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ORIGINAL RESEARCH

Platelet-to-Lymphocyte Ratio (PLR) as the Prognostic Factor for Recurrence/Residual Disease in HSIL Patients After LEEP

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Purpose: The platelet-to-lymphocyte ratio (PLR) is considered correlated with cancer prognosis including cervical cancer, in addition to high-risk papillomavirus (HR-HPV) infection, of which the predictive value in prognosis of high-grade squamous intraepithelial lesions (HSILs) remains unknown. Here, the prognostic predictive value of PLR in HSIL after loop electrosurgical excision procedure (LEEP) was evaluated.

Patients and Methods: This study included 335 nonpregnant participants with histopathologically confirmed HSIL and 3- and 5-year follow-ups from the Fujian Cervical Lesions Screening Cohorts (FCLSCs) between September 2016 and September 2018. PLR and other variables were evaluated to identify the factors related to the recurrence/residual cervical intraepithelial neoplasia (CIN)-free survival (RFS), namely, the time from LEEP at baseline to first detection of recurrence/residual CIN or end of follow-up, by logistic and Cox regression.

Results: In the Kaplan–Meier analysis, HR-HPV infection (p=0.049/0.012), higher PLR (p=0.031/0.038), and gland invasion (p=0.047) had a higher risk for recurrence/residual CIN at the 3-/5-year follow-up. The univariate logistic and Cox regression analyses showed significant differences and a higher cumulative risk in patients with HR-HPV infection (OR=3.917, p=0.026; HR=3.996, p=0.020) and higher PLR (OR=2.295, p=0.041; HR=2.161, p=0.030) at the 5-year follow-up. The findings by multivariate Cox regression analysis were similar, indicating a poor prognosis for patients with HR-HPV infection (HR=3.901, p=0.023) and higher PLR (HR=2.082, p=0.038) at the 5-year follow-up. The calibration plot showed a better model fit for RFS at the 3-year follow-up.

Conclusion: Preoperative PLR level and HR-HPV infection could be available markers for predicting recurrence/residual disease of HSIL after LEEP. Clinically, combining PLR with HR-HPV tests may provide novel evaluation method and reference for management in post-treatment patients with cervical precancerous lesions.

Keywords: systemic inflammatory response, cervical intraepithelial neoplasia, human papillomavirus, HPV, conization, prognosis

Introduction

Cervical cancer is a kind of cancer that seriously endangers the health of the female reproductive system. Approximately 604,000 new cases and 340,000 deaths from cervical cancer were reported worldwide in 2020 according to the 2020

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GLOBOCAN statistics.[1] Cervical intraepithelial neoplasia (CIN) is a precancerous lesion that can lead to malignancies in the cervix. The carcinoma in situ are not strictly distinguished from HSIL in cytology.[2] While in histopathology, HSIL are divided into CIN II and CIN III. The 2019 American Society for Colposcopy and Cervical Pathology (ASCCP) Risk-Based Management Consensus Guidelines for Abnormal Cervical Cancer Screening Tests and Cancer Precursors additionally stated that histopathology reports for HSIL based on Lower Anogenital Squamous Terminology (LAST) or World Health Organization (WHO) recommendations should include CIN II or CIN III qualifiers. Also, the immediate and 5-year CIN III+ risk are regarded as an important indicator of clinical decisions (colposcopic referral, expedited treatment, surveillance, etc.).[3] Thus, HSIL confirmed by histopathology (CIN II/III) suggested the high risk for progression to cervical cancer and the relevant clinical decisions leading to recommendations of treatment. In developed countries, approximately 1.5 out of every 1000 women are diagnosed with lesions designated as CIN II or worse every year.[4] However, in another prospective cohort of women aged 18–62 years, 40% of CIN II cases resolved within two years compared with an estimated 32% of CIN III cases.[5,6] Invasive cervical cancer in China develops mostly from HSIL.[7] Thus, surgical or physical treatment is generally recommended for higher stages of precancerous lesions, such as cold knife conization (CKC), large loop excision of the transformation zone (LLETZ) or loop electrosurgical excision procedure (LEEP).

The excision was preferred to ablative treatment for most patients with HSIL confirmed by histopathology, according to 2019 ASCCP guidelines.[3] And LEEP is one of the excisional treatments clinically for both diagnostic and therapeutic purposes. It is a minimally invasive surgical method that uses an electrotome for cutting tissues and a microwave for stopping bleeding in the cervix and is especially suitable for those who need to be discharged quickly from the day-ward units. However, the prognostic evaluation and prediction of recurrence after CIN treatment remain limited. After CIN II or CIN III treatment, the majority of patients (82.3%) were negative for HPV infection at the first follow-up, and the 5-year risk for recurrence of CIN III+ was approximately 0.3% and 2.0%, respectively.[3] A meta-analysis revealed that the rates of CIN recurrence at the 12-month follow-up after LEEP were approximately 26.6% and 5.3% at the long-term follow-up.[8,9] Co-testing combining high-risk papillomavirus (HR-HPV) and cytology tests is now considered the routine review method during posttreatment follow-up for HSIL patients. Our previous studies have demonstrated that HR-HPV genotypes and viral loads are independent predictors for recurrence/residual CIN.[10,11] Other studies have also shown that the margin status of intraoperative specimens can be used for predicting the persistence and recurrence of CIN II+ lesions.[12,13] However, the ASCCP guideline stated that the 5-year recurrence/residual rate of CIN III+ after treatment is approximately 0.9% in HSIL patients who are HPV-negative at the first-time follow-up, while the rate of recurrence or residual HSIL is higher in patients with positive HPV and/or cytology results.[3] This suggests that the establishment of new models for prognostic prediction is needed, which requires research on multiple factors associated with HSIL recurrence/residual disease.

