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Prognostic Prediction for Recurrent/Residual CIN in HSIL Patients After Conization: An Updated Retrospective Study Based on Ambulatory Surgery

Guanxiang Huang[1–3,*], Wenyu Lin[1–3,*], Hangjing Gao[1–3], Yuan Ren[1–3], Jun Shen[4], Shuxia Xu[5], Dabin Liu[6], Yuanjun Cai[6], Chengbin Lin[6], Xite Lin[1–3], Tingting Jiang[1–3], Binhua Dong[1–3], Pengming Sun[1–3]

[1]College of Clinical Medicine for Obstetrics & Gynecology and Pediatrics, Fujian Medical University, Fuzhou, Fujian, 350001, People's Republic of China; [2]Fujian Key Laboratory of Women and Children's Critical Diseases Research, Fujian Maternity and Child Health Hospital (Fujian Women and Children's Hospital), Fuzhou, Fujian, 350001, People's Republic of China; [3]Fujian Clinical Research Center for Gynecological Oncology, Fujian Maternity and Child Health Hospital (Fujian Obstetrics and Gynecology Hospital), Fuzhou, Fujian, 350001, People's Republic of China; [4]Fujian Provincial Cervical Disease Diagnosis and Treatment Health Center, Fujian Maternity and Child Health Hospital, Fuzhou, Fujian, 350001, People's Republic of China; [5]Department of Pathology, Fujian Maternity and Child Health Hospital, Fuzhou, Fujian, 350001, People's Republic of China; [6]Department of Gynecology, Fujian Maternity and Child Health Hospital, Fuzhou, Fujian, 350001, People's Republic of China

*These authors contributed equally to this work

Correspondence: Pengming Sun; Binhua Dong, Laboratory of Gynecologic Oncology, Department of Gynecology, Fujian Maternity and Child Health Hospital, College of Clinical Medicine for Obstetrics & Gynecology and Pediatrics, Fujian Medical University, Fuzhou, Fujian, 350001, People's Republic of China, Tel +86-591-87558732; +86-13599071900, Fax +86-591-87551247, Email fmsun1975@fjmu.edu.cn, sunfemy@hotmail.com; dongbinhua86@fjmu.edu.cn, dbh18-jy@126.com

Background: There are currently few prognostic models for conization in patients with high-grade squamous intraepithelial lesion (HSIL) because it is a rapid procedure that typically collects less case information. The present study aimed to establish a rapid/accurate postoperative prognostic assessment model for these patients.

Methods: This study included 631 nonpregnant participants with HSIL confirmed by histopathology from January 2015 to January 2018. The recurrent/residual cervical intraepithelial neoplasia (CIN) were divided into residual CIN, simple recurrent CIN and recurrent CIN accompanied with CIN progression. The recurrence/residual-free survival (RFS) time was defined as the time span from the time of surgery (baseline) until the first lesion of CIN was detected or the 1-/3-/5-year follow-up endpoint was reached.

Results: After LASSO regression selection, the higher platelet-to-lymphocyte ratio (PLR) (OR = 1.006, p = 0.002), positive margin status (OR = 2.451, p = 0.021), HPV-16 (OR = 4.414, p < 0.001), −18 (OR = 3.040, p = 0.009), −56 (OR = 10.715, p=0.021), and non-HR-HPV (OR = 2.487, p = 0.028) infection showed significant difference in the Logistic model. And HPV-16 infection (OR = 6.159, p = 0.001) could promote recurrent CIN accompanied with CIN progression. In multivariate Cox regression models, the higher PLR (HR = 1.005/1.005/1.005, p = 0.020/0.002/0.003) and HPV-16 infection (HR = 2.758/2.836/2.674, p < 0.001) showed statistical difference during 1-/3-/5-year follow-up. While gland invasion (p = 0.081), margin status (p = 0.075) and HPV infection genotype (p = 0.150) did not showed statistical difference in multivariate Cox regression models based on LASSO regression. And gland invasion (p = 0.251/0.686) and HPV-58 infection (p = 0.148/0.813) also showed no statistical difference in optimized Logistic regression models.

Conclusion: HPV-16, −18, −56 and non-HR-HPV infection status can be considered as indicators for recurrent CIN during the 5-year follow-up, especially for HPV-16 infection, which also lead to a CIN recurrence accompanied with disease progression. And the preoperative PLR level, gland invasion, positive margin may be predictors for recurrent/residual CIN during 1-, 3- and 5-year follow-up.

Keywords: cervical intraepithelial neoplasia, higher platelet-to-lymphocyte ratio, human papillomavirus, conization, prognosis

Introduction

Cervical cancer and related precancerous lesions seriously endanger female health globally. Although there was a 65% drop in cervical cancer incidence during 2012 through 2019 among women in their early 20s due to the widespread of screening and

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vaccination, about 13,960 new cases and 4310 deaths from cervical cancer were still estimated in the United States in 2023, compared with a number of 604,000 in new cases and 342,000 in dead cases from cervical cancer worldwide in 2020.[1,2] Precancerous cervical lesions precede the development of cervical cancer by 10–20 years. To guide appropriate management strategies for precancerous lesions, Bethesda classification and histopathology are utilized. According to the Bethesda system, different stages of dysplasia (mild, moderate, severe) are classified as low-grade squamous intraepithelial lesions (LSIL) and high-grade squamous intraepithelial lesions (HSIL) based on cytology terminology. In histopathology, the progression to cervical cancer typically involves stages of CIN, such as CIN I, CIN II, and CIN III. According to the Lower Anogenital Squamous Terminology (LAST) system, CIN I is classified as LSIL, while CIN II and CIN III are classified as HSIL.[3,4] The human papillomavirus (HPV) infection is considered to be the primary pathogenic factor of cervical lesions. A total of 16 HPV genotypes (HPV-16, −18, −31, −33, −35, −39, −45, −51, −52, −56, −58, −59, −66, −68, −73 and −82) are defined as high-risk human papillomavirus (HR-HPV), suggesting a higher risk for carcinogenesis.[5] According to the 2019 American Society for Colposcopy and Cervical Pathology (ASCCP) Risk-Based Management Consensus Guidelines for Abnormal Cervical Cancer Screening Tests and Cancer Precursors, surgery was highly recommended for treatment of histologic HSIL patients except in special conditions, namely, CIN II in age less than 25 years old or pregnancy.[4] And there are also some options for medical treatment of CIN II or CIN III lesions in frail patients who might be unable to tolerate surgical treatments, including electric ironing, freezing, laser therapy and so on.[6] Before physical therapy, doctors will usually conduct detailed examination and diagnosis to ensure that patients do not have invasive cancer and other diseases, which is also the basic principle to ensure the therapeutic effect. Immunotherapy including HPV therapeutic vaccines, immune checkpoint inhibitors and advanced adoptive T cell therapy were also mentioned.[7]

Cervical conization plays a crucial role in the long-term management of CIN by effectively removing abnormal cells from the cervix, reducing the risk of CIN recurrence, and aiding in the clearance of HPV infection.[8] This procedure helps in preventing the progression of CIN to more severe stages of cervical cancer, ensuring better outcomes for patients in the long term.[9,10] Conization mainly contains cold knife conization (CKC), large loop excision of the transformation zone (LLETZ) or loop electrosurgical excision procedure (LEEP). Generally, LEEP and LLETZ could be considered as different names for the same procedure. CKC is a classically surgical method for the treatment of CIN. Its greatest advantage is that the operation does not produce thermal injury, has little impact on the tissue, and can retain complete tissue specimens, which is helpful for pathological diagnosis. LEEP/LLETZ has the advantages of simple operation, fewer postoperative complications, lower cost of treatment and preservation of cervical morphology. In summary, conization surgery is widely used in outpatient operating rooms, day surgery wards and other medical scenarios, because it can ensure that the length of stay of patients is reduced with less trauma and thus improve the utilization of medical resources.

