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ORIGINAL RESEARCH

Differential Mediating Roles of Immune-Inflammatory Cells in ≥HSIL Women: A Focus on HPV and CD4/CD8 or NLR

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Purpose: This study aims to investigate the relationship between inflammatory-immune cells and ≥high-grade squamous intraepithelial lesions (HSIL), and to clarify the role of immune-inflammatory cells mediating human papilloma virus (HPV) and ≥HSIL.

Methods: We retrospectively enrolled 427 patients with ≥HSIL and 357 ≤low-grade squamous intraepithelial lesions (LSIL) from June 1, 2013 to June 1, 2023. Clinical data such as age, peripheral blood inflammatory-immune cells, serum tumor markers, HPV infection status were collected to evaluate the relationship between clinical indicators and ≥HSIL mediated by inflammatory-immune cells.

Results: Compared with the ≤LSIL cohort, the patients with ≥HSIL exhibited a significantly higher prevalence of infection with ≥2 type HPV genotypes compared to those with a single HPV infection (34.55% vs 17.65%, $p = 0.045$). Multiple HPV infection and lower CD4/CD8 ratio were the independent risk factors for the patients with ≥HSIL besides HPV infection. Moreover, the top three HPV genotypes were HPV-16, HPV-18 and HPV-52 among the populations of ≥HSIL. Interestingly, HPV (1.29%), HPV-16 (2.48%) and HPV-52 (9.70%) infection were partially mediated by CD4/CD8 ratio to promote ≥HSIL ($p < 0.05$). Furthermore, compared with the HSIL cohort, SCC, HPV16, HPV52 infection and higher neutrophil and lymphocyte ratio (NLR) were the independent risk factors for cervical cancer (CC). Among women with CC, the top three HPV types are 16, 18, and 52. The relationship between HPV/HPV-16 and CC was partially mediated by the NLR, with mediation effect ratios of 1.87% and 2.85%, respectively ($p < 0.05$).

Conclusion: HPV-induced HSIL and CC are associated with immune-inflammatory status. Specifically, the CD4/CD8 ratio plays a significant mediating role during the HSIL stage, whereas NLR assumes a major mediating role in the CC stage. Thus, immune-inflammatory cells play distinct roles at different stages of CC progression.

Keywords: cervical lesion, inflammatory-immune cells, multiple infection, HPV

Introduction

China reported approximately 109,741 newly diagnosed cases of cervical cancer (CC) and 59,060 related deaths.[1] CC is characterized by human papillomavirus (HPV)-driven carcinogenesis and is thought to be immunogenic in nature.[2–4]

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During the development of CC, the infiltration type of inflammatory-immune cells is different.[5] A decrease in the number of CD8+ and CD4+ T cells indicates that immune function is impaired and that infected cells cannot be cleared in a timely and effective manner, which is associated with cervical intraepithelial neoplasias (CIN) and poor prognosis in patients with CC.[6–8]

Persistent infection with high risk (HR) -HPVs is widely proven to be the dominant cause of CC and precancerous lesions including high-grade squamous intraepithelial lesions (HSIL). Compared to single HPV infection, some studies indicate that multiple types of HPV infection is associated with higher risk of HSIL and prolonged infection persistence.[9] However, it was reported that women aged ≥30 years with single HPV infection exhibit a lower risk of HSIL than those co-infected with other HR-HPV types (excluding HPV-18).[10] Therefore, it's controversial that co-infection of HR-HPV represents a distinct pathogenic factor in HSIL, emphasizing the need for further research to shed light on its underlying roles.

Beyond the HPV types, chronic inflammation plays a crucial role from HSIL to CC.[11,12] Single-cell RNA sequencing (scRNA-seq) analysis reveals altered infiltration and function of immune cells (including T cells, B cells, and NK cells) within the TME of CC. Studies also indicate that chronic inflammation, especially under the condition of persistent HPV infection facilitates tumor proliferation and invasion, through the release of cytokine and inflammatory factors.[5] Furthermore, HPV-16 infection closely correlates with the host immune competence, emphasizing the dominance of inflammatory cells activity, influenced by HPV types, in CC progression.[13] Studies indicate that interactions between persistent HPV-16 infection and the host immune system promoting CC.[14] Inflammatory cells serve as dual role in TME, not only mediating tumor suppression by cytotoxic T cells, but secrete pro-tumorigenic factors (including VEGF and cytokines) as well, to promote HSIL and worse.[15] It was reported that the counts of total T cells, CTLs and NK cells in cervical tissue gradually decreases from low-grade squamous intraepithelial lesions (LSIL) to HSILs until the occurrence of CC.[16–18] However, other studies have reported that the counts of total T lymphocytes increases during the progression of LSIL to HSIL.[19–21] As cervical lesions progress, B-cell counts increase,[22] but others have reported that there are no changes in lymphocyte subsets associated with cervical lesions.[23,24] Thus, the relationships between lymphocyte subsets in peripheral blood and cervical lesions deserve further exploration. This study aims to investigate the role of lymphocyte subsets and inflammatory cells in promoting high-grade cervical lesions and worse, and to clarify the mediating relationships between different HPV types or the number of infection types, immune-inflammatory cells, and cervical lesions.

Materials and Methods

Study Population

The criteria for inclusion in the study were as follows: (a) patients who underwent surgery from 1 June 2013 to 1 June 2023 at Fujian Maternity and Child Health Hospital; (b) ≥ HSIL diagnosis was based on comprehensive physical examination and histopathology tests, which fully met the approved diagnostic and therapeutic criteria for patients with gynecological malignancies. Cancer staging is defined according to the International Federation of Gynecology and Obstetrics (FIGO) system. A total of 427 patients were studied, 253 of whom were diagnosed with cervical carcinoma and 174 with HSIL. Women who underwent gynecological examinations in the hospital during the same period and were diagnosed with ≤LSIL were randomly selected for age-frequency matching. The exclusion criteria were no cervical cytology screening or HPV test. All patients with a history of cancer, immune system disease, hematological disease, or active infection were excluded (Figure 1). This retrospective case-control study was approved by the Ethics Committee of the Fujian Maternal and Child Health Hospital (2022KYLLR03050). The Ethics Committee of the Fujian Maternal and Child Health Hospital waived written informed consent. All methods were performed in accordance with the relevant guidelines and regulations as well as in compliance with the requirements of the Declaration of Helsinki.