Accumulative evidence has shown that markers of the systemic inflammatory response (SIR) could be predictors of prognosis in many malignancies.[14,15] The index includes serum markers such as the neutrophil-to-lymphocyte ratio (NLR), the lymphocyte-to-monocyte ratio (LMR) and the platelet-to-lymphocyte ratio (PLR). Previous study showed that high PLR level could be a significant poor predictor for progression-free survival (PFS) in cervical cancer.[16] Among patients diagnosed with FIGO stages I–III cervical cancer after chemoradiotherapy, NLR and PLR were considered as the more precise biomarkers. Higher PLR and LMR showed significant prognosis predicting value in advanced stages of FIGO III–IV cervical cancer, reported in a meta-analysis.[17] Our previous studies also found similar clinical validation of LMR in FIGO stage (2009) IB-IIA cervical cancer.[18] Additionally, the diagnostic efficiency of NLR and PLR has been shown in discriminating precancerous pathological lesions (including both low- and high-grade squamous intraepithelial lesions) from cervical cancer.[19] The conclusions above strongly suggest that SIR markers might be potential indicators in relation to different stages of precancerous lesions in the cervix, which lead to prognostic prediction effectiveness. However, previous studies have shown the relationship only between SIR and the occurrence or progression of diseases. No studies have assessed the prognostic predictive value or SIR markers in the follow-up of HSIL patients after LEEP, especially PLR. Moreover, no study has reported the clinical efficiency of combining SIRI and HR-HPV tests for predicting the prognosis of HSIL patients after surgery.

This study aims to evaluate the effect of PLR as the indicator that affects the prognosis of patients with HSIL after LEEP at different stages of follow-up, to provide novel markers and strategies for the management of posttreatment CIN patients.

Materials and Methods

Participants and Study Design

In total, 381 female patients from Fujian Province Cervical Lesion Screening Cohorts (FCLSCs) between September 2016 and September 2018 were retrospectively selected. FCLSCs are composed of 120 cervical cancer screening cohorts established in Fujian, of which more than 200,000 cases were used to evaluate the 121 values of introducing HR-HPV testing into screening, including 1 provincial hospital, 9 municipal hospitals and more than 500 community health service centers.[10,11] The FCPP project was launched on the basis of FCLSCs, which involved 2 years of planning (from January 2012 to December 2013) and 3 years of screening (from January 2014 to December 2016). In January 2014, FCPP was launched, and all FCLSCs institutions changed the main method of cervical cancer screening from HR-HPV non-genotyping to HR-HPV genotyping. Women in the FCPP project voluntarily go to the hospital or community health service center for cervical cancer screening. All participants in this study were selected from FCLSCs. The inclusion criteria were as follows: 1) patients with a pathological diagnosis of HSIL (including CIN II and CIN III) and 2) patients who underwent LEEP treatment within 1 month after the diagnosis was confirmed by histopathology. Blood tests and vaginal microecology/leucorrhea routine tests were performed in all patients 1 week before surgery. Twenty-one patients were excluded according to the following criteria: 1) patients with a history of cervical lesions, malignancies, immune system diseases or other sexually transmitted diseases (gonorrhea, syphilis, human immunodeficiency virus infection/acquired immuno deficiency syndrome (HIV/AIDs), etc.); 2) patients with a history of initial hysterectomy, surgical treatment or chemotherapy in the cervix; 3) sexual activity, vaginal medication or flushing within 72 hours before sampling; 4) abnormal results for vaginal microecology or leucorrhea routine tests within 1 week before the operation; or 5) pregnancy. The normal results of vaginal microecology or leucorrhea routine tests was defined as: 1) vaginal flora density of grade II to III; 2) diversity of grade II to III; 3) Lactobacillus as the dominant bacteria; 4) vaginal pH of 3.8 to 4.5; 5) the normal Lactobacillus function (normal H2O2 secretion); 6) negative leukocyte esterase and sialidase; and 7) the cleanliness of grade I to II and no fungi was detected in leucorrhea routine tests. The abnormal results for any of the above indicators were defined as abnormal results for vaginal microecology or leucorrhea routine tests. Blood samples within 1 week before LEEP were collected and analyzed using semiconductor laser flow cytometry,[20] as well as the HR-HPV DNA test and liquid-based cytology test (TCT) within 3 months before LEEP. All 360 patients were followed up initially every 4–6 months after the treatment. Annual co-testing was conducted until at least 3 consecutive negative tests were obtained, which was followed by continued surveillance at 3-year intervals. The follow-up content included the HR-HPV DNA test, TCT and colposcopic biopsy if necessary, according to the 2019 ASCCP risk-based management consensus guidelines.[3] The study endpoint event was defined as the recurrence/residual CIN, and the last follow-up was conducted in December 2022. During follow-up, 25 patients were excluded for the following reasons: 1) invalid, unsatisfactory or missed specimens; 2) large amount of missing data; or 3) need to withdraw from the study. Finally, a total of 335 patients were eligible for data analysis. This study was approved by the Ethics Review Committee of Fujian Maternity and Child Health Hospital, Affiliated Hospital of Fujian Medical University, China (2020KY015), and all individuals participating in this study and undergoing colposcopic biopsy provided written informed consent.

Liquid-Based Cytology

The auto-imaging system (Hologic, Inc., San Diego, CA, USA) was used for cytology tests. Cytological specimens were collected and assessed independently by 2 pathologists with adequate certifications. If different diagnosis occurs, the samples would be reviewed again until consensus diagnosis was reached. The cytological results were classified according to the Bethesda system.[21,22]

Histopathology

Specimens were fixed in 10% formalin and embedded in paraffin. Tissue sections of 4 μm thickness were stained with hematoxylin and eosin routinely, and then evaluated by an experienced pathologist according to the standard of the 2014 World Health Organization (WHO) Classification of Tumors of the Female Genital Tract and the CIN system.[23,24] Discrepancy specimens were re-evaluated by another pathologist until the consensus was reached.

PLR and Other Definitions During Follow-Up

PLR was defined as the absolute platelet count relative to the absolute lymphocyte count. Recurrent disease was defined as CIN II+ (cervical lesions of CIN II or worse) more than 12 months after LEEP, and residual disease was defined as CIN I after LEEP or CIN II+ within 12 months after LEEP. Principally, higher grades of lesions in the cervix detected within 12 months after LEEP were considered progression in residual lesions. Recurrence/residual-free survival (RFS) was defined as the time from LEEP at baseline to first detection of recurrence/residual disease or the end of follow-up among participants (including loss to follow-up).

