BMC Cancer

RESEARCH Open Access

HPV integration status conversion and CIN2 + cancer risk stratification based on HPV integration levels among HPV integration-positive women: a 1-year follow-up study

Kexin Li[1], Fanwei Huang[1], Tao Zhang[1], Fan Yang[2], Weitao Duan[2], Shimin Chen[2], Ting Hu[1,3*†] and Xiaoyuan Huang[1,3*†]

Abstract

Background HPV integration is a crucial genetic step in cervical carcinogenesis and the level of HPV integration increases with the grade of precancerous lesion. This study aimed to conduct risk stratification based on HPV integration levels and HPV integration status conversion among HPV integration-positive women after 1 year of follow-up.

Methods This prospective cohort study was conducted in Tongji Hospital between June 2020 and August 2022 and included 1297 consecutive HPV-positive women. The level of integration reads was stratified for risk assessment.

Results In total, 194 women were HPV integration-positive and followed for at least 1 year. The immediate risk of cervical intraepithelial neoplasia grade 2 or worse (CIN2+) increased from 36.2% (25/69) among women with 6–20 integration reads to 93.8% (30/32) among women with more than 1000 integration reads ($P_{trend} < 0.001$). The 1-year cumulative risk of CIN2 + increased from 39.1% (27/69) among women with 6–20 integration reads to 96.9% (31/32) among women with more than 1000 integration reads ($P_{trend} < 0.001$). The 1-year cumulative risk of CIN2 + with HPV integration reads more than 40 was 93.8% (90/96), which was significantly higher than that of HPV integration reads less than 40 (38/85, $P < 0.001$). Among women with HPV integration reads more than 40, 99.0% (95/96) of women progressed with positive outcomes after one year of follow-up (persistent integration at the same site, immediate CIN2+, and 1-year CIN2+). The progression rate of women with persistent integration at the same site was 41.6% (5/12), which was significantly higher than those of HPV integration-negative conversion (0/41, 0%, $P < 0.001$).

†Xiaoyuan Huang and Ting Hu are co-corresponding authors and contributed equally to this work.

*Correspondence:
Ting Hu
tinghu_tj@163.com
Xiaoyuan Huang
huangxy@tjh.tjmu.edu.cn

Full list of author information is available at the end of the article

Conclusion The number of HPV integration reads may help CIN2 + risk stratification and facilitate the clinical management of high-risk patients.

Keywords Human papillomavirus, HPV integration, Risk stratification, Follow-up.

Background

Cervical cancer is the fourth most common malignancy worldwide, with a morbidity and mortality rate of 9.89 and 3.05 per 10,000, respectively, in China [1, 2]. Several studies have shown that human papillomavirus (HPV) infection is highly associated with the development of cervical cancer, with 95% of patients with cervical cancer being positive for HPV infection [3–5]. After infection with HPV, the virus can be integrated into the human genome under certain circumstances. For instance, a consistent HPV infection imposes a high viral load [6]. HPV integration can lead to various genetic alterations, including oncogene activation, tumor suppressor gene inactivation, inter-chromosomal or intra-chromosomal rearrangements, and genetic instability [7, 8]. This host genetic transformation can change the expression levels of proteins, promoting cancer cell viability while providing a selective growth advantage [9]. Therefore, HPV integration may be an important event in oncogenic progression.

The integration of HPV into the human genome was first detected in 1987 using a technique based on restriction digestion/Southern blot hybridization [10]. Many techniques have emerged in recent decades [11–13]. The advent of next-generation sequencing (NGS) enabled the detection of HPV-human reads through whole-genome sequencing (WGS) [14–16]. Using these techniques, many studies have found a significant difference in the number of HPV integration events between normal, precancerous lesions, and invasive cervical cancer, suggesting that HPV integration may be a potential marker for precancerous progression [17–19]. Furthermore, it has been reported that HPV virus integration levels (number of integrations per genome or relative number of the supporting reads) are positively correlated with the severity of cervical lesions [20, 21]. Therefore, HPV integration levels may contribute to risk stratification for cervical intraepithelial neoplasia (CIN).

HPV integration events can be detected in all stages of the natural progression of cervical cancer, even among HPV-positive women with normal lesions [22, 23]. Previous studies have shown that the HPV integration rate increases with the grade of CIN lesions [17–19]. Among patients with HPV-positive cervical cancer, the prevalence of HPV integration is 84.3–97.8% [21, 24]. However, whether the HPV integration status persists among HPV integration-positive women over time has not been reported.

