Performance of human papillomavirus (HPV) 16 and 18/45 genotyping combined with age stratification in the triaging of women with histologic low-grade squamous intraepithelial lesions preceded by HPV oncogenic mRNA-positive/normal or mildly abnormal cytology: A longitudinal study

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Abstract

Objective: To assess the clinical performance of the human papillomavirus (HPV) 16 18/45 genotype assay (AHPV-GT) combined with age stratification in triaging women with histologic low-grade squamous intraepithelial lesion/cervical intraepithelial neoplasia grade 1 (LSIL [CIN1]) preceded by HPV E6/E7 mRNA assay (Aptima HPV, AHPV) positive/normal or mildly abnormal cytology. **Design:** Longitudinal study. **Setting:** Gynaecological clinic in Huzhou Maternity & Child Health Care Hospital, China. **Population:** Women aged [?]21 years with histologic LSIL (CIN1) preceded by AHPV-positive/normal or mildly abnormal cytology. **Methods:** Women underwent AHPV-GT testing at baseline and were followed at 6-month intervals for up to 2 years. At each follow-up, women with abnormal cytology or AHPV positivity were referred for colposcopy and then biopsy if indicated. **Main outcome measures:** The 2-year cumulative incidence rates (CIRs) of CIN3+ stratified by baseline AHPV-GT genotyping. **Results:** Of 349 eligible women, 25 women (7.2%) progressed, 301 (86.2%) regressed, and 23 (6.6%) persisted during the follow-up. The 2-year CIRs of CIN3+ in AHPV-GT-positive women were both significantly higher than those in AHPV-GT-negative women overall (8.6% vs. 1.7%, $P = 0.014$) and in the [?]25-year-old group (10.9% vs. 1.5%, $P = 0.002$) but slightly higher in the 21–24-year-old group ($P > 0.05$). **Conclusions:** AHPV-GT testing with age stratification is effective for triaging women with histologic LSIL (CIN1) preceded by AHPV-positive/normal or mildly abnormal cytology. Immediate treatment is a rational recommendation for women [?] 25 years old with histologic LSIL (CIN1) preceded by AHPV-GT positivity when good surveillance is not assured.

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Design: Longitudinal study.

Setting: Gynaecological clinic in Huzhou Maternity & Child Health Care Hospital, China.

Population: Women aged [?]21 years with histologic LSIL (CIN1) preceded by AHPV-positive/normal or mildly abnormal cytology.

Methods : Women underwent AHPV-GT testing at baseline and were followed at 6-month intervals for up to 2 years. At each follow-up, women with abnormal cytology or AHPV positivity were referred for colposcopy and then biopsy if indicated.

Main outcome measures: The 2-year cumulative incidence rates (CIRs) of CIN3+ stratified by baseline AHPV-GT genotyping.

Results : Of 349 eligible women, 25 women (7.2%) progressed, 301 (86.2%) regressed, and 23 (6.6%) persisted during the follow-up. The 2-year CIRs of CIN3+ in AHPV-GT-positive women were both significantly higher than those in AHPV-GT-negative women overall (8.6% vs. 1.7%, $P = 0.014$) and in the [?]25-year-old group (10.9% vs. 1.5%, $P = 0.002$) but slightly higher in the 21–24-year-old group ($P > 0.05$).

Conclusions :AHPV-GT testing with age stratification is effective for triaging women with histologic LSIL (CIN1) preceded by AHPV-positive/normal or mildly abnormal cytology. Immediate treatment is a rational recommendation for women [?] 25 years old with histologic LSIL (CIN1) preceded by AHPV-GT positivity when good surveillance is not assured.