PCR-RDB HR-HPV DNA Test

Polymerase chain reaction-reverse dot blot (PCR-RDB) for HR-HPV testing was used for the detection of HR-HPV DNA, including 16 genotypes (HPV-16, −18, −31, −33, −35, −39, −45, −51, −52, −56, −58, −59, −66, −68, −73 and −82), in cervical exfoliated cells (Yaneng® Biosciences, ShenZhen, China). Procedures were strictly performed according to the manufacturer's instructions.[25]

Statistical Analysis

The data analysis was performed by SPSS v25.0. The markers from blood samples, PLR and other clinicopathological data were summarized by descriptive analysis. Continuous data were analysed across cohorts by the unpaired *t* test or nonparametric test, while categorical data were compared using the Pearson test, with the correction for continuity being applied if necessary. Univariate/multivariate logistic regression and Cox regression were used to identify independent prognostic factors in relation to recurrence/residual disease. The odds ratio (OR) and hazard ratio (HR) were calculated. The forest plots for the regressions above were plotted by GraphPad Prism v9.4.1, along with the Kaplan–Meier curve using the Log rank test. The receiver operating characteristic (ROC) curve was generated by GraphPad Prism v9.4.1, and the optimal cut-off value for PLR (176.15) was calculated using X-tile software (http://www.tissuearray.org/rimmlab/). The nomograms were created with R software v4.2.1 using the 'rms' package. Calibration plots and Harrell's concordance index (C-index) were generated to test the predictive accuracy. The difference was considered statistically significance when $p < 0.05$.

Results

Participant Characteristics

A total of 335 patients were finally included in this study. The clinicopathologic characteristics of the participants are shown in Table 1. The analysis of the ROC curve showed some serum markers associated with SIR. Only PLR (p=0.005) showed a significant difference when the results of the area under the curve (AUC) and p value were combined (Supplemental Table 1 and Figure 1A). The optimal cut-off level for PLR was 176.15, which was analysed by SPSS v25.0 and X-tile software.

Generally, the median age and median RFS of the patients were 37 years (21–69 years) and 40 months (2–72 months), respectively. The numbers of patients with recurrence/residual CIN I, CIN II and CIN III were 21, 12 and 10, respectively, at the 3-year follow-up and 26, 13 and 11, respectively, at the 5-year follow-up point, as shown in the flowchart of the study (Figure 2). Among patients with recurrence/residual CIN during the 5-year follow-up period, 47 (94.0%) patients had positive HR-HPV test results, and 40 (80.0%) patients had a higher PLR. The number of patients with gland invasion confirmed by histopathology was 35 (70.0%) (Table 1).

Table 1 Clinicopathologic Characteristics of Participants

Items	Number of Participants (%)		
	Recurrence/Residual CIN[a]	No Recurrence/Residual CIN[b]	*P* value[c]
Age (years)			0.747
< 50	43 (86.0%)	240 (84.2%)	
≥ 50	7 (14.0%)	45 (15.8%)	
Gland invasive			0.055
No invasion	15 (30.0%)	127 (44.6%)	
Invasion	35 (70.0%)	158 (55.4%)	
Margin status			>0.999
Negative	50 (100.0%)	280 (98.2%)	
Positive	0	5 (1.8%)	
Preoperative cytology			0.863
NILM	15 (30.0%)	89 (31.2%)	
ASC-US or worse	35 (70.0%)	196 (68.8%)	
Preoperative pathology			0.102
CIN II	39 (78.0%)	189 (66.3%)	
CIN III	11 (22.0%)	96 (33.7%)	
Preoperative HR-HPV infection			0.017
Negative	3 (6.0%)	57 (20.0%)	
Positive	47 (94.0%)	228 (80.0%)	
Preoperative HPV genotype			<0.001
None or single genotype	32 (64.0%)	218 (76.5%)	
Double or multiple genotype	18 (36.0%)	67 (23.5%)	
Preoperative serum markers			
White blood cell count	6.08 (3.25–12.57) ($\times 10^9$/L)	6.18 (3.21–12.25) ($\times 10^9$/L)	0.508
Neutrophil count	3.75 (1.77–10.76) ($\times 10^9$/L)	3.57 (1.44–9.53) ($\times 10^9$/L)	0.894
Monocyte count	0.41 (0.19–0.64) ($\times 10^9$/L)	0.43 (0.12–0.93) ($\times 10^9$/L)	0.148
Preoperative PLR			0.036
≤ 176.15	40 (80.0%)	257 (90.2%)	
> 176.15	10 (10.0%)	28 (9.8%)	

Notes: [a]Recurrence CIN was defined as CIN II or worse more than 12 months after operation, and the residual CIN was defined as CIN I after operation or CIN II+ within 12 months after operation; [b]no recurrence/residual CIN was defined as no CIN found after operation; [c]a *p* value<0.05 indicates a statistically significance.

Abbreviations: CIN, cervical intraepithelial neoplasia; NILM, no intraepithelial lesions or malignant cells; ASC-US, atypical squamous cells of undetermined significance; HR-HPV, high-risk human papillomavirus; PLR, platelet-to-lymphocyte ratio.

Factors in Relation to RFS

Pearson's test showed that there were significant differences in preoperative HR-HPV infection (p=0.017), HPV genotypes (p<0.001) and PLR (p=0.036) at the 5-year follow-up (Table 1). The Kaplan–Meier curves showed significant differences among groups with different HR-HPV infection statuses (p=0.049) and PLR values (p=0.031) at the 3-year follow-up, along with significant differences among groups with different HR-HPV infection statuses (p=0.012), PLR values (p=0.038) and gland invasion statuses (p=0.047), as analysed by the Log rank test (Figure 1). Given that some SIR markers could be predictors for discriminating LSIL/HSIL lesions,[19] we further analysed variables that might be associated with recurrence/residual HSIL/LSIL after treatment. No significant difference was found in relevant factors (Supplemental Table 2).

