Article

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Association Between Neutrophil-to-Lymphocyte Ratio and Lymphovascular Space Invasion in Endometrial Cancer: A Single-Center Cross-Sectional Study With Survival Analysis

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Background: Endometrial cancer (EC) is a common gynecologic malignancy marked by ever-increasing incidence. This study primarily aimed to evaluate the association between preoperative neutrophil-to-lymphocyte ratio (NLR) and lymphovascular space invasion (LVSI), with the secondary objective to assess NLR's prognostic significance for recurrence-free survival (RFS) in endometrial cancer.

Methods: This single-center retrospective cohort study included 335 endometrial cancer patients at Dongyang Hospital of Wenzhou Medical University from January 2012 to January 2024. Two distinct analyses were conducted: (1) Cross-sectional association analysis using ordinal logistic regression and restricted cubic spline (RCS) modeling to evaluate the relationship between preoperative NLR and LVSI status; (2) Survival analysis using Cox proportional hazards (CPH) modeling to assess NLR's prognostic significance for RFS and examine prognostic interactions between NLR and LVSI status.

Results: Multivariate logistic regression revealed no significant association between NLR and LVSI after comprehensive adjustment (odds ratio [OR] = 1.09, 95% confidence interval [CI]: 0.77–1.53, $p = 0.636$). Though exploratory spline modeling showed stable risk estimates across the NLR spectrum, formal statistical testing revealed no significant non-linear relationship ($p = 0.465$). Survival analysis showed borderline significance in patients with superficial invasion (hazard ratio [HR] = 1.56, 95% CI: 1.00–2.43) for RFS, with consistent prognostic patterns across clinicopathological subgroups (all interaction $p > 0.05$).

Conclusion: This study revealed no significant association with NLR and LVSI, indicating no independent predictive value of NLR in LVSI risk stratification. However, patients with superficial myometrial invasion may present borderline risk for recurrence LVSI. Multi-center validation studies are needed to verify the prognostic value of NLR for LVSI risk stratification in clinical settings.

Keywords: endometrial cancer; neutrophil-to-lymphocyte ratio; lymphovascular space invasion; multi-layered statistical analysis; prognostic prediction

Introduction

Endometrial cancer (EC) is one of the most common gynecological malignancies worldwide, with its incidence continuing to rise globally. According to the latest cancer statistics, an estimated 67,880 new cases of EC had been projected to occur in the United States in 2024, representing 7% of all new female cancer cases [1]. On the other side of the world, China is also facing a substantial share of the EC burden, with approximately 77,700 new EC cases reported in 2022 among the total 4,824,700 new cancer cases nationwide [2]. This rising incidence, coupled with the heterogeneous nature of EC and its variable clinical outcomes, underscores the urgent need for improved prognostic tools and treatment stratification methods to optimize patient management and therapeutic decision-making.

Lymphovascular space invasion (LVSI) has emerged as one of the most significant independent prognostic factors in EC, fundamentally influencing both survival outcomes and therapeutic strategies [3]. Accumulating clinical evidence has consistently demonstrated that LVSI-positive patients exhibit significantly elevated rates of lymph node metastasis, increased risks of local and distant recurrence, and reduced overall survival (OS) compared to their LVSI-negative counterparts [4–6]. The clinical significance of LVSI is further underscored by its incorporation into major international guidelines, including the National Comprehensive Cancer Network (NCCN) Clinical Practice Guidelines in Oncology: Uterine Neoplasms, Version 1.2023 and the European Society of Gynaecological Oncology (ESGO)/European Society for Radiotherapy and Oncology (ESTRO)/European Society of Pathology (ESP) Guidelines

for the management of patients with endometrial carcinoma. These guidelines consistently identify LVSI as a critical risk factor and recommend adjuvant therapy for LVSI-positive early-stage EC patients [7,8]. However, LVSI status is only available postoperatively through histopathological examination, limiting its utility for preoperative risk assessment and treatment planning.

The neutrophil-to-lymphocyte ratio (NLR), a readily accessible inflammatory biomarker derived from routine complete blood count, has gained considerable attention as a promising prognostic indicator across various malignancies, including gynecologic cancers [9]. The biological rationale for NLR's prognostic value lies in its ability to reflect the balance between systemic inflammation (neutrophils) and immune response (lymphocytes), both of which play crucial roles in cancer progression and metastasis. Specifically, recent meta-analyses regarding EC have demonstrated that elevated preoperative NLR is significantly correlated with poor OS (hazard ratio [HR] = 2.51, 95% confidence interval [CI]: 1.70–3.71) and progression-free survival (PFS) (HR = 1.71, 95% CI: 1.04–2.81) [10]. Furthermore, emerging evidence suggests that combining NLR with other inflammatory markers may enhance its prognostic accuracy, potentially offering a more comprehensive assessment of patient outcomes [11].

Despite the growing body of evidence supporting NLR's prognostic value, several critical knowledge gaps remain, limiting its clinical application. First, the predictive utility of preoperative NLR specifically for estimating LVSI status, a pathological parameter critical for informing treatment decisions, has not been thoroughly investigated in the context of EC. Second, it remains unknown as to whether population-specific validation as genetic polymorphisms, environmental factors, and lifestyle variations that may influence inflammatory responses could affect the resulting NLR values among different ethnic populations. Third, most existing studies have been conducted in Western populations, with limited data from Asian cohorts, particularly Chinese patients. Therefore, this single-center retrospective study aims to address these knowledge gaps by systematically evaluating the predictive accuracy of preoperative NLR for LVSI in Chinese patients with EC. Through comprehensive multivariate analysis while controlling for established clinicopathological confounding factors, we seek to determine whether NLR can serve as a reliable, cost-effective preoperative biomarker for predicting LVSI, with the potential to enhance treatment stratification and inform clinical decision-making in this patient population. As a secondary objective, we evaluated NLR's prognostic significance for recurrence-free survival (RFS) to assess whether this inflammatory biomarker provides independent prognostic value beyond established clinicopathological factors, thereby offering comprehensive biomarker assessment for both preoperative risk stratification and postoperative prognosis prediction.

Methods

Study Population and Design

We conducted a single-center retrospective cohort study at the Gynecologic Oncology Department of Dongyang Hospital of Wenzhou Medical University from January 2012 to January 2024. Data were collected using a medical record mining software platform (Le9 Healthcare Technology, Shanghai, China) designed specifically for clinical research applications, with built-in data validation algorithms to ensure extraction accuracy and completeness. Sample size calculation was initially performed using PASS software (version 15.0, NCSS Statistical Software, Kaysville, UT, USA) ($\alpha = 0.05$, $\beta = 0.20$, effect size = 0.3) based on literature-reported LVSI prevalence of approximately 15% [12,13], with the analysis output indicating a minimum requirement of 298 cases. However, we acknowledge that additional survival analyses and subgroup investigations were not included in the initial study power calculations.

