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Association of HPV Infection with Vaginal Microecology and Abnormal Cervical Cytology: Dose–Response Effects of Viral Load in a Cross-Sectional Study of 7,997 Women in Tianmen, China

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Abstract

Background

Cervical cancer remains a leading cause of cancer-related mortality among women worldwide, and persistent infection with high-risk human papillomavirus (HR-HPV) is its necessary cause. However, the contribution of the vaginal microenvironment to HPV acquisition and cervical carcinogenesis is not well characterised. We evaluated independent associations between vaginal microecological indicators and HPV infection and explored the dose–response relationship between HPV viral load and cervical cytological abnormalities.

Methods

This cross-sectional study included 7,997 women who underwent concurrent HPV genotyping and vaginal microecological assessment at The First People's Hospital of Tianmen between June 2024 and June 2026; 6,562 (82.06%) also underwent thin-layer cytology (TCT). Microecological indicators were compared between HPV-positive and HPV-negative women using the chi-square test, and binary logistic regression models (M1–M3) identified factors associated with HPV positivity, HR-HPV positivity, and TCT abnormality, respectively. Among HPV-positive women, viral load was stratified into four tiers and its relation to cytological abnormalities was tested with the Cochran–Armitage trend test.

Results

Overall HPV and HR-HPV positivity rates were 15.77% (1,261/7,997) and 12.82% (1,025/7,997), with age peaks at < 31 years (21.39%) and ≥ 61 years (24.85%); HPV52, 58, 53, and 16 predominated. Bacterial vaginosis (BV) was associated with HPV (odds ratio [OR] 1.759) and HR-HPV (OR 1.537); leukocyte esterase (LE) with HR-HPV (OR 1.307) and TCT abnormality (OR 1.371); Trichomonas vaginalis with HR-HPV (OR 2.450, 95% confidence interval [CI] 1.180–5.087); and Candida inversely with HPV (OR 0.634). Cytological abnormalities were about eightfold more frequent in HPV-positive than HPV-negative women (26.58% vs. 3.32%), and HR-HPV positivity was associated with an OR of 8.822 (95% CI 7.161–10.870). Among HPV-positive women, the TCT abnormality rate increased with viral load from 17.6% (< 10^3 copies/10,000 cells) to 42.3% (≥ 10^5; trend $P < 0.001$), with a similar gradient among HR-HPV-positive women (18.2% to 43.9%).

Conclusions

Vaginal dysbiosis—particularly BV and inflammatory markers—is independently associated with HPV infection, and higher viral load is associated with a graded increase in cytological abnormality risk. These

findings suggest that vaginal microecological assessment and viral-load quantification may inform risk stratification in cervical cancer screening.

Introduction

Cervical cancer remains one of the most common malignancies of the female reproductive system worldwide, imposing a substantial disease burden despite advances in prevention and treatment [1]. The recognition that persistent infection with high-risk human papillomavirus (HR-HPV) is a necessary cause of cervical cancer and its precursor lesions has reshaped cervical cancer prevention over recent decades [2, 3]. This etiological understanding has driven a paradigm shift in screening, with HPV-based testing increasingly endorsed as a primary screening strategy [2]. Integrating HPV detection into screening programmes reduces the incidence of invasive cervical cancer [2], positioning HPV genotyping as a core modality in contemporary screening algorithms.

The natural history of HPV infection is highly variable, however, and a substantial proportion of infections regress spontaneously; HR-HPV infection alone is therefore insufficient to drive cervical carcinogenesis [3]. Against this background, the vaginal microecosystem has emerged as a critical modifier of cervical health—a dynamic milieu maintained by complex interactions among resident microflora, endocrine regulation, and local mucosal immunity [4]. A state of eubiosis, typically characterised by Lactobacillus-dominance and the production of lactic acid, bacteriocins, and hydrogen peroxide, serves as a key innate defence against pathogen colonisation [5]. Conversely, dysbiosis involving depletion of these protective bacteria and enrichment of anaerobic taxa-often manifesting clinically as bacterial vaginosis (BV) —is increasingly recognised as a pivotal cofactor in the natural history of HR-HPV infection [5]. Microbial imbalance can compromise the cervical mucosal barrier, modulate host susceptibility to HPV acquisition, and impede viral clearance [6, 7]. Consistent with this view, higher vaginal microbial diversity and reduced Lactobacillus abundance have been linked to HPV infection and to the severity of cervical intraepithelial neoplasia [8]. For instance, women with BV show a higher prevalence of HR-HPV infection, and this dysbiotic state may act as a cofactor in viral persistence and the development of cervical squamous intraepithelial lesions [9, 10]. Work in European and South American cohorts further indicates that a permissive, low-Lactobacillus microbiota increases the likelihood of detecting HPV and high-grade lesions [11, 12], and longitudinal data link Lactobacillus iners enrichment to reduced HR-HPV clearance [61]; review-level evidence likewise supports a permissive role of dysbiotic microbiota in HPV persistence and lesion progression [13], and a Chinese hospital-based series showed that BV-related anaerobes were over-represented in women with HR-HPV infections [14].

Mechanistic studies suggest that HPV oncoproteins can directly disrupt the protective microbial barrier, fostering a milieu conducive to viral persistence [15]. The host immune response is vital for viral clearance, and compositional shifts in the local microbiome can profoundly influence the mucosal immune milieu, thereby shaping the natural history of infection [16, 17]. The relationship between specific microbial components and HPV is complex: some studies have reported an inverse association between Candida colonisation and HPV positivity [18], whereas others link Candida species to specific

high-risk genotypes or to HR-HPV detection [19]. Disruption of normal lactobacillus-dominant flora facilitates viral persistence and replication, as demonstrated by studies linking reduced lactobacilli and BV to markedly increased HIV-1 genital shedding [20, 21]. Likewise, anomalies in vaginal microecology—including BV and reduced lactobacilli—have been associated with a higher likelihood of persistent HPV infection and with lesion progression after treatment of high-grade squamous intraepithelial lesions [22]. Large cohort studies have revealed complex, even stage-dependent interactions between specific vaginal microorganisms and HPV—for example, the dual effect of Candida albicans in protecting against initial acquisition yet promoting viral persistence [18]; the precise role of broader microecological disturbances, however, remains inadequately characterised.