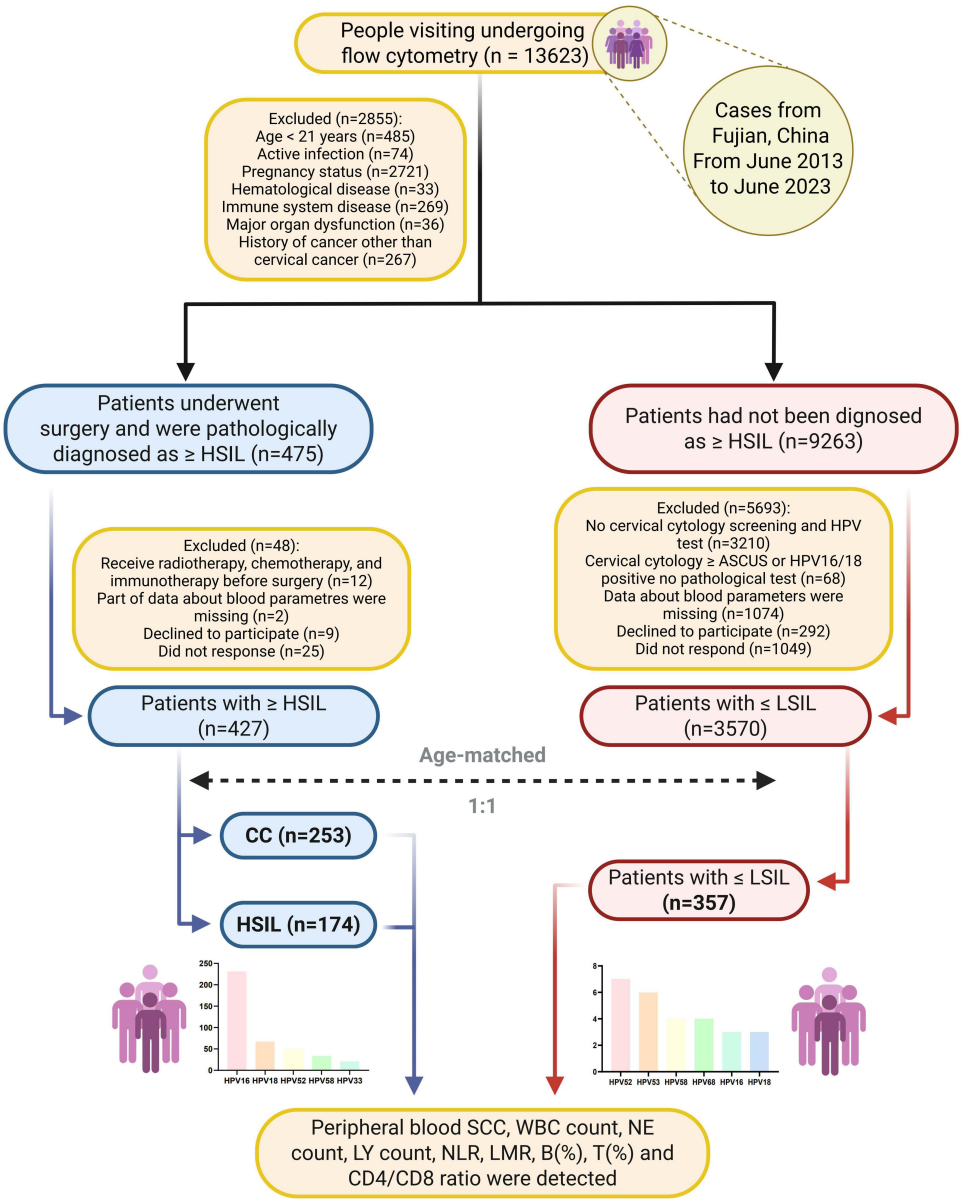

Figure 1 Flowchart for the selection of eligible participants.
Abbreviations: CC, Cervical cancer; HSIL, High-grade squamous intraepithelial lesion; LSIL, Low-grade squamous intraepithelial lesions.

Hematology Analysis

Fully automated complete blood counting (CBC) was performed in all study populations. On an empty stomach in the morning of the first or second day of admission, 2 mL of peripheral blood was collected from the cubital vein, and the serum was separated for analysis. The CBC test was completed within 4 hours. The neutrophil and lymphocyte ratio (NLR) was calculated by dividing the absolute neutrophil count by the absolute lymphocyte count; the lymphocyte-monocyte ratio (LMR) was calculated by dividing the absolute lymphocyte ratio count by the absolute monocyte count.

Assaying Serum Tumor Markers

Fasting venous blood (3 mL) was collected from each patient within 2 weeks before surgery and serum was collected by centrifugation. Serum carbohydrate antigen 125 (CA125) levels were measured via the Roche E70 automatic immunoassay and chemiluminescence method, and serum squamous cell carcinoma (SCC) levels were measured via the

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Abbott i2000SR automated immunoassay and microparticle enzyme-linked immunoassay (MEIA), which were performed according to the reagent instructions. An automatic chemiluminescence immunoanalyzer and supporting reagents were used for detection in strict accordance with the relevant operating specifications.

Flow Cytometry

Peripheral blood samples of 2 mL were collected in EDTA tubes. Approximately 100 μL of whole blood was mixed with anti-T/B/CD4+/CD8+/NK cell monoclonal antibodies and incubated at room temperature for 15 min (BD Biosciences, San Jose, USA). The red blood cells were lysed via FACS (BD Biosciences). T/B/CD4+/CD8+/NK cells from fresh blood samples were analyzed via automatic cytochemical flow cytometry and BD Canto II flow cytometry.

HR-HPV DNA Genotyping Test

The HR-HPV genotyping test was performed using the PCR-RDB HPV genotyping assay for 23 types (Yaneng® Biosciences, ShenZhen, China). This assay can identify 18 h-HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 82, and 83) and 6 Low Risk (LR-HPV) types (6, 11, 42, 43, and 81) in cervical exfoliated cells. The assay utilized the L1 consensus HPV PGMY09/PGMY11 primer set to amplify 5 μL of extracted HPV DNA or control (positive or negative) in a 24-μL reaction system. All detection procedures were performed according to the manufacturer's instructions provided by the kit.

Statistical Analyses

The means ± standard deviations represent continuous variables, and the frequencies (n) and percentages (%) represent categorical variables. Whether continuous variables were normally distributed was determined via the Kolmogorov–Smirnov test. Comparisons between groups were performed via single factor analysis of variance (ANOVA) and the Tukey's test. Multivariate logistic regression was used to analyze the risk factors for ≥HSIL. All tests were two-sided, and $p < 0.05$ was considered to indicate a statistically significant difference. Statistical analyses were performed using IBM SPSS Statistics 25.0 and R software (version 3.4.4). Mediation analysis was conducted using the R package "mediation" to estimate the average causal mediation effect (ACME), average direct effect (ADE), total effect (TE), and proportion of mediated effect (PME). Model specifications included linear/logistic regression (depending on outcome type), with significance of indirect effects validated via 1000 bootstrap resamples. Multiple comparisons were corrected using the Bonferroni method.

Results

Participant Characteristics

In this study, patients were categorized into three groups: ≤LSIL, ≥HSIL, and CC. Figure 2 displays the number of HPV infections across these groups. Within the ≤LSIL group, types 52 and 53 were the most prevalent HPV types, followed by types 58 (1.21%), 68 (1.21%), 16 (0.91%), and 18 (0.91%). Furthermore, the number of single infections was significantly higher than that of multiple infections. In contrast, the ≥HSIL and CC groups exhibited a distinct distribution pattern: the five most prevalent HPV types were 16 (55.40%; 62.45%), 18 (16.07%; 15.81%), 52 (11.98%; 6.72%), 58 (7.91%; 5.53%), and 33 (7.43%; 3.16%). Although multiple infections remained less frequent than single infections in these groups, their proportion was significantly higher compared to the ≤LSIL group.