Of 349 patients initially screened from institutional databases, we included adults aged 18 years or older who underwent comprehensive surgical staging, which included systematic lymphadenectomy. All cases underwent pathological confirmation according to the World Health Organization (WHO) Classification of Tumours of Female Reproductive Organs, 5th Edition (2020) for endometrial carcinoma diagnosis and grading, with disease staging based on the International Federation of Gynecology and Obstetrics (FIGO) 2009 classification system. We excluded patients with concurrent malignancies ($n = 4$), incomplete clinical data ($n = 6$), conditions affecting inflammatory markers ($n = 2$), or inadequate follow-up ($n = 2$). While these exclusions enhance analytical validity, they may also introduce selection bias by narrowing a study population to individuals with potentially homogeneous inflammatory profiles, thereby limiting the generalizability of our NLR-related findings to broader populations. The final analysis included 335 patients who completed standard follow-up through regular clinic visits or telephone interviews. The study flowchart is presented in Fig. 1.

Study Endpoints

The primary endpoint was the association between preoperative NLR and LVSI status in EC patients. Secondary endpoints included: (1) Evaluation of NLR's prognostic significance for RFS, defined as the time from surgery to first documented disease recurrence or death from any cause; (2) Assessment of NLR's performance across different clinicopathological subgroups. RFS analysis was included to evaluate whether NLR provides independent prognostic information beyond established pathological features, given that inflammatory biomarkers may influence both local invasion patterns and systemic disease progression.

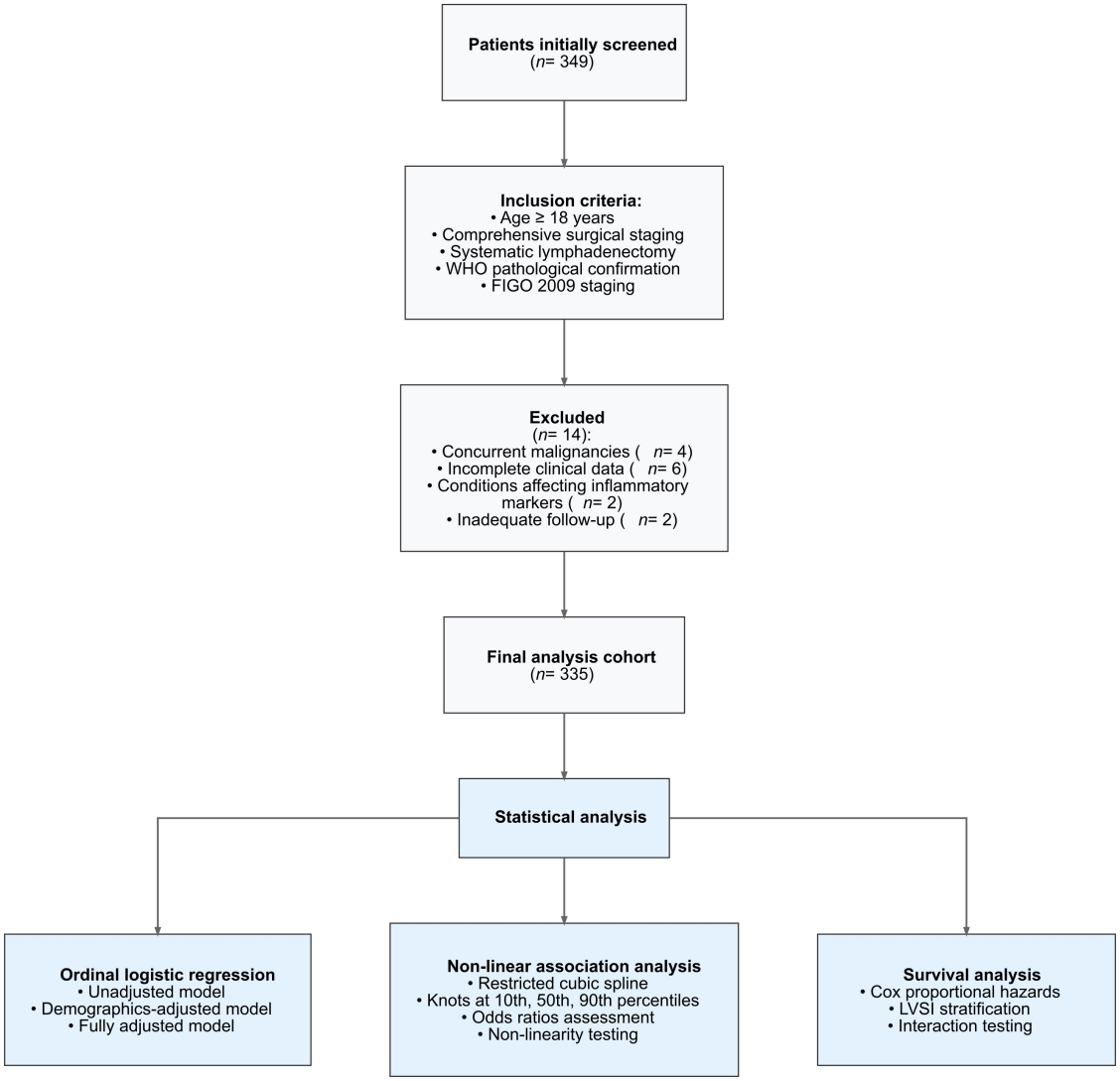

Fig. 1. Flow diagram of patient selection and statistical analysis strategy. Of the 349 patients from the initial screening, 335 were included in the final analysis after applying inclusion criteria and exclusion criteria. The inclusion criteria encompassed age ≥18 years, comprehensive surgical staging, systematic lymphadenectomy, WHO pathological confirmation, and FIGO 2009 staging. Fourteen patients were excluded due to concurrent malignancies (n = 4), incomplete clinical data (n = 6), conditions affecting inflammatory markers (n = 2), and inadequate follow-up (n = 2). The statistical analysis strategy comprised three main components: Ordinal logistic regression analysis (including unadjusted, demographics-adjusted, and fully adjusted models), non-linear association analysis (utilizing restricted cubic spline with knots at 10th, 50th, and 90th percentiles for odds ratios assessment), and survival analysis (incorporating Cox proportional hazards models, LVSI stratification, and interaction testing). Abbreviations: FIGO, International Federation of Gynecology and Obstetrics; LVSI, lymphovascular space invasion; WHO, World Health Organization.

Study Variables and Measurements

Key Variables

We assessed preoperative NLR as the primary exposure variable, derived from complete blood count analysis (Sysmex XN-3000 analyzer, Sysmex Corporation, Kobe, Japan). Blood samples were collected within 24 hours of admission and processed within 2 hours of collection, following standardized laboratory quality control procedures.