KEYWORDS

Human papillomavirus, E6/E7 mRNA, Genotyping, LSIL, CIN1

1 | INTRODUCTION

Histologic low-grade squamous intraepithelial lesions (cervical intraepithelial neoplasia grade 1) (LSIL [CIN1]) can be considered transient expression of human papillomavirus (HPV) infection.[1]Considering that histologic LSIL (CIN1) preceded by HPV-positive or mildly abnormal cytology (atypical squamous cells of undetermined significance [ASC-US] or LSIL cytology) is characterized by a low rate of progression to CIN2 or worse (CIN2+) of only 7.3–13.1% and a spontaneous degeneration rate of up to 60–80% within 2 years,[2-7] a consecutive visit for at least 2 years was recommended,[8-10] and treatment as an acceptable option was added in the 2019 American Society for Colposcopy and Cervical Pathology (ASCCP) guidelines.[11] However, the overall 2-year and 5-year risks of CIN2+ in these women were only 6.4% and 10.0%, respectively, in the Kaiser Permanente Northern California (KPNC) study.[12] Therefore, in addition to increasing the likelihood of cervical insufficiency in these women, treatment also increases the economic burden on the healthcare system.[13, 14]

HPV 16, 18 and 45 were found substantially more often in the genome-integrated state compared with other high-risk HPV (hrHPV) types and took substantially less time to progress to invasive cervical cancer from a precancerous state.[15]DNA-based HPV16/18 positivity, which was slightly less likely to be followed by a negative hrHPV test within 12 months of HPV16/18 infections than non-16/18 hrHPV infections,[16] has been reported to predict progression for women with histologic LSIL (CIN1) preceded by mildly abnormal cytology.[17] The mRNA-based hrHPV E6/E7 mRNA assay (Aptima HPV [AHPV]) test has been found to be more specific than DNA-based HPV tests in detecting high-grade disease, although it is similarly sensitive.[18-21] However, no previous report has presented a strategy for triaging histologic LSIL (CIN1) preceded by AHPV-positive/normal or mildly abnormal cytology. In the 18-month follow-up study of 318 AHPV-positive/cytology-negative women, the odds ratio of CIN2+ in AHPV 16 18/45 genotype assay (AHPV-GT)-positive vs. AHPV-GT-negative women was 3.5 (95% CI, 1.4–8.9).[22] In addition, AHPV-GT testing was reported to be a proper risk stratification method for women with AHPV-positive ASC-US cytology in our recent study.[23] Thus, the AHPV-GT test may have prognostic value in histologic LSIL (CIN1) preceded by AHPV-positive/normal or mildly abnormal cytology.

In this hospital-based longitudinal study, we evaluated the usefulness of AHPV-GT testing combined with age stratification as a triage for patients with histologic LSIL (CIN1) preceded by AHPV-positive/normal or mildly abnormal cytology.

2 | METHODS

2.1 | Setting and study design

From January 2017 to June 2019, women [?]21 years old with histologic LSIL (CIN1) by colposcopy-guided biopsy preceded by AHPV-positive/normal or mildly abnormal cytology were prospectively recruited in the Huzhou Maternity & Child Health Care Hospital. Women were excluded from the study based on the following criteria: 1) preceded by cytology of high-grade squamous intraepithelial lesion (HSIL), atypical squamous cell cannot exclude HSIL (ASC-H), atypical glandular cells (AGC), or adenocarcinoma in situ (AIS); 2) previous history of CIN, cervical cancer, or other malignancies; 3) previous therapeutic procedure performed on the cervix; 4) immunosuppression; and 5) pregnancy. After providing written informed consent, each woman completed a questionnaire administered by a trained gynaecologist regarding risk factors for hrHPV infection before clinical examination.

2.2 | Follow-up and final outcome

Eligible consenting patients were observed at 6-month intervals for up to 2 years with various procedures, including gynaecological examination, AHPV testing, and liquid-based cytology (LBC). During surveillance, patients with abnormal cytology or AHPV positivity were referred for colposcopy and then biopsy if indicated. The endpoints included histology-confirmed CIN2+ during the follow-up and were defined as follows: 1) progression: histology-confirmed CIN2+; 2) regression: both cytology and AHPV negative, abnormal

cytology or AHPV positive but histology negative; and 3) persistence: histology-confirmed LSIL (CIN1). Women reaching an endpoint would then be managed according to the 2012 ASCCP guidelines.[9]

2.3 | AHPV testing and liquid-based cytology

A cervical sample was collected from each patient and placed into PreservCyt collection medium (Hologic Inc., Marlborough, MA). LBC was categorized according to the 2014 Bethesda classification[24] by cytologists in our hospital. Cytologists were blinded to the AHPV and AHPV-GT test results, the subjects' clinical status, and the colposcopy and histology results. The AHPV and AHPV-GT tests (Gen-Probe Inc., San Diego, CA) were performed using an automated Panther System (Hologic, Inc., San Diego, CA) with residual PreservCyt specimens (1-mL aliquot) following the manufacturer's instructions. The AHPV test detects the E6/E7 oncogenic mRNA of 14 hrHPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68). The AHPV-GT test, which can detect HPV E6/E7 mRNA in hrHPV genotypes 16 and 18/45, was used for testing AHPV-positive samples.