However, there is an estimated recurrence rate of 5.3–12.0% in CIN after conization, with a median follow-up time of 10 years and a maximum follow-up time of 16 years.[9,10] Also, the recurrence rate of CIN was significantly correlated with preoperative HPV infection and the severity of CIN according to a systemic review.[9] Intensive follow-up surveillance and timely clinical intervention are very important for residual, recurrent and even developed lesions of CIN. In fact, only blood routine tests, coagulation function screening, and hepatic and renal function tests are conducted before the operation. Most conizations are performed in ambulatory departments in China, and this results in difficulties with follow-up and a large number of lost individuals. Therefore, identifying an accurate indicator that can pinpoint women at greater risk of recurrent CIN and/or future malignancy following treatment for cervical pre-cancer could enable tailored management according to the woman's individual risk, thereby avoiding over-treatment and reducing patient anxiety.

Our previous study demonstrated the correlation between the systematic inflammation response index (SIRI) and the prognosis of HSIL patients after LEEP. A higher PLR value suggested a poorer prognosis (recurrent or residual lesions) in HSIL patients combined with HR-HPV infection and gland invasion.[11] While in previous studies, we did not refine the stratification of infection indicators and the specific HPV genotype, which can lead to the limitation in study results. Given the findings above, combining SIRI and specific HPV infection may be the proper strategy. However, no relevant study was found both at home and abroad.

This study aims to assess the prognostic predictive factors for HSIL patients after conization, mainly including SIRI and HPV infection status, and to validate the efficiency of regression model in the previous study,[11] in order to provide novel predictive strategies for HSIL patients treated basically in ambulatory departments.

Materials and Methods

Participants and Study Design

A total of 704 participants from the Department of Gynecology in Fujian Maternity and Child Health Hospital was recruited from January 2015 to January 2018. The inclusion criteria was defined as: (1) unpregnant women with preoperative diagnosis of CIN II or CIN III confirmed by histopathology; (2) patients underwent cervical conization. Fifty-nine participants were excluded as the exclusion criteria was defined as: (1) history of cervical or vaginal lesions; (2) history of systematic diseases, other malignancies, sexually transmitted diseases or immune dysfunction; (3) history of initial hysterectomy, cervical treatment or chemotherapy; (4) sexual activity or abnormal results for vaginal micro-ecology or leucorrhea routine tests within 1 week before the operation; (5) reproductive tract treatment within 1 week. All individuals were under the 1-/3-/5-year follow-up and at each follow-up endpoint the data was recorded. During the follow-up, 14 participants were eliminated for: (1) missing data; (2) invalid specimens; (3) loss to follow-up; (4) required withdrawn from this study. Finally, 631 participants with complete data were included for statistical analysis (a p value of <0.05 indicates a statistical significance under all situations in this study). Blood samples were collected within 1 week before the operation and were analyzed using semiconductor laser flow cytometry.[12] And the HR-HPV DNA test and liquid-based cytology test (TCT) were conducted within 3 months before conization. Participants were followed up every 3–6 months after surgery. And the follow-up content mainly contained: (1) HR-HPV DNA test results during 1-/3-/5-year follow-up; (2) TCT results during 1-/3-/5-year follow-up; (3) histopathological results under colposcopy if necessary based on 2019 ASCCP guidelines.[4] The last follow-up was conducted at January 16th, 2024. And the study endpoint were set at 1-/3-/5-year from the baseline, which was defined as the date of surgery for any individual, respectively. 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.

PCR-RDB HR-HPV DNA Test

Polymerase chain reaction-reverse dot blot (PCR-RDB) was used for the detection of HR-HPV, which totally includes 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). Non-HR-HPV infection refers to other HPV genotype infection except for HR-HPV infection mentioned above. Procedures were strictly performed according to the manufacturer's instructions.[13]

Liquid-Based Cytology

The results for cytology tests were collected from an auto-imaging system (Hologic, Inc., San Diego, CA, USA). ThinPrep Cytologic Test (TCT) findings were assessed by two independent pathologists or, in the event of disagreement between the two pathologists, by a pathologist with a higher professional title. The Bethesda system were applied for classification of cytology test results.[14,15]

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.[16,17] When the pathology was not clear, it was evaluated by a pathologist with a higher professional title until a clear histological diagnosis was obtained.

Relevant Definitions During Follow-Up

PLR was defined as the absolute platelet count relative to the absolute lymphocyte count. Neutrophil-to-lymphocyte ratio (NLR) was defined as the absolute neutrophil count relative to the absolute lymphocyte count. Lymphocyte-to-monocyte ratio (LMR) was defined as the absolute lymphocyte count relative to the absolute monocyte count. Recurrent or residual CIN after surgery was defined as CIN or worse lesions confirmed by histopathology after conization. A residual CIN was defined as CIN I found within 12 months after conization. The definition of recurrent CIN were divided into 2 classifications: (1) simple recurrent CIN, which was defined as the lower or same grade of CIN II+ (lesions of CIN II or worse) found after conization compared with preoperative histopathological confirmation, or CIN I found more than 12 months after conization; (2) recurrent CIN accompanied with CIN progression, which was defined as the higher grade of CIN II+ found after conization, compared with preoperative CIN, within or more than 12 months after surgery. Principally, higher grades of lesions in the cervix detected after conization were considered progression in residual lesions. No recurrent/residual CIN found after conization was defined as completely negative results of cervical biopsy under colposcopy. The recurrence/residual-free survival (RFS) time was defined as the time span from the time of surgery (baseline) until the first lesion of CIN was detected or the 1-/3-/5-year follow-up endpoint was reached.

Statistical Analysis

SPSS v25.0 was used for general data analysis. The markers from blood samples, HPV infection status and other pathological 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 and multivariate Cox regression model has been established and the hazard ratio (HR) was calculated to select probable predictors for recurrent/residual CIN after conization, along with a LASSO regression model and Logistic regression model. The nomogram was plotted by R software v4.2.1. A log-rank analysis was performed and the forest plots for regression models above and the Kaplan–Meier curves were plotted both by GraphPad Prism 9.5.0 and the R software v4.2.1. A C-index was calculated for any Cox regression model built in this study. Besides, the optimal cut-off value for PLR (176.15) was calculated using X-tile software. A p value of <0.05 indicates a statistical significance.

