method of nodal assessment in apparent early-stage EC due to its lower morbidity compared to systemic lymphadenectomy. The National Comprehensive Cancer Network (NCCN) guidelines recommend SLN mapping for patients with low- to intermediate-risk disease and increasingly support its use in selecting high-risk histologic subtypes [4]. However, SLNB has several limitations, including technical difficulty, reliance on institutional expertise, and variable detection rates. Moreover, while SLN ultrastaging improves the detection of micrometastasis and isolated tumor cells, the prognostic significance and optimal management of these findings remain unclear. In high-risk populations, failed mapping or incomplete nodal assessment may lead to understaging and potentially inappropriate adjuvant therapy. Therefore, accurate preoperative identification of patients at high risk of LNM remains a clinical priority to inform therapeutic decision-making.

While most existing predictive models for LNM rely on postoperative factors, such as lymphovascular space invasion (LVSI), parametrial involvement, or final histologic type, which limits their utility in preoperative decision-making. Recent advances in molecular typing, as highlighted in The Cancer Genome Atlas (TCGA) framework and incorporated into international guidelines from International Federation of Gynaecology and Obstetrics (FIGO) (2023) and ESMO, have enhanced risk stratification in EC by correlating molecular subtypes with prognosis and treatment response [5]. Notably, the NCCN guidelines also highlight the prognostic importance of molecular markers, emphasizing their role in tailoring adjuvant therapy [4].

Despite this, few studies have developed preoperative predictive models that integrate molecular typing with other readily accessible clinical factors. Existing efforts are often limited by small sample sizes or narrow variable inclusion [6-8]. Therefore, there is a pressing need to establish a robust, clinically applicable model for preoperative LNM risk stratification.

In this study, we developed and externally validated a novel machine learning (ML)-based predictive model incorporating molecular typing and routinely available clinical parameters. By providing individualized risk estimates before surgery, the model aims to support clinical decision-making, facilitate patient counselling, and inform perioperative planning.

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MATERIALS AND METHODS

1. Study design and patients

This retrospective study included 465 patients with clinically early-stage endometrial cancer (EC), defined as no signs of LNM on preoperative imaging evaluation. All patients were treated at Qilu Hospital of Shandong University and were stratified into two cohorts: a training cohort (n=363; treated between January 2010 and December 2017) and a testing cohort (n=102; treated between January 2018 and February 2025). The inclusion criteria were as follows: 1) histopathological diagnosis of EC; 2) no evidence of LNM on preoperative imaging assessment; 3) underwent SLNB or systematic lymph node dissection (SLND); 4) availability of complete clinicopathological data, including parameters required for molecular typing; 5) concordance between preoperative magnetic resonance imaging (MRI) findings and postoperative pathological results, specifically regarding cervical involvement and depth of myometrial invasion. Exclusion criteria comprised: 1) concurrent ovarian cancer; 2) pathological slides of insufficient quality for molecular typing. The study was approved by the Ethics Committee of Qilu Hospital of Shandong University (KYLL-202209-029). A schematic flowchart of the study design and cohort selection process is provided in **Fig. 1**.

2. Molecular typing and preoperative assessment

Molecular subtyping was conducted retrospectively using TCGA-based classification [9]. Pathological slides were reviewed and categorized into four molecular subtypes: POLE-ultramutated, microsatellite instability-high (MSI-H), copy-number high (CN-H; p53-abnormal), and copy-number low (CN-L; p53-wild type). POLE mutations were identified by polymerase chain reaction. MSI status was determined via immunohistochemical staining for mismatch repair (MMR) proteins (MLH1, MSH2, MSH6, PMS2). p53 status was assessed via immunohistochemistry. Mutant-type p53 was defined as strong, diffuse nuclear staining in ≥80% of tumor cells, aberrant cytoplasmic staining, or complete absence (null pattern), while wild-type p53 was characterized by weak to moderate focal staining in <80% of tumor cells.

Due to the limited number of POLE-ultramutated cases, all non-CN-H subtypes (POLE, MSI-H, CN-L) were grouped together as 'non-CN-H,' in accordance with previous studies demonstrating p53 abnormalities are associated with poorer prognosis and increased LNM risk [10].