PLR and HR-HPV Status in Predicting the Prognosis of CIN

Logistic and Cox regression models were established to analyse the risk factors for recurrence/residual diseases. Both logistic and Cox regression showed significant differences and cumulative risks in patients with HR-HPV infection and a higher PLR at the 5-year follow-up, with ORs of 3.917 (p=0.026) and 2.295 (p=0.041) and HRs of 3.996 (p=0.020) and 2.161 (p=0.030), respectively, by univariate analysis. At the 3-year follow-up, only the higher PLR showed a significant

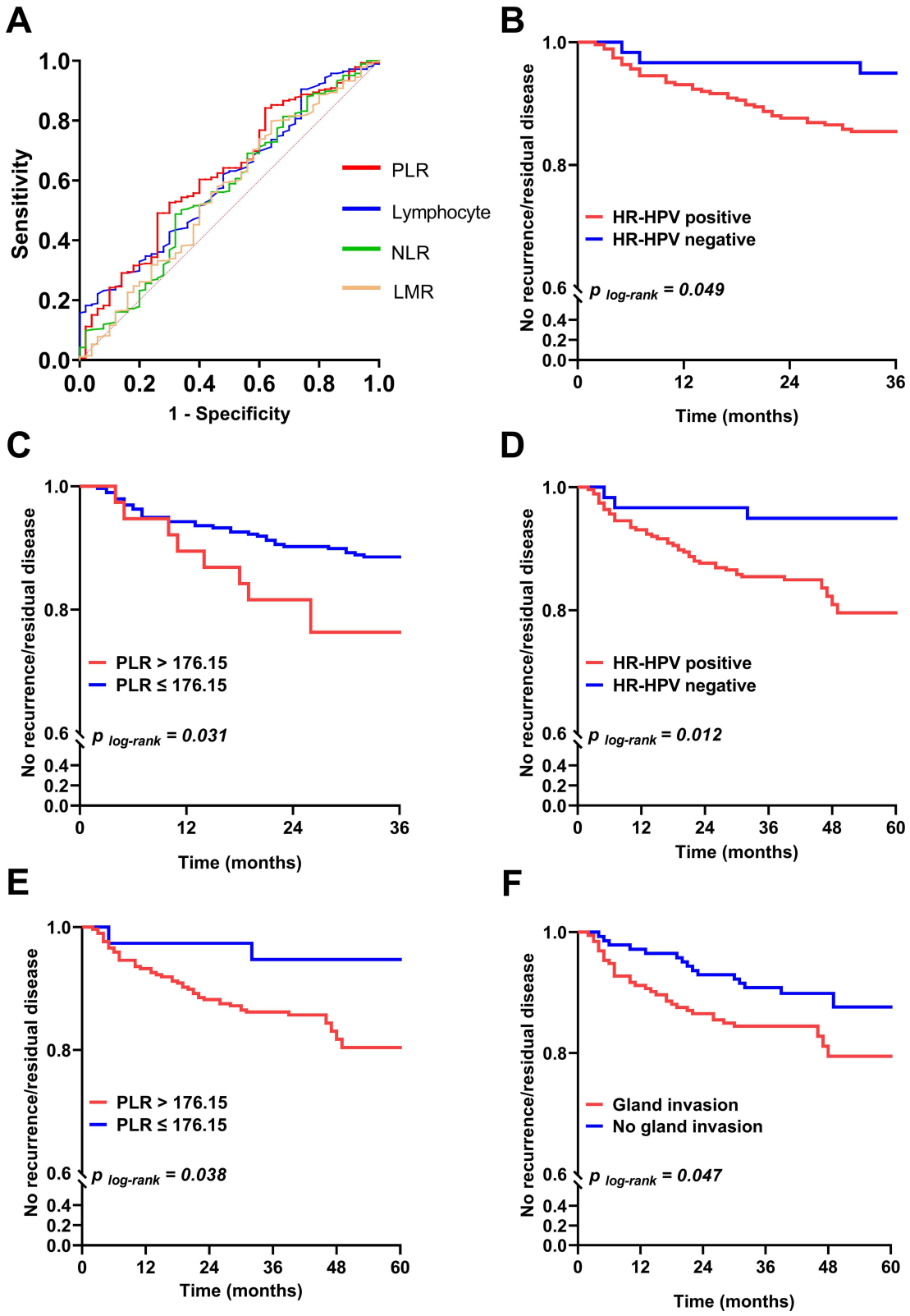

Figure 1 Kaplan–Meier curves for variables in relation to recurrence/residual CIN. (**A**) Some serum markers analysed by ROC curve analysis; (**B**) Preoperative HR-HPV infection promotes recurrence/residual CIN at the 3-year follow-up; (**C**) Preoperative high PLR level promotes recurrence/residual CIN at the 3-year follow-up; (**D**) Preoperative HR-HPV infection promotes recurrence/residual CIN at the 5-year follow-up; (**E**) Preoperative high PLR level promotes recurrence/residual CIN at the 5-year follow-up; (**F**) Gland invasive status promotes recurrence/residual CIN at the 5-year follow-up.

Abbreviations: PLR, platelet-to-lymphocyte ratio; NLR, neutrophil-to-lymphocyte ratio; LMR, lymphocyte-to-monocyte ratio; HR-HPV, high-risk human papillomavirus.