2.4 | Colposcopy and histological diagnosis

Colposcopy was performed for all patients with abnormal cytology or AHPV positivity during surveillance by a professional gynaecologist in a colposcopy clinic. A woman underwent combined cervical biopsy with endocervical curettage (ECC) if she presented with one of the following conditions: a high-grade cytology result (HSIL, ASC-H, AGC or AIS), an AHPV-GT-positive result, unsatisfactory colposcopy, or visible lesions. Women with low-grade cytology results (ASC-US or LSIL) and AHPV positivity other than AHPV-GT received a cervical biopsy only. Women with normal cytology/AHPV-negative results did not undergo colposcopy, and their histological diagnoses were considered normal based on ethical grounds. Colposcopists were made aware of the cytology, AHPV and AHPV-GT results before the colposcopy visit was performed. According to the 2014 World Health Organization (WHO) Classification of Tumours of the Female Genital Tract,[25] a histological diagnosis was obtained from at least 2 pathologists in the hospital. Immunohistochemical staining for $p16^{INK4A}$ was utilized to adjudicate any CIN2 interpretations.[26] If a disagreement with histopathology occurred, then a discussion was held by all pathologists in our hospital until a consensus was reached.

2.5 | Statistical analysis

R software 4.2.1 (Vienna, Austria) was used in this study. A Pvalue of less than 0.05 (two-sided) was considered indicative of statistical significance. Categorical variables are presented as the frequency (proportion). A Cox proportional hazards model was used to estimate the hazard ratio (HR) and 95% confidence interval (CI) for the risk factors for the 2-year cumulative incidence rate for CIN2+/CIN3+ in both univariate and multivariate analyses. Factors associated with progression at P <0.05 in univariate analysis were included in multivariate analysis and mutually adjusted. The 95% confidence intervals (CIs) of proportions of the 2-year cumulative incidence rates (CIRs) of CIN2+ and CIN3+ were calculated based on the exact binomial distribution. Pearson's chi-square or Fisher's exact probability test was used to compare the 2-year CIRs of CIN2+ and CIN3+ stratified by AHPV-GT genotyping at baseline, age at diagnosis and enrolment cytology.

3 | RESULTS

3.1 | Flowchart of the expectant management

A total of 349 women with histologic LSIL (CIN1) preceded by AHPV-positive/normal or mildly abnormal cytology were included and completed 2-year follow-up visits at 6-month intervals (Figure S1). Twenty-five (25) women (7.2%) progressed to CIN2+ (including 15 with CIN2, 9 with CIN3, and 1 with AIS), 301 (86.2%) regressed, and 23 (6.6%) persisted during the 2-year follow-up. In accordance with the 2012 ASCCP guidelines,[9] all women who progressed to CIN2+ were treated using a loop electrosurgical excision procedure (LEEP) or cold knife conization (CKC), all women who persisted with histologic LSIL (CIN1) for 2 years were treated with LEEP surgery, and all women who were diagnosed with no lesion at the 2-year follow-up visit were assigned to routine screening.

3.2 | Participant characteristics and demographic information

The baseline characteristics of the women are presented in Table 1. The mean age of the 349 enrolled women was 39.3 ± 10.6 years and did not differ significantly between the 3 outcome groups ($P > 0.05$). There was a significant difference between the 3 outcome groups in terms of AHPV-GT positivity ($P < 0.001$). The AHPV-GT positivity in the progression group was 44.0% (11/25), which was significantly higher than that in the regression group (14.3% [43/301]) ($P < 0.001$) but slightly higher than that in the persistence group (17.4% [4/23]) ($P > 0.05$). There was no significant difference between the 3 outcome groups in terms of cytology results and age groups (both $P > 0.05$).