The neutrophil-to-lymphocyte ratio (NLR) was dichotomized based on an optimal cutoff of 2.838, determined by maximizing the Youden index from receiver operating characteristic (ROC) analysis within the training cohort.

Additionally, preoperative MRI was used to evaluate the depth of myometrial invasion, cervical stromal involvement, and other tumor characteristics. MRI has demonstrated strong concordance with postoperative pathology and remains a reliable modality for preoperative evaluation in EC [11].

3. Statistical analyses

A total of 13 preoperative variables were selected for analysis: age, menopausal status, CA125 level (≤35 vs. >35 U/mL), NLR (≤2.838 vs. >2.838), tumor grade, histological subtype, myometrial invasion depth (≤50% vs. >50%), cervical involvement, and molecular typing. Descriptive statistics were used to summarize clinicopathological characteristics. The χ^2 test was applied to compare variables between the LNM and non-LNM groups. Least Absolute Shrinkage and Selection Operator (LASSO) regression was used to identify the most

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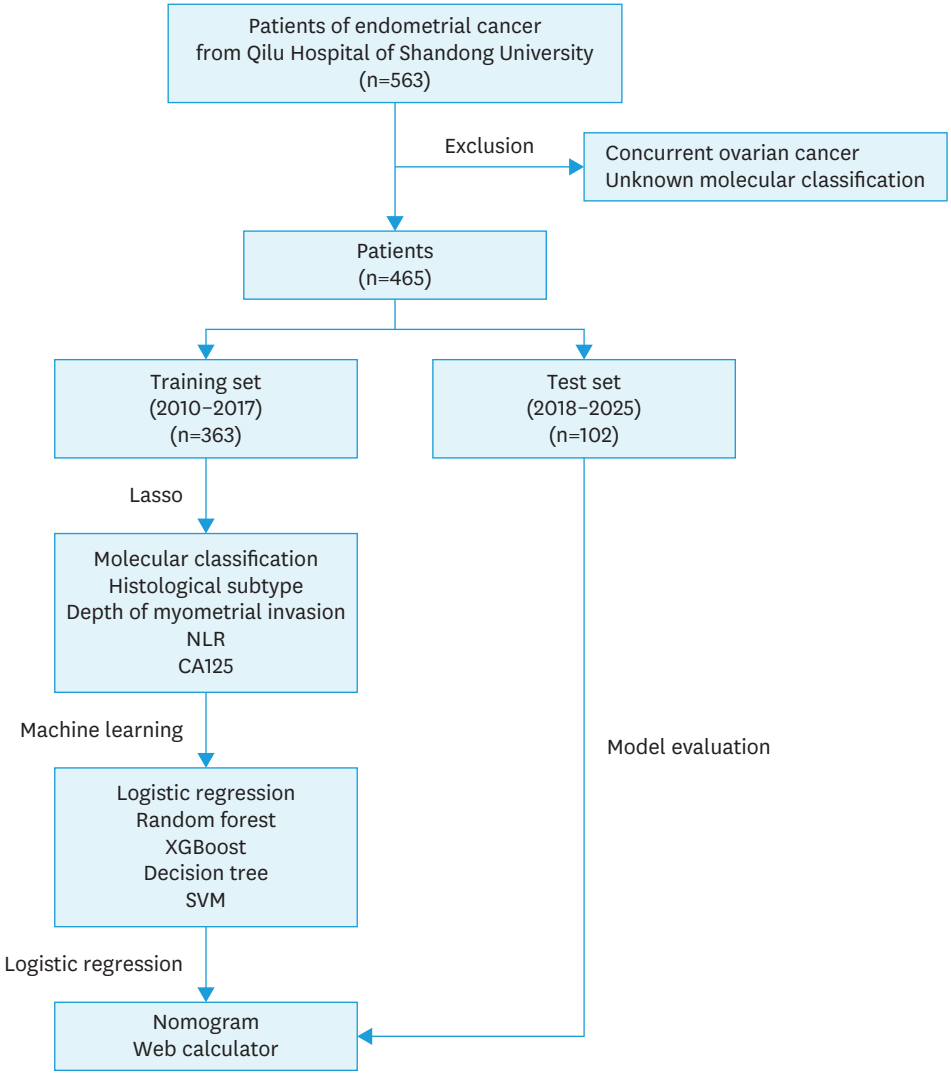

Fig. 1. The flowchart of this study.
NLR, neutrophil-to-lymphocyte ratio; SVM, support vector machine.

predictive features for LNM risk. Notably, LVSI was excluded from the analysis due to its reliance on postoperative pathology.