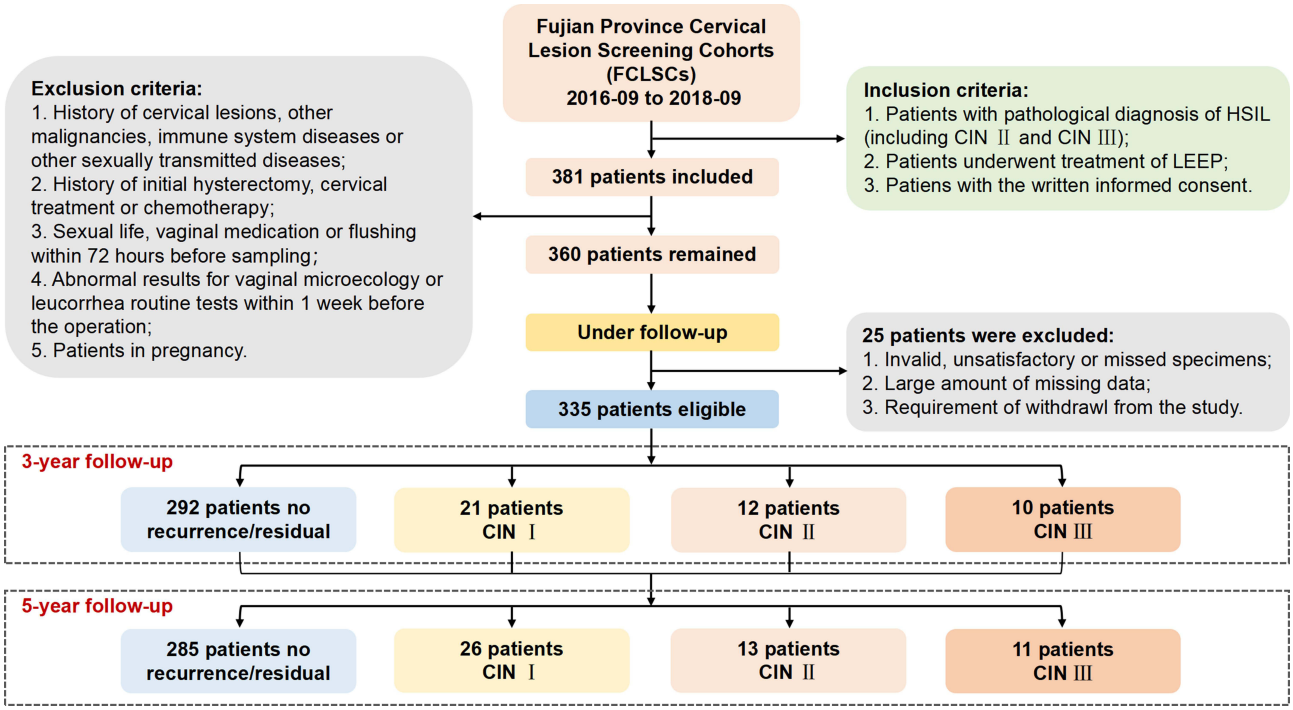

Figure 2 Flow chart of the study.
Abbreviations: CIN, cervical intraepithelial neoplasia; HSIL, high-grade squamous intraepithelial lesion; LEEP, loop electrosurgical excision procedure.

difference, with an OR of 2.401 (p=0.038) and an HR of 2.187 (p=0.037), by univariate analysis (Table 2 and Figure 3). The results of the crude OR and 95% confidence interval were revealed by forest plots (Figure 3). The multivariate Cox regression analysis indicated the difference in cumulative risk in groups with different HR-HPV infection statuses (HR=3.901, p=0.023) and PLRs (HR=2.082, p=0.038) at the 5-year follow-up (Figure 3). No significant difference was found in PLR (OR=2.212, p=0.053) compared with HPV infection status (OR=3.817, p=0.029) via logistic regression (Table 2).

The nomogram for predicting the 5-year risk of recurrence/residual disease along with RFS was plotted in the Cox regression models (Figure 4A). The calibration plots by Cox regression are also shown. The C-index was 0.612 (95% CI 0.549–0.675, p<0.001) in the logistic model and 0.603 (95% CI 0.541–0.665, p=0.001) in the Cox regression model. Basically, the degree of fitting was better at the 3-year follow-up than at the 5-year follow-up (Figure 4B and C).

Based on the findings above, the PLR-HR-HPV (P-H) score was defined as follows: 1) patients without preoperative HR-HPV infection or higher PLR (>176.15) were given a score of 0; 2) patients with preoperative HR-HPV infection or higher PLR (>176.15) were given a score of 1; and 3) patients with both preoperative HR-HPV infection and higher PLR (>176.15) were given a score of 2. The Kaplan–Meier curve showed that a higher P-H score might lead to a worse prognosis of participants (Figure 4D), with an HR of 2.503 (95% CI 1.461–4.288, p=0.001). The C-index was 0.597 (95% CI 0.535–0.627, p<0.001), suggesting a lower concordance than that of the Cox regression model above.

Discussion

In the present study, the correlation between the status of SIR/HR-HPV and the prognosis of HSIL is described. Pre- or post-operative HR-HPV persistent prevalence is an important factor in the prognosis of cervical lesions, which has been strongly demonstrated by studies and academic guidelines.[3,26] SIR has recently been demonstrated to be closely related to tumour progression and prognosis in various fields, including cervical cancer.[27–31] In precancerous lesions, the expression of SIR markers also differs from that in malignancies or normal tissues, including gynaecologic neoplasms such as endometrial and cervical carcinoma.[16–19,32] Among the SIR markers, PLR is considered a significant predictor of the prognosis of cervical cancer, including overall survival (OS), lymph node metastasis and postoperative recurrence,

Table 2 Clinicopathologic Variables Associated with or for Recurrence/Residual Disease

Items	At 3-Year Follow-Up				At 5-Year Follow-Up			
	Univariate[a]		Multivariate[b]		Univariate		Multivariate	
	OR	*P*[c]	OR	*p*	OR	*p*	OR	*p*
Age (years)		0.883		-		0.747		-
< 50	Reference		-		Reference		-	
≥ 50	1.067		-		0.868		-	
Gland invasive		0.087		-		0.057		-
Invasion	Reference		-		Reference		-	
No invasion	1.826		-		1.876		-	
Margin status		-		-		-		-
Negative	Reference		-		Reference		-	
Positive	-		-		-		-	
Cytology		0.818		-		0.863		-
NILM	Reference		-		Reference		-	
≥ASC-US[d]	0.923		-		0.923		-	
Pathology		0.194		-		0.106		-
CIN II	Reference		-		Reference		-	
CIN III	0.609		-		0.609		-	
Infected HR-HPV		0.057		-		0.026		0.029
Negative	Reference		-		Reference		Reference	
Positive	3.234		-		3.917		3.817	
HPV genotype		0.683		-		0.064		-
None/single	Reference		-		Reference		-	
Double/multiple	1.161		-		1.830		-	
Serum markers								
WBC ($\times 10^9$/L)	0.956	0.677	-	-	0.919	0.414	-	-
NeC ($\times 10^9$/L)	1.117	0.328	-	-	1.045	0.687	-	-
MoC ($\times 10^9$/L)	0.121	0.129	-	-	0.135	0.124	-	-
PLR		0.038		0.038		0.041		0.053
≤ 176.15	Reference		Reference		Reference		Reference	
> 176.15	2.401		2.401		2.295		2.212	

Notes: [a]Univariate logistic regression was applied for analysis of factors associated with recurrence/residual CIN; [b]multivariate analysis included variates with $p<0.05$, and was applied for analysis of factors associated with recurrence/residual CIN; [c]$p<0.05$ indicates a statistically significance; [d]the cytology test results of ≥ASC-US was defined as ASC-US or worse.

Abbreviations: OR, odds ratio; NILM, no intraepithelial lesions or malignant cells; ASC-US, atypical squamous cells of undetermined significance; CIN, cervical intraepithelial neoplasia; HR-HPV, high-risk human papillomavirus; WBC, white blood cell count; NeC, neutrophil count; MoC, monocyte count; PLR, platelet-to-lymphocyte ratio.

even at different FIGO stages.[18,29–31] Nevertheless, no study has confirmed the predictive value of PLR in LSIL/HSIL thus far, although the predictive effect of NLR and white blood cells (WBCs) has been reported before in patients with CIN after surgery.[33] The HPV test combined with TCT is still the dominant strategy in the management of postoperative patients with cervical precancerous lesions. Therefore, we hypothesized that PLR could be valuable for predicting the prognosis of CIN, which was consistent with our findings in this study.