Beyond the microbial community, HPV viral load is increasingly recognised as a factor closely linked to the severity of cervical lesions, with higher viral burden generally correlating with a greater risk of high-grade cervical intraepithelial neoplasia and invasive cancer [23]. Notably, accumulating evidence suggests that specific HPV genotypes may differ in pathogenicity and exhibit distinct relationships with cytological outcomes, with some non-high-risk genotypes, such as HPV53, demonstrating measurable—albeit slow—progression [24]. Age-related patterns of HPV prevalence and cytological lesion detection also exhibit regional variation, with older women often displaying a higher risk profile [25–27]. The prevalence of specific HPV genotypes and the composition of the vaginal microbiome show considerable geographic and ethnic variation, underscoring the need for region-specific epidemiological data [25, 28, 29]. Although numerous regional studies have been conducted in China, large-scale epidemiological data from central China—especially from primary-level hospitals serving rural and suburban populations—remain scarce, limiting the development of precisely tailored, region-specific strategies for cervical cancer prevention and management.

Despite these advances, existing studies have predominantly focused on targeted subpopulations, such as women with HR-HPV or established cervical lesions, or have relied on a single microecological parameter [10, 30]. Comprehensive evaluations that integrate multiple, clinically accessible vaginal microecological metrics with detailed HPV virological characteristics—including infection type and viral load—within a large, real-world screening population remain scarce. Previous investigations have largely focused on qualitative HPV positivity or specific high-risk genotypes [31], whereas the quantitative interplay between viral-load strata and microecological indicators has rarely been systematically evaluated. Substantial heterogeneity also exists across studies in the definitions of microecological abnormalities and in the detection platforms employed, complicating direct comparisons and the generalisability of findings [32, 33]. The independent relationships between a broad spectrum of microecological indicators and the specific patterns of HPV infection and replication—and the dose–response relationships between HPV viral load and both cervical cytological abnormalities and vaginal microecological status—remain to be fully delineated.

Given the potential geographical and ethnic variability in both vaginal microbiota composition and HPV genotype distribution, regional epidemiological investigations are warranted to inform localised preventive strategies. Accordingly, the present study leveraged a large, hospital-based dataset from

central China to systematically evaluate the association between vaginal microecological features and HPV infection status, as well as cervical cytological abnormalities. Among HPV-positive women, we further examined the dose–response relationships of viral-load strata with cervical cytological abnormalities and vaginal microecological indicators. We anticipated that the findings would provide region-specific epidemiological evidence clarifying the role of vaginal dysbiosis in HPV infection and cervical lesion development, thereby informing clinical strategies for microbiota-targeted adjunctive therapy to facilitate HPV clearance, and optimising cervical cancer screening protocols in this population.

Materials and methods

Study design and oversight

This cross-sectional study was conducted and reported in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement, and the completed STROBE checklist is provided as a supplementary file with this submission. Medical records of women who underwent concurrent HPV genotyping and vaginal microecological testing at The First People's Hospital of Tianmen between 1 June 2024 and 30 June 2026 were retrospectively reviewed. Data were de-identified before analysis.

Setting and study population

The study was performed at The First People's Hospital of Tianmen, a tertiary facility serving a county-level area in central China. Records were eligible if the woman underwent both HPV genotyping and vaginal microecological testing within the same clinical encounter during the study period, with complete key information on sex, age, and visit type. For women with multiple tests, only the earliest record was retained to avoid within-person duplication. Among the 9,179 records identified, the woman-level analysis included 7,997 unique women. Of these, 6,562 (82.06%) also underwent concurrent liquid-based thin-layer cytology (TCT) testing. Whether a woman underwent concurrent TCT reflected routine clinical practice at the participating institution rather than a study-defined testing protocol. The derivation of the analytic sample, the reasons for exclusion, and the analytic sub-cohorts (HPV-positive with viral load, TCT subgroup, sensitivity analysis) are summarised in Fig. 1.

Detection methods

HPV genotyping was performed using HPV nucleic acid genotyping kits (Jiangsu Bioperfectus Technologies Co., Ltd.) with real-time fluorescence polymerase chain reaction, detecting 21 HPV subtypes. Low-risk types comprised HPV6, 11, and 81; intermediate-risk types comprised HPV26, 53, 66, 73, and 82; and high-risk types comprised HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68. One

positive control and one blank control were amplified per batch, and viral load was quantified simultaneously (expressed as copies per 10,000 cells).

Vaginal microecology was assessed at the same time point following the Expert Consensus on Standardization of Clinical Testing and Result Reporting for Vaginal Microecological Evaluation. Secretions were tested on the automated reproductive tract secretion analyser AutowoMO W500 with matching reagents (Zhengzhou Autobio Diagnostics Co., Ltd.) using wet-mount microscopy combined with dry chemical enzyme assays. Recorded indicators included cleanliness grade, pH, white blood cells, red blood cells, Lactobacillus, Candida, Trichomonas, and the enzymes N-acetyl-β-D-glucosaminidase (NAG), leukocyte esterase (LE), coagulase, hydrogen peroxide (H_2O_2), glucosidase (GLU), proline aminopeptidase (PIP), and sialidase. Bacterial vaginosis was diagnosed according to the Amsel criteria (≥ 3 positive findings), and Candida and Trichomonas were identified by direct microscopy.

Liquid-based TCT slides were prepared and interpreted by cytopathologists according to The Bethesda System (TBS). NILM (negative for intraepithelial lesion or malignancy) was considered negative; all non-NILM categories—including ASC-US (atypical squamous cells of undetermined significance), ASC-H (atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion), LSIL (low-grade squamous intraepithelial lesion), HSIL (high-grade squamous intraepithelial lesion), AGC (atypical glandular cells), and malignant cells—were classified as cytological abnormalities.

Diagnostic criteria

Detection of any subtype was considered HPV-positive; detection of at least one high-risk subtype was considered HR-HPV-positive; and simultaneous detection of two or more subtypes was considered multiple infection. TCT abnormality referred to non-NILM results, with clinically significant cytological lesions defined as LSIL, HSIL, ASC-H, AGC, or malignant cells. Cleanliness grade III–IV was considered abnormal; pH ≥ 4.6 was considered elevated; LE results of + to 3 + were considered positive; leukocytes and erythrocytes reported as ± or above were considered positive; an H_2O_2 result of "−" was considered deficient; and a Lactobacillus result of "few" was considered reduced. BV was diagnosed according to the Amsel criteria (≥ 3 positive findings) using the routine laboratory panel described above. Viral load was defined as the highest value among detected subtypes (copies per 10,000 cells) and stratified into four tiers: $<10^3$ (low), 10^3–$<10^4$ (moderate), 10^4–$<10^5$ (high), and ≥ 10^5 (very high); values of exactly 10^3, 10^4, and 10^5 were assigned to the 10^3–$<10^4$, 10^4–$<10^5$, and ≥ 10^5 tiers, respectively.