3.3 | Prognostic demographic and clinicopathological characteristics

Demographic and clinicopathological characteristics in the women with LSIL (CIN1) preceded by AHPV positive/normal or mildly abnormal cytology cohort are summarized in Table 2. In univariate analysis, sexual partner (HR = 2.491; 95% CI, 1.073–5.784), enrolment cytology (HR = 2.339; 95% CI, 1.051–5.207) and AHPV-GT genotyping at baseline (HR = 4.155; 95% CI, 1.885–9.160) were all associated with the 2-year CIR of CIN2+ (all $P < 0.05$), while age at diagnosis, marital status, childbearing, contraception, menopausal status, sexual debut and enrolment cytology were excluded at $P > 0.05$. With mutual adjustment, only AHPV-GT genotyping at baseline (HR = 3.462; 95% CI, 1.546–7.749) was confirmed as an independent prognostic marker for progression in the multivariate analysis ($P = 0.003$). In univariate analysis, only AHPV-GT genotyping (HR = 5.153; 95% CI, 1.490–17.820) was associated with the 2-year CIR of CIN3+ ($P = 0.010$).

3.4 | 2-year CIRs for CIN2+ and CIN3+ by 6 months

Figure S2 shows the CIRs of CIN2+ (a) and CIN3+ (b) by 6 months according to AHPV-GT positivity status at baseline. During the 2-year follow-up, 10 patients of the 349 enrolled women (2.9%) progressed to CIN3+. Forty percent (4/10) of the cases of progression occurred at the first follow-up, 20.0% (2/10) at the second, 30.0% (3/10) at the third, and 10.0% (1/10) at the fourth. All assays showed a similar trend, with consistently higher risk for women with the HPV 16 genotype as well as higher risk for those with AHPV-GT-positive genotypes than those with AHPV-GT-negative genotypes every 6 months. Similar results were observed using a CIN2+ endpoint.

3.5 | 2-year CIRs for CIN2+ and CIN3+ by baseline AHPV-GT, age at diagnosis and enrolment cytology status

Figure 1 presents the 2-year CIRs of CIN2+ and CIN3+ by baseline AHPV-GT, age at diagnosis and enrolment cytology status. The 2-year CIR of CIN3+ in the group of AHPV-GT-positive women at baseline was significantly higher than that in the group of AHPV-GT-negative women (8.6% [95% CI; 2.9–19.0%] vs. 1.7% [95% CI; 0.6–4.0%],$P = 0.014$), with a CIR ratio of 5.0 (95% CI; 1.5–16.8). In the [?]25-year-old group, the 2-year CIR of CIN3+ in the group of AHPV-GT-positive women was significantly higher than that in the group of AHPV-GT-negative women (10.9% [95% CI; 3.6–23.6%] vs. 1.5% [95% CI; 0.4–3.8%],$P = 0.002$). However, there was no significant difference between the two subgroups in the 21–24-year-old group (8.3% [95% CI; 0.2–38.5%] vs. 0.0% [95% CI; 0.0–14.8%], $P = 0.343$). In the two groups of women with NILM and ASC-US/LSIL cytology at baseline, the 2-year CIRs of CIN3+ were both higher in the AHPV-GT-positive subgroup at baseline than in the AHPV-GT-negative subgroup, but there was no significant difference between the two subgroups ($P = 0.536$, $P = 0.097$, respectively). Similar results were achieved when comparing the 2-year CIRs of CIN2+.

4 | DISCUSSION

4.1 | Main findings

AHPV-GT positivity was confirmed as an independent prognostic marker for 2-year cumulative progression in the multivariate analysis ($P< 0.001$). After 2 years of follow-up of women with histological LSIL (CIN1) preceded by AHPV-positive/normal or mildly abnormal cytology, AHPV-GT-positive women were 3.9 times

(19.0% vs. 4.8%, $P < 0.001$) more likely to develop CIN2+ and 5.0 times (8.6% vs. 1.7%, $P = 0.014$) more likely to develop CIN3+ than AHPV-GT-negative women. The 2-year CIRs of CIN3+ in AHPV-GT-positive women were significantly higher than those in AHPV-GT-negative women in the [?]25-year-old group (10.9% vs. 1.5%, $P = 0.002$) but slightly higher in the 21–24-year-old group ($P > 0.05$). These results support the value of risk stratification using AHPV-GT testing in managing women with histologic LSIL (CIN1) preceded by AHPV-positive/normal or mildly abnormal cytology.