Here, we conducted a prospective cohort study to investigate risk stratification based on HPV integration levels and assess the HPV integration status conversion of HPV integration-positive women. To our knowledge, this is the first study to propose a critical threshold for HPV integration level and risk stratification.

Methods

Study design

This was a prospective cohort study conducted at Tongji Hospital, Tongji Medical College, and Huazhong University of Science and Technology in Wuhan, China. Between June 2020 and August 2022, we enrolled 1297 HPV-positive women aged from 21 to 75 in our outpatient department, all of whom underwent HPV integration testing. Among them, 194 women were HPV integration-positive and were invited to participate in this study. The exclusion criteria were as follows: (1) CIN1 with surgical treatment; (2) a history of cervical cancer; and (3) no colposcopy was conducted at the baseline. At baseline, women all underwent colposcopy and biopsy within 1 month after HPV integration testing. Women with histological results ≤ CIN1 at baseline were recommended to repeat cervical cancer screening and another HPV integration test at a one-year follow-up. We conducted a repeat HPV integration test on the discarded Thinprep cytology specimen at a one-year follow-up. The flow chart is shown in Fig. 1. Ethics Ethical approval was obtained from the Ethics Committee of Tongji Hospital of Huazhong University of Science and Technology, and patients' consent was waived (TJ-IRB20211110).

HPV Test

The HPV DNA test was conducted using the Cobas 4800 system (Roche Molecular Diagnostics), which detects HPV16, HPV18, and 12 other high-risk HPV types (HPV 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68).

HPV integration test

The sampling method for HPV integration test was the same as that of the HPV test, which used cervical exfoliated cells. After DNA extraction, 500 ng of genomic DNA was used from each patient for DNA library preparation. Based on our previous study, the HPV integration test was conducted using high-throughput viral integration detection (HIVID) [19]. This technique detected 18 HPV integration types, including HPV16, 18, and 12 other high-risk HPV types. Low-quality bases of each read were trimmed using an average Q value < 20. Window