Results

Clinical Characteristics of Patients During Follow-Up

A total of 631 patients finally included in the study were under a complete 1-/3-/5-year follow-up. At the time of 1-/3-/5-year the follow-up endpoint were set respectively and the relevant clinical information was collected (Figure 1). At the 1-year follow-up endpoint, there were 582 patients with no recurrent/residual CIN after conization, 16 patients with CIN I, 11 patients with CIN II and 22 patients with CIN III+ (lesions of CIN III or worse). At the 3-year follow-up endpoint, 544 cases with no recurrent/residual CIN, 33 cases with CIN I, 20 cases with CIN II and 34 cases with CIN III+ were recorded. And 535 patients without recurrent/residual CIN, 40 patients with CIN I, 21 patients with CIN II and 35 patients with CIN III+ were detected, respectively, at the 5-year follow-up endpoint. In general, there were 16 cases with residual CIN, 69 cases with simple recurrent CIN and 16 cases with recurrent CIN accompanied with the CIN progression, when the follow-up has been performed for over 5 years.

Pearson's test and mono-factor analysis (including unpaired *t*-test and nonparametric test) were applied in comparison for baseline data. The 5-year follow-up endpoint was set as the main outcome for patients. Under the 5-year follow-up, the median age was 41 (24–67) in the 5-year recurrence/residual CIN group, compared with a median age of 39 (21–71) in the 5-year no recurrence/residual CIN group ($p = 0.111$). The margin status ($p = 0.022$), preoperative HR-HPV infection ($p=0.007$), and preoperative HR-HPV genotype (None or simple compared with double or more, $p < 0.001$) showed statistical significance between different groups (Table 1). No significant differences were found among the blood cell analysis index including white blood cell, neutrophil count, lymphocyte count, monocyte count, eosinophil count, basophil count and red blood cell. While there is significant difference in PLR ($p = 0.012$) between different groups (Table 1). Based on findings above, an optimal cut-off of 163.46 for PLR was calculated as there was statistical significance for area under curve (AUC) of PLR (Supplementary Table 1).

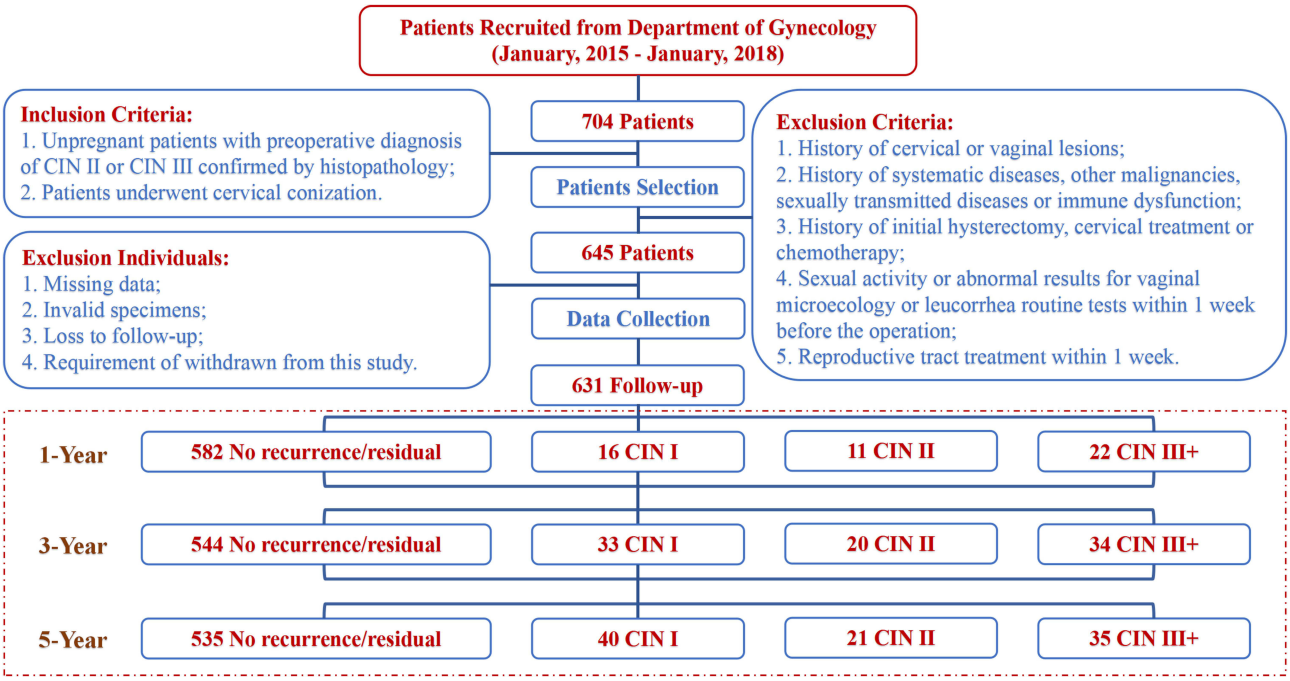

Figure 1 Flow chart of the study.
Abbreviation: CIN, cervical intraepithelial neoplasia.

SIRI and HPV Infection Status at Each Endpoint

The Kaplan-Meier curves were plotted at different follow-up endpoints to select the probably related factors for predicting recurrent/residual CIN after conization. A higher PLR (PLR > 163.46) suggested a higher recurrence/residual

Table 1 Clinicopathologic Characteristics of Patients

Items	Number of Participants		
	5-Year Recurrence/ Residual CIN[a]	5-Year No Recurrence/ Residual CIN[b]	P value[c]
Age (years)	41 (24–67)	39 (21–71)	0.111
Preoperative histopathology			
≤ CIN II	55	319	0.668
> CIN II	41	216	
Preoperative cytology			
NILM	24	135	0.961
≥ ASC-US	72	400	
Gland invasion status			
No invasion	29	213	0.075
Invasion	67	322	
Margin Status			
Margin negative	83	499	0.022
Margin positive	13	36	
Preoperative HR-HPV infection			
No infection	7	99	0.007
Infection	89	436	

(*Continued*)

Table 1 (Continued).

Items	Number of Participants		
	5-Year Recurrence/ Residual CIN[a]	5-Year No Recurrence/ Residual CIN[b]	P value[c]
Preoperative HR-HPV genotype			
None or single	67	453	<0.001
Double or multiple	29	82	
Blood routine test index (×10^9/L)			
White blood cell	6.39 (3.25–12.57)	6.11 (3.00–13.95)	0.543
Neutrophil count	3.65 (1.26–10.76)	3.55 (0.81–12.75)	0.498
Lymphocyte count	1.88 (0.82–3.81)	1.95 (0.39–4.10)	0.225
Monocyte count	0.41 (0.19–0.78)	0.41 (0.05–1.03)	0.678
Eosinophil count	0.08 (0.01–0.55)	0.07 (0.00–0.97)	0.254
Basophil count	0.02 (0.00–0.08)	0.03 (0.00–0.09)	0.140
Red blood cell	4.34 (3.31–6.76)	4.35 (2.47–6.09)	0.654
Systematic inflammation index			
Platelet-to-lymphocyte ratio	128.29 (64.57–375.23)	117.86 (6.88–496.30)	0.012
Neutrophil-to-lymphocyte ratio	4.57 (1.14–12.74)	4.73 (0.80–44.60)	0.309
Lymphocyte-to-monocyte ratio	1.94 (0.77–9.61)	1.78 (0.55–24.06)	0.146

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 or carcinoma 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.

rate during the 1- (p = 0.032), 3- (p = 0.001) and 5-year (p = 0.001) follow-up. Among all HPV genotypes, HPV-16 infection was correlated with the poorer prognosis at the endpoint of 1- (p < 0.001), 3- (p < 0.001), 5-year (p < 0.001) follow-up. Besides, HPV-56 (p = 0.004) and non-HR-HPV (the other HPV genotype except for HR-HPV) (p = 0.008) infection were considered the predictors for recurrent/residual CIN during the 5-year follow-up, along with the positive margin status (p = 0.019) confirmed by histopathology (Figure 2). Also, the double or multiple HPV genotype infection significantly suggested the higher recurrent/residual CIN rate after surgery at the 3- (p = 0.009) and 5-year (p = 0.001) follow-up. In addition to SIRI and HPV infection status, pathological conditions also lead to different prognosis. The gland invasion significantly promotes the recurrence/residual disease during the 1-year (p=0.018) and 3-year (p = 0.042) follow-up (Figure 2).