Statistical analysis

Data analysis was performed in Python 3.12 using the pandas, NumPy, SciPy and statsmodels libraries. Continuous variables are presented as mean ± SD for normally distributed data and as median (interquartile range, IQR) otherwise, with between-group comparisons performed using the t-test or non-parametric tests as appropriate. Categorical variables are presented as counts (%), with between-group comparisons performed using the chi-square test; rates for two-group comparisons are based on

Pearson’s chi-square calculated from corresponding 2 × 2 contingency tables. Univariate comparisons were exploratory; no multiplicity adjustment was applied to these descriptive contrasts.

Three binary logistic regression models (M1–M3) were fitted with HPV positivity, HR-HPV positivity, and TCT abnormality as outcomes, respectively, using the forced entry method and adjusting for age group, visit type, and a pre-specified panel of microecological indicators. Results are reported as odds ratios (ORs) with 95% confidence intervals (CIs). Because sialidase, coagulase, and PIP were highly correlated with BV, including all of them simultaneously could induce multicollinearity; only BV was therefore retained in the multivariable models. In our cohort, the numbers of BV-positive and sialidase-positive cases were identical (both 140), confirming near-complete concordance between sialidase positivity and BV diagnosis on this platform. Because H_2O_2 deficiency and reduced Lactobacillus were almost perfectly concordant (concordance 99.6%), only the latter was retained. GLU was measured as part of the routine panel but was not included in the analyses because it was not a pre-specified study variable; its results are therefore not presented. Erythrocyte positivity was reported descriptively in univariate analyses (Table 2) but was not entered into the multivariable models, as it was not a pre-specified analysis variable. Collinearity diagnostics showed variance inflation factors (VIFs) < 2.5 for all included variables (LE 2.02, leukocytes 1.98, BV 1.07), indicating no severe collinearity.

Among HPV-positive women, age-stratified HR-HPV-specific rates and viral-load strata were examined in relation to microecological indicators and TCT abnormalities, with trends tested using the Cochran–Armitage trend test. A sensitivity analysis was performed at the record level by including all 9,179 records to assess robustness. To address the strong correlation between LE and leukocyte positivity ($\varphi = 0.679$), Models M1 and M2 were refitted after excluding the leukocyte indicator. Because Trichomonas positivity was rare (35 affected women), Model M2 was additionally refitted using Firth’s penalised likelihood to reduce sparse-data bias, with penalised profile-likelihood 95% CIs. To evaluate the potential for selection bias arising from clinically indicated TCT ordering, baseline characteristics were additionally compared between women with and without concurrent TCT results using Pearson’s chi-square tests (Fisher’s exact test where any expected count was < 5) for categorical variables and Welch’s t-test for age (Table 5). For all analyses, two-sided $P < 0.05$ was considered statistically significant.

Ethics approval

This study was conducted in accordance with the Declaration of Helsinki (as revised in 2024) and the Measures for Ethical Review of Life Science and Medical Research Involving Human Subjects (2023) issued by the National Health Commission of the People's Republic of China, and was reviewed and approved by the Ethics Committee of The First People's Hospital of Tianmen (approval number: 20260288; approved on September 12, 2026). Because the study used de-identified routine clinical laboratory data, the requirement for individual written informed consent was waived. Among the 7,997 enrolled women, 18 were younger than 18 years (2 aged 15, 7 aged 16, and 9 aged 17 years); because the study used only de-identified routine laboratory data and involved no direct participant contact, the ethics approval and the consent waiver applied to these participants as well.

Results

Overall HPV infection status and genotype distribution

The enrolled women were aged 15–90 years (mean 45.08 ± 11.75 years; median 43 years). Of the 7,997 women, 6,840 (85.5%) were outpatients, 1,120 (14.0%) were inpatients, and 37 (0.5%) attended for a health check-up. HPV was detected in 1,261 women (15.77%), of whom 1,025 (81.28%) were HR-HPV-positive and 182 (2.28% of the total cohort) were positive for HPV16/18. Multiple infections were identified in 346 HPV-positive women (27.44%), with up to nine genotypes detected in a single sample. The most frequent genotypes were HPV52 (345 cases; 27.36% of HPV-positive women), HPV58 (166; 13.16%), HPV53 (145; 11.50%), HPV16 (137; 10.86%), and HPV39/HPV81 (115 each; 9.12%).

HPV infection by age group and visit type (Table 1)

HPV infection exhibited a bimodal age pattern: 21.39% (126/589) in women aged < 31 years, decreasing to 11.76% (316/2,688) in the 31–40-year group, 11.92% (248/2,081) in the 41–50-year group, rebounding to 20.14% (363/1,802) in the 51–60-year group, and reaching 24.85% (208/837) in women aged ≥ 61 years ($\chi^2 = 147.805$, $P < 0.001$). The mean age of HPV-positive women (47.33 ± 13.43 years) was higher than that of HPV-negative women (44.66 ± 11.35 years; $t = 6.635$, $P < 0.001$). Positivity rates also differed by visit type ($\chi^2 = 28.046$, $P < 0.001$): 16.65% (1,139/6,840) in outpatients, 10.45% (117/1,120) in inpatients, and 13.51% (5/37) in health check-up attendees. Because the health check-up group was small (n = 37), the visit-type comparison was additionally verified using Fisher's exact test (Monte Carlo simulation with 200,000 replicates), which confirmed the significant difference ($P < 0.001$).

Table 1
HPV infection by age group and visit type

Group	n	HPV-positive, n	Positivity rate, %	χ^2	P
Age group (years)					
< 31	589	126	21.39		
31–40	2,688	316	11.76		
41–50	2,081	248	11.92		
51–60	1,802	363	20.14		
≥ 61	837	208	24.85		
Comparison across age groups				147.805	< 0.001
Visit type					
Outpatient	6,840	1,139	16.65		
Inpatient	1,120	117	10.45		
Health check-up	37	5	13.51		
Comparison across visit types				28.046	< 0.001

The χ^2 values refer to the overall comparison across the five age groups and three visit types. The visit-type comparison was additionally verified with Fisher's exact test (Monte Carlo simulation, 200,000 replicates), $P < 0.001$.

