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Original Article

Comparison of ThinPrep cytology and high-risk human papillomavirus testing with different triage protocols for cervical cancer screening

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Abstract: Objective: To evaluate and compare the diagnostic performance of four cervical cancer screening triage strategies based on the ThinPrep cytologic test (TCT) and high-risk human papillomavirus (HR-HPV) testing, using histologically-confirmed cervical intraepithelial neoplasia grade 2 or worse (CIN2+) as the reference standard. Methods: This retrospective analysis included 1,582 consecutive women who underwent concurrent TCT and HR-HPV testing, followed by colposcopy and histopathologic evaluation, between January 2020 and December 2024. Four screening triage strategies were compared: Strategy A (co-testing, either positive), Strategy B (co-testing, both positive), Strategy C (HPV primary with TCT triage), and Strategy D (TCT primary with HPV triage). Using histologically confirmed CIN2+ as the gold standard, we calculated sensitivity, specificity, positive and negative predictive values, colposcopy referral rate, and the area under the receiver operating characteristic curve (AUC). Results: Among 42 confirmed CIN2+ cases, Strategy A achieved the highest colposcopy referral rate (20.10%) and sensitivity (97.62%), but had lower specificity (85.18%). Strategy B had the highest specificity (97.40%) and the lowest referral rate (2.34%), but substantially lower sensitivity (59.52%). Strategy C demonstrated balanced performance, with a sensitivity of 88.10%, specificity of 91.36%, and a referral rate of 9.42%; its AUC (0.897) was similar to that of Strategy A (0.914, P = 0.163). HPV16/18 positivity was the strongest predictor of CIN2+ (OR = 12.39). Conclusion: HPV primary screening with TCT triage offers a clinically effective cervical cancer screening strategy, achieving a favorable balance between diagnostic accuracy and colposcopy workload. HPV16/18 genotyping further enhances risk stratification.

Keywords: Cervical cancer screening, high-risk human papillomavirus, ThinPrep cytologic test, triage strategy, diagnostic accuracy

Introduction

Cervical cancer is one of the most common malignant tumors among women worldwide, and its high incidence and mortality pose a significant threat to women's health. The age-standardized incidence rate of this disease is approximately 13.3 cases per 100,000 people per year, ranking fourth among the most common cancers in women worldwide, and is also one of the leading causes of cancer-related deaths [1]. Multiple studies have shown that cervical cancer is primarily caused by persistent infection with high-risk human papillomavirus (HR-HPV). More than 97% of cervical cancer cases are associated with HR-HPV infection, with HPV types 16 and 18 accounting for about 70% of such cases [2, 3]. It usually takes several years for HR-HPV infection to develop into invasive cervical cancer, during which precancerous lesions may appear [3], which provides a crucial opportunity for early detection and intervention through routine screening. Therefore, developing efficient and accurate cervical cancer screening methods is essential to reduce the disease burden.

HR-HPV testing and ThinPrep cytologic test (TCT) are currently the main technologies for cervical cancer screening. However, both methods have their limitations. HR-HPV testing has relatively low specificity, often leading to unnecessary colposcopy referrals and increasing follow-up burden; while TCT has limited sensitivity, possibly causing missed diagnoses [4-6]. To improve screening efficiency, various combined

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screening and triage schemes have been developed, including risk stratification management based on HPV16/18 genotyping, cytologic triage after HR-HPV initial screening, and combined TCT and HR-HPV testing (combined screening) [7, 8]. The differences in sensitivity, specificity, and colposcopy referral rates among these technologies directly affect the cost-effectiveness and clinical feasibility of screening [9, 10]. Colposcopy, as a core technology for direct assessment and biopsy of cervical lesions, is a key means of confirming positive cases. However, since this examination is invasive, a reasonable positive triage scheme is needed to achieve a balance between medical resource use and diagnostic benefit [9, 11]. Therefore, optimizing the combined application of TCT and HR-HPV testing and constructing an efficient and accurate triage pathway are core issues in current screening work.

This diagnostic accuracy study aimed to evaluate and compare the effectiveness of four cervical cancer screening triage schemes based on TCT and HR-HPV. These schemes include sequential triage processes based on different primary screening methods (HPV or TCT), and referral processes based on different positive criteria (any positive or dual positive) after co-testing. This study intended to comprehensively evaluate and compare the effectiveness of the above four schemes in clinical screening pathways, and by combining HPV genotyping and multivariate risk analysis, provide evidence-based medicine for optimizing screening processes, improving diagnostic accuracy, and more rationally allocating medical resources.

Materials and methods

Study design and participants

This was a retrospective study. Data were extracted from the electronic medical record system of the People's Hospital of Qilihe District, Lanzhou City, spanning from January 2020 to December 2024. Initially, 1,837 female patients who underwent TCT and HR-HPV testing followed by colposcopy and histopathological biopsy, were included. After strict inclusion and exclusion criteria, 1,582 eligible participants were ultimately included. All women meeting the criteria for colposcopy referral (TCT positive or HR-HPV positive) underwent colposcopic biopsy. Since this study utilized all available eligible data within a specific time period, no pre-calculated sample size was performed; the sample size was determined based on the completeness of the clinical medical records. Following inclusion and exclusion criteria, a total of 1,582 participants were ultimately included in the analysis.