4.2 | Strengths and limitations

To our knowledge, this is the first population-based longitudinal prospective study in which the prognostic value of AHPV-GT combined with age stratification for triaging women with histological LSIL (CIN1) preceded by AHPV-positive/normal or mildly abnormal cytology has been assessed. A major strength of this study was that we used a larger sample size to compare the 2-year CIRs of CIN2+ and CIN3+ stratified by baseline AHPV-GT genotyping. Moreover, based on the threshold of CIN3+ risk in the 2019 ASCCP guidelines,[11, 27] we recommend a management algorithm for women with histologic LSIL (CIN1) preceded by AHPV-positive/normal or mildly abnormal cytology.

This study has several limitations. First, the 2-year follow-up period in this study is relatively short compared to the 2019 ASCCP guidelines,[11] which recommend that women with histological LSIL (CIN1) should have consecutive visits for at least 2 years. Second, according to the 2012 ASCCP guidelines,[9] women with histology-confirmed CIN2+ underwent treatment instead of follow-up up to 2 years, which may have resulted in an underestimation of the 2-year CIR of CIN3+. Third, an inherent weakness of cohort studies is the difficulty of distinguishing new diseases from baseline-related progression. As in previous studies, it was unclear whether the HPV genotyping status of women who progressed to CIN2+ lesions was completely consistent at diagnosis and baseline.

4.3 | Interpretation

Although previous studies have revealed higher regression rates and lower progression to CIN2+ of histologic LSIL (CIN1),[2-7] the results varied due to different study populations, inclusion criteria, ages, hrHPV testing methods and results, and cytological results at baseline and follow-up intervals. A cohort study from India with 177 CIN1 women who were HPV positive but had no baseline cytological results yielded findings of a 15.3% progression rate of CIN2+ within 2 years.[28] In a study of 1031 histological LSIL (CIN1) women without HPV infection status and cytological results at baseline after 2 years of follow-up, the progression rate of CIN2+ was 6.9%.[29] In this study, the 2-year risk of CIN2+ was 7.2%, which was basically consistent with previously reported results.[28, 29] The risk of progression to CIN3+ among women with CIN1 was linked to prior cytology in the 2012 ASCCP guidelines[9] and HPV infection status.[30] A Chinese cohort study of 124 women with histological LSIL (CIN1) found a significant difference in the progression rate of CIN2+ within 6 years between hrHPV-positive and hrHPV-negative women (12.7% vs. 0.0%).[30] Considering that women with histologic LSIL (CIN1) preceded by HPV-positive or mildly abnormal cytology are characterized by low progression rates to CIN3+ and high regression rates within 2 years,[2-7] a consecutive visit for at least 2 years was recommended in the 2012 ASCCP guidelines.[9] In this study, the 2-year CIR of CIN3+ in this study was 2.9%, similar to the 2.3–2.8% in the KPNC study.[12, 27]

DNA-based HPV16/18 positivity has been reported to predict progression in women with histologic LSIL (CIN1) preceded by mildly abnormal cytology.[5, 17] In the ASC-US/Low-Grade Squamous Intraepithelial Lesion Triage Study (ALTS) of 574 women patients with histologic LSIL (CIN1) preceded by DNA-based HPV-positive/mildly abnormal cytology, the 2-year risk of CIN3 for women positive for HPV16, HPV18, or other carcinogenic HPV genotypes was 22.1%, 17.7%, and 6.1%, respectively.[5] In a 2-year follow-up study of 273 patients with histologic LSIL (CIN1) preceded DNA-based HPV-positive/normal or mildly abnormal cytology, the 2-year cumulative progression rate in the HPV16/18-positive group was significantly higher than that in the HPV16/18-negative group (34.0% vs. 13.0%, $P< 0.001$).[17]

HPV 16, 18 and 45, which are more likely to be integrated into the human genome than other HPV types, account for approximately 76% of invasive cervical cancers worldwide.[15, 31] In addition, the mRNA-based