Influence Factors and Independent Risk Factors for HSIL

A comparison between the ≥HSIL (n = 427) and ≤LSIL (n = 357) groups revealed that the overall HPV positivity rate was significantly higher in the ≥HSIL group than in the ≤LSIL group (91.61% vs 10.30%, $p < 0.001$). Similarly, the positivity rates for HPV-16 (60.47% vs 8.82%, $p < 0.001$), HPV-52 (11.98% vs 2.12%, $p < 0.001$), and multiple HPV infections (34.55% vs 17.65%, $p = 0.045$) were significantly higher in the ≥HSIL group. However, no significant difference was observed in HPV-18 positivity between the two groups ($p = 0.238$). Furthermore, Gravida and Parity were significantly higher in the ≥HSIL group compared to the ≤LSIL group (3.45 ± 1.52 vs 2.78 ± 1.65; 2.21 ± 1.16 vs

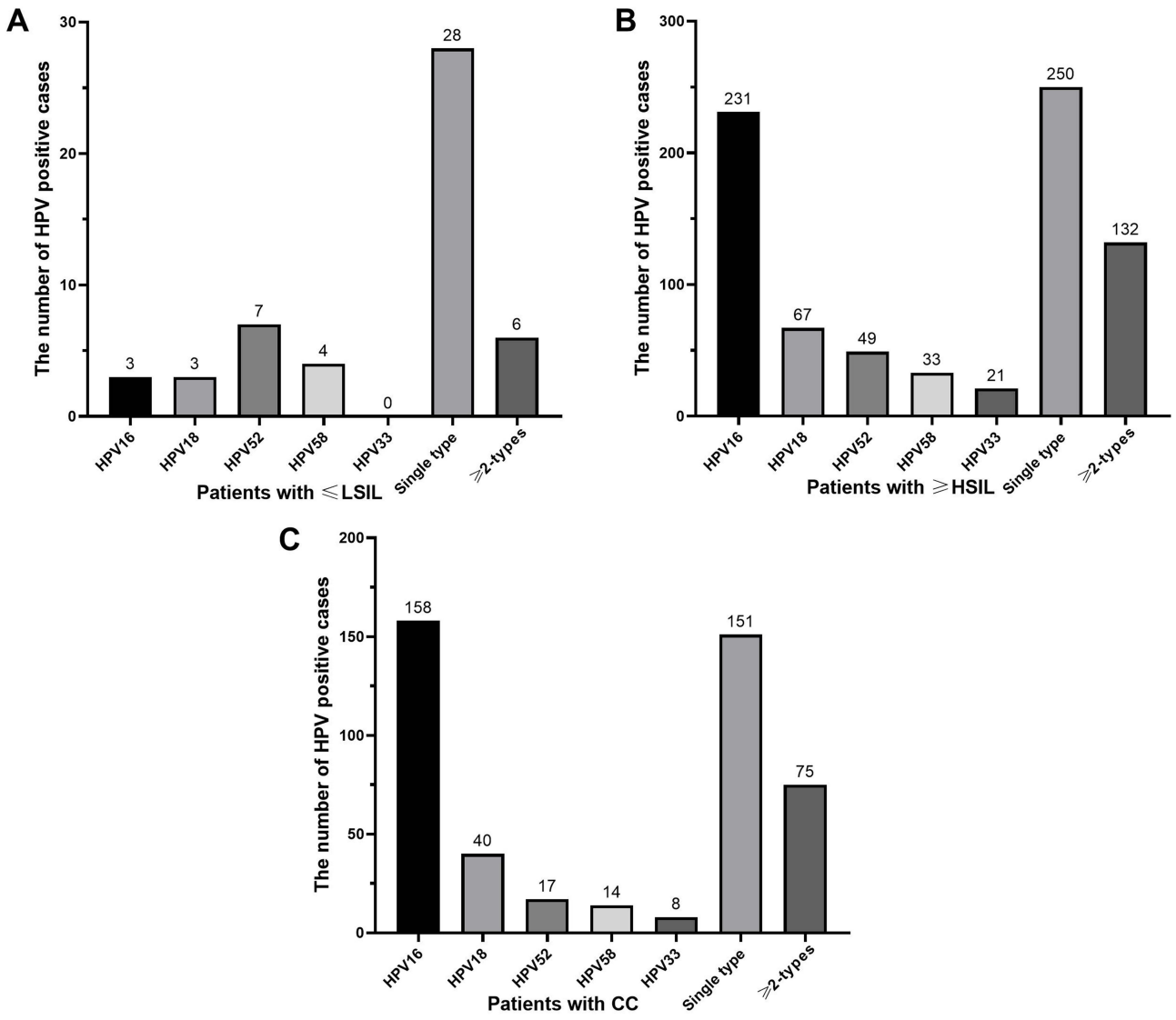

Figure 2 HPV infection status among different populations. (**A**) Number of HPV-infected patients with ≤LSIL; (**B**) Number of HPV-infected patients with ≥HSIL; (**C**) Number of HPV-infected patients with CC.
Abbreviations: CC, Cervical cancer; HSIL, High-grade squamous intraepithelial lesion; LSIL, Low-grade squamous intraepithelial lesions.

1.71 ± 1.12; all $p < 0.001$). The tumor markers SCC (2.68 ± 4.97 vs 1.54 ± 1.30, $p < 0.001$) and CEA (3.90 ± 16.04 vs 1.76 ± 1.22, $p = 0.019$) also exhibited significant differences. In contrast, no significant differences were found in age ($p = 0.878$) or menopausal status ($p = 0.710$) between the groups. Following adjustment for relevant covariates (p-value adjustment), the overall HPV positivity rate ($p < 0.001$), HPV-16 positivity rate ($p = 0.001$), and the presence of multiple HPV infections ($p = 0.045$) were identified as independent risk factors for ≥HSIL (Table 1).

Furthermore, the ≥HSIL group exhibited significantly lower percentages of B cells (13.08% ± 5.09% vs 13.94% ± 6.26%, $p = 0.033$) and CD4+ T cells (1.73 ± 0.36 vs 2.48 ± 3.07, $p < 0.001$) compared to the ≤LSIL group. Conversely, NLR was significantly higher in the ≥HSIL group (2.16 ± 1.25 vs 1.91 ± 1.18, $p = 0.008$). Additionally, the CD4+/CD8+ ratio was significantly lower in the ≥HSIL group than in the ≤LSIL group (1.67 ± 0.58 vs 1.97 ± 0.82, $p < 0.001$). After adjustment for multiple comparisons (p-value correction), the CD4+/CD8+ ratio remained significantly lower in the ≥HSIL group ($p = 0.013$) and was identified as an independent risk factor for HSIL (Table 2).