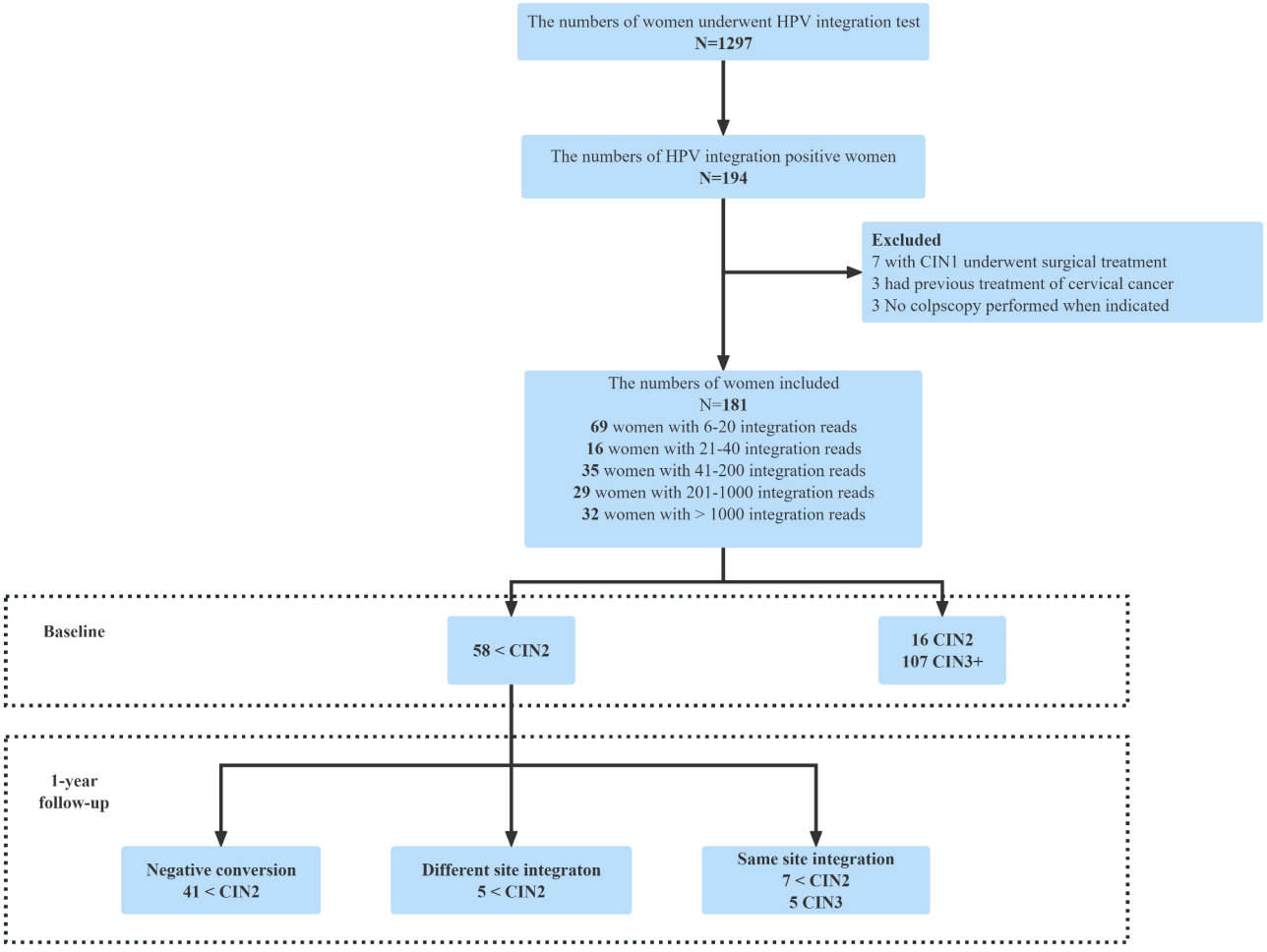

Fig. 1 The flow chart of study enrollment. Abbreviations: CIN2, cervical intraepithelial neoplasia grade 2; CIN3+, cervical intraepithelial neoplasia grade 3 or worse; HPV, Human papillomavirus

size = 4 bases from left and right ends, and reads with length > 100 bp were retained for downstream analysis. These clean reads were filtered using BWA and paired-end reads (partial read sequence aligned to human genome and partial read sequence aligned to HPV genome) were reserved [25]. All integrated sites were verified using PCR and Sanger sequencing. Sequencing reads containing both HPV and human genome sequences (both not shorter than 20 bp in length) was labeled as 1 integrated read. Samples with more than 5 reads were considered HPV integration positive. Samples with discrepant HPV infection results between the HPV test and HPV integration test were excluded.

Study endpoint

All subjects underwent at least one colposcopy examination. Histologic diagnosis was considered the gold standard, and the lesions were categorized based on the CIN grade: no indication for biopsy, normal, CIN grade 1 (CIN1), CIN grade 2 (CIN 2), CIN grade 3 (CIN3), adenocarcinoma in situ (AIS), and cervical cancer. Two pathologists at our hospital reviewed all histological results. The clinical endpoint included CIN2+ (including CIN2, CIN3, AIS and cervical cancer) and CIN3+ (including CIN3, AIS and cervical cancer).

In this study, we divided patients' outcomes into positive outcomes and negative outcomes. The positive outcomes included: (1) immediate CIN2+: women with CIN2 + at the baseline; (2) 1-year CIN2+: women with normal or CIN1 at baseline and histologically diagnosed with CIN2 + at 1-year follow-up; (3) persistent integration at the same site: women with the same breakpoint in two consecutive HPV integration tests at different time points (at a minimum of 3 months). The negative outcomes included negative conversion and HPV integration at a different site. Women with positive HPV integration results at baseline and negative results at 1-year follow-up were defined as a negative conversion. Women with a different breakpoint in two consecutive HPV integration detection sessions (at a minimum interval of 3 months)

were regarded as having HPV integration at a different site.

Statistical analysis

All analyses were conducted using SPSS (version 26). All *P* values are from a two-sided test, and the results were deemed statistically significant in the presence of $P < 0.05$. In the present study, integration reads were defined as the number of supporting reads of the most frequent breakpoint per 5,000,000 sequencing reads. Contingency tables and χ [2] trend tests were used to determine the percentage of positive outcomes and 1-year cumulative CIN2 + rate among HPV integration reads (6–20, 21–40, 41–200, 201–1000, and >1000) strata. The CIN2 + progression rates of different HPV integration statuses (negative conversion, HPV integration at a different site, and persistent integration at the same site) were also measured. Categorical variables were analyzed using Pearson's chi-square test or Fisher's exact test. The odds ratio (OR) with its 95% confidence interval (95% CI) was used to express the statistical correlation between the number of HPV integration reads and 1-year cumulative risk of CIN2+.