Cox Regression Model for Predictors of Recurrent/Residual CIN

Univariate and multivariate Cox regression analysis were both performed. The univariate Cox regression analysis showed that under the 1-year follow-up the higher PLR value (p = 0.026), gland invasion (p=0.021), and HPV-16 infection (p = 0.001) suggested a poorer prognosis of HSIL patients after conization. While under the 3-year follow-up the PLR value (p = 0.005), the number of HPV genotype (p = 0.010), gland invasion (p = 0.045), HPV-16 infection (p < 0.001) showed significant difference. And during the 5-year follow-up, the higher PLR value (p = 0.005), the larger number of HPV genotype (p = 0.001), positive margin status (p = 0.022), HPV-16 infection (p < 0.001), HPV-56 infection (p=0.009), and non-HR-HPV infection (p = 0.009) led to a higher recurrent/residual disease rate (Table 2).

The multivariate Cox regression analysis results were shown by the forest plots. During the 1-, 3- and 5-year follow-up, respectively, the higher PLR (HR = 1.005, p = 0.020; HR = 1.005, p = 0.002; HR = 1.005, p = 0.003) and HPV-16 infection (HR = 2.758, p < 0.001; HR = 2.836, p < 0.001; HR = 2.674, p < 0.001) showed accuracy for predicting recurrent/residual CIN. And the gland invasion showed statistical significance during the 1-year and 3-year follow-up, with the HR of 2.212 (p = 0.020) and 1.627 (p = 0.040). While during the 5-year follow-up, the positive margin status

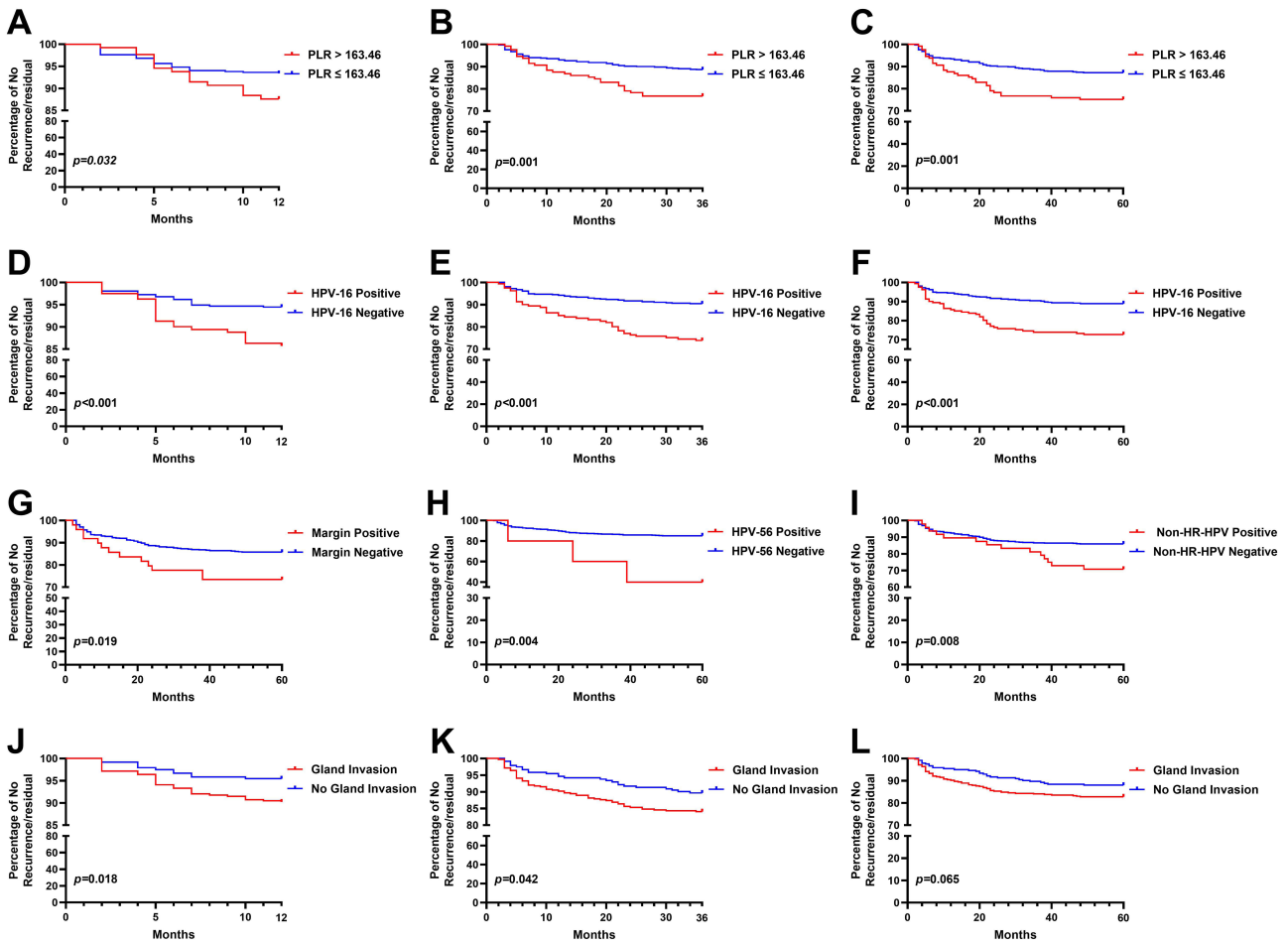

Figure 2 Kaplan–Meier curves for recurrence/residual CIN: (**A**) The higher PLR for 1-year follow-up; (**B**) The higher PLR for 3-year follow-up; (**C**) The higher PLR for 5-year follow-up; (**D**) HPV-16 infection for 1-year follow-up; (**E**) HPV-16 infection for 3-year follow-up; (**F**) HPV-16 infection for 5-year follow-up; (**G**) Margin status for 5-year follow-up; (**H**) HPV-56 infection for 5-year follow-up; (**I**) Non-HR-HPV infection for 5-year follow-up; (**J**) Gland invasion for 1-year follow-up; (**K**) Gland invasion for 3-year follow-up; (**L**) Gland invasion for 5-year follow-up.