Vaginal microecological indicators by HPV status (Table 2)

In univariate analysis, the HPV-positive group had significantly higher positivity rates than the HPV-negative group for BV (11.10% vs. 6.41%), LE (76.92% vs. 69.74%), cleanliness grade III–IV (56.86% vs. 53.04%), sialidase (11.10% vs. 6.31%), PIP (1.35% vs. 0.65%), Trichomonas (0.87% vs. 0.36%), and leukocytes (81.44% vs. 76.10%) (all $P < 0.05$). Conversely, positivity rates for Candida (8.33% vs. 13.41%) and coagulase (9.20% vs. 13.70%) were significantly lower in the HPV-positive group ($P < 0.05$). No statistically significant differences were observed for pH ≥ 4.6 (91.67% vs. 90.75%), H_2O_2 deficiency (84.54% vs. 83.55%), reduced Lactobacillus (85.25% vs. 83.89%), NAG (3.09% vs. 2.73%), or erythrocyte positivity (26.72% vs. 25.01%) ($P > 0.05$).

Table 2
Vaginal microecological indicators by HPV status

Indicator	HPV-positive (n = 1,261)	HPV-negative (n = 6,736)	χ^2/t	P
Age (years, mean ± SD)	47.33 ± 13.43	44.66 ± 11.35	6.635	< 0.001
Cleanliness grade III–IV	717 (56.86)	3,573 (53.04)	6.221	0.012
BV positivity	140 (11.10)	432 (6.41)	35.165	< 0.001
pH ≥ 4.6	1,156 (91.67)	6,113 (90.75)	1.091	0.296
LE positivity	970 (76.92)	4,698 (69.74)	26.516	< 0.001
H_2O_2 deficiency	1,066 (84.54)	5,628 (83.55)	0.756	0.385
Reduced Lactobacillus	1,075 (85.25)	5,651 (83.89)	1.464	0.226
NAG positivity	39 (3.09)	184 (2.73)	0.511	0.475
Coagulase positivity	116 (9.20)	923 (13.70)	19.056	< 0.001
Sialidase positivity	140 (11.10)	425 (6.31)	37.161	< 0.001
PIP positivity	17 (1.35)	44 (0.65)	6.776	0.009
Candida positivity	105 (8.33)	903 (13.41)	24.871	< 0.001
Trichomonas positivity	11 (0.87)	24 (0.36)	6.491	0.011
Leukocyte positivity	1,027 (81.44)	5,126 (76.10)	17.102	< 0.001
Erythrocyte positivity	337 (26.72)	1,685 (25.01)	1.644	0.200

Categorical data are presented as n (%); the age row shows the t value and all other rows show chi-square values.

Multivariable analysis of microecology and HPV infection (Table 3)

In Model M1 (outcome: HPV positivity), independent positive associations were identified for BV (OR 1.759, 95% CI 1.422–2.176), age < 31 years (OR 1.971, 95% CI 1.564–2.483), age 51–60 years (OR 1.802, 95% CI 1.519–2.138), and age ≥ 61 years (OR 2.620, 95% CI 2.131–3.219), whereas hospitalisation (OR 0.546, 95% CI 0.444–0.672) and Candida positivity (OR 0.634, 95% CI 0.504–0.796) showed inverse

associations. In Model M2 (outcome: HR-HPV positivity), independent associations were identified for BV (OR 1.537, 95% CI 1.216–1.942), LE (OR 1.307, 95% CI 1.047–1.631), Trichomonas (OR 2.450, 95% CI 1.180–5.087), age < 31 years (OR 2.123, 95% CI 1.649–2.734), age 51–60 years (OR 2.038, 95% CI 1.687–2.461), and age ≥ 61 years (OR 2.988, 95% CI 2.390–3.736), with Candida remaining inversely associated (OR 0.666, 95% CI 0.519–0.856). Age was referenced to the 31–40-year group and visit type to outpatient visits. In a Firth penalised-likelihood sensitivity analysis, the Trichomonas–HR-HPV association was virtually unchanged (OR 2.501, 95% CI 1.178–5.013).

Table 3
Multivariable analysis of microecology and HPV infection

Variable	M1 OR (95% CI)	M1 P	M2 OR (95% CI)	M2 P
< 31 years	1.971 (1.564–2.483)	< 0.001	2.123 (1.649–2.734)	< 0.001
31–40 years	1.000	—	1.000	—
41–50 years	1.038 (0.868–1.241)	0.684	1.143 (0.936–1.394)	0.190
51–60 years	1.802 (1.519–2.138)	< 0.001	2.038 (1.687–2.461)	< 0.001
≥ 61 years	2.620 (2.131–3.219)	< 0.001	2.988 (2.390–3.736)	< 0.001
Outpatient	1.000	—	1.000	—
Inpatient	0.546 (0.444–0.672)	< 0.001	0.569 (0.455–0.713)	< 0.001
Health check-up	0.965 (0.371–2.510)	0.941	0.980 (0.342–2.802)	0.969
Cleanliness grade III–IV	1.072 (0.918–1.251)	0.381	1.055 (0.892–1.247)	0.531
BV positivity	1.759 (1.422–2.176)	< 0.001	1.537 (1.216–1.942)	< 0.001
pH ≥ 4.6	0.861 (0.678–1.094)	0.222	0.874 (0.671–1.137)	0.315
LE positivity	1.196 (0.979–1.462)	0.080	1.307 (1.047–1.631)	0.018
NAG positivity	0.778 (0.540–1.123)	0.180	0.893 (0.609–1.309)	0.562
Reduced Lactobacillus	0.972 (0.807–1.173)	0.770	0.916 (0.748–1.122)	0.395
Candida positivity	0.634 (0.504–0.796)	< 0.001	0.666 (0.519–0.856)	0.002
Trichomonas positivity	1.887 (0.910–3.914)	0.088	2.450 (1.180–5.087)	0.016
Leukocyte positivity	1.006 (0.811–1.248)	0.957	0.989 (0.779–1.255)	0.926

Age was referenced to the 31–40-year group and visit type to outpatient visits; because sialidase, coagulase, and PIP were highly correlated with BV, only BV was retained in the multivariable models.