The progression of malignant tumours and precancerous lesions is associated with the inflammatory response in individuals, which refers to the tumour microenvironment (TME), tumour necrosis induction, inflammasomes, relative cytokines, tumour immune-cell infiltration and response of acute-phase proteins, leading to tissue invasion and angiogenesis. The process is closely related to biomarkers such as STATs, NF-κB, HIF-1α, CTLA, c-KIT and CSF1.[34] Previous studies provided clinical evidence with severe tissue inflammation detected in the higher grades of cervical precancerous lesions, while most findings focused on the NLR and the anomaly of tumour-associated macrophages (TAMs) instead of platelets (PLTs) or lymphocytes.[35,36] In some cases, PLTs and lymphocytes were reported to promote tumour progression by immune escape, tissue invasion and angiogenesis,[37] and the increased PLTs could predict the high

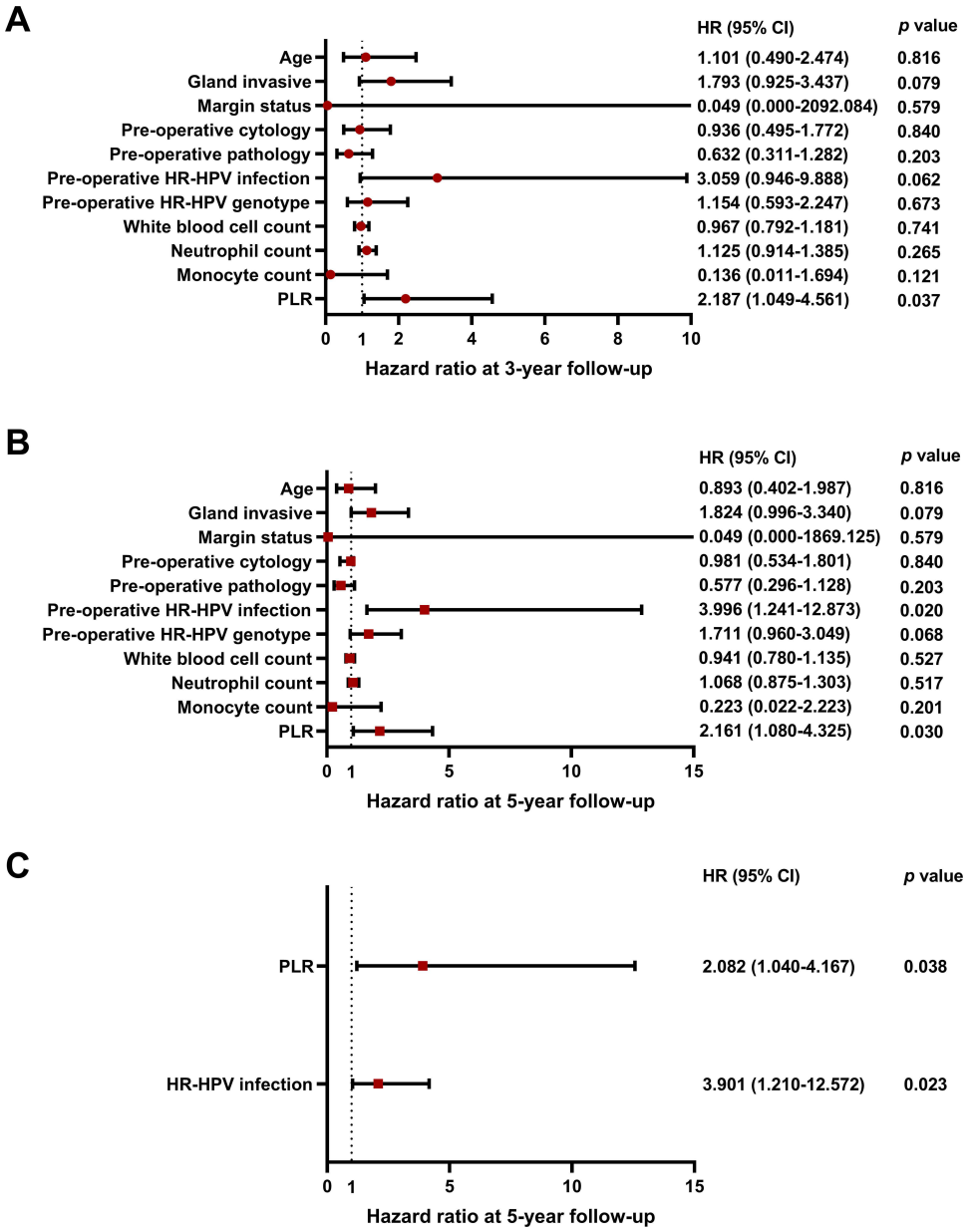

Figure 3 The Cox regression analysis during the follow-up. (**A**) The forest plot of HR for recurrence/residual disease at the 3-year follow-up (univariate analysis); (**B**) The forest plot of HR for recurrence/residual disease at the 5-year follow-up (univariate analysis); (**C**) Forest plot of HR for recurrence/residual disease at the 5-year follow-up (multivariate analysis). A *p* value<0.05 indicates a statistically significance.

Abbreviations: HR, hazard ratio; 95% CI, 95% confidence interval; HR-HPV, high-risk human papillomavirus; PLR, platelet-to-lymphocyte ratio.

incidence of recurrence CIN III after cervical excision.[38] However, few studies have precisely revealed the mechanism underlying the clinical findings above in cervical precancerous lesions. Additionally, there are no studies about the relevance between PLR and recurrence/residual CIN, especially for studies about HSIL, as mentioned above.