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

suggested the higher recurrence/residual rate instead of the gland invasion status (p = 0.040) (Figure 3). The corresponding nomogram was plotted, with the C-index of 0.674, 0.682 and 0.667 for the models built during 1-, 3- and 5-year follow-up (Figure 3). The calibration plots were also shown (Supplementary Figure 1).

Table 2 Univariate Cox Regression Analysis of Predictors for Recurrence/Residual CIN[a]

Items	1-Year Follow-up Endpoint		3-Year Follow-up Endpoint		5-Year Follow-up Endpoint	
	HR (95% CI)	P value[b]	HR (95% CI)	P value	HR (95% CI)	P value
PLR	1.005 (1.001–1.009)	0.026	1.004 (1.001–1.008)	0.005	1.004 (1.001–1.007)	0.005
HPV Genotype	1.712 (0.908–3.229)	0.096	1.852 (1.157–2.964)	0.010	2.121 (1.372–3.279)	0.001
Gland Invasion	2.197 (1.123–4.299)	0.021	1.607 (1.010–2.557)	0.045	1.502 (0.971–2.322)	0.067
Margin Status	2.020 (0.908–4.497)	0.085	1.816 (0.965–3.417)	0.065	1.983 (1.105–3.559)	0.022
HPV-16	2.659 (1.517–4.661)	0.001	2.947 (1.935–4.488)	<0.001	2.699 (1.806–4.033)	<0.001
HPV-18	0.621 (0.151–2.558)	0.510	1.482 (0.716–3.066)	0.289	1.724 (0.895–3.318)	0.103
HPV-31	0.812 (0.112–5.886)	0.837	1.407 (0.445–4.450)	0.561	1.720 (0.632–4.680)	0.288
HPV-33	1.671 (0.520–5.374)	0.389	1.242 (0.456–3.389)	0.671	1.122 (0.412–3.053)	0.822

(*Continued*)

Table 2 (Continued).

Items	1-Year Follow-up Endpoint		3-Year Follow-up Endpoint		5-Year Follow-up Endpoint	
	HR (95% CI)	P value[b]	HR (95% CI)	P value	HR (95% CI)	P value
HPV-35	1.219 (0.168–8.828)	0.845	0.673 (0.094–4.829)	0.693	1.859 (0.589–5.868)	0.291
HPV-39	1.497 (0.207–10.843)	0.690	0.823 (0.115–5.910)	0.846	0.741 (0.103–5.312)	0.765
HPV-45	0.049 (0.000-NaN)	0.642	0.049 (0.000-NaN)	0.528	0.049 (0.000-NaN)	0.505
HPV-51	1.903 (0.592–6.120)	0.280	1.832 (0.743–4.520)	0.189	1.658 (0.674–4.078)	0.271
HPV-52	1.228 (0.552–2.734)	0.615	1.325 (0.735–2.389)	0.349	1.291 (0.732–2.275)	0.378
HPV-56	2.693 (0.372–19.513)	0.327	3.239 (0.797–13.165)	0.100	4.605 (1.458–14.545)	0.009
HPV-58	1.298 (0.583–2.888)	0.523	1.672 (0.959–2.916)	0.070	1.613 (0.943–2.759)	0.081
HPV-59	0.049 (0.000-NaN)	0.642	2.340 (0.576–9.508)	0.235	2.168 (0.534–8.800)	0.279
HPV-66	0.049 (0.000-NaN)	0.704	1.806 (0.252–12.969)	0.557	1.656 (0.231–11.876)	0.616
HPV-68	0.049 (0.000-NaN)	0.615	0.049 (0.000-NaN)	0.495	0.049 (0.000-NaN)	0.472
HPV-73	0.005 (0.000-NaN)	0.850	0.050 (0.000-NaN)	0.797	0.050 (0.000-NaN)	0.786
HPV-82	0.049 (0.000-NaN)	0.742	0.049 (0.000-NaN)	0.656	0.049 (0.000-NaN)	0.638
Non-HR-HPV	1.379 (0.547–3.478)	0.496	1.419 (0.712–2.828)	0.321	2.122 (1.204–3.741)	0.009

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, and no recurrence/residual CIN was defined as no CIN or carcinoma found after operation; [b]A p value<0.05 indicates a statistically significance; references during analysis were set as negative gland invasion, negative margin status and negative HPV infection, and reference of HR-HPV genotype was set as none or single HR-HPV infection.
Abbreviations: CIN, cervical intraepithelial neoplasia; HR-HPV, high-risk human papillomavirus; PLR, platelet-to-lymphocyte ratio; HR, hazard ratio; 95% CI, 95% confidence interval.

LASSO Regression Selecting HPV-Genotype and Comprehensive Factors

In order to exclude the effect of collinearity caused by HPV co-infection status, the LASSO regression model was established to select factors related to 5-year recurrent/residual CIN combing comprehensive and focal conditions. When only HPV genotypes were included, HPV-16, −18, −56, −58 and non-HR-HPV were selected with the coefficient of 0.105, 0.004, 0.139, 0.014 and 0.048, respectively. While combining with PLR and histopathological factors, the gland

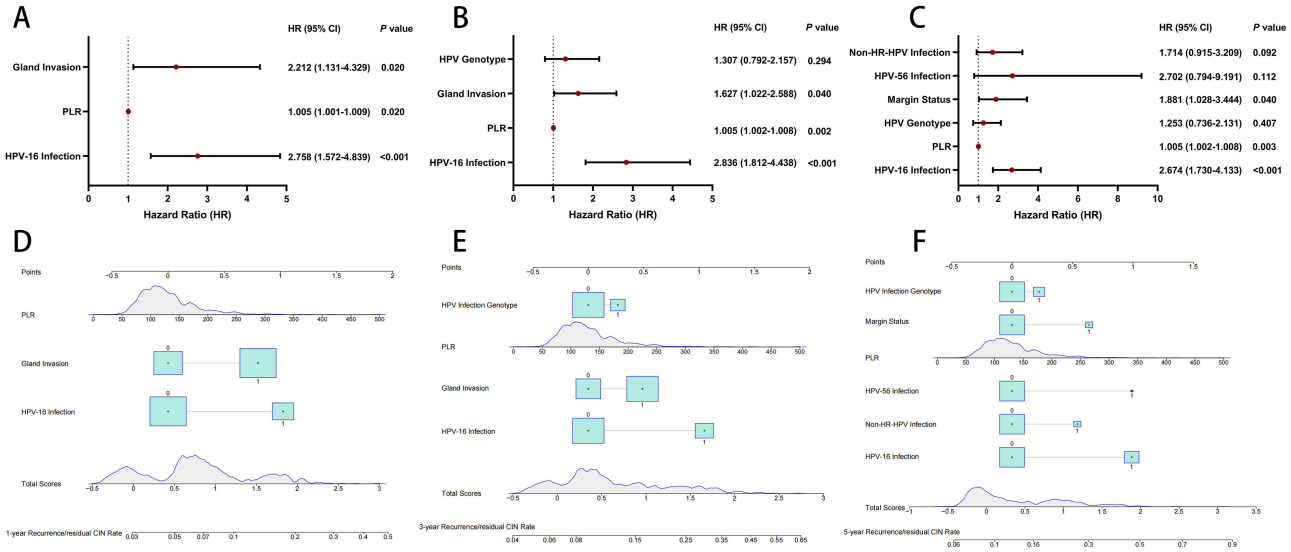

Figure 3 The multivariate Cox regression analysis during the follow-up. (**A**) The forest plot of HR for recurrence/residual disease at the 1-year follow-up; (**B**) The forest plot of HR for recurrence/residual disease at the 3-year follow-up; (**C**) The forest plot of HR for recurrence/residual disease at the 5-year follow-up; (**D**) The nomogram for recurrence/residual disease at the 1-year follow-up; (**E**) The nomogram for recurrence/residual disease at the 3-year follow-up; (**F**) The nomogram for recurrence/residual disease at the 5-year follow-up.
Notes: 0 represents for negative testing results or none/single HPV genotype, and 1 represents for positive testing results or double/multiple HPV genotype.
Abbreviations: HR, hazard ratio; 95% CI, 95% confidence interval; PLR, platelet-to-lymphocyte ratio; HR-HPV, high-risk human papillomavirus.