Various ML algorithms were employed on the training cohort, including logistic regression (LR), random forest (RF), gradient boosting machine (GBM), decision tree, and support vector machine (SVM). Model performance was assessed via ROC analysis, area under the curve (AUC), sensitivity, specificity, and bootstrap validation. The best-performing model was further tested in the testing cohort (n=102). Calibration curves, decision curve analysis (DCA), and clinical impact curves (CICs) were generated to evaluate model calibration and clinical utility.

Statistical analyses were performed using IBM SPSS Statistics (v.29.0; IBM Corp., Armonk, NY, USA) and R software (v.4.2.3; R Foundation for Statistical Computing, Vienna, Austria). A two-sided p-value <0.05 was considered statistically significant.

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RESULTS

1. Clinical characteristics of patients

In this study, we analyzed the clinicopathological characteristics of 465 patients (**Table 1**). Among them, 90.3% had no LNM, while 9.7% exhibited nodal involvement. A total of 1,371 pelvic lymph nodes were resected. Among them, 619 nodes were from the left pelvic region with a metastasis rate of 11.8% (73/619), and 576 nodes were from the right pelvic region with a higher rate of 15.6% (90/576). Additionally, 176 para-aortic lymph nodes were resected, and 45 were positive, resulting in a metastasis rate of 25.6% (**Table S1**). Molecular typing revealed that 50.8% of patients had CN-H tumors, while 49.2% were classified as non–CN-H.

No significant differences were observed in age at diagnosis, cervical involvement, tumor grade or menopausal status between the LNM and no-LNM groups ($p>0.05$). However, serum CA125 levels ($p<0.001$), LVSI ($p<0.001$), NLR ($p=0.035$), molecular typing ($p=0.025$), histological subtype ($p<0.001$), and depth of myometrial invasion ($p<0.001$) were significantly associated with LNM.

Table 1. Patient clinicopathological characteristics

Characteristics	Total	LNM (n=45)	No-LNM (n=420)	p-value
Age at diagnosis (yr)				0.328
≤60	329 (70.8)	29 (64.4)	300 (71.4)	
>60	136 (29.2)	16 (35.6)	120 (28.6)	
Menopausal status				0.062
No	162 (34.8)	10 (22.2)	152 (36.2)	
Yes	303 (65.2)	35 (77.8)	268 (63.8)	
Serum CA125 (U/mL)				<0.001
≤35	283 (73.7)	14 (40.0)	269 (77.1)	
>35	101 (26.3)	21 (60.0)	80 (22.9)	
NA	81			
NLR				0.035
≤2.838	358 (77.0)	29 (57.1)	329 (76.8)	
>2.838	107 (23.0)	16 (42.9)	91 (23.2)	
Grade				0.091
G1-G2	345 (75.7)	28 (65.1)	317 (76.8)	
G3	111 (24.3)	15 (34.9)	96 (23.2)	
Unknown	9			
Histological subtype				<0.001
Endometrioid carcinoma	414 (89.0)	28 (62.2)	386 (91.9)	
Non-endometrioid carcinoma	51 (11.0)	17 (37.8)	34 (8.1)	
Depth of myometrial invasion				<0.001
≤1/2	360 (77.4)	19 (42.2)	341 (81.2)	
>1/2	105 (22.6)	26 (57.8)	79 (18.8)	
Cervical involvement				0.084
Negative	409 (88.0)	36 (80.0)	373 (88.8)	
Positive	56 (12.0)	9 (20.0)	47 (11.2)	
LVSI				<0.001
Negative	407 (89.3)	28 (57.1)	379 (93.1)	
Positive	49 (10.7)	21 (42.9)	28 (6.9)	
Unknown	9			
Molecular typing				0.025
Non-CN-H (MSI, CN-L)	229 (49.2)	15 (33.3)	214 (51.0)	
CN-H	236 (50.8)	30 (66.7)	206 (49.0)	

Values are presented as number (%).
CN-H, copy-number high; CN-L, copy-number low; LNM, lymph node metastasis; LVSI, lymph-vascular space invasion; MSI-H, microsatellite instability-high; NLR, neutrophil to lymphocyte ratio.