The CD4+/CD8+ ratio plays a crucial role in HPV infection progression. Overall, HPV-positive patients exhibited a significantly lower CD4+/CD8+ ratio compared to HPV-negative individuals (1.66 ± 0.62 vs 1.95 ± 0.80, $p < 0.001$).

Table 1 Clinicopathological Characteristics of Patients with Different Degrees of Cervical Lesions

Variable	≥HSIL (n = 427)	≤LSIL (n = 357)	*p*-value	OR $_{adjusted}$ (95% CI)	*p*-value $_{adjusted}$
Age	48.18 ± 9.44	48.07 ± 10.21	0.878		
Menopausal status	161 (37.7%)	130 (36.4%)	0.710		
Gravida	3.45 ± 1.52	2.78 ± 1.65	< 0.001*	1.159 (0.794–1.693)	0.445
Parity	2.21 ± 1.16	1.71 ± 1.12	< 0.001*	1.125 (0.694–1.825)	0.632
SCC	2.68 ± 4.97	1.54 ± 1.30	< 0.001*	0.947 (0.862–1.041)	0.260
CEA	3.90 ± 16.04	1.76 ± 1.22	0.019*	1.157 (0.896–1.493)	0.264
HPV-positive (n, %)	382 (91.6%)	34 (10.3%)	< 0.001*	68.239 (38.306–121.563)	< 0.001*
HPV-negative (n, %)	35 (8.4%)	296 (89.7%)			
HPV-16					
Positive	231 (60.5%)	3 (8.8%)	< 0.001*	48.361 (5.050–463.150)	0.001*
Negative	151 (39.5%)	31 (91.2%)			
HPV-18					
Positive	67 (17.5%)	3 (8.8%)	0.238		
Negative	315 (82.5%)	31 (91.2%)			
HPV-52					
Positive	49 (12.0%)	7 (2.1%)	< 0.001*	0.961 (0.331–2.793)	0.942
Negative	360 (88.0%)	323 (97.8%)			
HPV-infection					
≥ 2-types	132 (34.6%)	6 (17.6%)	0.045*	3.264 (1.028–10.357)	0.045*
Single type	250 (65.4%)	28 (82.4%)			

Note: *Significant *p*-value < 0.05.
Abbreviations: HSIL, High-grade squamous intraepithelial lesion; LSIL, Low-grade squamous intraepithelial lesions; SCC, squamous cell carcinoma antigen; CEA, carcinoembryonic antigen.

Table 2 Relationship Between Different Grades of Cervical Lesions and Lymphocyte Subsets

	≥HSIL (n = 427)	≤LSIL (n = 357)	*p*-value	*p*-value $_{adjusted}$
WBC	6.13 ± 1.97	6.11 ± 3.28	0.923	
NE	3.62 ± 1.69	3.40 ± 1.65	0.090	
LY	1.86 ± 0.69	1.94 ± 0.60	0.111	
B (%)	13.08 ± 5.09	13.94 ± 6.26	0.033*	0.662
NK (%)	15.91 ± 7.95	16.25 ± 8.45	0.590	
T (%)	69.55 ± 8.93	69.61 ± 8.92	0.918	
NLR	2.16 ± 1.25	1.91 ± 1.18	0.008*	0.216
LMR	4.68 ± 2.21	4.98 ± 3.80	0.203	
CD4+ T cells	1.73 ± 0.36	2.48 ± 3.07	< 0.001*	0.005*
CD8+ T cells	3.00 ± 0.78	2.97 ± 1.02	0.709	0.161
CD4/CD8 ratio	1.67 ± 0.58	1.97 ± 0.82	< 0.001*	0.013*

Notes: *Significant *p*-value < 0.05. *p*-value $_{adjust}$: After adjustment for gravida, parity, SCC, CEA and HPV-Positive.
Abbreviations: HSIL, High-grade squamous intraepithelial lesion; LSIL, Low-grade squamous intraepithelial lesions; WBC, white blood cell; NE, neutrophil; LY, lymphocyte; NLR, Neutrophil-to-lymphocyteratio ratio; LMR, Lymphocyteratio-to-monocyte ratio.

Analysis of single infections by specific HPV types revealed that patients positive for HPV-16 (1.63 ± 0.61 vs 1.87 ± 0.75, p < 0.001) and HPV-52 (1.50 ± 0.61 vs 1.82 ± 0.72, p < 0.001) also demonstrated a significantly reduced CD4+/CD8+ ratio compared to their respective HPV-negative counterparts. However, this significant difference was not observed for HPV-18 (p = 0.111) or HPV-58 (p = 0.237). Regarding multiple infections, the overall CD4+/CD8+ ratio was significantly lower in individuals with multiple HPV infections than in those with single infections (1.54 ± 0.63 vs 1.73 ± 0.61, p = 0.004). However, when analyzing multiple infections involving specific HPV types, a significantly lower