Results

Study population

We included 194 HPV integration-positive women in this study. In this cohort, we excluded 7 women with CIN1 who received surgical treatment, 3 women with a history of cervical cancer, and 3 women without colposcopy at the baseline. Finally, 181 women were enrolled in this study (Fig. 1). Baseline characteristics of participants are shown in Table S1. The median age of participants was 42 years (interquartile range, 35–52 years). The median follow-up duration was 10 months (interquartile range, 5.5–13 months). Among patients, 28 had AIS, 79 had CIN3, 16 had CIN2, 13 had CIN1, and 45 had benign lesions at baseline. The most common integrated HPV types were HPV16 (99/181, 54.7%), HPV18 (18/181, 9.9%), and HPV52 (18/181, 9.9%) (Table S1).

Risk stratification for CIN2 + and CIN3 + based on HPV integration reads

The immediate risk of CIN2 + increased as the number of HPV integration reads increased. The immediate risk of CIN2 + was 36.2% (25/69) among women with 6–20 integration reads, 62.5% (10/16) among women with 21–40 integration reads, 85.7% (30/35) among women with 41–200 integration reads, 96.6% (28/29) among women with 201–1000 integration reads, and 93.8% (30/32) among women with more than 1000 integration reads ($P_{trend} < 0.001$, Fig. 2; Table 1). The same patterns were found for the immediate risk of CIN3+, which was 30.4% (21/69) among women with 6–20 integration reads, 50.0% (8/16) among women with 21–40 integration reads, 74.3% (26/35) among women with 41–200 integration reads, 82.8% (24/29) among women with 201–1000 integration reads, and 87.5% (28/32) among women with more than 1000 integration reads ($P_{trend} < 0.001$).

The 1-year cumulative risk of CIN2 + and CIN3 + also increased with the number of HPV integration reads. The 1-year cumulative risk of CIN2 + was 39.1% (27/69) among women with 6–20 integration reads, 68.8% (11/16) among women with 21–40 integration reads, 88.6% (31/35) among women with 41–200 integration reads, 96.6% (28/29) among women with 201–1000 integration reads, and 96.9% (31/32) among those with more than 1000 reads ($P_{trend} < 0.001$, Fig. 2; Table 1). The 1-year cumulative risk of CIN3 + was 33.3% (23/69) among women with 6–20 integration reads, 56.3% (9/16) among women with 21–40 integration reads, 77.1% (27/35) among women with 41–200 integration reads, 86.2% (25/29) among women with 201–1000 integration reads, and 90.6% (29/32) among women with more than 1000 integration reads ($P_{trend} < 0.001$).

After 1 year of follow-up, the 1-year cumulative CIN2 + risk of women with integration reads more than 40 was 93.8% (90/96), which was much higher than that of women with integration reads less than 40 (38/85, 44.7%, OR = 18.6, 95%CI, 7.3–47.0, $P < 0.001$).

Effect of integrated HPV type on the immediate/1-year cumulative CIN2 + risk of women stratified based on HPV integration reads

Among women with immediate CIN2 + risk, we observed no significant differences between HPV16/18 integration women and non-HPV16/18 integration women with 6–20 integration reads ($P = 0.206$), 21–40 integration reads ($P > 0.99$), 41–200 integration reads ($P = 0.139$), 201–1000 integration reads ($P > 0.99$), and more than 1000 integration reads ($P > 0.99$, Table 2). The same patterns were observed for 1-year cumulative risk of CIN2 + between HPV16/18 integration women and non-HPV16/18 integration women with 6–20 integration reads ($P = 0.204$), 21–40 integration reads ($P > 0.99$), 41–200 integration reads ($P = 0.477$), 201–1000 integration reads ($P > 0.99$), and more than 1000 integration reads ($P > 0.99$).

HPV integration status conversion of HPV integration-positive women after 1 year of follow-up

After 1 year of follow-up, 71.4% (5/7) of women with persistent integration at the same site had more than 40 integration reads. In addition, 97.6% (40/41) of women with negative conversion and 100% (5/5) of women with HPV integration at a different site had less than 40 integration reads (Table 3).