Inclusion criteria: (1) Age 25-65 years; (2) Valid and qualified TCT and HR-HPV test results; (3) Underwent colposcopic biopsy or endocervical curettage; (4) Complete clinical, cytological, HPV, and histopathological data.

Exclusion criteria: (1) History of cervical conization or hysterectomy; (2) Pregnancy; (3) Substandard TCT or HPV specimen quality; (4) Invisible transformation zone under colposcopy or unclear biopsy diagnosis; (5) Missing key clinical or pathological data.

Indications for colposcopy referral included positive or atypical TCT and/or HR-HPV screening results, and other suspicious clinical manifestations. Furthermore, this study protocol has been reviewed and approved by the Medical Ethics Committee of the People's Hospital of Qilihe District, Lanzhou City.

Screening strategy

This study evaluated four triage schemes: Strategy A (co-testing, either positive): Patients underwent both TCT and HPV testing simultaneously; colposcopy was done if either test was positive. Strategy B (co-testing, dual positive): Patients underwent both TCT and HPV testing simultaneously; colposcopy was done only if both tests were positive. Strategy C (HPV primary with TCT triage): HPV testing was performed first; HPV-positive patients underwent TCT, and colposcopy was done only if the TCT was also positive. Strategy D (TCT primary with HPV triage): TCT was performed first; TCT-positive patients underwent reflex HPV testing, and colposcopy was referred only if HPV was also positive.

Test methods

For TCT, liquid-based cytology specimens were prepared using the ThinPrep® 2000 testing system (Hologic, Inc., Marlborough, Massachusetts, USA). Patients were advised to abstain from sexual intercourse for at least 72 hours before specimen collection and to stop using

vaginal medications or vaginal douches. Subsequently, a gynecologist used a special cervical brush to rotate clockwise 5 times in the squamocolumnar junction of the cervix to obtain a cervical sample. The collected cells were immediately placed in ThinPrep® preservation solution for preservation and transport. The TCT specimens were independently reviewed and evaluated by two pathologists, both with more than five years of experience in cervical cytology diagnosis, who were unaware of the patient's clinical data. The diagnostic results were graded according to the criteria listed in the 2014 Bethesda System for reporting cervical cytology [12]. First, the specimen adequacy was assessed, and the diagnostic categories included no intraepithelial lesion or malignant lesion, atypical squamous cells of undetermined significance (ASC-US), atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion (ASC-H), low-grade squamous intraepithelial lesion, high-grade squamous intraepithelial lesion, and squamous cell carcinoma. In this study, ASC-US and above cytological results were defined as the positive threshold [13]. If there was a diagnostic discrepancy, the slides were reviewed by a third senior pathologist at the chief physician level to determine the final diagnosis.

HR-HPV DNA was detected using the Roche Cobas® 4800 HPV detection system. This detection method applied real-time quantitative PCR technology and can simultaneously identify 14 HR-HPV types, specifically types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68. In addition, this testing method can report the genotyping results for HPV types 16 and 18 separately. All laboratory procedures, including nucleic acid extraction, PCR amplification, and cycle threshold (CT) interpretation, strictly adhered to the manufacturer's standard operating procedures.

Colposcopy and pathologic diagnosis

All study subjects underwent colposcopic biopsy, which was the gold standard for diagnosis. The procedures were performed by senior gynecologists with over five years of experience in colposcopy. Following standard protocol, cervical epithelial and vascular morphology were assessed using electronic colposcopy after treatment with 3%-5% acetic acid solution and Lugol's iodine solution. Targeted biopsies were performed on areas showing thickened acetic acid-white epithelium, coarse punctate vessels, coarse mosaic changes, or suspected invasive lesions. If no obvious abnormalities were found under colposcopy, random four-point biopsies were performed at the 3, 6, 9, and 12 o'clock in the cervical transformation zone. After the collected biopsy tissues were fixed in 4% neutral buffered formalin, they were successively embedded in paraffin, sectioned and stained with hematoxylin and eosin (HE). The pathologic evaluation was completed independently by two pathologists at the level of associate chief physician or above, neither of whom were aware of the corresponding TCT and HPV test results. The diagnosis was established according to the 2020 World Health Organization (WHO) Classification of Female Genital Tumors [14], and the lesions were graded using the cervical intraepithelial neoplasia (CIN) grading system. If there were discrepancies in the initial diagnosis, a final consensus diagnosis was reached through joint review of the slides or consultation with the chief physician and pathologist.

Definition of study endpoints

This study used histopathologic diagnosis as the definitive diagnostic criterion. The primary positive endpoint was defined as a histopathologically confirmed cervical intraepithelial neoplasia grade 2 or higher (CIN2+), including CIN2, CIN3, and invasive cervical cancer. Cases with pathologic diagnoses of normal cervical tissue, chronic inflammation, or CIN1 were defined as negative endpoints.