invasion (coefficient = 0.023), margin status (coefficient = 0.052), HPV genotype (coefficient = 0.002), HPV-16 (coefficient = 0.132), −18 (coefficient = 0.055), −52 (coefficient = 0.018), −56 (coefficient = 0.243), −58 (coefficient = 0.052), −59 (coefficient = 0.046), −68 (coefficient = −0.031), non-HR-HPV (coefficient = 0.076), and PLR (coefficient < 0.001) were selected (Figure 4).

Further Regression Models Based on LASSO Regression Results

Combining results above (Table 2 and Figure 4), the further and optimized Cox regression model was built for 5-year follow-up, which showed a statistical difference for higher PLR (HR = 1.005, p = 0.003), HPV-16 infection (HR = 3.154, p < 0.001), HPV-18 infection (HR = 2.144, p = 0.034), HPV-56 infection (HR = 3.728, p = 0.039), HPV-58 infection (HR = 2.118, p=0.013), and non-HR-HPV infection (HR = 2.139, p = 0.020) (Table 3).

Our previous study showed no correlation between the specific HR-HPV infection and recurrence/residual CIN.[11] In the present study, based on the LASSO optimized model, the univariate Logistic regression showed the odds ratio (OR) of 1.006, 2.452, 2.458, 3.776, 2.481, 10.695 and 2.861 for higher PLR (p = 0.004), positive margin status (p = 0.012), double or multiple HPV infection genotypes (p = 0.001), HPV-16 infection (p < 0.001), HPV-18 infection (p = 0.019), HPV-56 infection (p = 0.010) and non-HR-HPV infection (p = 0.003), respectively, in the prediction for recurrent CIN. While only HPV-16 infection (OR = 6.159, p = 0.001) showed statistical difference in distinguishing the recurrent CIN accompanied with CIN progression and other lesions (Table 4). Under the multivariate logistic regression analysis, the higher PLR (OR = 6.159, p = 0.001), positive margin (OR = 2.451, p = 0.021), HPV-16 infection (OR = 4.414, p < 0.001), HPV-18 infection (OR = 3.040, p = 0.009), HPV-56 infection (OR = 10.715, p = 0.021) and non-HR-HPV infection (OR = 2.487, p = 0.028) were considered to be risk factors for recurrent CIN prediction during the 5-year follow-up (Figure 4), with an Akaike information criterion (AIC) of 506.819.

Discussion

The present study analyzed probably related predictors for recurrent/residual CIN after conization in HSIL patients. In addition to HPV infection, particularly HR-HPV infection, the positive gland and margin status confirmed by pathology indicated the more extensive intraepithelial neoplasia and residual virus infection than before operation, thus leading to a poorer prognosis. Clinically, a considerable number of conization operations can be completed in the outpatient operating room or day ward (ambulatory surgery) because this operation causes the smaller wound, lower cost and faster wound healing. Therefore, the preoperative workup for conization is relatively less complete. Research reported a recurrence/residual rate of 6.6% in CIN II+, 6.1% in CIN III and 9.0% in adenocarcinoma in situ (AIS).[10,18] Postoperative monitoring and management are of great clinical significance. Due to less baseline information, a novel and precise predicting model should be established. Our previous study indicated the prognosis predicting value of PLR,[11] which was consistent with the findings in the present study. Study also showed that NLR and total white blood cell (WBC) count might be prognostic factors involved in the prediction of recurrence in CIN patients underwent excisional procedure.[19] While in the present study, NLR (p = 0.150/0.093/0.146) and LMR (p = 0.782/0.291/0.309) values did not show statistical difference in predicting recurrence/residual CIN. Combining the findings above, only PLR was included in final analysis. Thus, the clinical prognostic factors were selected combining SIRI, HPV infection and pathological conditions in the present study, which is not only a validation of the previous study finding but also the establishment for novel models, based on ambulatory surgery.

A meta-analysis including 11 studies with 3065 patients revealed that the positive endocervical margins suggested risk for recurrence/residual disease after CIN excision. The rate of residual/recurrence with positive margins was significantly higher than in that of patients with negative ones, with an OR of 3.99 (p < 0.001).[20] While few studies reported the clinical efficiency of gland invasion found before surgery by colposcopy. Most studies considered the HPV infection and positive margin status as indicators of poor prognosis.[8,21] The present study found that the margin positivity was correlated with recurrent/residual CIN during the 5-year follow-up, and gland invasion could predict recurrent/residual CIN for 1-year and 3-year surveillance. From the perspective of pathological detection, the clinical predictive efficiency of preoperative TCT is not accurate enough compared with gland invasion and margin status. This may be due to the fact that the cytology examination is limited to the surface of the cervix and part of the internal orifice of the cervix