Consistency between the higher PLR and HR-HPV infection was observed in participants with postoperative recurrence/residual CIN, suggesting a correlation between viral infection and SIR. Peripheral lymphocyte activity has been demonstrated to be closely associated with viral infection status. Persistent HR-HPV infection has also been reported as an important predictor for CIN prognosis. The median HPV infection rates for patients with CIN after treatment were approximately 27.0%, 21.0%, 15.0% and 10.0% at the 3-month, 6-month, 12-month and 24-month follow-ups, respectively.[26] HR-HPV infection, including HPV-16, HPV-18, HPV-31, HPV-33, HPV-56, and HPV-58, significantly promotes the progression and recurrence of HSIL according to our previous studies.[10,11] A recent study

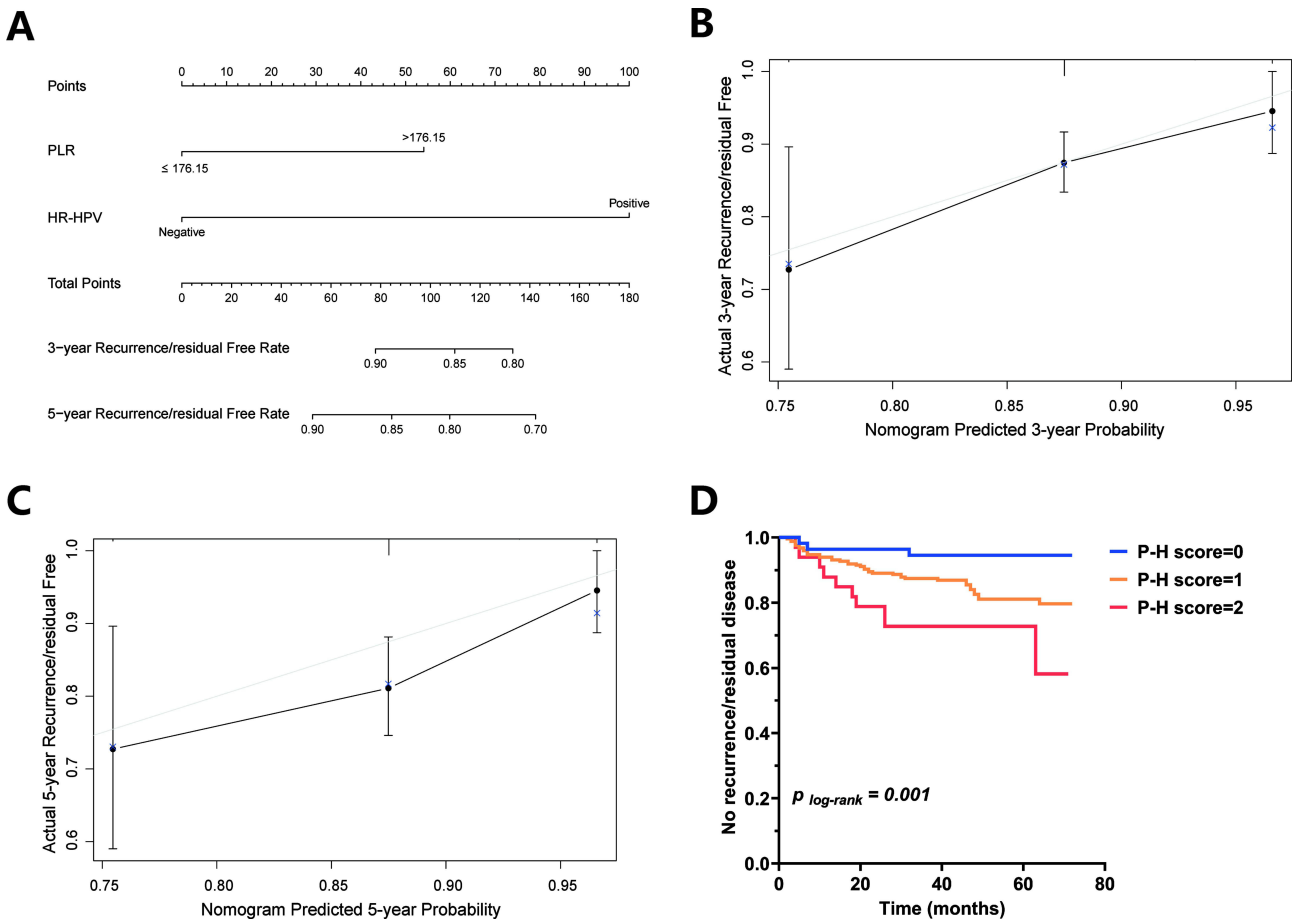

Figure 4 Established models for predicting the prognosis in patients. (**A**) Nomogram for predicting recurrence/residual disease by Cox regression; (**B**) Calibration plot at the 3-year follow-up; (**C**) Calibration plot at the 5-year follow-up; (**D**) Higher PLR & HR-HPV (P-H) score led to worse prognosis at the 5-year follow-up.
Abbreviations: PLR, platelet-to-lymphocyte ratio; HR-HPV, high-risk human papillomavirus.

found significant increases in NLR, MLR, PLR and systemic inflammation response index (SIRI) values in patients with persistent HPV infection after control tests.[39] In some HPV-related malignancies, SIR markers were linked to OS along with HPV infection status. Basically, high values of SIR markers and positive HPV test results are considered adverse prognostic factors.[30,40] Biomarkers associated with the development of malignancies and other nonbenign lesions through immune-relevant pathways include interleukins (ILs) including IL-6, IL-8, and IL-10, tumor necrosis factor α (TNF-α), CD4+/CD8+ T cells, and Toll-like receptors (TLRs), which were confirmed in recent studies.[39] Additionally, it has been reported that SIR inhibits the increase in CD4+/CD8+ T cells in the TME, resulting in the deregulation of cell proliferation, which is essential for the disturbance of the cell cycle.[41] Based on other studies about peripheral SIR markers, our study illustrated the interdependency in PLR and recurrence/residual precancerous lesions, providing supplementary evidence for recent inflammation-carcinoma transformation research. In addition, epidemiological studies indicate that in most parts of countries, the HPV prevalence follows a "double-peak" curve, with the first infection peak representing women aged 17–24 years and the second representing women aged 40–44 years. A similar survey in China concludes that the infection peak for older age at 61–65 years is due to features of population ageing. The susceptibility of younger patients to HPV results mainly from sexual activity, while susceptibility to HPV among elderly patients may result from decreased oestrogen levels, the altered microenvironment of the reproductive tract flora, reduced immunity, and a history of previous deliveries or abortions. All the factors resulting in susceptibility to HPV above are related to SIR or local inflammation in the female lower genital tract. In general, much evidence has indicated the effect of systemic inflammation leading to persistent HPV infection, which ultimately promotes the development of cervical lesions.