HPV infection and cytological abnormalities (Table 4)

TCT results were available for 6,562 women, including 6,101 NILM and 461 (7.03%) cytological abnormalities. The abnormality rate in the HPV-positive group (26.58%; 278/1,046) was about eightfold higher than that in the HPV-negative group (3.32%; 183/5,516; $\chi^2 = 724.731$, $P < 0.001$); the rates of clinically significant cytological lesions were 13.19% (138/1,046) and 1.85% (102/5,516), respectively. In the HR-HPV-positive group, the abnormality rate was 28.52% (243/852) versus 3.82% (218/5,710) in the HR-HPV-negative group ($\chi^2 = 688.881$, $P < 0.001$), with clinically significant lesions at 14.55% (124/852) and 2.03% (116/5,710), respectively. Regarding abnormality categories, the HPV-positive group showed higher proportions of ASC-US, LSIL, and malignant cells compared with the HPV-negative group.

Table 4
HPV infection and cytological abnormalities

Group	n	NILM, n (%)	Cytological abnormality, n (%)	Clinically significant lesions, n (%)	χ^2	P
HPV-positive	1,046	768 (73.42)	278 (26.58)	138 (13.19)	724.731	< 0.001
HPV-negative	5,516	5,333 (96.68)	183 (3.32)	102 (1.85)		
HR-HPV-positive	852	609 (71.48)	243 (28.52)	124 (14.55)	688.881	< 0.001
HR-HPV-negative	5,710	5,492 (96.18)	218 (3.82)	116 (2.03)		

Chi-square values compare the HPV-positive and HPV-negative groups (or the HR-HPV-positive and HR-HPV-negative groups) based on 2 × 2 tables of positivity/negativity by cytological abnormality/NILM; clinically significant cytological lesions include LSIL, HSIL, ASC-H, AGC, and malignant cells.

Comparison of baseline characteristics between women with and without concurrent TCT testing (Table 5)

Among 7,997 women in the woman-level analysis, 6,562 (82.06%) had concurrent TCT results and 1,435 (17.94%) did not. To assess the potential for selection bias arising from clinically indicated TCT ordering, women with and without available TCT results were compared. HPV (15.9% vs. 15.0%; P = 0.367) and HR-HPV (13.0% vs. 12.1%; P = 0.341) positivity rates were virtually identical between the two groups, indicating that HPV infection status—the principal determinant of the cytological analyses—was essentially unrelated to TCT ordering. Women who underwent TCT were slightly older (mean age 45.4 vs. 43.6 years; P < 0.001) and more often had inflammation-related findings (leukocyte positivity, 78.5% vs. 70.0%; erythrocyte positivity, 26.5% vs. 19.9%; pH ≥ 4.6, 91.9% vs. 86.3%; cleanliness grade III–IV, 54.7% vs. 48.9%; all P < 0.001; N-acetyl-β-D-glucosaminidase, 3.0% vs. 1.8%; P = 0.013), whereas the prevalences of BV, Candida, Trichomonas, LE positivity, H_2O_2 deficiency, reduced Lactobacillus, sialidase, coagulase, and PIP were similar (all P > 0.05).

Table 5
Comparison of baseline characteristics between women with and without concurrent TCT testing

Characteristic	With TCT (n = 6,562)	Without TCT (n = 1,435)	P value
Age group, years			
Age (years), mean ± SD	45.41 ± 11.74	43.60 ± 11.65	< 0.001
Age < 31 years	451 (6.87)	138 (9.62)	< 0.001
Age 31–40 years	2,163 (32.96)	525 (36.59)	0.008
Age 41–50 years	1,735 (26.44)	346 (24.11)	0.069
Age 51–60 years	1,494 (22.77)	308 (21.46)	0.284
Age ≥ 61 years	719 (10.96)	118 (8.22)	0.002
Visit type			
Outpatient	5,589 (85.17)	1,251 (87.18)	0.050
Inpatient	938 (14.29)	182 (12.68)	0.111
Health check-up	35 (0.53)	2 (0.14)	0.046
HPV and microecological characteristics			
HPV positivity	1,046 (15.94)	215 (14.98)	0.367
HR-HPV positivity	852 (12.98)	173 (12.06)	0.341
BV	481 (7.33)	91 (6.34)	0.188
LE positivity	4,641 (70.73)	1,027 (71.57)	0.525
Candida	818 (12.47)	190 (13.24)	0.423
Trichomonas	27 (0.41)	8 (0.56)	0.448
Cleanliness grade III–IV	3,589 (54.69)	701 (48.85)	< 0.001
Sialidase	477 (7.27)	88 (6.13)	0.128
H2O2 deficiency	5,481 (83.53)	1,213 (84.53)	0.351
Reduced Lactobacillus	5,497 (83.77)	1,229 (85.64)	0.079
NAG	197 (3.00)	26 (1.81)	0.013
Coagulase	842 (12.83)	197 (13.73)	0.360
PIP	48 (0.73)	13 (0.91)	0.491

Characteristic	With TCT (n = 6,562)	Without TCT (n = 1,435)	P value
Age group, years			
Leukocyte positivity	5,149 (78.47)	1,004 (69.97)	< 0.001
Erythrocyte positivity	1,737 (26.47)	285 (19.86)	< 0.001
pH ≥ 4.6	6,030 (91.89)	1,239 (86.34)	< 0.001

Data are n (%) unless otherwise stated. P values are from Pearson's χ^2 tests (Fisher's exact test where any expected count was < 5) and Welch's t-test for age. HPV, human papillomavirus; HR-HPV, high-risk human papillomavirus; TCT, thin-layer cytology; BV, bacterial vaginosis; LE, leukocyte esterase; NAG, N-acetyl-β-D-glucosaminidase.

Multivariable analysis of factors associated with TCT abnormalities (Model M3, Table 6)

Using TCT abnormality as the outcome (n = 6,562), independent associations were identified for HR-HPV positivity (OR 8.822, 95% CI 7.161–10.870), age 41–50 years (OR 1.438, 95% CI 1.025–2.016), age 51–60 years (OR 2.844, 95% CI 2.094–3.862), age ≥ 61 years (OR 5.005, 95% CI 3.597–6.962), and LE positivity (OR 1.371, 95% CI 1.030–1.827). Other variables (age < 31 years, visit type, cleanliness grade, BV, and Candida) were not independently associated.