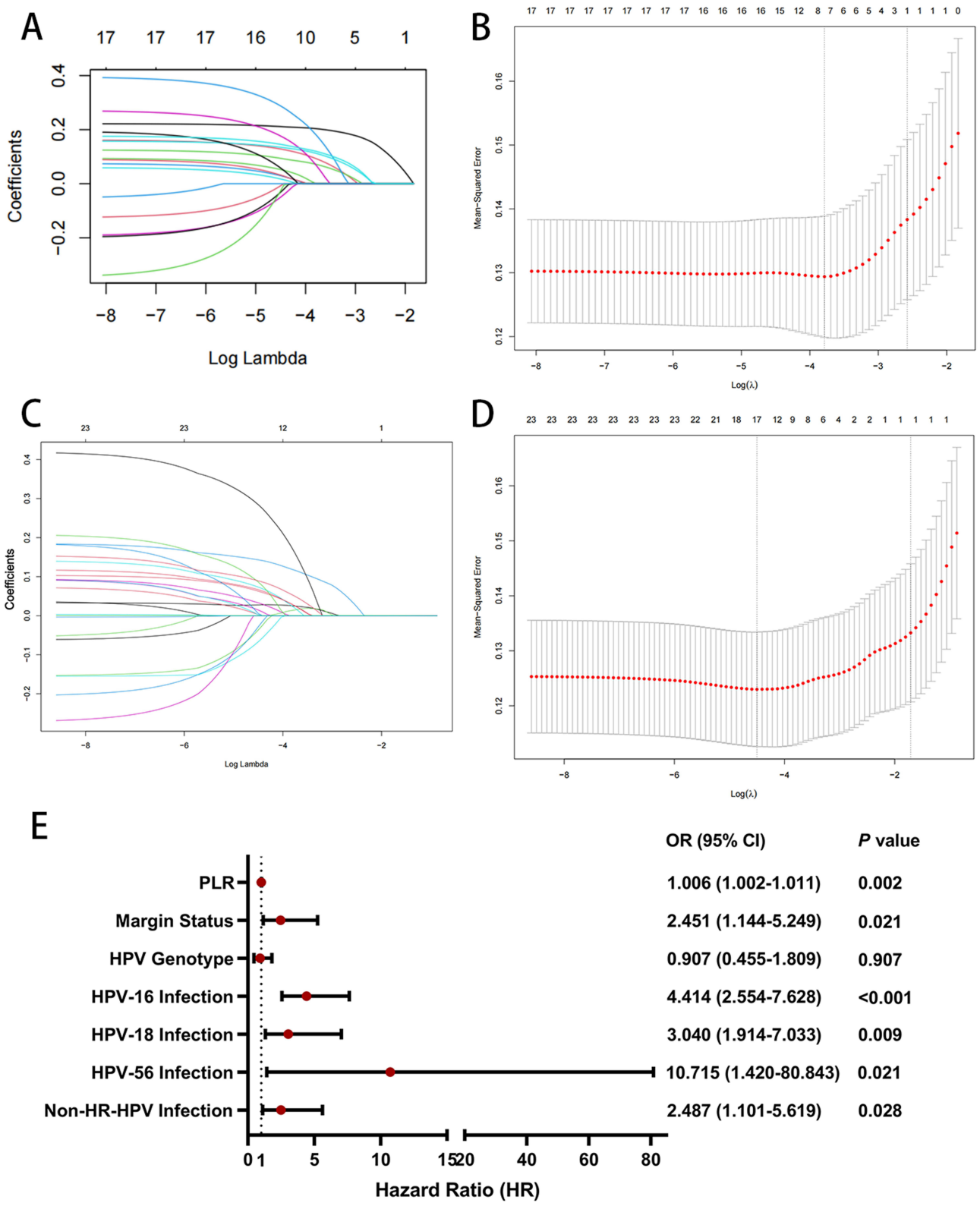

Figure 4 LASSO regression and optimized Logistic regression models. (**A**) The plot for LASSO coefficient path including HPV infection; (**B**) The LASSO cross-validation diagram including HPV infection; (**C**) The plot for LASSO coefficient path including HPV infection and other indicators; (**D**) The LASSO cross-validation diagram including HPV infection and other indicators; (**E**) The forest plot for the optimized Logistic regression model.

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

Table 3 Multivariate Cox Regression Models Based on LASSO Regression

Items	Hazard Ratio	95% Confidence Interval	P value
PLR	1.005	1.002–1.008	0.003
Gland Invasion			
No Invasion	Reference	–	0.081
Invasion	1.487	0.953–2.323	
Margin Status			
Negative	Reference	–	0.075
Positive	1.746	0.945–3.223	
HPV Infection Genotype			
None or Single	Reference	–	0.150
Double or multiple	0.891	0.497–1.598	
HPV-16 Infection			
Negative	Reference	–	<0.001
Positive	3.154	1.997–4.982	
HPV-18 Infection			
Negative	Reference	–	0.034
Positive	2.144	1.058–4.344	
HPV-56 Infection			
Negative	Reference	–	0.039
Positive	3.728	1.071–12.976	
HPV-58 Infection			
Negative	Reference	–	0.013
Positive	2.118	1.173–3.825	
Non-HR-HPV Infection			
Negative	Reference	–	0.020
Positive	2.139	1.128–4.059	

Notes: Multivariate Cox regression was applied for analysis of factors associated with recurrence/residual CIN, and $p<0.05$ indicates a statistically significance.
Abbreviations: PLR, platelet-to-lymphocyte ratio; HR-HPV, high-risk human papillomavirus.

Table 4 Optimized Logistic Regression Model for Recurrent/Residual/Progressed CIN Prediction

Items	Recurrent CIN			Recurrent CIN Accompanied with CIN Progression		
	Odds Ratio	95% Confidence Interval	P value	Odds Ratio	95% Confidence Interval	P value
PLR	1.006	1.002–1.010	0.004	0.999	0.998–1.009	0.785
Gland Invasion						
No Invasion	Reference	–	0.251	Reference	–	0.686
Invasion	1.339	0.814–2.204		1.251	0.422–3.704	
Margin Status						
Negative	Reference	–	0.012	Reference	–	0.088
Positive	2.452	1.219–4.929		3.098	0.844–11.371	
HPV Infection Genotype						
None or Single	Reference	–	0.001	Reference	–	0.356
Double or multiple	2.458	1.453–4.158		1.730	0.541–5.536	
HPV-16 Infection						
Negative	Reference	–	<0.001	Reference	–	0.001
Positive	3.776	2.330–6.119		6.159	2.073–18.302	

(*Continued*)

Table 4 (Continued).

Items	Recurrent CIN			Recurrent CIN Accompanied with CIN Progression		
	Odds Ratio	95% Confidence Interval	P value	Odds Ratio	95% Confidence Interval	P value
HPV-18 Infection						
Negative	Reference	–	0.019	Reference	–	0.274
Positive	2.481	1.163–5.294		2.340	0.510–10.747	
HPV-56 Infection						
Negative	Reference	–	0.010	Reference	–	0.999
Positive	10.695	1.759–65.025		<0.001	<0.001-NaN	
HPV-58 Infection						
Negative	Reference	–	0.148	Reference	–	0.813
Positive	1.618	0.843–3.107		1.200	0.265–5.429	
Non-HR-HPV Infection						
Negative	Reference	–	0.003	Reference	–	0.405
Positive	2.861	1.441–5.678		1.906	0.417–8.705	

Note: A p value<0.05 indicates a statistically significance.
Abbreviations: CIN, cervical intraepithelial neoplasia; PLR, platelet-to-lymphocyte ratio; HR-HPV, high-risk human papillomavirus.

while pathological biopsy and diagnostic conization can cover the muscular layer of the cervix. Thus, a HPV-based risk prediction has been recommended by 2019 ASCCP guidelines.[3,4] Also, the positive margin and gland invasion might suggest a higher rate for incomplete excision which results in residual or progressed lesions. A meta-analysis showed that the proportion of positive margins was about 23.1% among 44,446 patients, indicating high risks for disease recurrence/residual of CIN after operation.[18] Therefore, preoperative pathological examination is helpful to predict the postoperative prognosis. The growth pattern of the epithelium, the distribution of the atypia, nuclear spacing, and the degree of anisokaryosis and the presence of enlarged hyperchromatic nuclei might also help distinguish a non-neoplastic from a neoplastic process, as well as immunohistochemistry (IHC) indexed such as P16 and Ki67.[22,23]