AHPV test has been found to be more specific than DNA-based HPV tests in detecting high-grade disease, although it is similarly sensitive.[18-20] The immediate risk of CIN3+ in women with AHPV-GT-positive ASC-US cytology was significantly higher than that in other hrHPV-positive women (10.4% vs. 2.7%, $P < 0.001$) as reported in our previous study.[23] In a follow-up study of 318 women with AHPV-positive/cytology-negative results, the 18-month CIR of CIN2+ in the AHPV-GT-positive group was significantly higher than that in the other hrHPV-positive women group (11.5% vs. 3.6%, $P < 0.001$).[22] In this study, the 2-year CIRs of CIN2+ and CIN3+ were both significantly higher in AHPV-GT-positive women at baseline than in AHPV-GT-negative women (19.0% vs. 4.8%, $P < 0.001$; 8.6% vs. 1.7%,$P = 0.014$, respectively). Therefore, the AHPV-GT test was shown to be an effective triaging method for women with histologic LSIL (CIN1) preceded by AHPV-positive/normal or mildly abnormal cytology.

Few age-stratified data on the accuracy of HPV genotyping status for triaging women with histologic LSIL (CIN1) have supported any definitive conclusion, and no algorithm has been reported for triaging such women using AHPV-GT genotyping combined with age stratification. In this study, the 2-year CIR of CIN3+ in the 25-year -or-older & AHPV-GT-negative subgroup was 1.5%, which was similar to the 0.53–1.74% and 2.3–2.8% for 1-year and 5-year CIN3+ risk, respectively, in women with histologic LSIL (CIN1) preceded by HPV-positive/normal or mildly abnormal cytology in the KPNC study,[12, 27] so the recommended management is 1-year follow-up according the 2019 ASCCP guidelines.[11, 27] In addition, the 2019 ASCCP guidelines also stated that treatment was an acceptable option based on patient preferences after shared decision-making.[11]Considering that the 2-year CIR of CIN3+ was up to 10.9% in the 25-year-or-older & AHPV-GT-positive subgroup, which was 7.3 times (10.9% vs. 1.5%, $P = 0.002$) that of the 25-year-or-older & AHPV-GT-negative subgroup, 1-year follow-up is a rational recommended management for those women, and treatment (either ablative or excisional methods) may be a better option if good surveillance is not assured. In the 21–24-year-old group, the 2-year CIR of CIN3+ in the AHPV-GT-negative subgroup was 0.0%, which was lower than the CIN3+ risk value at the 1-year follow-up recommended by the 2019 ASCCP guidelines.[11, 27] Therefore, the intervals recommended for follow-up can be extended to 2 years for the 21–24-year & AHPV-GT-negative subgroup. The algorithm's recommended management of AHPV-GT testing combined with age stratification in triaging women with histologic LSIL (CIN1) preceded by AHPV-positive/normal or mildly abnormal cytology is shown in Figure 2.

5 | CONCLUSION

AHPV-GT testing in conjunction with age stratification may be a suitable prognostic method for women with histologic LSIL (CIN1) preceded by AHPV-positive/normal or mildly abnormal cytology. Immediate treatment is a rational recommendation for women [?] 25 years old with histologic LSIL (CIN1) preceded by AHPV-GT positivity when good surveillance is not assured.

AUTHOR CONTRIBUTIONS

JJW designed the study, obtained funding, performed database analysis, collected the data, performed statistical analyses and drafted the manuscript. JD, JLT, MP, PFW, and YRZ performed the clinical and experimental work related to cervical cancer screening.

ZXD and YRZ participated in the cytological and histological experiments, data collection and statistical analyses. WGL conceived the study, participated in its design and coordination and revised the final paper. All the authors have read and approved the final version of the article.

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CONFLICT OF INTEREST

None of the authors declare any conflict of interest.

ETHICS APPROVAL

All the authors report adherence to ethical standards in the conception of the study, data collection and writing of the manuscript. IRB-status: This study was conducted in accordance with the tenets of the Declaration of Helsinki and was approved by the ethics committee of Huzhou Maternity & Child Health Care Hospital (IRB-2017-R-010).