Table 6
Multivariable analysis of factors associated with TCT abnormalities

Variable	OR (95% CI)	P
< 31 years	1.062 (0.637–1.772)	0.817
31–40 years	1.000	—
41–50 years	1.438 (1.025–2.016)	0.036
51–60 years	2.844 (2.094–3.862)	< 0.001
≥ 61 years	5.005 (3.597–6.962)	< 0.001
Outpatient	1.000	—
Inpatient	0.866 (0.636–1.177)	0.358
Health check-up	1.870 (0.535–6.533)	0.327
Cleanliness grade III–IV	0.950 (0.745–1.213)	0.683
BV positivity	0.798 (0.539–1.181)	0.259
LE positivity	1.371 (1.030–1.827)	0.031
Candida positivity	0.689 (0.449–1.057)	0.088
HR-HPV positivity	8.822 (7.161–10.870)	< 0.001

Viral load, microecology, and TCT abnormalities (Table 7)

All 1,261 HPV-positive cases had quantitative viral-load results, with a median (IQR) of 8,460 (533–97,700) copies per 10,000 cells (median $\log_{10}$ viral load 3.93). Stratified by viral load, the TCT abnormality rate increased monotonically: 17.6% (54/306) in the < 10^3 group, 21.7% (55/253) in the 10^3–<10^4 group, 26.5% (62/234) in the 10^4–<10^5 group, and 42.3% (107/253) in the ≥ 10^5 group ($\chi^2 = 47.56$, $P < 0.001$; Cochran–Armitage trend $P < 0.001$). Among HR-HPV-positive women with available TCT results, a similar gradient emerged: 18.2% (43/236), 23.5% (47/200), 28.7% (56/195), and 43.9% (97/221) across the same four strata ($\chi^2 = 40.37$, $P < 0.001$). Log_{10} viral load was higher in women with TCT abnormalities than in those with NILM (4.52 vs. 3.66, $P < 0.001$). BV positivity did not differ across viral-load strata (10.4%, 10.8%, 11.9%, 11.5%; trend $P = 0.565$), but LE positivity declined (85.0% to 67.7%) and abnormal cleanliness overall declined (66.7% to 47.9%, with non-monotonic variation across intermediate strata) with increasing viral load (both trend $P < 0.001$).

Table 7
Viral load, microecology, and TCT abnormalities

Viral load stratum (copies/10,000 cells)	n	BV positivity	LE positivity	Cleanliness grade III–IV	TCT abnormality
$< 10^3$	366	38 (10.4)	311 (85.0)	244 (66.7)	54/306 (17.6)
10^3–$<10^4$	296	32 (10.8)	229 (77.4)	159 (53.7)	55/253 (21.7)
10^4–$<10^5$	286	34 (11.9)	218 (76.2)	164 (57.3)	62/234 (26.5)
$\geq 10^5$	313	36 (11.5)	212 (67.7)	150 (47.9)	107/253 (42.3)
P for trend		0.565	< 0.001	< 0.001	< 0.001

Viral load is expressed as copies/10,000 cells, taking the highest value among detected subtypes; percentages for TCT abnormality were calculated using women with available TCT results as the denominator; trend tests were performed using the Cochran–Armitage trend test. The HR-HPV-specific dose–response analysis was additionally restricted to HR-HPV-positive women with available TCT results, with each stratum-specific denominator comprising these women. In this subgroup (n = 852), the stratum-specific TCT abnormality rates were 43/236 (18.2%) in the $< 10^3$ stratum, 47/200 (23.5%) in the 10^3–$<10^4$ stratum, 56/195 (28.7%) in the 10^4–$<10^5$ stratum, and 97/221 (43.9%) in the $\geq 10^5$ stratum.

Sensitivity analyses

When all 9,179 records were analysed, the HPV positivity rate was 19.33% (1,774/9,179) and the HR-HPV positivity rate was 16.00% (1,469/9,179); both were slightly higher than the woman-level estimates, and the directions of associations were consistent with those of the primary analysis. After excluding the leukocyte indicator due to its strong correlation with LE ($\varphi = 0.679$), refitting Models M1 and M2 left the direction and magnitude of LE associations stable (M1: OR 1.200, 95% CI 1.016–1.417, P = 0.031; M2: OR 1.299, 95% CI 1.081–1.560, P = 0.005).

Discussion

Principal findings

In this large, hospital-based cross-sectional study of 7,997 women from central China, BV, LE, and Trichomonas showed independent positive associations with HPV or HR-HPV infection, whereas Candida was inversely associated with HPV. HR-HPV positivity was associated with markedly elevated odds of cytological abnormality (adjusted OR 8.822), with a graded relationship between viral load and TCT abnormality that was evident both overall and among HR-HPV-positive women (trends P < 0.001). Together, these findings support integrating vaginal microecological assessment and viral-load quantification into risk stratification of HPV-positive women.

HPV prevalence, age distribution, and genotype profile

The overall HPV (15.77%) and HR-HPV (12.82%) positivity rates are consistent with the broader epidemiological landscape in China, where prevalence typically ranges from roughly 7% to over 16% depending on the population and region [34, 35]. The predominance of HPV52, HPV58, and HPV16 mirrors the genotype profile repeatedly documented in Chinese cohorts [26, 36–38], and the notable HPV53 burden observed here is likewise consistent with reports from Nanjing and Northeast China [26, 37]. The substantial residual burden of HPV infection in older women, with overall positivity reaching 24.85% in the ≥ 61-year age group, reinforces the need for age-inclusive screening and follow-up strategies.

A bimodal age distribution was apparent: a first peak in women younger than 31 years and a second, higher peak from age 51 onward. The risk of cytological abnormality also rose independently with age in multivariable analysis (OR 5.005 for ≥ 61 years, Model M3). This convergence suggests that a substantial part of the disease burden in older women reflects persistent infection acquired earlier in life rather than new acquisition, a pattern increasingly reported in Chinese screening populations [26, 38]. The perimenopausal transition—characterised by hormonal change, epithelial atrophy, and shifts in the vaginal ecosystem—may render the transformation zone more vulnerable to the cytopathic effects of established infection; however, cross-sectional data cannot fully disentangle age effects from cohort or duration effects.