LVSI status served as the primary outcome, evaluated according to the WHO Classification of Female Genital Tu-

mours (2020, 5th Edition) [14]. In pathological assessment, 4-µm-thick, hematoxylin-eosin (H&E)-stained sections derived from surgical specimens were used. Degree of LVSI in the patients was classified using a pre-specified three-tier ordinal system to preserve biological gradient and facilitate dose-response analysis: Absent (no involved vessels), focal (1–4 foci of invasion), or extensive (≥5 foci of invasion) [15]. These assessments were conducted independently in a blinded manner by two senior pathologists, with an inter-observer agreement of Cohen's $\kappa = 0.85$. Any discrepancies were resolved through a third-party expert consultation.

Potential confounding variables included demographic factors (age, body mass index [BMI]), clinical indices (age-adjusted Charlson Comorbidity Index [aCCI]), laboratory parameters (platelet-to-lymphocyte ratio [PLR], fibrinogen, albumin), and pathological characteristics (histological type/grade, myometrial invasion depth, FIGO stage). The aCCI was calculated by assigning age-related points based on established discrete age categories: ≤40 years (0 points), 41–50 years (1 point), 51–60 years (2 points), 61–70 years (3 points), and >70 years (4 points); Those were then added to the standard Charlson comorbidity scores, which were assigned according to validated criteria [16,17]. Individual patient aCCI scores were calculated as integers, and relevant results from group comparisons are reported as median and interquartile range (IQR). Data collection followed standardized institutional protocols with predefined requirements: Complete pathological documentation including systematic LVSI assessment, pre-operative inflammatory marker measurements within 72 hours of surgery, and minimum 6-month follow-up data. Patients with incomplete primary variable data (LVSI status, inflammatory markers, or survival endpoints) were excluded from analysis.

Statistical Analysis

All analyses were performed using R software (version 4.4.1, R Foundation for Statistical Computing, Vienna, Austria). Data distribution characteristics were examined, with continuous variables presented as median (interquartile range) and categorical variables as frequencies and percentages.

The Shapiro–Wilk test was employed to assess normality of data distribution. All continuous variables showed significant departure from normality (all $p < 0.05$); Therefore, continuous variables were compared using the Kruskal–Wallis test (H statistic).

Primary Analysis (NLR-LVSI Association)

The relationship between NLR and LVSI was investigated using ordinal logistic regression models, with degree of LVSI analyzed as an ordered categorical variable (negative < focal < extensive). This pre-specified three-tier original approach was consistently utilized in all analyses to preserve the biological gradient. This regression models used include unadjusted, demographic-adjusted (age, BMI), and fully adjusted (including inflammatory indices and clinicopathological factors). The proportional odds assumption was verified using the Brant test.

Secondary Analysis (NLR-RFS Prognostic Assessment)

(1) Non-linear association analysis: Restricted cubic spline (RCS) analysis was performed to examine potential non-linear associations between NLR and LVSI status, with knots placed at 10th, 50th, and 90th percentiles. Formal statistical testing for non-linearity was conducted using likelihood ratio tests comparing cubic spline models to linear models [18]. Linearity was assessed using likelihood ratio tests comparing spline models to linear models. Hazard ratios were calculated for survival outcomes with corresponding 95% confidence intervals. The model was optimized in accordance with the Akaike Information Criterion (AIC) and the Bayesian Information Criterion (BIC) [19].

(2) Prognostic interaction analysis: Cox proportional hazards models examined how NLR effects on RFS vary across LVSI categories (negative, focal, extensive). Variables with $p < 0.10$ in univariate analysis were included in multivariate models. Interaction effects were evaluated through likelihood ratio tests. Results were visualized using forest plots for subgroup-specific HRs. The proportional hazards assumption was assessed using Schoenfeld residuals analysis.

All models were adjusted for potential confounders including clinical, demographic, and pathological factors. Statistical significance was set at $p < 0.05$ (two-sided).

Results

Baseline Characteristics and LVSI Prevalence

Among the 335 patients analyzed, LVSI was identified in 41 patients (12.2%), with 35 (10.4%) showing focal LVSI (1–4 foci of invasion) and 6 (1.8%) showing extensive LVSI (≥5 foci of invasion). Table 1 presents the baseline characteristics stratified by LVSI status.

Patients with more severe LVSI were older [55.0 (51.0, 60.0), 59.0 (55.0, 61.5), 59.5 (54.2, 66.2) years; H = 9.28, $p = 0.010$] and had higher aCCI scores [1.0 (1.0, 3.0), 3.0 (1.0, 5.5), 7.0 (4.0, 8.5); H = 17.19, $p < 0.001$]. Preoperative NLR varied modestly across LVSI categories [2.2 (1.6, 2.8), 2.2 (1.7, 3.3), 2.5 (2.1, 3.8); H = 2.21, $p = 0.332$], while fibrinogen showed an upward trend [3.2 (2.9, 3.7), 3.4 (3.2, 4.1), 3.9 (3.2, 4.5) g/L; H = 5.11, $p = 0.078$] (Table 1).

LVSI severity strongly correlated with aggressive tumor features: Non-endometrioid histology (6.8%, 22.9%, 66.7%; $\chi^2 = 32.4$, $p < 0.001$), lymph node metastasis (2.0%, 28.6%, 50.0%; $\chi^2 = 63.59$, $p < 0.001$), and deep myometrial invasion (14.3%, 42.9%, 83.3%; $\chi^2 = 33.95$, $p < 0.001$). Advanced-stage disease (FIGO III–IV) occurred

Table 1. Baseline characteristics of 335 endometrial cancer patients according to LVSI status.