Statistical analysis

Data analysis was performed using SPSS version 26.0 (IBM, Armonk, NY, USA) and R version 4.5.2 (R Foundation for Statistical Computing, Vienna, Austria). Normally distributed continuous data were expressed as mean ± standard deviation (SD), and differences between groups were assessed using paired t-tests. Non-normally distributed continuous data were analyzed using the Mann-Whitney U test and expressed as median (interquartile range, IQR; p25, p75). Categorical data were expressed as percentages, and comparisons between groups were performed using chi-square tests or Fisher's exact test, depending on the circumstances.

Histopathologic diagnosis was used as the gold standard, with a positive result defined as CIN2+. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (PLR), negative likelihood ratio (NLR), and their corresponding 95% confidence intervals (CIs) were calculated for four screening protocols. Colposcopy referral rates for each protocol were also calculated. McNemar test was used to analyze differences in sensitivity and specificity among different protocols, and the DeLong test was used to assess differences in the area under the receiver operating characteristic curve (AUC).

To evaluate diagnostic efficacy across different age groups, subgroup analyses were performed by age stratification. Age, HR-HPV genotype, and TCT grading were incorporated into a multivariate logistic regression model to screen for independent predictors of CIN2+. In all two-sided statistical tests, a *P*-value < 0.05 was considered significant.

Results

Baseline characteristics

This study included 1,582 participants, of whom 1,335 were HPV-negative (84.39%) and 247 were HPV-positive (15.61%). There were no significant differences between the two groups in baseline characteristics such as age, ethnicity, marital status, parity, smoking and drinking history, contraceptive methods, or HPV vaccination status (all P > 0.05). Compared with HPV-negative women, HPV-positive women had a higher proportion of those with a junior high school education or below (65.59% vs. 56.78%, P = 0.031), a lower average age at first sexual intercourse (21.23 ± 2.32 years vs. 21.66 ± 2.26 years, P = 0.006), and a higher proportion of those who had had two or more sexual partners in the past three years (3.64% vs. 0.97%, P = 0.003). Detailed baseline characteristics are presented in **Table 1**.

Detection efficacy of single screening indicators

The positive rate of TCT (defined as ASC-US or higher) was 6.19% (98/1,582), and the positive rate of HR-HPV was 15.61% (247/1,582). Using histopathologic diagnosis of CIN2+ as the ground truth, a total of 42 positive cases were diagnosed, with a detection rate of 2.65%. The detection rate of CIN2+ varied among different diagnostic markers. It was 37.93% (22/58) among women positive for HPV types 16/18, 18.37% (18/98) among those positive for TCT, and 8.47% (16/189) among those positive for other HR-HPV types. See **Table 2**.

Comparison of diagnostic efficacy of four screening protocols

Using histopathologic diagnosis of CIN2+ as the ground truth, the diagnostic accuracy of the four screening schemes was evaluated, and the results are summarized in **Table 3**. Strategy A had the highest sensitivity, reaching 97.62% (41/42), with a negative predictive value of 99.90%, specificity of 85.18%, and a colposcopy referral rate of 20.10% (318/1,582). This protocol missed one case of CIN2+ confirmed by histopathology, whose TCT and HR-HPV test results were both negative. Strategy B yielded the highest specificity (97.40%) and positive predictive value (39.13%), along with the lowest referral rate (2.34%, 37/1,582), and a sensitivity of only 59.52%. The sensitivity, specificity, positive predictive value, negative predictive value, and referral rate of Strategy C were 88.10%, 91.36%, 21.57%, 99.60%, and 9.42% (149/1,582), respectively. Pairwise comparisons using the McNemar test revealed that the sensitivity ranking was A > C > D > B, with significant differences between all groups except B and D; the specificity ranking was B > D > C > A, with significant differences between all groups.

The sensitivity, specificity, PPV, NPV, and referral rate of Strategy D were 76.19%, 95.84%, 28.57%, 99.40%, and 6.07% (96/1,582), respectively. The AUC values for Strategies A through D was 0.914, 0.784, 0.897, and 0.860, respectively. **Figure 1A** visually compares the core performance indicators of the four protocols, namely sensitivity, specificity, and colposcopy referral rate; the corresponding ROC curves are depicted in **Figure 1B**.