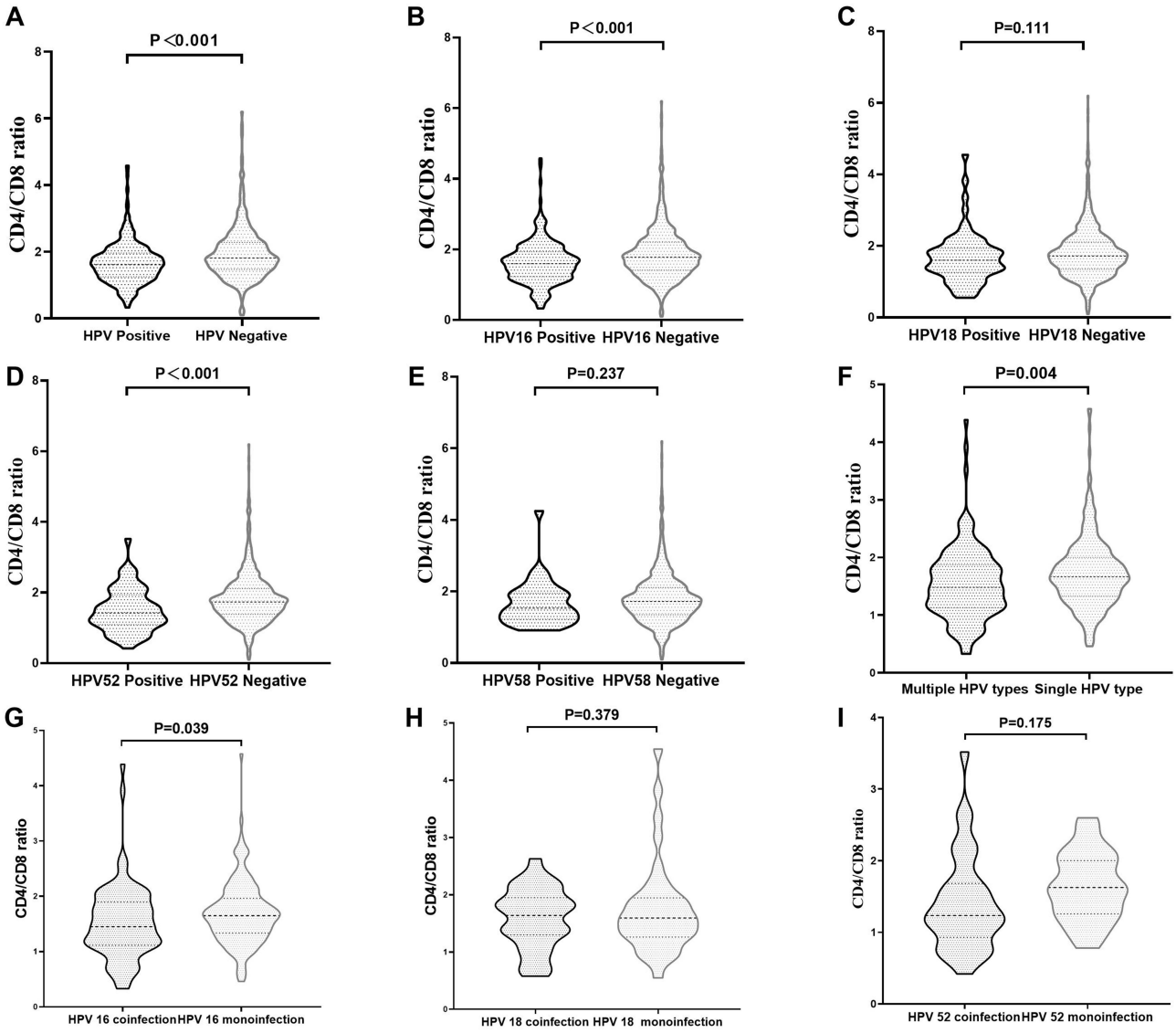

Figure 3 Comparison of the CD4/CD8 ratio under different HPV infection states. (**A**) Compare CD4/CD8 ratios between HPV positive and negative patients; (**B**) Compare CD4/CD8 ratios between HPV 16 positive and negative patients; (**C**) Compare CD4/CD8 ratios between HPV 18 positive and negative patients; (**D**) Compare CD4/CD8 ratios between HPV 52 positive and negative patients; (**E**) Compare CD4/CD8 ratios between HPV 58 positive and negative patients; (**F**) Compare CD4/CD8 ratios in patients infected with different HPV numbers; (**G**) Compare CD4/CD8 ratios between patients with monotypic and polytypic HPV 16 infections; (**H**) Compare CD4/CD8 ratios between patients with monotypic and polytypic HPV 18 infections; (**I**) Compare CD4/CD8 ratios between patients with monotypic and polytypic HPV 52 infections.

ratio was found in multiple infections involving HPV-16 (p = 0.039). And no significant differences were observed for multiple infections involving HPV-18 (p = 0.379) or HPV-52 (p = 0.175) (Figure 3).

Influence Factors and Independent Risk Factors for CC

A comparison between the CC group (n = 253) and the HSIL group revealed significant differences in specific HPV type prevalence. The HPV-16 infection rate was significantly higher in the CC group (64.49% vs 42.44%, p < 0.001). Conversely, the HPV-52 infection rate was significantly lower in the CC group (7.17% vs 18.60%, p = 0.001). However, no significant differences were observed in the overall HPV infection rate (p = 0.914), HPV-18 infection rate (p = 0.863), or the rate of multiple HPV infections (p = 0.498) between the two groups. Furthermore, the serum level of the tumor marker SCC was significantly elevated in the CC group compared to the HSIL group (3.68 ± 6.35 vs 1.35 ± 0.98, p < 0.001). In contrast, no significant differences were found between the groups for Age (p = 0.644), Menopausal status (p =

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Table 3 Clinicopathological Characteristics of Patients with CC and HSIL

Variable	Cervical Cancer	n	HSIL	n	*p*-value	OR $_{adjust}$ (95% CI)	*p*-value $_{adjust}$
Age	48.36 ± 9.64	253	47.93 ± 9.17	174	0.644		
Menopausal status	101 (39.9%)	253	60 (34.5%)	174	0.255		
Gravida	3.44 ± 1.44	219	3.45 ± 1.64	128	0.952		
Parity	2.18 ± 1.11	219	2.26 ± 1.25	128	0.562		
SCC	3.68 ± 6.35	213	1.35 ± 0.98	160	< 0.001*	1.465 (1.234–1.740)	0.001*
CEA	4.49 ± 17.82	196	2.93 ± 12.56	119	0.405		
HPV-positive	89.3%	226	89.7%	156	0.914		
HPV-negative	10.7%	27	10.3%	18			
HPV16							
Positive	64.5%	158	42.4%	73	<0.001*	2.469 (1.561–3.904)	0.004*
Negative	35.5%	87	57.6%	99			
HPV18							
Positive	16.3%	40	15.7%	27	0.863		
Negative	83.7%	205	84.3%	145			
HPV52							
Positive	7.2%	17	18.6%	32	0.001*	0.428 (0.213–0.862)	0.017*
Negative	92.8%	220	81.4%	140			
HPV-positive							
≥ 2-types	33.2%	75	36.5%	57	0.498		
Single type	66.8%	151	63.5%	99			

Note: *Significant *p*-value < 0.05.
Abbreviations: HSIL, High-grade squamous intraepithelial lesion; LSIL, Low-grade squamous intraepithelial lesions; SCC, squamous cell carcinoma antigen; CEA, carcinoembryonic antigen.

0.255), Gravida (p = 0.952), Parity (p = 0.562), or the tumor marker CEA (p = 0.405). Following adjustment for multiple comparisons (p-value correction), elevated SCC (p = 0.001), HPV-16 positivity (p = 0.004), and HPV-52 positivity (p = 0.017) were identified as independent risk factors for CC (Table 3).

Furthermore, the CC group exhibited a significantly higher NLR compared to the HSIL group (2.37 ± 1.40 vs 1.88 ± 1.10, p < 0.001). Conversely, LY (1.83 ± 0.76 vs 1.94 ± 0.58, p = 0.031) and the CD4+/CD8+ ratio (1.63 ± 0.58 vs 1.89 ± 0.76, p < 0.001) were significantly lower in the CC group. Following adjustment for multiple comparisons (p-value correction), NLR (p < 0.001) was identified as an independent risk factor for CC (Table 4).