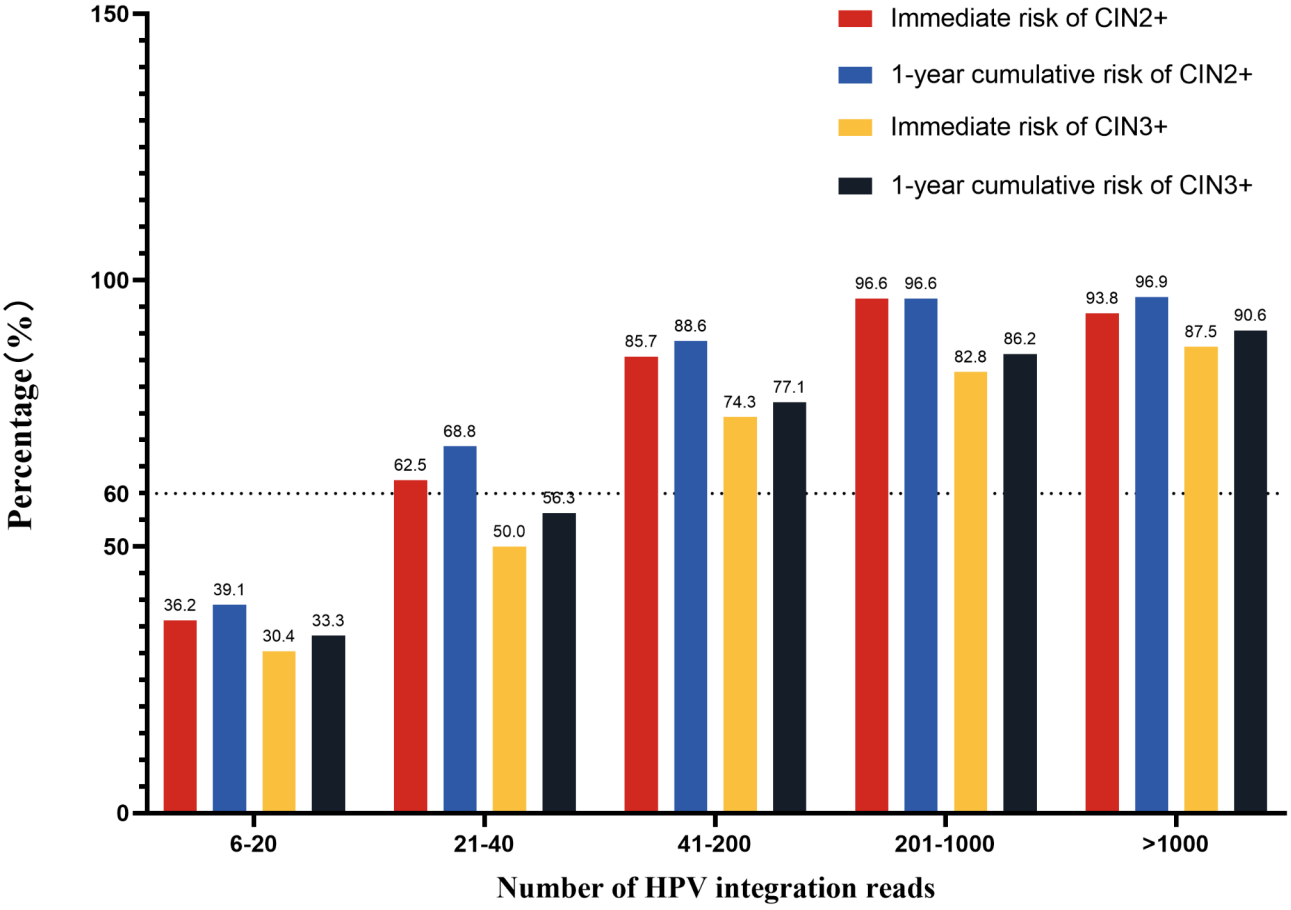

Fig. 2 The immediate risk of CIN2 + and CIN3 + and the 1-year cumulative risk of CIN2 + and CIN3 + at different number of HPV integration reads. Abbreviations: CIN2+, cervical intraepithelial neoplasia grade 2 or worse; CIN3+, cervical intraepithelial neoplasia grade 3 or worse; HPV, Human papillomavirus. The dashed line corresponds to the treatment referral threshold

Table 1 The number of immediate risk of CIN2+, 1-year cumulative risk of CIN2+, immediate risk of CIN3+, 1-year cumulative risk of CIN3 + of different HPV integration reads strata