Stratified analysis of screening strategies by age group

Stratified analysis by age group (**Table 4**) shows that Strategy A maintained the highest sensitivity across all age cohorts (25-34 years, 35-44 years, 45-54 years, and 55-65 years), ranging

Table 1. Baseline characteristics of the study population (N = 1,582)

Variable	HPV-positive (n = 247)	HPV-negative (n = 1335)	t/χ^2	P
Age (years, $\bar{x}\pm s$)	44.85 ± 7.92	45.44 ± 8.26	1.038	0.300
Ethnicity [n (%)]			0.386	0.534
Han Chinese	239 (96.76)	1301 (97.45)		
Others	8 (3.24)	34 (2.55)		
Marital status [n (%)]			1.150	0.284
Married	216 (89.07)	1198 (89.74)		
Unmarried/Divorced/Widowed	31 (10.93)	137 (10.26)		
Education [n (%)]			6.966	0.031
Junior high school or below	162 (65.59)	758 (56.78)		
High school/Secondary school	61 (24.70)	395 (29.59)		
College or above	24 (9.71)	182 (13.63)		
Occupation [n (%)]			4.783	0.092
Farmer/Worker	145 (58.70)	698 (52.28)		
Clerk/Teacher	55 (22.27)	384 (28.76)		
Others/Unemployed	47 (19.03)	253 (18.95)		
Gravidity [n (%)]			1.785	0.618
0	25 (10.12)	158 (11.84)		
1	88 (35.63)	508 (38.05)		
2	98 (39.68)	476 (35.66)		
≥ 3	36 (14.57)	193 (14.46)		
Parity [n (%)]			2.117	0.548
0	31 (12.55)	207 (15.51)		
1	143 (57.89)	715 (53.56)		
2	63 (25.51)	352 (26.37)		
≥ 3	10 (4.05)	61 (4.57)		
Age at menarche (years, $\bar{x}\pm s$)	14.12 ± 1.93	14.34 ± 1.84	1.713	0.087
Age at first intercourse (years, $\bar{x}\pm s$)	21.23 ± 2.32	21.66 ± 2.26	2.736	0.006
Number of sexual partners in the past 3 years [n (%)]			8.976	0.003
0-1	238 (96.36)	1322 (99.03)		
≥ 2	9 (3.64)	13 (0.97)		
Smoking [n (%)]			1.518	0.218
Yes	8 (3.24)	24 (1.80)		
No	239 (96.76)	1311 (98.20)		
Alcohol consumption [n (%)]			3.334	0.068
Yes	11 (4.45)	32 (2.40)		
No	236 (95.55)	1303 (97.60)		
Contraception method [n (%)]			5.236	0.155
Condom	87 (35.22)	541 (40.52)		
Oral contraceptives	34 (13.77)	141 (10.56)		
Intrauterine device	83 (33.60)	391 (29.29)		
Other/None	43 (17.41)	262 (19.63)		
History of HPV vaccination [n (%)]			1.183	0.277
Yes	15 (6.07)	108 (8.09)		
No	232 (93.93)	1227 (91.91)		

Note: $\bar{x}\pm s$, mean ± standard deviation.

Table 2. Summary of screening results [n (%)]

Test item	Positive cases	Negative cases	CIN2+ cases detected (proportion among positive cases of that test)
TCT	98 (6.19)	1484 (93.81)	18 (18.37)
HR-HPV	247 (15.61)	1335 (84.39)	38 (15.38)
HPV 16/18	58 (3.67)	1524 (96.33)	22 (37.93)
Other HR-HPV	189 (11.95)	1393 (88.05)	16 (8.47)
Colposcopic biopsy	42 (2.65)	1540 (97.35)	42 (100.00)

Note: TCT, ThinPrep cytologic test; HR-HPV, high-risk human papillomavirus; CIN2+, cervical intraepithelial neoplasia grade 2 or worse (includes CIN2, CIN3, and invasive carcinoma).

Table 3. Comparison of the diagnostic performance of four screening strategies for CIN2+ detection

Metric	Strategy A	Strategy B	Strategy C	Strategy D
Sensitivity (%, 95% CI)	97.62 (87.41-99.93)[a]	59.52 (43.27-74.38)[b]	88.10 (74.37-96.02)[c]	76.19 (60.55-87.94)[d]
Specificity (%, 95% CI)	85.18 (83.29-86.95)[a]	97.40 (96.43-98.17)[b]	91.36 (89.81-92.71)[c]	95.84 (94.71-96.78)[d]
PPV (%, 95% CI)	15.72 (11.53-20.90)	39.13 (26.57-52.91)	21.57 (15.71-28.57)	28.57 (20.00-38.65)
NPV (%, 95% CI)	99.90 (99.49-100.00)	98.51 (97.74-99.05)	99.60 (99.08-99.85)	99.40 (98.79-99.73)
PLR	6.58	22.92	10.17	18.33
NLR	0.03	0.42	0.13	0.25
Colposcopy Referral Rate (%)	20.10 (318/1582)	2.34 (37/1582)	9.42 (149/1582)	6.07 (96/1582)
AUC (95% CI)	0.914 (0.882-0.946)	0.784 (0.731-0.837)	0.897 (0.861-0.933)	0.860 (0.818-0.902)

Note: PPV, positive predictive value; NPV, negative predictive value; PLR, positive likelihood ratio; NLR, negative likelihood ratio; AUC, area under the receiver operating characteristic curve; Strategy A, co-testing, refer to colposcopy if either test is positive; Strategy B, co-testing, refer to colposcopy if both tests are positive; Strategy C, HPV primary with TCT triage; Strategy D, TCT primary with HPV triage. For pairwise comparisons using the McNemar test, different superscript letters (a, b, c, d) indicate significant differences at $P < 0.05$, while the same letter indicates no significant difference.