Characteristics	Negative LVSI (n = 294)	Focal LVSI (n = 35)	Extensive LVSI (n = 6)	Test statistic	p-value
Patient characteristics					
Age (years)	55.0 (51.0, 60.0)	59.0 (55.0, 61.5)	59.5 (54.2, 66.2)	H = 9.28**	0.010
BMI (kg/m^2)	24.6 (21.8, 27.1)	23.3 (21.6, 26.6)	24.2 (22.4, 26.1)	H = 0.76	0.684
aCCI	1.0 (1.0, 3.0)	3.0 (1.0, 5.5)	7.0 (4.0, 8.5)	H = 17.19***	<0.001
Clinical parameters					
Menopausal status				$\chi^2 = 1.0^{\ddagger}$	0.653
Premenopausal	117 (39.8)	11 (31.4)	2 (33.3)		
Postmenopausal	177 (60.2)	24 (68.6)	4 (66.7)		
Comorbidities					
Hypertension	111 (37.8)	16 (45.7)	3 (50.0)	$\chi^2 = 1.16^{\ddagger}$	0.514
Diabetes mellitus	49 (16.7)	7 (20.0)	3 (50.0)	$\chi^2 = 4.66^{\ddagger}$	0.100
Laboratory parameters					
Preoperative NLR	2.2 (1.6, 2.8)	2.2 (1.7, 3.3)	2.5 (2.1, 3.8)	H = 2.21	0.332
Preoperative PLR	141.5 (115.1, 185.2)	141.8 (120.1, 192.7)	146.1 (113.2, 224.1)	H = 0.22	0.895
Fibrinogen (g/L)	3.2 (2.9, 3.7)	3.4 (3.2, 4.1)	3.9 (3.2, 4.5)	H = 5.11	0.078
Albumin (g/L)	43.2 (40.9, 45.6)	42.8 (39.7, 45.3)	43.8 (37.9, 45.3)	H = 1.00	0.605
Pathological features					
Histological type				$\chi^2 = 32.4^{\ddagger}$***	<0.001
Endometrioid	274 (93.2)	27 (77.1)	2 (33.3)		
Non-endometrioid	20 (6.8)	8 (22.9)	4 (66.7)		
Lymph node metastasis	6 (2.0)	10 (28.6)	3 (50.0)	$\chi^2 = 63.59^{\ddagger}$***	<0.001
Deep myometrial invasion	42 (14.3)	15 (42.9)	5 (83.3)	$\chi^2 = 33.95^{\ddagger}$***	<0.001
High-grade histology	37 (12.6)	18 (51.4)	4 (66.7)	$\chi^2 = 42.65^{\ddagger}$***	<0.001
FIGO stage				$\chi^2 = 57.59^{\ddagger}$***	<0.001
I	256 (87.1)	21 (60.0)	1 (16.7)		
II	19 (6.5)	2 (5.7)	1 (16.7)		
III	18 (6.1)	11 (31.4)	3 (50.0)		
IV	1 (0.3)	1 (2.9)	1 (16.7)		
Treatment and outcomes					
Adjuvant therapy				$\chi^2 = 61.12^{\ddagger}$***	<0.001
None	225 (76.5)	8 (22.9)	1 (16.7)		
Chemotherapy	15 (5.1)	4 (11.4)	2 (33.3)		
Radiotherapy	22 (7.5)	5 (14.3)	1 (16.7)		
Chemoradiotherapy	32 (10.9)	18 (51.4)	2 (33.3)		
RFS (months)	59.2 (34.6, 88.5)	31.9 (16.2, 66.9)	19.4 (16.4, 22.6)	H = 21.93***	<0.001
Recurrence/death	27 (9.2)	13 (37.1)	3 (50.0)	$\chi^2 = 29.39^{\ddagger}$***	<0.001

Notes: Data are presented as median (interquartile range) for continuous variables and n (%) for categorical variables. Normality was assessed using the Shapiro–Wilk test. All continuous variables showed significant departure from normality (all $p < 0.05$); therefore, continuous variables were compared using Kruskal–Wallis test (H statistic). Categorical variables were compared using chi-square test. ‡ Chi-square statistic shown but p-values calculated using Fisher's exact test due to expected frequencies <5. **$p < 0.01$, ***$p < 0.001$. Abbreviations: aCCI, age-adjusted Charlson Comorbidity Index; BMI, body mass index; FIGO, International Federation of Gynecology and Obstetrics; LVSI, lymphovascular space invasion; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; RFS, recurrence-free survival.

more frequently with extensive LVSI (66.7%) versus focal (34.3%) or negative cases (6.5%; $\chi^2 = 57.59$, $p < 0.001$) (Table 1).

Treatment patterns and outcomes differed significantly by LVSI status. Chemoradiotherapy was administered more often in LVSI-positive cases (51.4% focal, 33.3% extensive vs 10.9% negative; $\chi^2 = 61.12$, $p < 0.001$). Median RFS decreased with LVSI severity [59.2 (34.6, 88.5), 31.9 (16.2, 66.9), 19.4 (16.4, 22.6) months; H = 21.93, $p < 0.001$], with higher recurrence/death rates in LVSI-positive patients (37.1% focal, 50.0% extensive vs 9.2% negative; $\chi^2 = 29.39$, $p < 0.001$) (Table 1).

NLR-LVSI Association Analysis

Table 2 shows the ordinal logistic regression analysis examining the NLR-LVSI relationship using continuous

and categorical approaches. In continuous analysis, each unit increase in NLR was associated with 31% higher odds of severe LVSI in unadjusted models (OR = 1.31, 95% CI: 1.08–1.60, p = 0.007). This association weakened after adjustment for demographic factors (OR = 1.26, 95% CI: 1.03–1.55, p = 0.023) and became non-significant following comprehensive adjustment (OR = 1.09, 95% CI: 0.77–1.53, p = 0.636) (Table 2).

Quartile-based analysis (Q1: <1.60, Q2: 1.60–2.19, Q3: 2.19–2.86, Q4: ≥2.86) suggested a dose-response pattern in unadjusted analysis, with risk increasing from Q2 (OR = 1.26, 95% CI: 0.47–3.37) to Q4 (OR = 1.74, 95% CI: 0.68–4.45). However, this pattern reversed in fully adjusted models, with Q4 showing an inverse association (OR = 0.62, 95% CI: 0.18–2.21) (Table 2).

Restricted cubic spline analysis with 3 degrees of freedom revealed no significant non-linear relationships across all adjustment levels (p for non-linearity = 0.719, 0.887, and 0.465) (Table 2, Fig. 2). Linear trend analyses confirmed no significant associations (p_{trend} = 0.248, 0.324, and 0.528) (Table 2). These findings indicate that apparent NLR-LVSI associations in unadjusted analyses likely reflect confounding by established clinicopathological factors rather than independent biological relationships.

These findings demonstrate that while NLR showed promise in unadjusted analyses, the loss of significance after comprehensive adjustment (OR = 1.09, 95% CI: 0.77–1.53, p = 0.636) indicates that any apparent NLR-LVSI association is likely confounded by established clinicopathological factors. Therefore, NLR cannot currently be recommended as an independent predictor of LVSI status.

Prognostic Interaction Between NLR and LVSI

As a secondary objective, we evaluated NLR's prognostic significance for RFS to assess its independent prognostic value beyond the established NLR-LVSI association analysis. Fig. 3 illustrates the prognostic interaction between NLR and LVSI status for recurrence-free survival ($p_{interaction}$ < 0.001). NLR's prognostic significance varied markedly across LVSI categories. Negative LVSI cases showed minimal risk variation (HRs: 0.77–1.53). In focal LVSI cases, elevated NLR (>6.0) corresponded to substantially increased recurrence risk (HR reaching 10.0). Extensive LVSI cases exhibited complex patterns with heightened risk at both low (<3.0) and high (>6.0) NLR values (Fig. 3). These relationships persisted after comprehensive adjustment, suggesting potential clinical utility in combining NLR and LVSI for prognostic assessment.