HPV integration reads	Total. No	Immediate risk of CIN2+ (%, No)	P_{trend} value	1-year cumulative risk of CIN2+ (%, No)	P_{trend} value	Immediate risk of CIN3+ (%, No)	P_{trend} value	1-year cumulative risk of CIN3+ (%, No)	P_{trend} value
6–20	69	36.2 (25/69)	< 0.001	39.1 (27/69)	< 0.001	30.4 (21/69)	< 0.001	33.3 (23/69)	< 0.001
21–40	16	62.5 (10/16)		68.8 (11/16)		50.0 (8/16)		56.3 (9/16)	
41–200	35	85.7 (30/35)		88.6 (31/35)		74.3 (26/35)		77.1 (27/35)	
201–1000	29	96.6 (28/29)		96.6 (28/29)		82.8 (24/29)		86.2 (25/29)	
> 1000	32	93.8 (30/32)		96.9 (31/32)		87.5 (28/32)		90.6 (29/32)	

Abbreviations: CIN2+, cervical intraepithelial neoplasia grade 2 or worse; CIN3+, cervical intraepithelial neoplasia grade 3 or worse; HPV, Human papillomavirus

In this study, persistent integration at the same site, immediate CIN2 + and 1-year CIN2 + were considered positive outcomes, and HPV integration at a different site and negative conversion were considered negative outcomes. Of 181 participants, after 1 year of follow-up, the proportion of positive outcomes was 42.0% (29/69) among women with 6–20 integration reads, 68.8% (11/16) among women with 21–40 integration reads, 100% (35/35) among women with 41–200 integration reads, 96.6% (28/29) among women with 201–1000 integration reads, and 100% (32/32) among women with more than 1000 integration reads ($P_{trend} < 0.001$, Table 3). We observed that the proportion of negative outcomes decreased as the integration reads increased. In contrast, the percentage of positive outcomes increased with the increase in HPV integration reads (Fig. 3). After 1 year of follow-up, 99.0% (95/96) of women had positive outcomes among women with more than 40 integration reads ($P < 0.001$). Among women with negative outcomes,

Table 2 The relationship between HPV integration type and CIN2 + risk in each HPV integration reads strata

HPV integration reads	Women No. (%)			*P* value
	Total (*N* = 181)	Non-HPV16/18 integration (*n* = 64)	HPV16/18 integration (*n* = 117)	
6–20	69 (38.1)	43 (67.2)	26 (22.2)	-
Immediate CIN2+	25 (36.2)	13 (30.2)	12 (46.2)	0.206
1-year cumulative CIN2+	27 (39.1)	14 (32.6)	13 (50.0)	0.204
21–40	16 (8.8)	3 (4.6)	13 (11.1)	-
Immediate CIN2+	10 (62.5)	2 (66.7)	8 (61.5)	> 0.99
1-year cumulative CIN2+	11 (68.8)	2 (66.7)	9 (69.2)	> 0.99
41–200	35 (19.3)	5 (7.8)	30 (25.6)	-
Immediate CIN2+	30 (85.7)	3 (60.0)	27 (80.0)	0.139
1-year cumulative CIN2+	31 (88.6)	4 (80.0)	27 (80.0)	0.477
201–1000	29 (16.0)	6 (9.4)	23 (19.7)	-
Immediate CIN2+	28 (96.6)	6 (100.0)	22 (95.7)	> 0.99
1-year cumulative CIN2+	28 (96.6)	6 (100.0)	22 (95.7)	> 0.99
> 1000	32 (17.7)	7 (11.0)	25 (21.4)	-
Immediate CIN2+	30 (93.8)	7 (100.0)	23 (92.0)	> 0.99
1-year cumulative CIN2+	31 (96.9)	7 (100.0)	24 (96.0)	> 0.99

Abbreviations: CIN2+, cervical intraepithelial neoplasia grade 2 or worse; HPV, Human papillomavirus

87.0% (40/46) of women had integration reads less than 20.

The association between persistent HPV integration at the same site and the development of precancerous lesions

Among 181 participants, 58 women had < CIN2 histologic results at baseline. After 1 year of follow-up, the CIN2 + progression rate of women with negative conversion and the CIN2 + progression rate of women with HPV integration at a different site were 0%. The CIN2 + progression rate of women with negative conversion was significantly lower than that of women with persistent integration at the same site (5/12, 41.6%, $P < 0.001$, Table S2). However, there were no significant differences in CIN2 + progression rates between women with persistent integration at the same site and women with HPV integration at a different site.