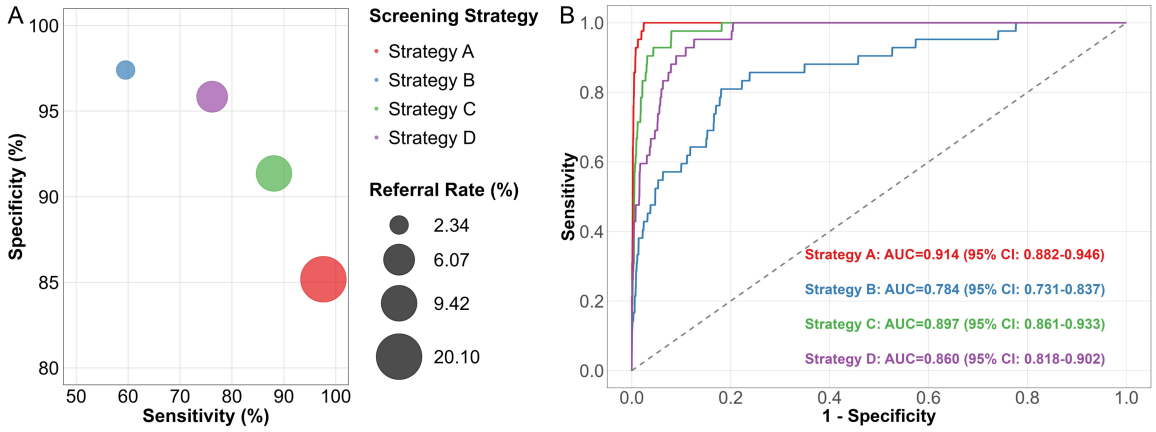

Figure 1. Performance comparison of four cervical cancer screening strategies for CIN2+ detection. A. Bubble plot comparing sensitivity, specificity, and colposcopy referral rate. B. Receiver operating characteristic (ROC) curves with corresponding area under the receiver operating characteristic curve (AUC) values and 95% confidence intervals. Strategy A (co-testing, either positive): Patients underwent both TCT and HPV testing simultaneously; colposcopy was referred if either test was positive. Strategy B (co-testing, dual positive): Patients underwent both TCT and HPV testing simultaneously; colposcopy was referred only if both tests were positive. Strategy C (HPV primary with TCT triage): HPV testing was performed first; HPV-positive patients underwent TCT, and colposcopy was referred only if TCT was also positive. Strategy D (TCT primary with HPV triage): TCT was performed first; TCT-positive patients underwent reflex HPV testing, and colposcopy was referred only if HPV was also positive.

from 92.86% to 100%. Strategy C had a specificity between 90.06% and 93.31%, while its sensitivity remained stable across all age groups, ranging from 85.71% to 91.67%. Strategy B and Strategy D showed relatively low sensitivity across all age groups.

Table 4. Sensitivity and specificity of four screening strategies for CIN2+ detection across different age groups

Age (years)	Total Cases	CIN2+ Cases	Strategy A (Se/Sp)	Strategy B (Se/Sp)	Strategy C (Se/Sp)	Strategy D (Se/Sp)
25-34	324	8	100.00/83.33	62.50/98.72	87.50/90.06	75.00/96.47
35-44	516	12	100.00/84.65	58.33/97.44	91.67/90.75	83.33/95.87
45-54	479	14	92.86/86.47	57.14/97.42	85.71/92.06	71.43/95.90
55-65	263	8	100.00/88.98	62.50/97.64	87.50/93.31	75.00/96.46

Note: Se, sensitivity (%); Sp, specificity (%); CIN2+, cervical intraepithelial neoplasia grade 2 or worse; Strategy A, co-testing, refer if either positive; Strategy B, co-testing, refer if both positive; Strategy C, HPV primary with TCT triage; Strategy D, TCT primary with HPV triage.

Table 5. Comparison of AUC among the four screening strategies (DeLong test)

Comparison	AUC difference (95% CI)	Z	P
Strategy A vs. Strategy B	0.130 (0.086-0.174)	5.842	< 0.001
Strategy A vs. Strategy C	0.017 (-0.007-0.041)	1.396	0.163
Strategy A vs. Strategy D	0.054 (0.024-0.084)	3.571	< 0.001
Strategy B vs. Strategy C	-0.113 (-0.155- -0.071)	5.246	< 0.001
Strategy B vs. Strategy D	-0.076 (-0.116- -0.036)	3.758	< 0.001
Strategy C vs. Strategy D	0.037 (0.010-0.064)	2.682	0.007

Note: AUC, area under the receiver operating characteristic curve; CI, confidence interval; Strategy A, co-testing, refer to colposcopy if either test is positive; Strategy B, co-testing, refer to colposcopy if both tests are positive; Strategy C, HPV primary with TCT triage; Strategy D, TCT primary with HPV triage.