Based on previous studies above, we combined SIR status and virus infection as prognostic indicators. Various P-H score groups showed significant difference in accumulative risks for recurrence/residual CIN after surgery. At present, HPV detection combined with cytology tests (colposcopic referral or biopsy if necessary) are still the main contents of follow-up for HSIL patients after excision treatments. Our study suggested that adding the assessment of SIR as part of follow-up measures, or as part of the reference, may be of clinical interests. The HR-HPV detection methods mainly include testing of viral DNA/mRNA, viral load and HPV methylation detection. The previous study revealed that in HPV-associated oropharyngeal squamous cell carcinoma, the higher level of nucleophosmin, which is considered the activator of inflammatory pathways, were significantly related to higher level of HR-HPV E6/E7 mRNA, along with the higher viral load in some cases.[42] Also, the methylation signatures of 5'-cytosine-phosphate-guanosine-3' (CpG) sites were found to be associated with changes of inflammatory or immunologic status in Crohn's disease tissues and the human placenta, in which the NF-κB pathway might participate.[43,44] No study about HPV methylation and SIR has been reported, while it's noted that LAX1 and ZNF582, the biomarkers of methylation detection, has been reported to be probably correlated with difference of transformation from cervical inflammation to carcinoma.[45,46] Applying p16/ki67 dual staining also showed prognostic value in triaging women with HPV infection associated diseases.[47,48] These findings suggested that combining various HR-HPV infection status tests and SIR biomarkers may contribute to disease surveillance and outcome prediction, and even the triage of clinical populations. In patients with high-grade glioma, an prognostic model has been established combining preoperative SIRI, MGMT methylation status, age, resection extent and the tumor number.[49] While there were no relevant studies in recent years for cervical cancer or precancerous lesions. Given the high rate of recurrence/residual disease after surgery of HSIL, the triage may also apply to CIN patients, targeted on the study population.

The 2019 ASCCP risk-based management consensus guideline strongly recommends excisional treatments for HSIL patients as the threshold of treatment in different CIN stages.[3] Compared with other surgeries, the wound scale of LEEP is relatively small, and the intraoperative bleeding can be coagulated by electrocautery without surgical sutures compared with CKC. Additionally, the shorter operation time, less trauma and enhanced recovery are also advantages of LEEP. Therefore, most LEEP treatments for CIN patients are conducted in ambulatory wards considering the effective and adequate utilization of medical resources. Under these circumstances, a novel and simplified prognostic predictor should be applied in clinical practice. The present study has provided a simple index for the prediction of recurrence/residual disease, the effectiveness of which needs to be further studied and verified.

Clinically, the immediate LEEP is not recommended for HSIL patients with genital tract infections mainly containing vaginitis or pathogens detected in cervical secretions, due to the increased risks of infection spread which may result in the acute or chronic endometritis, salpingitis and pelvic inflammatory diseases, as well as increasing the risk of poor wound healing and secondary infertility under surgical procedures.[50,51] The elective surgery after infection control is acceptable Thus, only non-pregnant patients without anomaly in vaginal microecology or leucorrhea routine tests were final included, which may, however, lead to inadequate discussion between local/systemic inflammation and the state of vaginal microenvironment. Studies have indicated the complex interactions between microbiota, HPV, inflammation and cervical cancer.[52] And our previous researched found that Prevotella overgrowth in the vagina may influence the development of cervical lesions associated with persistent HR-HPV infection through host NF-κB and C-myc signaling pathways.[53] And sialidase secreted by Gardnerella and Prevotella may promote in the progression from HPV primary infection to cervical lesions.[54] While no recent study has indicated a significant correlation between PLR and vaginal microecology. And only the NLR level has been reported to be higher in bacterial vaginosis patients.[55] This may be due to the fact that bacterial or fungal infections do not usually cause significant changes in platelet and lymphocytes counts compared with neutrophils, suggesting that the specificity of PLR may be higher than that of other SIRI in prognostic prediction of CIN, which are mostly associated with HPV persistent infection. Indeed, the correlation between PLR and other microecological indicators needs to be further studied.

Clearly, the univariate logistic analysis indicated an adequate association between recurrence/residual disease and variables including HR-HPV infection and PLR, while the multivariate logistic analysis did not show corresponding results, which might be due to the lack of time-dependent information (RFS) for analysis. Based on the above, we conducted further analysis with the Cox regression model, taking into account the survival-relevant data, and the results strongly supported the

conclusions in our study (Figure 3). The calibration plot for the 3-year follow-up showed a better model-fitting effect than that for the 5-year follow-up (Figure 4), with the same upwards trend for predicting recurrence/residual CIN. We attribute this to the inclusion of the censored value in the study, given that the censored value provided a nonnegligible reference of survival-relevant data. The results combining the multiple model validation above further supported our findings in this study.

There are some other limitations in this study. First, potential selection bias might exist because this is a retrospective study in which the participants were selected from the disease screening cohort in Fujian Province, China. Second, confounding factors such as smoking, drinking or region were not included in the final analysis, which might influence the SIR status and demographic characteristics before LEEP treatment. Thus, more prospective multi-centre studies and the establishment of prognosis-predicting models are needed in the future for further research. Third, the follow-up time span for some participants was less than 5 years, so we used different regression models and correlation analyses for calibration to obtain more accurate results.

Conclusion

In conclusion, our study suggests that the preoperative PLR level and HR-HPV infection could be available markers for predicting recurrence/residual disease of HSIL after LEEP. Clinically, combining PLR with HR-HPV tests may provide novel evaluation method for predicting and managing the recurrence/residual disease of HSIL patients after LEEP treatment.

Data Sharing Statement

The datasets generated during and/or analysed during the current study are not publicly available due to the restrictions to medical records and patient privacy, but are available from the corresponding author on reasonable request.

Ethics Approval and Informed Consent

This study was approved by the Ethics Review Committee of Fujian Maternity and Child Health Hospital, Affiliated Hospital of Fujian Medical University, China (2020KY015). The procedures followed were in accordance with the ethical standards of the Declaration of Helsinki of the WHO. and all individuals participating in this study provided written informed consent.

Consent for Publication

All authors reviewed the article and agreed to submit the manuscript to this journal for publication.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

No potential conflict of interest relevant to this article was declared.

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