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Figure legend

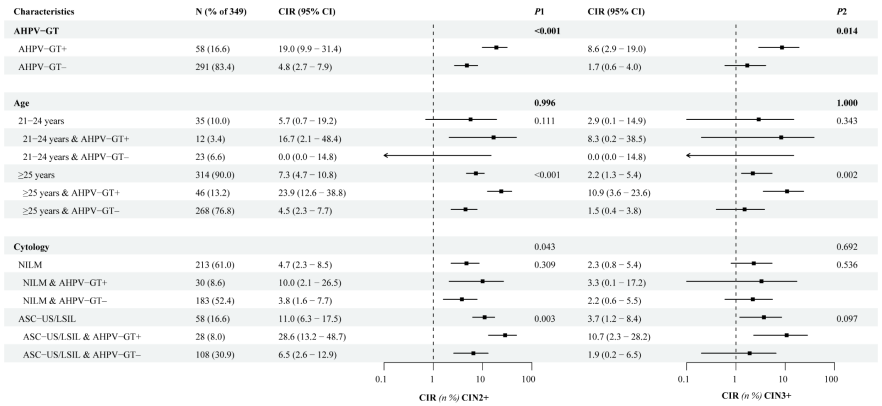

FIGURE 1 Cumulative incidence rates (CIRs) for CIN2+ and CIN3+ during 2 years of follow-up by baseline AHPV-GT status, age at diagnosis and enrolment cytology status. CIN, cervical intraepithelial neoplasia; AHPV-GT+, Aptima HPV 16 18/45 genotype assay positive; AHPV-GT–, AHPV E6/E7 mRNA assay positive other than 16 and 18/45; NILM, negative for intraepithelial lesion or malignancy; ASC-US, atypical squamous cells of undetermined significance; LSIL, low-grade squamous intraepithelial lesion.

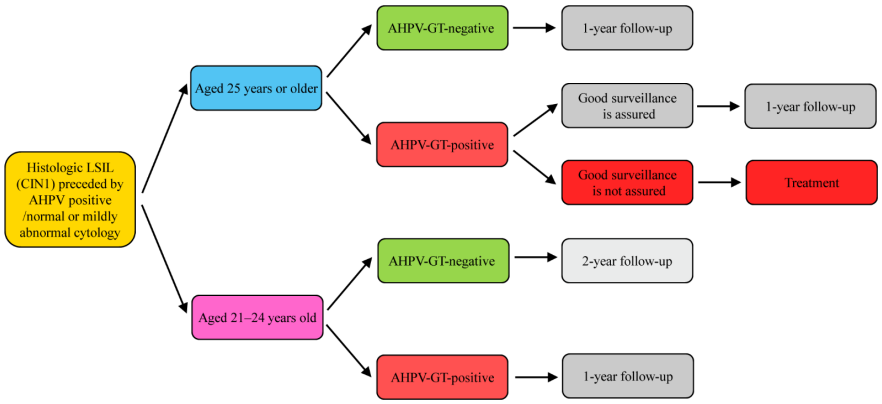

FIGURE 2 Recommended management of women with histologic LSIL (CIN1) preceded by AHPV-positive/normal or mildly abnormal cytology algorithm. LSIL (CIN1), low-grade squamous intraepithelial lesion/cervical intraepithelial neoplasia grade 1; AHPV, human papillomavirus E6/E7 mRNA assay; AHPV-GT, Aptima HPV 16 18/45 genotype assay.

TABLES

TABLE 1 Baseline characteristics of the 349 women from the cohort study on expectant management of histologic LSIL (CIN1)

Characteristic	All women	Regression	Persistence	Progression	Progression
	(N = 349)	Normal (n = 301)	LSIL (CIN1) (n = 23)	CIN2 (n = 15)	CIN3+ (n = 10)
Age (mean±SD), y	39.3±10.6	39.1±10.5	38.4±10.9	40.2±10.9	40.6±12.4
Age groups, n (%)					
21-24 years	25 (7.2%)	20 (6.6%)	3 (13.0%)	1 (6.7%)	1 (10.0%)?¿?
25 years	324 (92.8%)	281 (93.4%)	20 (87.0%)	14 (93.3%)	9 (90.0%)
Cytology, n (%)					
NILM	213 (61.0%)	192 (63.8%)	11 (47.8%)	5 (33.3%)	5 (50.0%)
ASC-US	58 (16.6%)	49 (16.3%)	3 (13.0%)	4 (26.7%)	2[a] (20.0%)
LSIL	78 (22.3%)	60 (19.9%)	9 (39.1%)	6 (40.0%)	3 (30.0%)
AHPV, n (%)					
AHPV-GT+	58 (16.6%)	43 (14.3%)	4 (17.4%)	6 (40.0%)	5 (50.0%)
HPV 16+	37 (10.6%)	27 (9.0%)	2 (8.7%)	4 (26.7%)	4 (40.0%)
HPV 18/45+	21 (6.0%)	16 (5.3%)	2 (8.7%)	2 (13.3%)	1[a] (10.0%)
AHPV-GT–	291 (83.4%)	258 (85.7%)	19 (82.6%)	9 (60.0%)	5 (50.0%)