Discussion

Previous studies have suggested that HPV infections can be classified into productive infections that clear spontaneously and transforming infections that lead to cervical precursor lesions [26]. HPV integration is a crucial event in HPV-mediated malignant transformation and is associated with transforming infections [27, 28]. Previous studies have demonstrated that HPV integration can lead to genome instability, structural rearrangement, and copy number variation [29–31]. The level of HPV integration increases with the grade of CIN and has been suggested as a biomarker for cancer progression [32–34]. Moreover, recent studies have found that the number of HPV integration reads increases with the severity of CIN [21, 35]. Therefore, HPV integration reads may be a potential marker for risk stratification among HPV-positive women.

HPV integrated testing has the advantages of easy sampling, simple operation, and minimal trauma, as it uses cervical exfoliated cells similarly to standard HPV testing. Therefore, it can be effectively used as a cervical cancer screening method in clinical practice. In this study, we investigated the prognostic role of HPV integration reads in cervical precancer risk prediction. As the number of HPV integration reads increased, the immediate risk of CIN2 + and CIN3 + and the 1-year cumulative risk of CIN2 + and CIN3 + increased. In our study, compared to non-HPV16/18 infection, HPV16/18 type is more easily integrated into the human genome, which is consistent with what has been reported before [36, 37]. However, in each integration read strata, there were no significant differences in CIN2 + risk between women with HPV16/18 integration and non-HPV16/18 integration. This finding raises the question of whether the oncogenicity of different HPV types is solely associated with their ability to integrate, rather than the number of integration reads. This question warrants further studies. Furthermore, we found that the 1-year cumulative CIN2 + risk with HPV

Table 3 The relationship between participants' positive outcomes and HPV integration reads

HPV integration reads	Total. No	Positive outcomes, No.			Negative outcomes, No.		Proportion of positive outcomes	P_{trend} value
		Immediate CIN2+	1-year CIN2+	Persistent integration at the same site	Negative conversion	Integration at a different site		
6–20	69	25	2	2	37	3	42.0%	< 0.001
21–40	16	10	1	0	3	2	68.8%	
41–200	35	30	1	4	0	0	100%	
201–1000	29	28	0	0	1	0	96.6%	
> 1000	32	30	1	1	0	0	100%	

Abbreviations: CIN2+, cervical intraepithelial neoplasia grade 2 or worse; HPV, Human papillomavirus

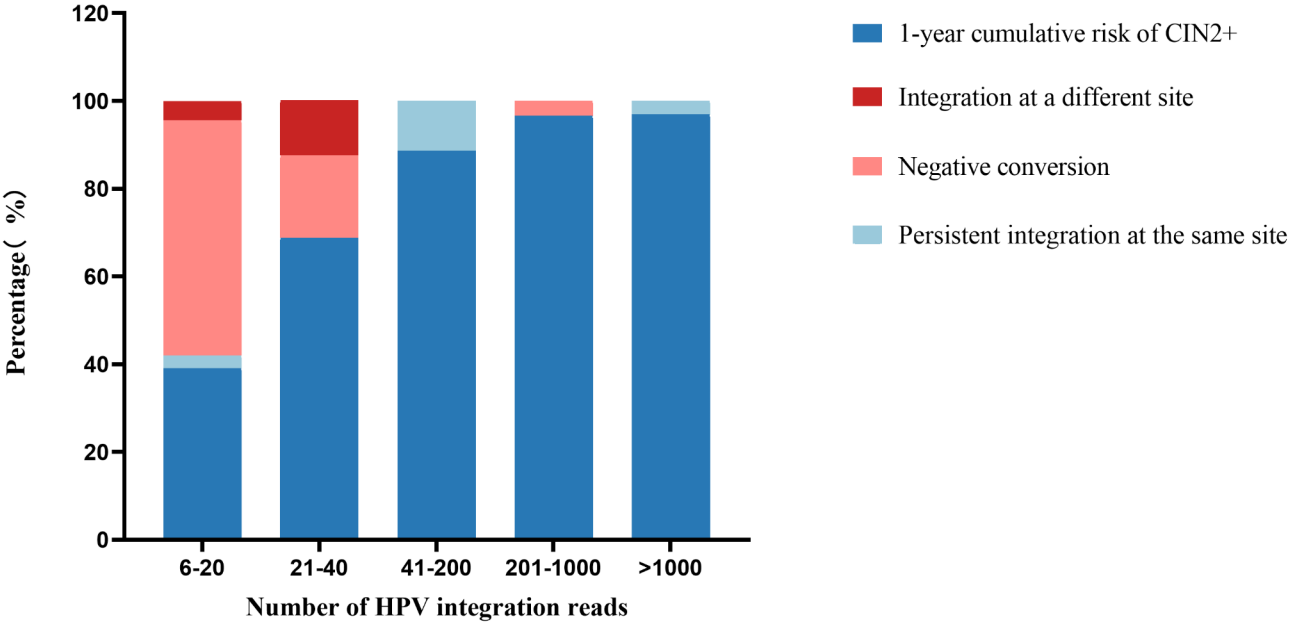

Fig. 3 The 1-year outcomes of HPV integration-positive women at different integration reads Abbreviations: CIN2+, cervical intraepithelial neoplasia grade 2 or worse; HPV, Human papillomavirus