Comparative analysis of diagnostic efficacy of screening strategies

The DeLong test was used to compare the AUC values of the four strategies (**Table 5**). The results showed that the AUC of strategy A was significantly higher than that of strategy B (difference 0.130, P < 0.001) and strategy D (difference 0.054, P < 0.001), but there was no significant difference compared to Strategy C (difference 0.017, P = 0.163). The AUC value of Strategy C was significantly higher than that of Strategy B (difference -0.113, P < 0.001) and Strategy D (difference 0.037, P = 0.007).

HPV genotype distribution and lesion risk among 247 subjects diagnosed with HPV positivity, the detection frequency distribution of specific HR-HPV genotypes is as follows: other high-risk (HR) HPV types (non-16/18) accounted for the highest proportion (34.82%), followed by HPV16 (17.00%), HPV31 (14.17%), HPV52 (11.34%), HPV58 (8.91%), and HPV18 (6.48%). The positive rates for CIN2+ corresponding to each genotype were as follows: HPV16 42.86%, HPV18 25.00%, HPV31 13.64%, HPV33 11.11%, HPV-52 11.43%, HPV58 10.71%, and other HR-HPV types 6.98%. The overall CIN2+ positive rate for all HPV-positive cases was 16.19%. See **Figure 2**.

Multivariate analysis of risk factors for CIN2+

Multivariate logistic regression analysis (**Figure 3**) showed that HPV 16/18 infection (OR = 12.386, 95% CI: 6.853-22.392), TCT results of ASC-H or higher (OR = 8.907, 95% CI: 4.503-17.614), infection with other HR-HPV types (OR = 4.563, 95% CI: 2.352-8.851), ≥ 2 sexual partners in the past 3 years (OR = 2.342, 95% CI: 1.132-4.846), and age at first sexual intercourse < 20 years (OR = 1.844, 95% CI: 1.023-3.323) were significantly associated with the occurrence of CIN2+. Age ≥ 45 years was not significantly associated with the risk of CIN2+ (P = 0.304).

Discussion

This study comprehensively evaluated four cervical cancer screening triage strategies combining liquid-based cytology testing with HR-HPV testing. The results showed that the HPV screening combined with cytology triage achieved the optimal balance between clinical benefit and resource use. This method combined high sensitivity, acceptable specificity, and a manageable colposcopy referral rate. Overall, its diagnostic accuracy was comparable to the most sensitive co-testing method, but the latter required significantly more clinical procedures.

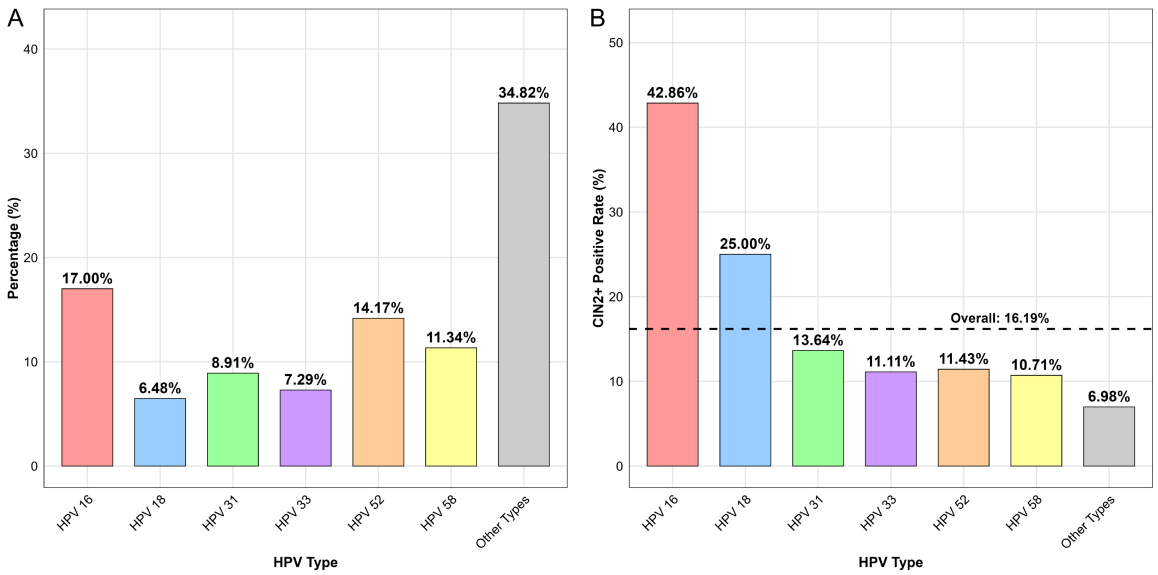

Figure 2. Distribution of high-risk human papillomavirus (HR-HPV) genotypes and their cervical intraepithelial neoplasia grade 2 or worse (CIN2+) positive rates. A. Proportion of different HR-HPV genotypes among 247 HPV-positive women. B. CIN2+ positive rate for each HR-HPV genotype.