Abbreviations: LSIL (CIN1), low-grade squamous intraepithelial lesion/cervical intraepithelial neoplasia grade 1; NILM, negative for intraepithelial lesion or malignancy; ASC-US, atypical squamous cells of undetermined significance; AHPV, high-risk human papillomavirus E6/E7 mRNA assay; AHPV-GT+, Aptima HPV 16 18/45 genotype assay positive; AHPV-GT–, AHPV E6/E7 mRNA assay positive other than 16 and 18/45; CIN, cervical intraepithelial neoplasia.

[a] Includes one cases of adenocarcinoma in situ.

TABLE 2 Univariate/multivariate analysis of prognostic factors for 2-year cumulative incidence rate for CIN2+ and CIN3+

Characteristics	**CIN2+**	**CIN2+**	**CIN2+**	**CIN2+**	**CIN2+**
	n/N (%)	**Univariate analysis HR (95% CI)**	**Univariate analysis** P		**Multivariate analysi HR (95% CI)**
Age at diagnosis					
21-24 years	2/35 (5.7%)	0.686 (0.162-2.910)	0.609		
25 years	23/314 (7.3%)	Reference			
Marital status					
Married	21/274 (7.7%)	Reference			
Single	4/75 (5.3%)	0.449 (0.765-6.497)	0.142		
Childbearing					
1	20/273 (7.3%)	1.096 (0.411-2.920)	0.855		
0	5/76 (6.6%)	Reference			
Contraception					
Oral contraceptive	6/50 (12.0%)	2.364 (0.931-6.007)	0.0705		
Condom	5/90 (5.6%)	0.785 (0.478-3.965)	0.629		
Other	14/209 (6.7%)	Reference			
Menopausal status					
Premenopause	20/296 (6.8%)	Reference			
Menopause	5/53 (9.4%)	1.954 (0.779-4.902)	0.153		
Sexual debut					
<20 years old	6/57 (10.5%)	1.809 (0.722-4.535)	0.206		
20 years old	19/292 (6.5%)	Reference			
Sexual partner					
2	8/65 (12.3%)	2.491 (1.073-5.784)	0.034		1.982 (0.843-4.663)

Characteristics	CIN2+	CIN2+	CIN2+	CIN2+	CIN2+
1	17/284 (6.0%)	Reference			
Enrollment cytology					
ASC-US/LSIL	15/136 (11.0%)	2.339 (1.051-5.207)	0.037		2.081 (0.932-4.650)
NILM	10/213 (4.7%)	Reference			
AHPV genotyping					
AHPV-GT+	11/58 (19.0%)	4.155 (1.885-9.160)	<0.001		3.462 (1.546-7.749)
AHPV-GT–	14/291 (4.8%)	Reference			

Abbreviations: CIN, cervical intraepithelial neoplasia grade; ASC-US, atypical squamous cells of undetermined significance; LSIL, low-grade squamous intraepithelial lesion; NILM, negative for intraepithelial lesion or malignancy; AHPV, human papillomavirus E6/E7 mRNA assay; AHPV-GT+, Aptima HPV 16 18/45 genotype assay positive; AHPV-GT–, AHPV E6/E7 mRNA assay positive other than 16 and 18/45; HR: hazard ratio; CI: confidence interval.

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`Supplementary Figures.docx` available at https://authorea.com/users/585559/articles/624144-performance-of-human-papillomavirus-hpv-16-and-18-45-genotyping-combined-with-age-stratification-in-the-triaging-of-women-with-histologic-low-grade-squamous-intraepithelial-lesions-preceded-by-hpv-oncogenic-mrna-positive-normal-or-mildly-abnormal-cytology-a-longitudinal-study