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Patients were treated at Qilu Hospital of Shandong University and were categorized into two cohorts: a training cohort (n=363; treated between January 2010 and December 2017) and an testing cohort (n=102; treated between January 2018 and February 2025). The baseline levels of the training cohort and the validation set are comparable (**Table S2**).

2. Identification of key predictive factors

LASSO regression was applied in the training cohort to identify relevant predictors of LNM. The variable selection process is demonstrated in **Fig. S1**. Only preoperative variables were included in the analysis. Significant factors identified included molecular typing, histological subtype, depth of myometrial invasion, CA125, and NLR. Coefficient distribution and parameter plots are shown in **Fig. S2**.

3. Development, validation, and performance of the predictive model

Varied ML models including (LR, RF, GBM, Decision Tree and SVM, were developed using the selected factors (**Fig. S3** and **Table S3**). Both LR and RF achieved the highest AUC (0.804) in the training cohort. Given their comparable performance, LR was chosen for its interpretability, simplicity, and ease of clinical translation. It also enables straightforward visualization and integration into nomograms and web-based tools.

The optimal LR model was established and the final nomogram is presented in **Fig. 2**. This visual representation delineates the relative contribution of multiple variables to LNM occurrence. The training cohort demonstrated robust discriminative capacity with an AUC of 0.843 (**Fig. 3**). Subsequently, external validation was performed utilizing the independent testing cohort. In this testing cohort, the ROC curve exhibited strong predictive performance, yielding an AUC of 0.809 (**Fig. 4**). The calibration curve revealed substantial concordance between predicted probabilities and observed frequencies. DCA and CIC further substantiated the model's clinical utility and favorable decision-making impact. Additionally, we conducted a comprehensive feature importance analysis for the model variables, which quantitatively elucidates the relative contribution of each predictor to the overall model performance (**Fig. 5**).

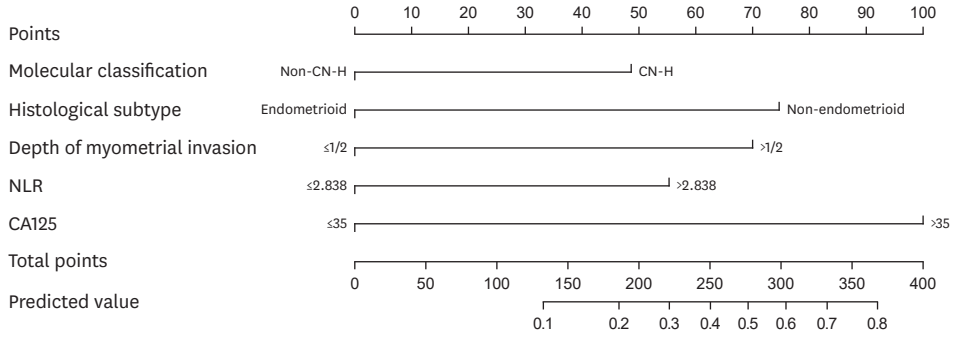

Fig. 2. The nomogram to predict LNM.
CA125, serum carbohydrate antigen 125; LNM, lymph node metastasis; NLR, neutrophil-to-lymphocyte ratio.