Vaginal microecology and HPV infection

Among the microecological indicators examined, BV showed the most consistent association, being independently linked to both overall HPV positivity (OR 1.759) and HR-HPV positivity (OR 1.537). This finding aligns with meta-analytic estimates from case–control studies [39] and with odds ratios of comparable magnitude reported in large single-centre series [40]; similar associations have been observed in large regional cohorts [41, 42]. Specific BV-associated anaerobes, such as Atopobium vaginae, BVAB1, and BVAB3, have also been linked to prevalent HR-HPV and may serve as biomarkers of HPV risk [43], and the protective role of a Lactobacillus-dominant flora is further supported by reports that Lactobacillus crispatus-dominant communities are inversely associated with HPV detection [44]. LE positivity was independently associated with HR-HPV positivity (OR 1.307) and cytological abnormality (OR 1.371); the association with overall HPV positivity was attenuated in the primary model (P = 0.080) but became significant after excluding the correlated leukocyte indicator (OR 1.200, P = 0.031), suggesting that LE captures an inflammatory component partly overlapping with leukocyte counts [45–47]. The association between LE positivity and cytological abnormality (OR 1.371) warrants particular caution: because LE and cervical cytology were assessed concurrently at a single visit, elevated LE may either accompany epithelial abnormality or, alternatively, reflect inflammation that compromises specimen quality or cytological interpretation, whereas coexisting cervicitis or vaginitis may itself elevate LE. The cross-sectional design cannot distinguish these directions, and the modest effect size indicates that LE is best interpreted as an inflammatory correlate of abnormal cytology rather than a specific marker of epithelial lesion. Together, these findings support dysbiosis and mucosal inflammation as measurable cofactors in HPV persistence and cervical lesion development.

Trichomonas positivity was independently associated with HR-HPV positivity (OR 2.450, 95% CI 1.180–5.087) but not with overall HPV positivity in the primary model, consistent with the mixed literature on concurrent sexually transmitted infections [48, 49]. Because only 11 Trichomonas-positive women were HPV-positive and 24 were HPV-negative, this estimate rests on a small number of events and the wide confidence interval indicates limited precision; the association should therefore be regarded as exploratory, although a Firth penalised-likelihood sensitivity analysis yielded a virtually identical estimate (OR 2.501, 95% CI 1.178–5.013), indicating that the finding is unlikely to reflect sparse-data bias. The inverse association between Candida positivity and HPV infection (OR 0.634 for overall HPV; OR 0.666 for HR-HPV) echoes cohort and multicentre clinical data showing no increased HR-HPV risk with vulvovaginal candidiasis [18, 50] and nationwide data linking treated candidal vulvovaginitis to a lower subsequent risk of cervical cancer [50]. Some reports have linked Candida positivity to infection with alpha-9 high-risk HPV genotypes [19] or described Candida among infectious agents detected in cervical cytology screening populations [51], and population differences or diagnostic methods may explain these inconsistencies. Whether the net inverse association reflects competitive microbial ecology, differences in host immunity, or diagnostic artefacts remains uncertain; experimental evidence indicates that Candida components can modulate local inflammatory signalling [52], clinical observational data link BV to cervical neoplasia [53], and lactobacilli can inhibit Candida albicans [54]. The dual role of Candida in HPV natural history merits dedicated longitudinal and mechanistic studies [18].

Viral load and cytological abnormality

HPV-positive women had an approximately eightfold higher rate of cytological abnormality than HPV-negative women (26.58% vs. 3.32%), and HR-HPV positivity was associated with an adjusted OR of 8.822 for abnormal cytology—substantially larger than any other covariate in the model. This magnitude aligns with the central role of persistent oncogenic HPV infection in cervical carcinogenesis [3, 55] and reinforces the rationale for HPV-based screening with appropriate triage of HPV-positive women.

Among HPV-positive women, the rate of cytological abnormality increased monotonically with viral load, from 17.6% in the lowest stratum ($< 10^3$ copies per 10,000 cells) to 42.3% in the highest ($\geq 10^5$), with a parallel gradient among HR-HPV-positive women (18.2% to 43.9%; trend $P < 0.001$). These findings support viral-load quantification as a candidate adjunct for risk stratification and triage [56–58]. In exploratory analyses, BV prevalence did not differ across viral-load strata, whereas LE positivity and abnormal cleanliness declined overall with increasing viral load. Because these analyses were confined to HPV-positive women and were cross-sectional, this inverse pattern may reflect differences in infection timing, lesion stage, or sample composition rather than a causal relationship; we therefore do not interpret it as evidence against a role of the vaginal milieu in HPV persistence.

Limitations

Several limitations should be acknowledged when interpreting these findings.

1. This was a single-centre, hospital-based study in which 85.5% of participants were outpatients; selection bias may limit the generalisability of our estimates to community-based populations, and unmeasured confounders—including HPV vaccination status, sexual behaviour, hormonal contraception, and socioeconomic position—could not be addressed [59].

2. The cross-sectional design precludes causal or temporal inference, which is particularly important given that HPV infection and the vaginal microbiota appear to influence each other bidirectionally [33, 60, 61].

3. Viral-load and microecological indicators were each measured at a single time point; serial sampling would be required to capture clearance, persistence, and intra-individual variability [61–63].

4. The routine enzymatic and morphological markers available on this platform offer less species-level resolution than sequencing-based methods, and the strong interrelations among indicators complicate the interpretation of individual biomarkers [31, 60]. For example, LE and leukocyte positivity were strongly correlated ($\varphi = 0.679$), whereas LE and BV showed no severe collinearity (VIF 1.07); after excluding the leukocyte indicator, LE associations remained stable in direction and magnitude.

5. Viral-load assays are not yet standardised across HPV genotypes or platforms, and the four-tier stratification used here, while clinically pragmatic, may obscure genotype-specific patterns such as those described for HPV52 [58].

6. No sample-size calculation was performed a priori; however, the sensitivity analysis at the record level (n = 9,179) yielded consistent effect directions, supporting the robustness of the primary findings.

7. TCT was performed in 82.06% of the study population and reflected routine clinical ordering rather than a study-defined protocol; women who underwent cytological testing may differ systematically from those who did not (for example, in age, visit type, or clinical indication). A direct comparison showed that women with and without TCT results had virtually identical HPV (15.9% vs. 15.0%; P = 0.367) and HR-HPV (13.0% vs. 12.1%; P = 0.341) positivity rates (Table 5), but women tested with TCT were slightly older and more often had inflammation-related findings, so residual selection on age and inflammatory conditions cannot be excluded in the Model M3 estimates or in the viral-load dose–response analyses, which were restricted to this subgroup.

8. Future multicentre prospective studies with serial sampling and standardised detection platforms should clarify the temporal ordering of these associations; integrative analytical approaches, including machine learning applied to high-dimensional microbiome data, may further improve risk stratification as such methods mature, following precedents set in preterm-birth prediction [64, 65].