Table 4 Relationship Between Cervical Lesions and Lymphocyte Subsets

	Cervical Cancer (n = 253)	≤HSIL (n = 531)	*p*-value	*p*-value $_{adjusted}$
WBC	6.33 ± 2.14	6.02 ± 2.93	0.977	
NE	3.84 ± 1.86	3.36 ± 1.55	0.140	
LY	1.83 ± 0.76	1.94 ± 0.58	0.031*	0.252
B (%)	12.70 ± 5.15	13.85 ± 5.87	0.312	
NK (%)	16.26 ± 8.23	16.00 ± 8.20	0.791	
T (%)	69.71 ± 9.44	69.51 ± 8.67	0.478	
NLR	2.37 ± 1.40	1.88 ± 1.10	< 0.001*	< 0.001*
LMR	4.50 ± 2.46	4.98 ± 3.37	0.056	
CD4+ T cells	1.74 ± 0.38	2.28 ± 2.66	< 0.001*	0.118
CD8+ T cells	2.93 ± 0.75	3.01 ± 0.97	0.223	
CD4/CD8 ratio	1.63 ± 0.58	1.89 ± 0.76	< 0.001*	0.127

Notes: *Significant *p*-value < 0.05. *p*-value $_{adjust}$: After adjustment for gravida, parity, SCC, CEA and HPV-Positive.
Abbreviations: HSIL, High-grade squamous intraepithelial lesion; WBC, white blood cell; NE, neutrophil; LY, lymphocyte; NLR, Neutrophil-to-lymphocyteratio ratio; LMR, Lymphocyteratio-to-monocyte ratio.

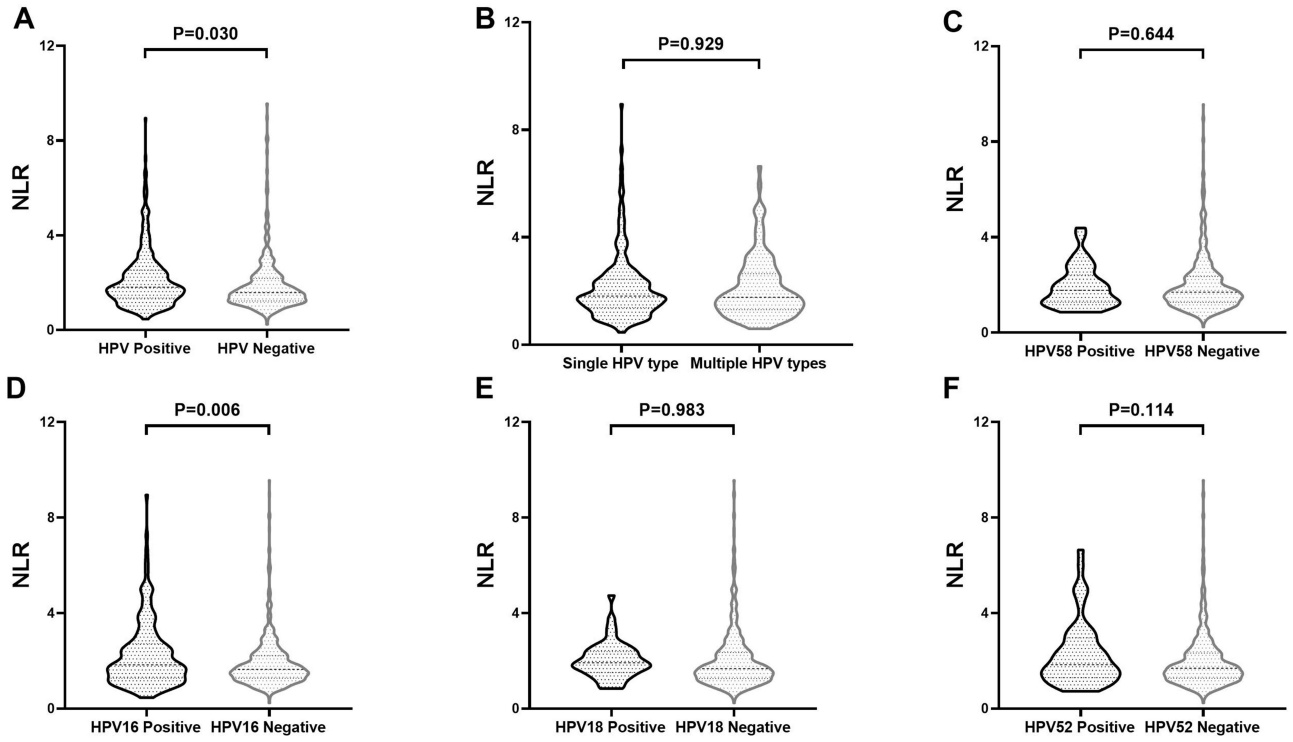

Figure 4 Comparison of the NLR under different HPV infection states. (**A**) Compare NLR between HPV positive and negative patients; (**B**) Compare NLR between patients with monotypic and polytypic HPV infections; (**C**) Compare NLR between HPV 58 positive and negative patients; (**D**) Compare NLR between HPV 16 positive and negative patients; (**E**) Compare NLR between HPV 18 positive and negative patients; (**F**) Compare NLR between HPV 52 positive and negative patients.
Abbreviations: NLR: Neutrophil-to-lymphocyteratio ratio.

NLR plays a critical role in the progression to CC. Overall, HPV-positive patients exhibited significantly higher NLR levels compared to HPV-negative individuals (p = 0.030). When analyzed by specific HPV type, however, significantly elevated NLR was observed only in HPV-16-positive patients (p = 0.006). In contrast, no significant differences were found between positive and negative patients for HPV-58 (p = 0.644), HPV-18 (p = 0.983), or HPV-52 (p = 0.114). Furthermore, no significant difference in NLR was observed between patients with multiple HPV infections and those with single infections (p = 0.929) (Figure 4).

Mediation Analysis

As mentioned above, the CD4+/CD8+ ratio exhibits distinct roles depending on both the specific HPV type and the infection status. Mediation analysis revealed that in the progression to ≥HSIL, HPV infection (1.29%, p = 0.014), HPV-16 infection (2.48%, p < 0.001), and HPV-52 infection (9.7%, p < 0.001) were all partially mediated by the CD4+/CD8+ ratio, albeit to varying degrees. Among these, HPV-52 infection exhibited the strongest mediation effect, with the CD4+/CD8+ ratio accounting for 9.7% of its total effect (Figure 5A–C).

In the progression to CC, HPV infection was partially mediated by NLR, accounting for 1.87% of the total effect (p = 0.048). Notably, significant mediation by NLR was observed only for HPV-16 infection, contributing to 2.85% of its effect on CC progression (p = 0.002). In contrast, no significant mediation effect was found for HPV-52 infection (p = 0.97) (Figure 5D–F).

Discussion

In this cross-sectional study, we found that HPV infection and peripheral blood inflammatory-immune cell profiling were associated with progression to ≥HSIL and CC. Notably, the CD4/CD8 ratio and NLR may play a mediating role in HPV-mediated cervical lesions and worse.