Non-Linear Relationship Assessment

Restricted cubic spline regression analysis (Fig. 2) was performed to comprehensively evaluate the relationship between NLR and LVSI, with knots positioned at the 10th, 50th, and 90th percentiles of the NLR distribution (1.05, 2.19, and 4.60, respectively). After adjustment for all significant covariates including age, BMI, aCCI score, PLR, fibrinogen, albumin, histological type, depth of myometrial invasion, tumor grade, and FIGO stage, no statistically significant association was observed between NLR and LVSI (OR = 1.09, 95% CI: 0.77–1.53, p = 0.636).

Formal testing for non-linearity using the likelihood ratio test revealed no evidence of a non-linear relationship (p for non-linearity = 0.465), indicating that any potential association, if present, would be predominantly linear. The RCS curve demonstrated stable risk estimates across the entire NLR spectrum, with ORs consistently hovering around the null value of 1.0. The 95% CIs remained relatively narrow in the central range of NLR values but appropriately

Table 2. Association of NLR with LVSI status: Ordinal logistic regression analysis.

Characteristics	OR (95% CI)	p-value	p_{trend}
Continuous Analysis			
Per unit increase	1.31 (1.08–1.60)	0.007†	-
Per unit increase[1]	1.26 (1.03–1.55)	0.023*	-
Per unit increase[2]	1.09 (0.77–1.53)	0.636	-
Categorical Analysis			
Unadjusted			0.248
Q1 (<1.60)	1.00 (Reference)	-	
Q2 (1.60–2.19)	1.26 (0.47–3.37)	0.643	
Q3 (2.19–2.86)	1.33 (0.50–3.56)	0.568	
Q4 (≥2.86)	1.74 (0.68–4.45)	0.245	
Model 1[1]			0.324
Q1 (<1.60)	1.00 (Reference)	-	
Q2 (1.60–2.19)	1.21 (0.45–3.26)	0.705	
Q3 (2.19–2.86)	1.40 (0.52–3.78)	0.505	
Q4 (≥2.86)	1.58 (0.61–4.10)	0.349	
Model 2[2]			0.528
Q1 (<1.60)	1.00 (Reference)	-	
Q2 (1.60–2.19)	1.14 (0.35–3.64)	0.831	
Q3 (2.19–2.86)	1.25 (0.40–3.88)	0.704	
Q4 (≥2.86)	0.62 (0.18–2.21)	0.464	

Notes: p for non-linearity assessed using likelihood ratio test comparing RCS (3 df) vs linear models. p for non-linearity (RCS): Unadjusted, 0.719; Model 1, 0.887; Model 2, 0.465. [1]Adjusted for age and BMI; [2]Adjusted for age, BMI, aCCI, PLR, fibrinogen, albumin, histological type, deep myometrial invasion, histological grade, and FIGO stage. Formal testing using restricted cubic spline regression revealed no statistically significant non-linear association between NLR and LVSI across all models (p for non-linearity > 0.05), justifying the use of linear modeling. The loss of significance in the fully adjusted model (Model 2) indicates that any apparent NLR-LVSI association observed in unadjusted analyses is likely mediated through established clinicopathological factors rather than representing an independent relationship. *p < 0.05, †p < 0.01. Abbreviations: CI, confidence interval; LVSI, lymphovascular space invasion; NLR, neutrophil-to-lymphocyte ratio; OR, odds ratio; Q, quartile; RCS, restricted cubic spline.

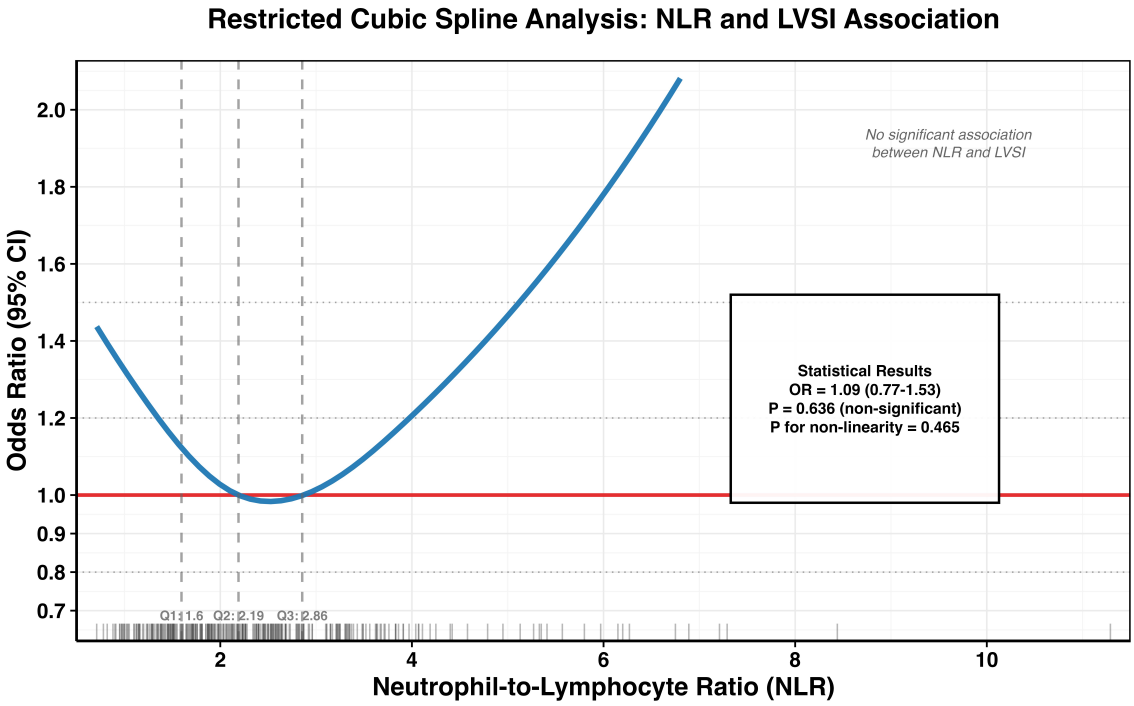

Fig. 2. Restricted cubic spline analysis of the association between NLR and LVSI in endometrial cancer. The solid blue line represents the odds ratio with the median NLR value (2.19) as the reference point (OR = 1.0, indicated by the horizontal red line). The shaded gray area depicts the 95% CI. Dashed vertical lines indicate quartile positions (Q1: 1.60, Q2: 2.19, Q3: 2.86). The rug plot at the bottom shows the distribution of individual NLR values. The curve demonstrates no statistically significant association between NLR and LVSI (overall OR = 1.09, 95% CI: 0.77–1.53, $p = 0.636$), with formal testing confirming the absence of non-linear relationships (p for non-linearity = 0.465). Abbreviations: aCCI, age-adjusted Charlson Comorbidity Index; BMI, body mass index; CI, confidence interval; FIGO, International Federation of Gynecology and Obstetrics; LVSI, lymphovascular space invasion; NLR, neutrophil-to-lymphocyte ratio; OR, odds ratio; PLR, platelet-to-lymphocyte ratio.

widened at the extremes where data density was lower, reflecting the expected uncertainty in sparse data regions.