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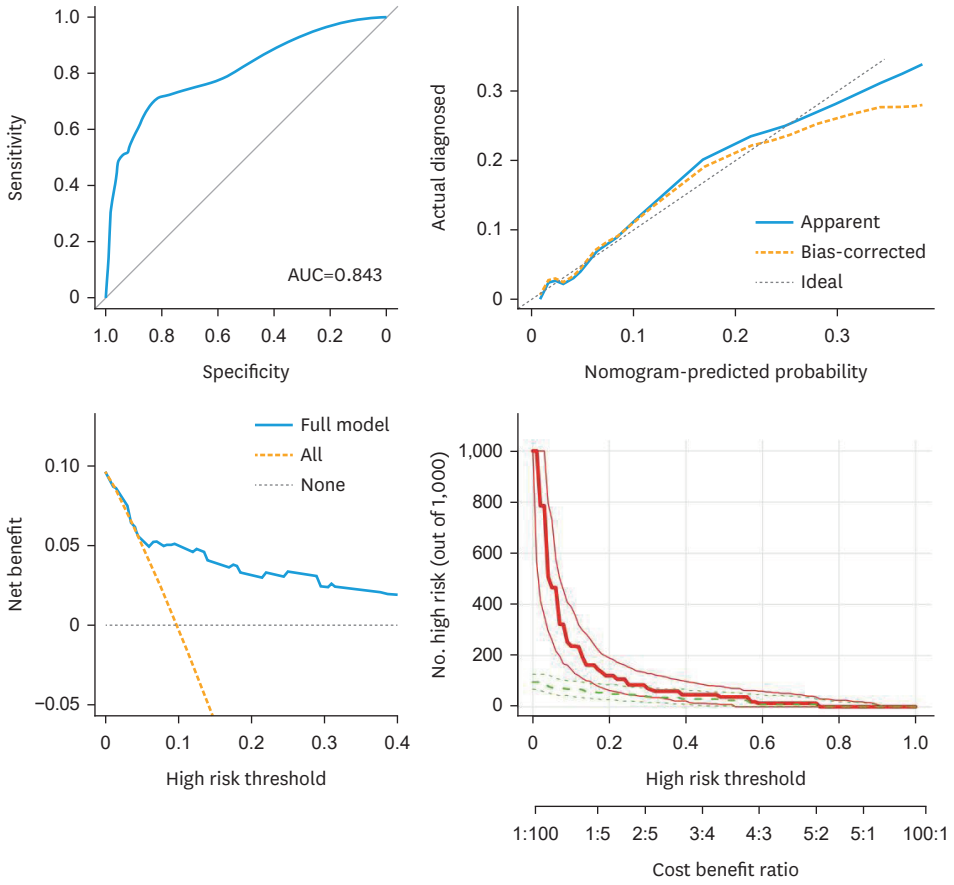

Fig. 3. Evaluation of the prediction model in the training cohort.
ROC curve for evaluating discrimination performance in the training cohort. The calibration curves of model. Clinical validity assessment: DCA curves; clinical validity assessment: CIC curves.
AUC, area under the ROC curve; CIC, clinical impact curve; DCA, decision curve analysis; ROC, receiver operating characteristic.

4. Clinical application and risk stratification

To enhance usability, a web-based nomogram was developed using the Shiny framework: https://fzfxlbjzy.shinyapps.io/JGODynNomapp/. Clinicians can input clinical data to obtain real-time individualized LNM risk estimates, facilitating informed preoperative planning. Nomogram scores were calculated for each patient, and a threshold value of 151.934 was identified through ROC curve analysis (**Fig. S3**). This threshold effectively stratifies patients into high-risk (>151.934) and low-risk (≤151.934) categories for LNM. Statistical analysis confirmed a significant difference between these groups ($p<0.001$), validating the stratification method. For high-risk patients, more comprehensive lymph node evaluation is recommended, including SLND.

5. Model sensitivity analysis

To evaluate the robustness of our predictive model, we conducted a sensitivity analysis by excluding SLNB cases, addressing potential concerns related to the false-negative rate associated with SLNB procedures. When assessing model performance on the subset containing only SLND cases, the discriminative ability yielded an AUC of 0.710, compared to an AUC of 0.809 in the full testing cohort. The difference was not statistically significant ($p=0.502$), demonstrating the model's stability across varying lymph node assessment methodologies.