In the study, the participants were categorized into three groups: ≤LSIL, HSIL, and CC. The highest infection rates in the ≤LSIL group were observed for HPV-52 and HPV-53. However, extensive literature reports indicate that HPV16 is

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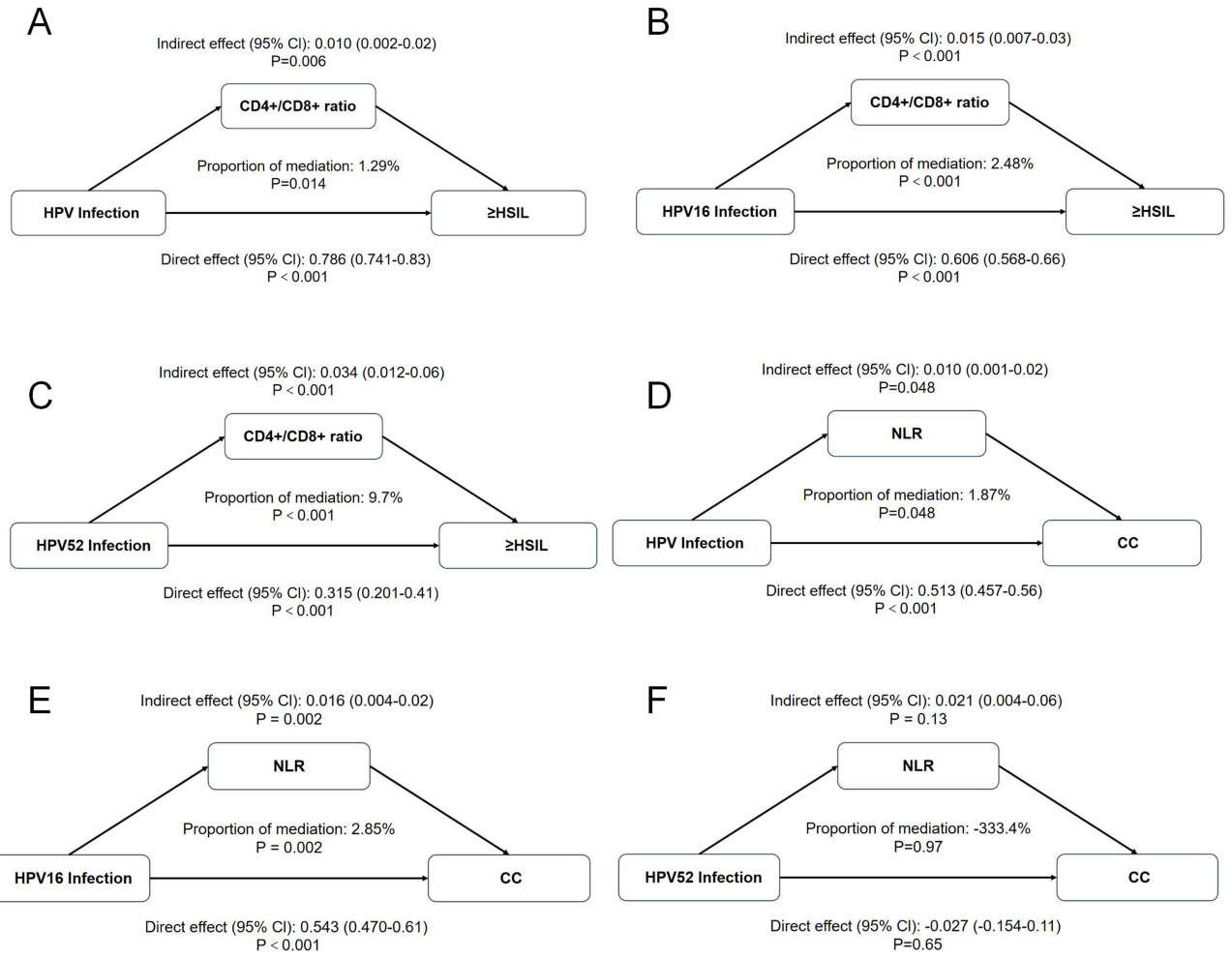

Figure 5 Mediating Effect. (**A**) Adjusted direct and indirect associations of HPV infection with ≥ HSIL by CD4/CD8 ratios. (**B**) Adjusted direct and indirect associations of HPV 16 infection with ≥ HSIL by CD4/CD8 ratios. (**C**) Adjusted direct and indirect associations of HPV 52 infection with ≥ HSIL by CD4/CD8 ratios. (**D**) Adjusted direct and indirect associations of HPV infection with CC by NLR. (**E**) Adjusted direct and indirect associations of HPV 16 infection with CC by NLR. (**F**) Adjusted direct and indirect associations of HPV 52 infection with CC by NLR.

Abbreviations: HSIL, High-grade squamous intraepithelial lesion; CC, Cervical cancer; NLR, Neutrophil-to-lymphocyteratio ratio.

the most prevalent genotype in LSIL. This discrepancy could be due to the low HPV positivity rate (10.30%) among the LSIL patients in our cohort, which resulted in a limited number of events and thus precluded the detection of a statistically significant predominance of HPV16 over other genotypes.[25,26] Notably, a distinct pattern of HPV prevalence emerged in the ≥HSIL and CC groups, where HPV-16, HPV-18, HPV-52, HPV-58, and HPV-33 were the most prevalent genotypes, which is consistent with previous reports focusing on.[27] Additionally, comparative analysis revealed significant differences in HPV-16 and HPV-52 positivity rates in the ≤LSIL/≥HSIL and HSIL/CC contracts development by inducing proliferation, angiogenesis, and metastasis;[28] inhibiting the adaptive immune system; and altering responses to hormones and chemotherapy drugs.[29] Previous studies have indicated that infiltration of intra-tumoral lymphocytes is associated with improved clinical outcomes.[30,31]

We observed a greater density of CD4+ cells in ≥HSIL than in ≤LSIL. Indeed, since HPV infection is most critical in the progression to HSIL, and CD4 immune cells play a vital role in the clearance of HPV, consequently, impaired immune function can lead to persistent HPV infection, increasing the risk of progression from LSIL to HSIL. Furthermore, CD4 cells could activate cytotoxic CD8+ T cells, to destroy tumor cells in two ways: through the perforin-granzyme pathway or through the apoptotic Fas ligand pathway.[23] Under conditions of chronic viral infection and cancer, the accumulation of Tregs release immunosuppressive cytokines (including IL-10 and TGF-β) to suppress the activity of effector CD4+ T cells.

Meanwhile, MDSCs inhibit CD4+ T cell function by producing reactive oxygen species (ROS), depleting arginine, and expressing PD-L1.[32] In the context of HSIL progression, immunosuppressive factors can also impair CD8+ T cell function, thereby facilitating the advancement of HSIL.[5] Alterations in the state of CD4+ T cells can lead to an imbalance in the CD4/CD8 ratio, which may consequently result in immunosuppression and promote tumor growth.