Conclusions

In this large hospital-based cross-sectional study of 7,997 women in central China, BV positivity was independently associated with HPV and HR-HPV infection, LE positivity was independently associated

with HR-HPV infection and abnormal cervical cytology, and HR-HPV positivity was strongly associated with cytological abnormalities (OR 8.822). The clear dose–response relationship between HPV viral load and the risk of cytological abnormality supports incorporating viral-load quantification into risk stratification of HPV-positive women. Vaginal microecological assessment may thus add useful information to cervical screening; enhanced screening should be considered for women aged < 31 years, in whom infection risk—but not cytological abnormality risk—was elevated, as well as for women aged ≥ 51 years, in whom both risks were increased. Because these data are observational, microbiota-directed interventions warrant evaluation in prospective intervention studies before being incorporated into HPV prevention and control strategies.

Abbreviations

AGC
atypical glandular cells
ASC-H
atypical squamous cells,cannot exclude high-grade squamous intraepithelial lesion
ASC-US
atypical squamous cells of undetermined significance
BV
bacterial vaginosis
CI
confidence interval
GLU
glucosidase
HPV
human papillomavirus
HR-HPV
high-risk human papillomavirus
HSIL
high-grade squamous intraepithelial lesion
LE
leukocyte esterase
LSIL
low-grade squamous intraepithelial lesion
NAG
N-acetyl-β-D-glucosaminidase
NILM
negative for intraepithelial lesion or malignancy
OR
odds ratio

PIP
proline aminopeptidase
STROBE
Strengthening the Reporting of Observational Studies in Epidemiology
TBS
The Bethesda System
TCT
ThinPrep cytologic test
VIF
variance inflation factor.

Declarations

Ethics approval and consent to participate

This retrospective study was conducted in accordance with the Declaration of Helsinki (as revised in 2024) and the Measures for Ethical Review of Life Science and Medical Research Involving Human Subjects (2023) issued by the National Health Commission of the People's Republic of China, and was reviewed and approved by the Ethics Committee of The First People's Hospital of Tianmen (Tianmen, Hubei, China; approval number: 20260288; approved on September 12, 2026). Because the study used de-identified routine clinical laboratory data, the requirement for individual written informed consent was waived by the Ethics Committee.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Authors’ information

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Author Contribution

Meijun Chen contributed to study conception and design, data interpretation, and drafting of the manuscript. Huashun Li contributed to analyze the cytological results. Weiwei Cao contributed to collect data. Tingting Ma contributed to laboratory analyses and biomarker measurements. Feibo Guo contributed to supervision, study design, and critical review of the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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Figures

Figure 1. Flow chart of the study population

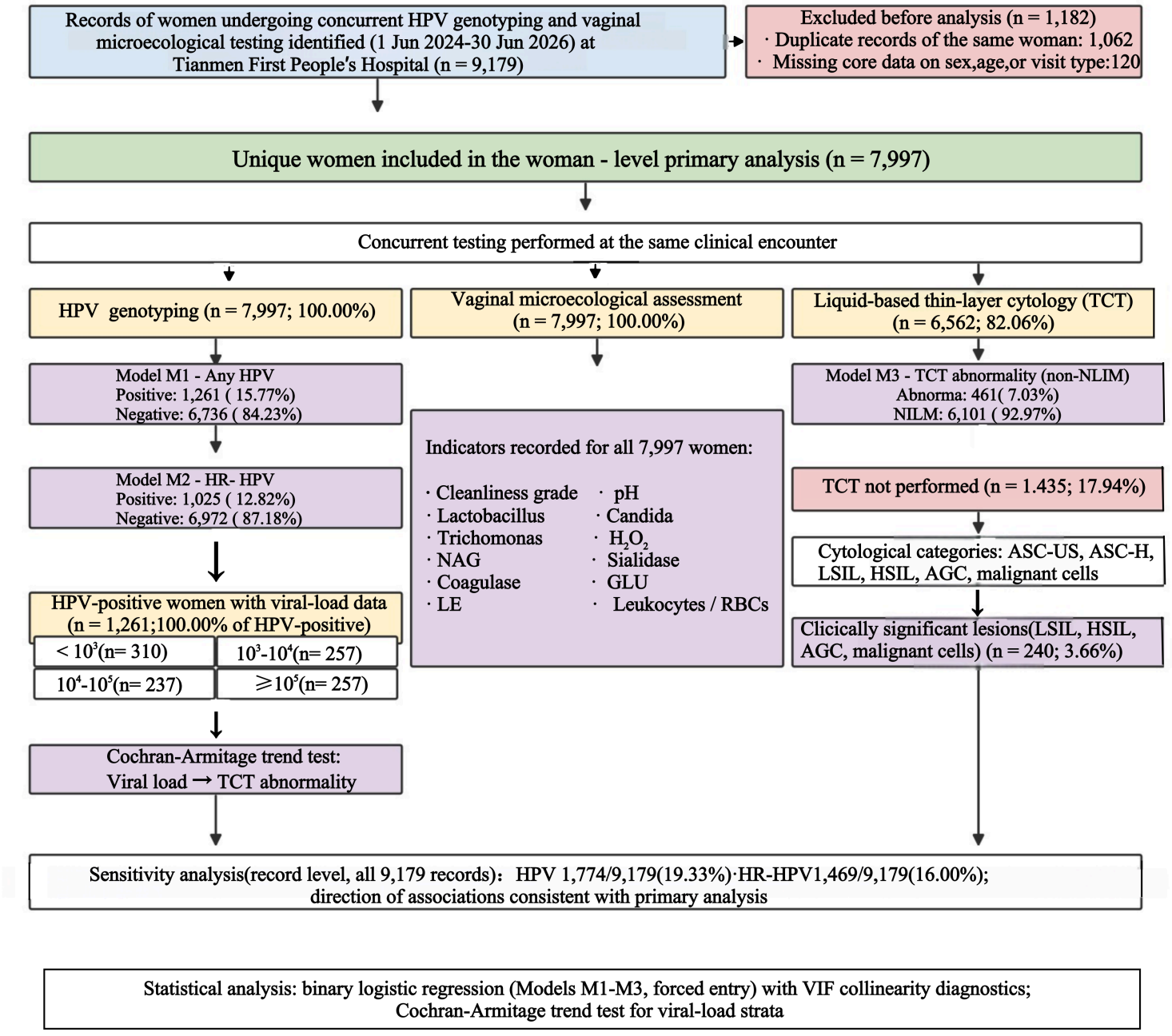

Figure 1

Flow chart of the study population. Numbers are derived from the woman-level primary analysis; the sensitivity analysis included all 9,179 records. NILM = negative for intraepithelial lesion or malignancy; BV = bacterial vaginosis; LE = leukocyte esterase; NAG = N-acetyl-β-D-glucosaminidase; GLU = glucosidase.