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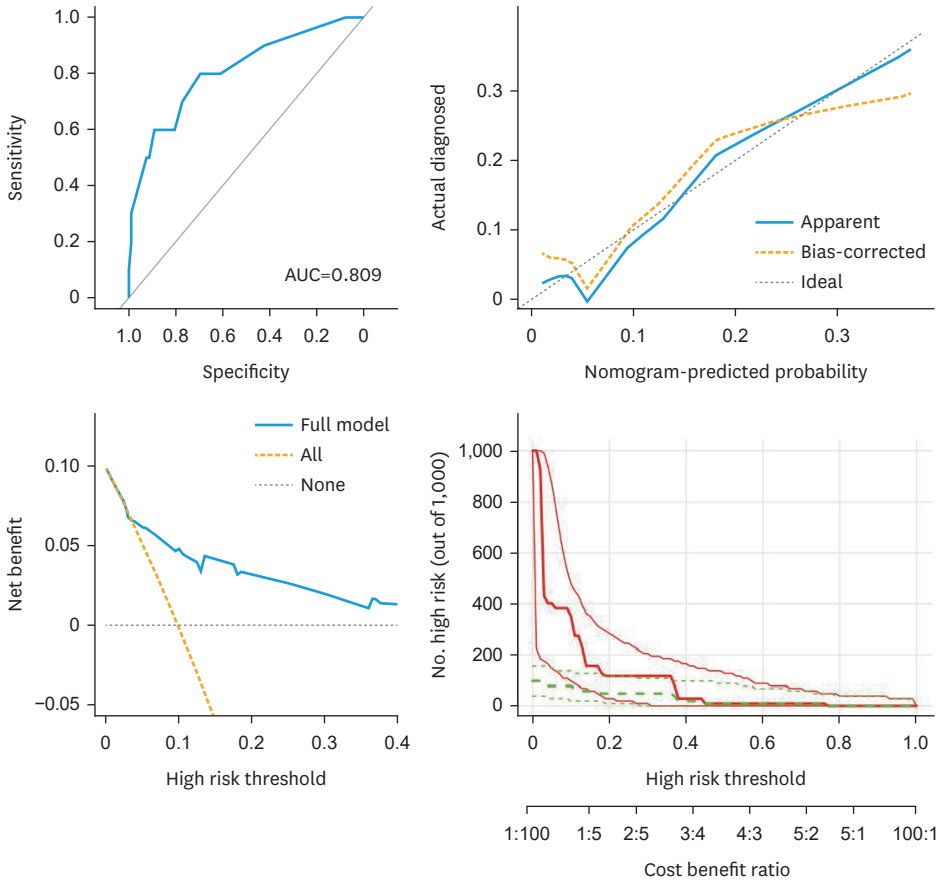

Fig. 4. Evaluation of the prediction model in the testing cohort.
ROC curve for evaluating discrimination performance in the testing cohort. The calibration curves of model. Clinical validity assessment: DCA curves; clinical validity assessment: CIC curves.
AUC, area under the ROC curve; CIC, clinical impact curve; DCA, decision curve analysis; ROC, receiver operating characteristic.

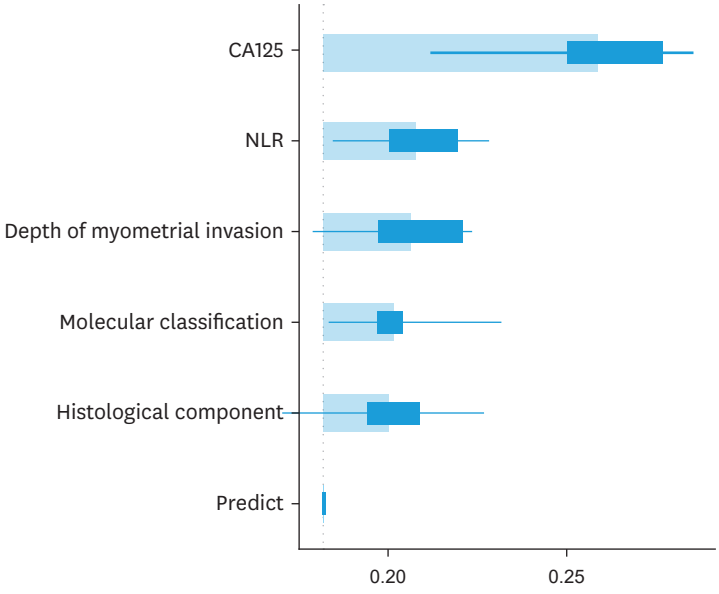

Fig. 5. Feature importance analysis of predictive variables in the lymph node metastasis model.
CA125, serum carbohydrate antigen 125; NLR, neutrophil-to-lymphocyte ratio.