Interestingly, no statistically significant difference was observed for HPV-18, which probably boils down to more frequency of the SCC in our patient population (%). HPV-18 is more commonly associated with adenocarcinoma of the cervix, whereas HPV-16 and HPV-52 are predominantly linked to squamous cell carcinoma.[33,34] Adenocarcinomas and squamous cell carcinomas differ in their histopathological features, molecular pathogenesis, and tumor microenvironment. The NLR pathway may play a less prominent role in the development and progression of adenocarcinoma compared to squamous cell carcinoma. HPV-52 has a higher prevalence in certain ethnic groups, and it is the most predominant genotype in the general Chinese population.[35] HPV-52, a high-risk genotype with distinct genomic features (E6/E7 oncoprotein variants), may induce a more pronounced adaptive immune response during the HSIL stage.[36] HSIL represents a pre-invasive lesion where viral replication is active but tumorigenesis is not fully established. CD4+ T cells (Th1/Th17 subsets) and CD8+ cytotoxic T lymphocytes play critical roles in eliminating virus-infected cells by recognizing HPV-derived antigens. HPV-52's oncoproteins might have higher immunogenicity or lower immune evasion capacity compared to HPV-16, leading to robust T cell infiltration and activation in HSIL. This adaptive immune pressure could directly mediate lesion regression or progression, explaining the dominant role of CD4+/CD8+ T cells in HPV-52-associated HSIL. HPV16 is mainly mediated by NLR in the CC stage, which may be related to the advanced stage of cervical cancer. At this stage, the tumor microenvironment undergoes significant changes, including massive infiltration of inflammatory cells and increased release of pro-inflammatory cytokines. The oncoproteins of HPV16 (especially E6 and E7) can induce the continuous activation of the NF-κB signaling pathway and promote the production of inflammatory factors such as IL-6 and TNF-α.[37] These factors further stimulate the increase of neutrophils and the decrease of lymphocytes, leading to a significant elevation of NLR. HPV-16's E6/E7 may specifically upregulate neutrophil chemoattractants or downregulate T cell recruitment, shifting the immune balance toward innate inflammatory mediators.[32] By CC stage, adaptive immunity (CD4+/CD8+ T cells) is often exhausted or sequestered, making NLR a more prominent mediator of disease progression for HPV-16.

Moreover, we also observed that multitype HPV infection is associated with the development of HSIL. Different HPV types may exhibit a synergistic effect, working together to promote the abnormal proliferation of cervical epithelial cells and the development of lesions. Co-infection with multiple high-risk HPV types increases the probability of malignant transformation and accelerates the development of HSIL.[38] In addition, HPV evades immune surveillance through mechanisms such as regulating cytokine secretion and interfering with antigen presentation. When multiple infections occur, the virus can cooperatively down-regulate the expression of MHC-I molecules, inhibit the activation of CD8+ T cells, and at the same time, the abnormal CD4/CD8 ratio further weakens the immune clearance ability, jointly promoting the progression of the lesion.[9,13] In our study, however, no significant association was observed between multiple HPV infections and CC development, which is consistent with previous studies.[39] This may be because multiple HPV infections, compared to single infections, are subject to viral competition and interference, leading to a higher clearance rate. Consequently, the infection status often reverts to a single infection before progression to cervical cancer (CC), thus diminishing the observable risk from the initial multiple infection.[40,41]

We also observed that the progression from HPV16-induced HSIL to CC involves a shift in the primary mediating mechanisms, from immune-mediated responses in HSIL and early stages to inflammation-driven progression in late-stage squamous cell carcinoma, as measured by the NLR. Research suggests that an elevated NLR may serve as a marker for a profoundly immunosuppressive tumor microenvironment, a condition driven by neutrophil activity that facilitates tumor growth and metastasis and is most characteristic of mid-to-late-stage progression.[42] Consistent with this observation, multiple studies have identified NLR and its derivative ratios as robust predictors of tumor progression and treatment outcomes in CC.[43,44] Chronic inflammation represents a well-established hallmark of cancer development and progression. Within this framework, elevated NLR serves as a systemic indicator of pro-tumorigenic inflammation: neutrophils promote angiogenesis, tissue remodeling, and immunosuppression, while lymphocytes mediate antitumor

immune surveillance. Consequently, an increased NLR reflects an imbalance favoring pro-tumor inflammatory processes over adaptive immunity.[45]

Our study has several limitations: the study included only inpatient surgical patients at the same hospital, and the possibility of selection bias is inevitable The analysis of the results was only based on peripheral hemolymph subgroups and clinical data. Peripheral blood immune indicators cannot fully represent the local immune microenvironment of the cervix. Although mediation analysis suggests a causal path, retrospective design cannot establish a causal relationship. Therefore, further study of the underlying mechanisms may be necessary to clarify the importance of these molecular processes.

Conclusion

Overall, when immunity "fails" and the infection persists, cervical lesions may develop. Previous studies have shown that high-risk HPV infection in patients with CC is associated with immune function.[46] *Chlamydia trachomatis*/HPV coinfection decreases the CD4+ T/CD8+ T-cell ratio to below 1, impairing cell-mediated immunity and accelerating the progression to CC.[47] The immune response might be a key element in the progression or regression of HPV infection in the stroma of the uterine cervix. Therefore, the CD4/CD8 ratio serves as a biomarker of localized adaptive immune surveillance against HPV infection, where CD8+ T-cell-mediated clearance of infected keratinocytes is critical for preventing HSIL development. Conversely, elevated NLR reflects systemic pro-tumor inflammation that fuels cancer progression, characterized by neutrophil-driven angiogenesis and lymphopenia-induced immunosuppression.

Abbreviations

HPV, Human Papilloma virus; CC, cervical cancer; LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesions; CIN, cervical intraepithelial neoplasia; FIGO, International Federation of Gynecology and Obstetrics; CBC, complete blood counting; NLR, neutrophil and lymphocyte ratio; LMR, lymphocyte-monocyte ratio; CA125, antigen 125; SCC, squamous cell carcinoma; CEA, carcinoembryonic antigen; MEIA, microparticle enzyme-linked immunoassay; ACME, average causal mediation effect; ROS, reactive oxygen species; VEGF, vascular endothelial growth factor.

Data Sharing Statement

Due to the privacy policy, the datasets analyzed in this study are not publicly available, but they are available from the corresponding author upon reasonable request.

Ethics Approval and Informed Consent

This retrospective case-control study was approved by the Ethics Committee of the Fujian Maternal and Child Health Hospital (2022KYLLR03050). The Ethics Committee of the Fujian Maternal and Child Health Hospital waived written informed consent. All methods were performed in accordance with the relevant guidelines and regulations as well as in compliance with the requirements of the Declaration of Helsinki.

Author Contributions

Yao Tong - Conceptualization, Methodology, Writing - Original Draft; Formal analysis, Writing – reviewing & editing, Data curation

Yuqin Gao - Data curation, Methodology, Investigation

Xiaodan Mao - Conceptualization, Methodology; Funding acquisition, Project administration, Supervision

Hui Zhong - Conceptualization, Supervision; Formal analysis, Methodology, Project administration, Resources,

Xuanhao Wu - Software, Resources, Formal analysis, Visualization, Writing – reviewing & editing

Yafang Kang - Resources; Data curation, Methodology

Huanrui Zheng – Methodology, Formal analysis

Rong Yu – Software, Formal analysis

All authors 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

The authors declare no competing interests. The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the paper.

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