SIRI including PLR, LMR and NLR can independently predict OS in cervical cancer patients undergoing radical resection and is therefore superior to existing systemic inflammatory markers.[24] Among SIRI, PLR has been proven to be a predictive value of hematological markers in prognosis of cervical cancer and related precancerous lesions.[25–27] This suggests that inflammatory markers are important markers of inflammation to cancer transformation in the body. Studies have shown that HPV infection can speed up the cell micro-environment and facilitate the production of interleukin 6 and interleukin 8, which in turn interact with 80% of CIN II+.[28] The changes of NLR and PLR indicate a neutrophils-platelet dependent inflammatory response and lymphocyte-mediated anti-tumor immune response, and the change of the balance between them and the increase of the ratio may indicate the increase of the body's pro-tumor inflammatory response and the decrease of the anti-tumor immunity. A study found that among groups of abnormal TCT specimens, there was a higher detection rate of inflammation (23.86% vs 2.0%, $p < 0.001$), with an increased rate of cytological abnormality by 12.598 times and HSIL by 756.47 times.[29] Actually, the tumor micro-environment (TME) is not only associated with changes of cytokines and chemokines including IL-1, IL-6, CXCL1, −2, −5, −12, CCL-2 and −3 but also related to the stimuli including TGF-β, Notch signaling and reactive oxygen species.[30] Relative changes mentioned above from tissue resident fibroblasts, epithelial cells, endothelial cells and bone marrow-derived mesenchymal stem cells may result in activation of tumor metabolism, tumor-associated inflammation (TAI) and adaptive immune responses.[31] IL-1, HMGB-1, toll-like receptor (TLR)-2, tumor associated macrophages, $CD4^+$ T-cells, tumor necrosis factor (TNF)-α and epidermal growth factor receptor (EGFR) ligands are also involved in progress of carcinogenesis, thus lead to differentiation of SIRI like PLR.[30,31]

The recurrence rate of advanced CIN after surgery could reach about 3.5%, and pre- and post-operative HPV-16 infection was considered as the most important factor for recurrence.[21] Studies have revealed that the risk of CIN II+ recurrence is significantly reduced when the result of HPV testing combined with cytology is negative after treatment,

regardless of the results of preoperative screening. But it is difficult to achieve the five-year follow-up risk at present, suggesting difficulties in virus clearance and complete lesion excision.[32] So far, no study has revealed the correlation between post-operative CIN recurrence or progression. The present study showed that PLR, HPV-16, HPV-18, HPV-56 and non-HR-HPV infection might promote CIN recurrence, and HPV-16 infection could be the significant predictive factor for higher grade CIN progression after conization. Based on findings above, applying pre-operative risk predicting models with HPV-based strategy and HPV vaccination may lead to better prognosis. It is hypothesized that HPV vaccination stimulates local antibodies, increases the immune response, prevents the virus from entering uninfected cells in the basal layer, and thus prevents disease recurrence. It has also been hypothesized that surgical treatment may reduce the local inflammatory response, induce more intense and durable local cellular immunity, and restore the naive microenvironment of HPV, which accounts for the higher PLR value in cervical precancerous lesions.[30,31,33] Studies have demonstrated that prophylactic HPV vaccination before surgery can significantly reduce the recurrence/persistence rate of CIN I+ or HSIL.[34,35] Growing evidence has shown that the optimal timing for vaccination may be 3–6 months before conization, as the pre-treatment vaccination might lead to a better prognosis than the post-treatment vaccination did.[33,36–38] Based on findings above, different HPV vaccination strategies can be triaged preoperatively according to the risk of recurrence or progression of CIN. Besides, various HPV testings can be introduced for pre-treatment examination as HPV viral load testing, $p16^{INK4a}$/Ki-67 testing and corresponding methylation testing provide predictive value for recurrence/residual CIN.[39–41]

Studies have revealed the correlation between HPV-infection and disease recurrence, while no study has focused on the association between HPV-infection and relevant disease progression in cervix after conization, which is one of the most important findings in the present study. HPV-16 infection has been demonstrated to be the key reason for disease from CIN I+ to CIN II+ before treatment,[42] and is also considered to be the most persistent and difficult genotype to eradicate. This refers to HPV E-protein-associated pathways such as PI3K/Akt, Wnt, Notch and other cell signaling pathways, and plays an important role in the occurrence, cell proliferation, metastasis and drug resistance of CIN or cervical carcinoma,[43] which may account for the fact that among 631 participants in this study 15 cases were detected with an increased level of lesions after surgery when the recurrence had been found. And 5 of them were even diagnosed with cervical carcinoma. E6 and E7, as oncoproteins, have been implicated in the progression of cancer. And E5 regulates cell proliferation, apoptosis and promotes E6 and E7 activity, which suggest that differentiation of E proteins among various HPV genotypes may influence the promotional capacity for CIN progression.[43] Besides, the deep infiltration and extensive spread of HPV-16 in lesions may also lead to incomplete surgical resection resulting in subsequent tissue malignancy. As studies declared active cell division (as it occurs during wound healing) is necessary for the viral genome to enter the nucleus and for exosomal maintenance, and the presence of cuboidal stem-like cells at the squamo-columnar junction, which may be prone to cancer progression, may account for high recurrence rate in HPV-16 infection related lesions and surgical resection sites.[44,45]

The Cox regression, Logistic regression and LASSO regression models were established in the present study for assessment of predictors to residual/recurrent/progressed CIN. During the Cox regression analysis the higher PLR, double or multiple HPV genotype, gland invasion and margin status showed the promoting effect of CIN with a p value < 0.05 (Table 2). While in LAASO regression considering the precise HPV infection status the infection of HPV-16, −18, −56, −58 and non-HR-HPV were considered as the predictors when the collinearity effects were excluded. Thus, the Logistic model for predicting post-treatment lesions were then built including PLR, gland invasion, margin status, HPV genotype, HPV-16, HPV-18, HPV-56, HPV-58 and non-HR-HPV. Interestingly, HPV-16 infection were demonstrated to be predictors for not only recurrent CIN but also the recurrent CIN accompanied with progression which lead to a much poorer prognosis. Based on our previous studies, the 1-year follow-up process was evaluated with specific HPV genotype testing and the findings of the study will allow rapid and accurate assessment of ambulatory surgical patients and provide important direction for later risk triage and treatment strategies.

There are some limitations in the present study. Firstly, this study was a retrospective single-center study based on department of gynecology which might lead to a selection bias. Secondly, given that we did not include patients with abnormal vaginal cleanliness, the discussion for the relationship of the local microbial environment to inflammation may have been overlooked. Thirdly, the study did not analyzed the effect of different ways of conization on the

prediction for recurrent/residual disease. A Cochrane review comparing surgical techniques for treatment of CIN concluded that no technique was clearly superior in terms of treatment failure or associated morbidity.[4] And studies also showed that there was no statistically significant difference in the risk of residual disease at 36 months between women who received LLETZ and those who received CKC.[46] Additionally, LEEP is an in-office procedure with less discomfort and fewer complications than CKC.[47] While in the present study, some patients' outpatient surgical records cannot be followed up, which currently hinders us from reanalyzing. Further researches would be conducted in the future.

Conclusion

In conclusion, our study suggests that the preoperative PLR level, gland invasion, and positive margin may be predictors for recurrent/residual CIN. And HPV-16, −18, −56 and non-HR-HPV infection status can be considered as indicators for recurrent CIN during the 5-year follow-up, especially for HPV-16 infection, which also lead to a CIN recurrence accompanied with disease progression.

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

Binhua Dong and Pengming Sun are co-corresponding authors. 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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