These findings suggest that NLR, despite its established role as a systemic inflammatory marker in various oncological contexts, does not serve as an independent predictor of LVSI in EC patients in our cohort.

Subgroup Analysis of NLR Prognostic Effects

Fig. 4 presents subgroup survival analyses examining NLR effects on RFS across clinicopathological characteristics. Meta-analysis of eight subgroups revealed low heterogeneity ($I^2 = 0.0\%$, $p = 0.768$) with overall significant effect (HR = 1.42, 95% CI: 1.15–1.76, $p = 0.001$).

Elevated NLR significantly increased recurrence risk in patients with <50% myometrial invasion (HR = 1.56, 95% CI: 1.00–2.43, $p = 0.048$). Similar trends appeared in low-grade tumors (HR = 2.10, 95% CI: 0.89–4.91, $p = 0.088$) and FIGO stage I disease (HR = 1.83, 95% CI: 0.93–3.59, $p = 0.081$), though not statistically significant (Fig. 4). Other subgroups showed varied associations: endometrioid histology (HR = 1.39, 95% CI: 0.65–2.99, $p = 0.395$), non-endometrioid histology (HR = 1.41, 95% CI: 0.78–2.57, $p = 0.259$), high-grade tumors (HR = 1.21, 95% CI: 0.79–1.84, $p = 0.378$), FIGO stage III (HR = 1.87, 95% CI: 0.82–4.24, $p = 0.135$), and ≥50% myometrial invasion (HR = 0.93, 95% CI: 0.48–1.80, $p = 0.822$) (Fig. 4).

The consistent NLR impact across subgroups, indicated by low heterogeneity, suggests that its prognostic value is independent of clinicopathological features. NLR appears most valuable as a prognostic marker in patients with superficial myometrial invasion.

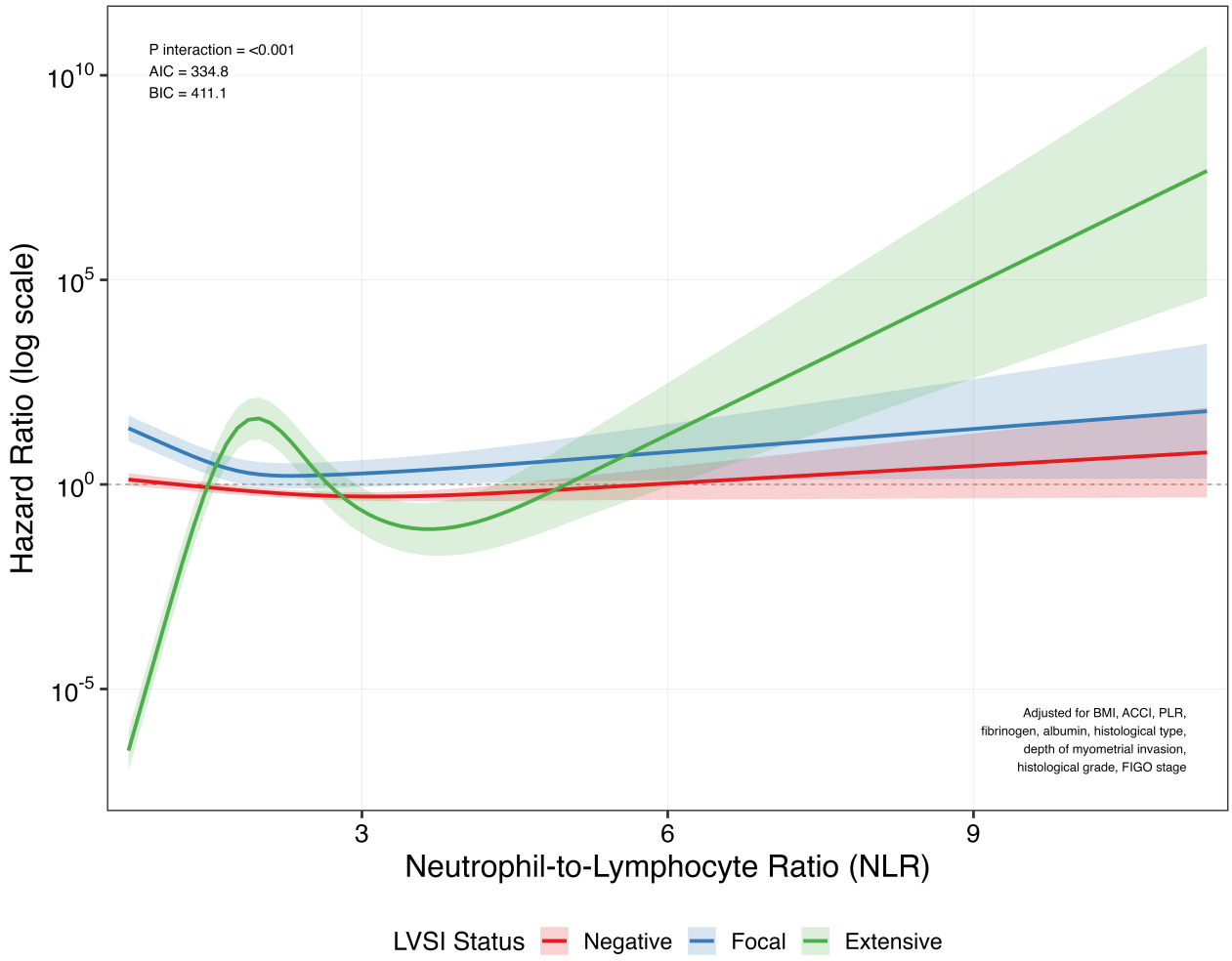

Fig. 3. Prognostic interaction between NLR and LVSI status in relation to recurrence-free survival, assessed using Cox proportional hazards modeling. The graph highlights the non-linear relationship between NLR and recurrence risk (presented as log hazard ratio) across different LVSI categories. Solid lines represent the estimated log HRs, and shaded areas indicate the 95% CIs. The reference line at HR = 1 (log HR = 0) represents the overall population average risk. The model was adjusted for BMI, aCCI, PLR, fibrinogen, albumin, histological type (endometrioid vs non-endometrioid carcinoma), depth of myometrial invasion (<50% vs ≥50%), histological grade (low-grade vs high-grade endometrial carcinoma), and FIGO stage. Knots were placed at the 5th, 35th, 65th, and 95th percentiles of NLR distribution. A significant interaction was observed between NLR and LVSI status ($p_{\text{interaction}} < 0.001$), suggesting that the prognostic value of NLR varies substantially according to LVSI severity. In this analysis of 335 patients, the model yield an AIC of 334.8 and a BIC of 411.1, suggesting a good overall fit. Abbreviations: aCCI, age-adjusted Charlson Comorbidity Index; AIC, Akaike Information Criterion; BIC, Bayesian Information Criterion; BMI, body mass index; CI, confidence interval; FIGO, International Federation of Gynecology and Obstetrics; HR, hazard ratio; LVSI, lymphovascular space invasion; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio.