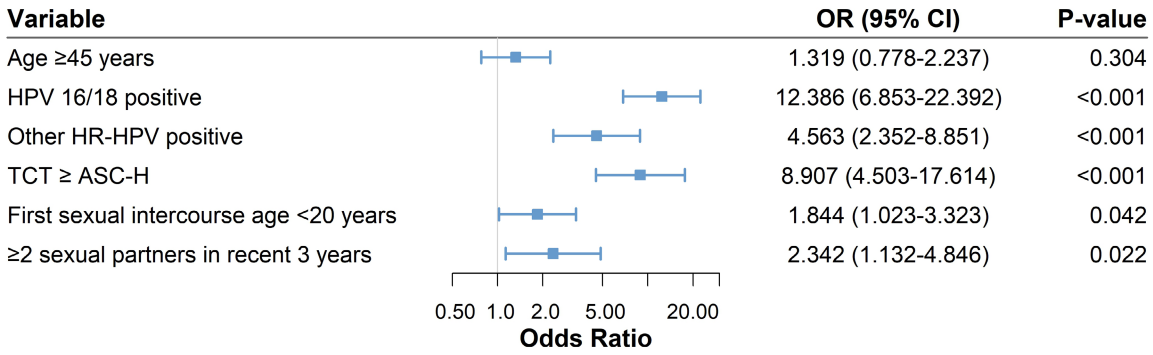

Figure 3. Forest plot of multivariable logistic regression analysis for factors associated with CIN2+ in cervical cancer screening. Notes: OR, odds ratio; CI, confidence interval; HPV, human papillomavirus; HR-HPV, high-risk human papillomavirus; TCT, ThinPrep cytologic test; ASC-H, atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion.

Baseline differences between HPV-positive and HPV-negative women provided important context for the results of this study. A higher proportion of women with lower levels of education were found in the HPV-positive group, a finding consistent with numerous global studies linking socioeconomic factors with healthcare accessibility and HPV exposure [15, 16]. Women who tested positive for HPV reported more recent sexual partners and earlier age at first intercourse, both of which are proven behavioral risk factors for HPV infection [16]. Therefore, the population in this study is representative of a typical HR clinical cohort, and the absolute incidence of CIN2+ and cytologic abnormalities may be higher in this population. Nevertheless, the comparison of the relative efficacy of the four strategies is still valid because the comparison was conducted in the same population and under the same conditions. Sensitivity, specificity, and AUC were mainly determined by the detection parameters, rather than the disease prevalence. Therefore, similar clinical populations can refer to the comparison results of this study [9].

The results of this study indicate that HPV testing and cytology alone have complementary effects. A higher proportion of CIN2+ patients were found in TCT-positive individuals, suggest-

ing that cytologic abnormalities are a specific indicator of precancerous lesions. However, the low overall positive rate of TCT also confirmed its insufficient sensitivity in early diagnosis. In contrast, HR-HPV testing can screen for more HR individuals, but its positive predictive value for CIN2+ was low. These findings support the view that HPV testing is a mainstream detection technology with high sensitivity and low specificity [4, 6].

In this study, the sensitivity of Strategy D was relatively low (76.19%), reflecting that even with combined HPV triage testing, cytology as a primary screening method still has inherent performance limitations. According to the 2021 WHO guidelines, based on moderately credible evidence that HPV testing can more significantly reduce the incidence and mortality of cervical cancer, HPV DNA testing is recommended as the preferred primary screening method, rather than cytology [17]. The WHO has clearly stated that existing cytology-based screening protocols can continue to be used only before HPV testing becomes widely available [18]. Similarly, the 2019 American Society for Colposcopy and Cervical Pathology consensus guidelines on risk-based management have shifted the screening model from “evidence-based” to “risk stratification” algorithms, recognizing that primary screening HPV testing has better sensitivity in detecting high-grade cervical lesions [13].

The limited sensitivity of TCT-based screening protocols stems primarily from the following factors. First, cytology examination can only detect pathologic morphologic changes caused by persistent HPV infection, while HPV testing can directly identify the pathogenic virus itself. This time lag means that cytology-based screening inevitably misses early infections and some existing lesions [4]. Second, the accuracy of cytology diagnosis itself varies, mainly affected by sampling errors and the subjectivity of smear interpretation, and up to 30% of CIN2+ lesions may not be accompanied by abnormal cytology [4, 19]. Studies have shown that the sensitivity of cytology examination for CIN3+ fluctuates between 34% and 94%, with a typical sensitivity of about 70% to 80% in routine clinical application [4]. Therefore, even though HPV triage testing is used in Strategy D, the overall sensitivity is still limited by the initial cytology examination, and subsequent triage testing cannot completely make up for this limitation.

These findings align with the current global consensus that HPV testing-based initial screening, combined with cytology or other triage methods, is the optimal approach that balances testing accuracy with colposcopy resource utilization [13, 18]. This inherent trade-off makes the sequential triage strategy reasonable. First, a broad screening is conducted using highly sensitive HPV testing, followed by more targeted cytology to identify individuals requiring further clinical intervention [20, 21].