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DISCUSSION

This study proposes and validates a novel preoperative prediction model for LNM in early-stage EC, integrating clinical and molecular features. Five independent preoperative predictors were identified: molecular typing, histological subtype, depth of myometrial invasion, NLR, and CA125. Among the ML algorithms evaluated, LR demonstrated the most favorable balance of performance and clinical utility, achieving an AUC of 0.809 in the testing cohort.

A key innovation of this study lies in the integration of molecular typing into a preoperative prediction model for LNM. While p53 abnormalities and other molecular subtypes have been associated with LNM in previous studies [7,8,12], their clinical application has largely been limited to postoperative settings. Our findings reinforce the feasibility and value of preoperative molecular stratification. According to NCCN guidelines, molecular testing—including MMR IHC and MSI analysis—can be performed on biopsy or D&C specimens, supporting real-world implementation [4,5].

Additionally, this study reinforces the prognostic significance of CA125 as a routinely available biomarker in EC. Its correlation with LNM, as shown here and in prior literature [13,14], supports its role in predictive modeling. Histological subtype also emerged as relevant predictors, with non-endometrioid subtypes tumors showing elevated LNM risk, consistent with recent FIGO classification updates. Notably, our study is one of the first studies to explore the predictive value of preoperative NLR for LNM for early-stage EC. While elevated NLR has previously been associated with worse outcomes in recurrent or advanced EC, particularly in immunotherapy settings [15], our findings suggest its broader utility as a preoperative inflammatory marker. Tumor-associated inflammation and immune suppression may underlie this association warranting further mechanistic exploration [16].

Based on these predictors, we developed a clinically applicable nomogram and an accessible web-based calculator to facilitate real-time risk assessment. By enabling personalized LNM risk stratification preoperatively, this tool supports evidence-based surgical planning, informed patient counselling, and optimized perioperative management, serving as a valuable adjunct to established clinical decision-making frameworks. The proposed nomogram and web-based calculator provide effective risk discrimination, guiding individualized management strategies that align with current best practices. For high-risk patients, comprehensive lymph node evaluation including systematic lymphadenectomy is recommended, while for low-risk patients, more conservative approaches may be appropriate, thereby potentially reducing morbidity from unnecessary interventions while enhancing overall clinical decision-making efficiency.

Despite these strengths, our study has several limitaions. First, although the inclusion of an testing cohort enhances the model's generalizability, all data were derived from a single institution, potentially limiting applicability in other clinical settings. Second, although binary molecular typing was effective, the small number of POLE-mutated cases limits conclusions about rare subtypes. Third, while MRI was used for standardized preoperative evaluation, imaging-based evaluation can vary in accuracy depending on technique and interpretation, which may influence model inputs. Additionally, molecular typing was performed retrospectively on available archival specimens, and variations in specimen quality or testing protocols may have influenced subtype classification. These potential biases

should be considered when interpreting the molecular distribution and its association with LNM in this study.

Future research should focus on multi-center validation of this model in diverse populations and prospective cohorts. Further refinement of molecular integration, such as inclusion of additional genomic alterations or immunologic markers may enhance predictive power.

In this study, we developed and externally validated a novel ML-based model to predict LNM in early-stage EC. By integrating molecular typing with key preoperative clinical parameters, the model demonstrated high predictive accuracy and clinical utility.

The resulting nomogram and user-friendly web-based calculator enable individualized, real-time risk assessment prior to surgery, supporting informed clinical decision-making and perioperative planning. This model offers a practical approach to improving preoperative risk stratification in EC and represents a step forward toward more personalized, data-driven patient management.

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SUPPLEMENTARY MATERIALS

Table S1
Lymph nodes metastasis statistics

Table S2
Baseline level comparison of training cohort and validation set

Table S3
Machine learning model training and evaluation index

Fig. S1
The coefficient distribution diagram and parameter diagram of LASSO.

Fig. S2
ROC curves for machine learning model.

Fig. S3
ROC curve analysis for determining the optimal cut-off value for lymph node metastasis risk stratification.

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