Discussion

This study comprised two complementary analyses: (1) Cross-sectional evaluation of NLR-LVSI association, and (2) Survival analysis examining prognostic interactions between NLR and LVSI with respect to RFS in 335 EC patients over 12 years (2012–2024). Cross-sectional analysis showed elevated NLR was initially correlated with LVSI risk (OR = 1.31, 95% CI: 1.08–1.60; p = 0.007), though significance was lost after full adjustment (OR = 1.09, 95% CI: 0.77–1.53, p = 0.636). Survival analysis revealed prognostic significance in patients with superficial myometrial invasion (<50% depth) (HR = 1.56, 95% CI: 1.00–2.43; p = 0.048).

While our findings align with previous studies in certain respects, they also uncover notable differences that advance current understanding. Takahashi *et al.*'s single-center study [20] (n = 320) first demonstrated that elevated

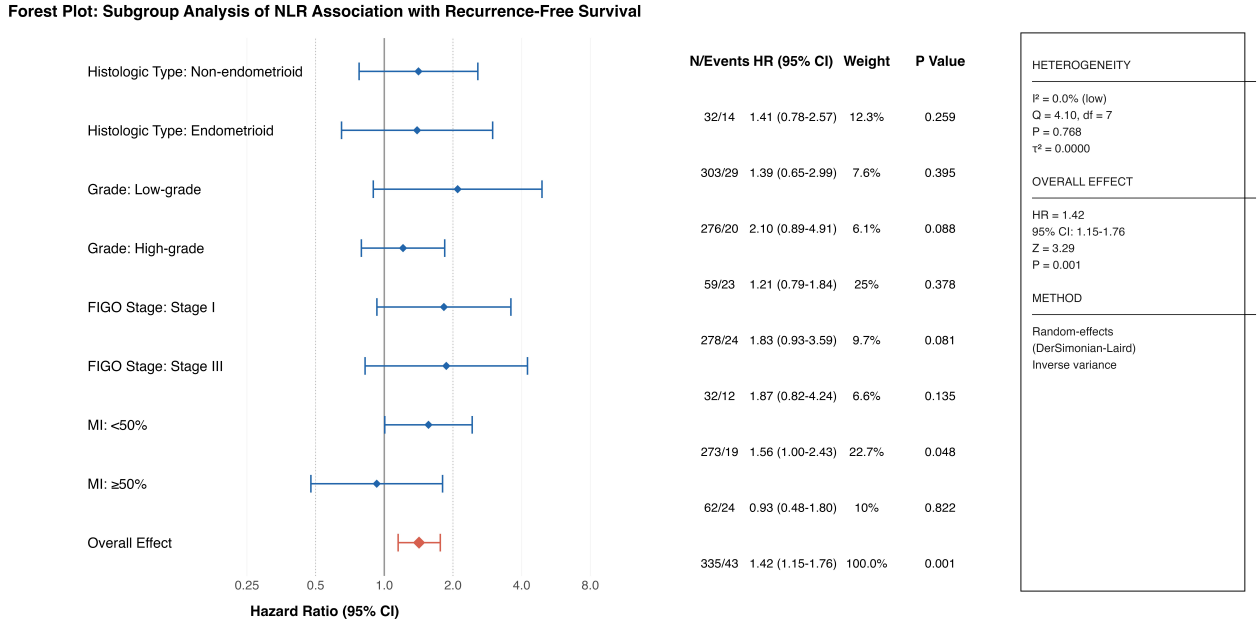

Fig. 4. Subgroup analysis of NLR's prognostic effects on recurrence-free survival stratified by clinicopathological characteristics. Hazard ratios (HRs) with 95% confidence intervals (CIs) in this forest plot were calculated using multivariate Cox proportional hazards models adjusted for age, BMI, aCCI, PLR, fibrinogen, and albumin. Random-effects meta-analysis was performed using the DerSimonian–Laird method with inverse variance weighting. The vertical dashed line represents HR = 1.0. The size of diamonds is proportional to the effect size. Horizontal lines represent 95% CIs. Individual subgroup weights are shown as percentages. Abbreviations: aCCI, age-adjusted Charlson Comorbidity Index; BMI, body mass index; FIGO, International Federation of Gynecology and Obstetrics; MI, myometrial invasion; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio.

neutrophil count was significantly associated with LVSI (p = 0.0003), providing important pathological basis for the NLR-LVSI association. Leng *et al.*'s meta-analysis [10] further supported this finding, showing significant correlation between NLR and OS (HR = 2.51, 95% CI: 1.70–3.71). However, Cong *et al.*'s study [11] in Chinese population suggested that the relationship between NLR and prognosis might be more complex, requiring integration with other inflammatory indicators. Song *et al.*'s research [21] in non-endometrioid carcinoma indicated that NLR's predictive value might vary by histological type. Our exploratory RCS analysis showed stable risk estimates across the NLR spectrum, but formal testing of likelihood ratio revealed no statistically significant non-linear relationship (p = 0.465), which may concur with the linear association hypothesis proposed by Marin *et al.* [22]. However, formal statistical testing confirmed no significant associations in both linear (p = 0.636) and non-linear (p = 0.465) modeling approaches, in line with the stable risk estimates observed across the entire NLR spectrum. These differences might be attributed to variations in study populations, clinicopathological parameters, and statistical methodologies. While studies by Leng *et al.* [10] (HR = 2.51, 95% CI: 1.70–3.71) and Ni *et al.* [23] (HR = 2.22, 95% CI: 1.77–2.78) reported significant associations between NLR and survival outcomes, our study focused specifically on LVSI association and found no significant relationship (OR = 1.09, 95% CI: 0.77–1.53, p = 0.636). This difference may reflect distinct biological pathways between NLR's effects on long-term survival versus LVSI progression. Additionally, our larger sample size (n = 335) and comprehensive adjustment for confounders may provide more robust assessment compared to smaller studies. These methodological differences highlight the importance of rigorous validation before clinical implementation.