This study, through systematic comparison, clarified the trade-offs between the advantages and disadvantages of various screening strategies in clinical application. Strategy A (cotesting, referral based on any positive result) can serve as a highly sensitive and safe screening approach, missing only one case of CIN2+. However, this strategy has low specificity and imposes a significant burden on colposcopy. This strategy is not suitable for routine screening, but may have some application value in extremely HR populations [5]. Strategy B exhibits the opposite characteristics: high specificity and few referrals, but low sensitivity, so it may not meet clinical needs. Relying solely on double-positive results for referral carries the risk of missing true precancerous lesions [19]. Strategy D shows good specificity, but its effectiveness is limited by the insufficient sensitivity of the initial cytology screening step itself, ranking second to last among the four strategies. This result supports the general consensus that HPV-based screening programs are superior to cytology-based initial screening programs [3, 22].

In summary, Strategy C achieves the best balance in all aspects. Its theoretical basis stems from the biologic mechanism of the disease: initial HPV screening can detect almost all high-risk individuals, while subsequent cytology retesting can identify individuals with viral-induced cellular morphologic changes. This process maintains excellent detection capabilities while improving specificity [20, 21]. Age stratification analysis in this study also confirmed that the strategy remained effective across different age groups.

This study conducted a comprehensive risk assessment based on HPV genotypes. Women positive for HPV16/18 had a significantly increased probability of developing CIN2+, and multivariate analysis confirmed that HPV16/18 was an important independent risk factor. This result provides strong evidence for current guidelines recommending direct referral of HPV16/18-positive patients for colposcopy [7, 8, 23]. Other high-risk HPV types (such as 31, 33, 52, and 58) showed varying disease risks, suggesting that expanding the scope of genotype testing could help optimize risk stratification management [10, 24].

This study found that high-grade cytologic abnormalities were an important independent predictor of CIN2+, and their predictive effect was not affected by virological variables. This result highlights the continued value of cytological examination as a biomarker of disease severity [18]. Behavioral characteristics such as multiple sexual partners and early age at first intercourse remain important predictive factors, and their mechanism of action may be related to increased HPV exposure probability and prolonged infection duration [16]. It is noteworthy that, after adjusting for HPV and cytology test results, age ≥ 45 years was not an independent risk factor. This suggests that in populations with well-established screening systems, biomarkers may be more significant than actual age.

In this study, one case of CIN2+ (accounting for 2.38%) was negative by both TCT and HR-HPV testing, which brought the sensitivity of Strategy A to 97.62%. This result is consistent with previous studies, namely that co-testing may miss 1% to 3% of high-grade lesions [19, 25]. The patient was a 52-year-old woman with a transformation zone of type 3. Colposcopy showed no obvious abnormalities, and she was diagnosed with CIN2 by random biopsy. There may be a variety of reasons for the occurrence of such cases: sampling errors (especially for lesions in the endocervical canal) [4], false negative results of HPV testing due to low viral load or rare genotypes not covered by the test [6], and the subjectivity of TCT itself, with up to 30% of cervical intraepithelial neoplasia grade 2 or higher lesions not showing typical cytologic abnormalities [19]. The results suggest that even with co-testing, there is still a residual risk of missing a small number of high-grade lesions, highlighting the importance of standardized screening intervals and appropriate management of patients with unclear colposcopy results [13, 17].

This study had several advantages. It directly compared several commonly used clinical screening protocols in a large, well-defined cohort and conducted a multivariate risk analysis based on comprehensive HPV genotyping. However, this study also had several limitations. First, the single-center retrospective study design may introduce validation bias. Since all participants underwent colposcopy, the estimated specificity and positive predictive value may have been overestimated. Second, the lack of racial and geographical diversity in the study population limited the extrapolation of the obtained HPV genotyping characteristics and the absolute efficacy results of each protocol. Third, this study lacked long-term follow-up data, making it impossible to assess the impact of each screening protocol on cervical cancer incidence and mortality [9]. Fourth, this study did not include novel biomarkers such as p16/Ki-67 double staining, even though existing studies have confirmed that these biomarkers can improve the specificity of triage diagnosis in HPV-positive individuals [18-20]. Future research could explore whether including such biomarkers could further optimize triage efficiency.

Future research should address these shortcomings. The results of this study need to be validated through prospective, multicenter trials in real-world screening scenarios across different populations. Including novel biomarkers such as p16/Ki-67 double staining is expected to further optimize triage protocols [18, 26]. The implementation of precise screening pathways must rely on research evidence including standardized health economics assessments and long-term clinical follow-ups [27].

Conclusion

The best screening strategy for cervical cancer is to use HPV testing as the initial screening method, followed by TCT triage for positive cases. This approach achieves a good balance between screening sensitivity, specificity, and referral burden, and is consistent with the pathophysiologic development of the disease. Combined HPV genotyping of types 16 and 18

can further achieve accurate risk stratification and clinical triage.

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Disclosure of conflict of interest

None.

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