Our study's observation of potential non-linear patterns between NLR and LVSI provides insights for clinical risk assessment in EC patients. However, our primary multivariate analysis demonstrated no significant independent association after comprehensive adjustment (OR = 1.09, 95% CI: 0.77–1.53, p = 0.636), indicating that NLR's predictive value may be limited once established clinicopathological factors are taken into account. Our spline analysis showed stable risk estimates, but both formal non-linearity testing (p = 0.465) and traditional regression analysis (p = 0.636) consistently demonstrated no significant associations, confirming the absence of clinically meaningful relationships. As these findings are considered exploratory and hypothesis-generating, validation in larger cohorts with sufficient statistical power are required to detect and confirm potential associations. This finding aligns with the meta-analysis by Ni *et al.* [23], which confirmed the sig-

nificant association between NLR and OS (HR = 2.22, 95% CI: 1.77–2.78), though survival outcomes may differ from LVSI prediction. Notably, subgroup survival analysis showed borderline significance for recurrence risk in early-stage patients with superficial myometrial invasion, consistent with Cong *et al.*'s findings [24] that identified NLR as an independent prognostic factor for EC outcomes. Future prospective studies should rigorously investigate whether NLR could complement existing preoperative risk assessment protocols as an additional stratification parameter. Any potential clinical applications would require robust multicenter validation demonstrating clear incremental benefit before implementation. This NLR-based approach may warrant cautious further investigation in prospective validation studies to determine whether it offers meaningful clinical utility beyond current standard assessment methods.

Our study implemented systematic methodological approaches to ensure result reliability. In terms of statistical analysis, we employed an innovative multilayered strategy: Utilizing one sequential logistic regression analysis for comprehensive association assessment, which incorporated exploration of non-linear relationship between NLR and LVSI through RCS analysis, and validating result stability through stratified and subgroup analyses. The study also incorporated comprehensive adjustment for confounders, such as clinical characteristics and inflammatory indices, to enhance the credibility of our conclusions. This systematic analytical approach provides a robust methodological foundation for exploring the association between NLR and LVSI in EC.

Retrospective power analysis revealed that our study achieved adequate power (80%) for the primary NLR-LVSI association analysis. However, with 41 LVSI events (12.2% incidence), the achieved power for detecting the observed small effect size (OR = 1.09) was approximately 65%. For survival analysis with 43 events, the statistical power was estimated at 60%. Subgroup analyses were substantially underpowered (<50% for most subgroups), which may explain the borderline significance observed in superficial invasion cases (HR = 1.56, 95% CI: 1.00–2.43, $p = 0.048$).

This study has several limitations: (1) Statistical power limitations. Our study was adequately powered for NLR-LVSI association analysis but was underpowered for survival endpoints and subgroup analyses. With only 41 LVSI events and 43 survival events, detection of smaller but clinically meaningful effect sizes may have been limited. Future studies require larger cohorts to adequately power both association and survival analyses. Both traditional regression analysis and exploratory non-linear modeling consistently showed no significant associations (OR = 1.09, 95% CI: 0.77–1.53, $p = 0.636$; p for non-linearity = 0.465), demonstrating methodological consistency across different analytical approaches. Study design constraints include restrictions to adults undergoing systematic lymphadenectomy, excluding those with concurrent malignancies and inflammatory conditions, which may introduce selection bias by creating an artificially 'clean' study population that does not reflect real-world clinical complexity. These exclusions, while necessary for NLR interpretability, may limit applicability to patients with comorbidities commonly encountered in clinical practice, limiting extrapolation to broader patient populations. (2) Generalizability limitations. As a single-center retrospective study with solely Chinese patients, our findings require multicenter validation across diverse populations and healthcare systems. NLR values and their clinical significance may vary across different ethnic populations due to genetic polymorphisms in inflammatory pathways and environmental factors such as dietary patterns, infectious disease exposure, and lifestyle differences that may influence baseline inflammatory status [25]. Therefore, our findings cannot be directly extrapolated to non-Chinese populations without population-specific validation. (3) Analytical standardization limitations. NLR measurements may exhibit inter-laboratory variability due to differences in automated hematology analyzers, counting methodologies, and quality control standards. Pre-analytical factors including sample processing protocols and storage conditions can also influence neutrophil and lymphocyte counts. This analytical variability could impact the reproducibility of our findings across healthcare settings and poses challenges for establishing universal NLR cut-off values. Our single-center design using standardized laboratory protocols may not reflect measurement variability in multicenter settings, limiting direct applicability to other institutions without local validation. (4) Statistical limitations. Future studies with larger sample sizes may help rule out smaller effect sizes, though current evidence across multiple analytical approaches consistently does not support NLR's clinical utility for LVSI prediction.

Conclusion

Our investigation evaluated NLR's relationship with LVSI and its prognostic significance through cross-sectional association and survival analyses. For LVSI prediction, both RCS analysis and multivariate regression showed no significant association after adjustment, indicating NLR provides no independent predictive value beyond established clinicopathological factors. For survival outcomes, subgroup analysis revealed borderline significance for recurrence risk in patients with superficial myometrial invasion, though this requires cautious interpretation given borderline statistical significance.

As an observational study, we demonstrated associations rather than causality, and specific clinical recommendations cannot be definitively proposed based on these preliminary findings. Despite extensive covariate adjustment, the possibility of residual confounding by unmeasured fac-

tors cannot be excluded. At the current stage, these results do not support the use of NLR for LVSI risk stratification; However, its potential prognostic value for recurrence prediction in specific subgroups warrants further investigation with appropriately powered prospective studies that can address these inherent limitations of observational research design.

Availability of Data and Materials

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

Author Contributions

LX and ZH designed the research study, drafted and revised the manuscript, and analyzed the data. JD contributed to the conception and design of the study, provided critical supervision throughout the research process, participated in data interpretation, revised the manuscript critically for important intellectual content, supervised the overall project, and submitted the paper for publication. Each author contributed significantly to the article and approved the submitted version. All authors read and approved the final manuscript and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

This study was approved by the Ethics Committee of Dongyang Hospital of Wenzhou Medical University (approval number: DRY2024-YX-391). The requirement for informed consent was waived by the institutional review board due to the retrospective nature of this study. The study was conducted in accordance with the Declaration of Helsinki and the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines for observational studies. All patient data were strictly de-identified, securely stored, and exclusively used for research purposes. Data management and statistical analysis were conducted independently by the Department of Clinical Research Center to ensure objectivity.

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Conflict of Interest

The authors declare no conflict of interest.

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