integration reads more than 40 was 93.8%, which was significantly higher than the 1-year cumulative CIN2 + risk with HPV integration reads less than 40. Therefore, our findings suggested that HPV integration reads more than 40 can indicate an extremely high risk for CIN2+. These women should be referred to expedited treatment based on the guideline [38].

We explored the integration status conversion among HPV integration-positive women during a 1-year follow-up period. The results showed that women with less than 40 integration reads were more likely to have negative conversion or HPV integration at a different site. On the contrary, women with integration reads more than 40 were more likely to maintain persistent HPV integration at the same site. For women with persistent integration at the same site, the number of integration reads increased during the follow-up. On this basis, we investigated whether this persistent integration status was associated with cervical cancer progression. The results demonstrated that women with persistent integration at the same site had a significantly higher progression rate than those with negative conversion. Thus, persistent integration at the same site may be a potential biomarker for CIN2 + progression. Previous studies have shown that HPV integration occurs in two forms: productive integration and silent integration [39]. The former is closely associated with the development of cervical precancerous lesions, while the latter rarely progresses to cervical cancer. Therefore, we hypothesized that HPV integration at different sites may be the result of natural clearance of a silent and non-oncogenic integration site. However, in our study, the difference in progression rates between women with persistent integration at the same site and women with HPV integration at a different site was not statistically significant, probably due to the small sample size. After one year of follow-up, 99.0% of women with more than 40 integration reads had persistent integration at the same site, immediate CIN2 + or 1-year CIN2+, suggesting that women with integration reads more than 40 might have a high risk of CIN2+. Therefore, close attention should be paid to women with more than 40 integration reads or persistent integration at the same site in clinical settings.

HPV integration test may help with risk management after colposcopy. In our study, we discovered two women with negative cytology results and normal histology, one with HPV35 infection and the other with HPV16 infection. Based on the guidelines, they were referred to 1-year follow-up. However, both patients had more than 40 integration reads and progressed to AIS within 1 year. Several researchers have found that AIS has rapid progression, is difficult to identify, and can be easily misdiagnosed [40–42]. The immediate CIN3 + risk for these two women was more than 70% based on our risk stratification results, while the strategy recommended by the American Society for Colposcopy and Cervical Pathology (ASCCP) suggested that women with an immediate CIN3 + risk of 60% were referred to treatment instead of colposcopy [38]. Therefore, based on our results, women with more than 40 HPV integration reads should be referred to cervical conization, and women with integration reads between 20 and 40 should be referred to diagnostic conization if

follow-up is inconvenient. Li et al. reported that the HPV integration test can prevent the misdiagnosis of cervical cancer [43]. Besides, some rare combinations, such as high-grade squamous intraepithelial lesion (HSIL) or atypical squamous cells, cannot rule out HSIL (ASC-H) cytologic results with histological results ≤ CIN1, the risk assessment according to the guideline can be less reliable, because of the occult diseases after any high-grade cytology [44]. For these rare combinations, HPV integration reads can help colposcopists assess the risk of lesions, and together with biopsy, can prevent missed diagnoses.

To our knowledge, this is the first study to evaluate the CIN2 + risk and HPV integration status conversion among HPV integration-positive women. The strengths of this study were as follows. Previous techniques for identifying integration events based on the E2/E6 ratio may be inaccurate, as the E2 gene may not be affected in some events, while HIVID could overcome this problem in this study [45, 46]. Compared to WGS, identifying HPV integration events using HIVID can detect more HPV integration sites in cervical exfoliated cells [19]. Therefore, our approach provided accurate integration data for CIN2 + risk assessment and HPV integration status, suggesting that HPV integration can precisely predict the risk of developing cervical precancerous lesions. Technically, the number of integration supporting reads correlates with the number of cells in which HPV integration occurs, while cervical squamous epithelial cells were usually exfoliated once every 4–5 days [47]. Thus, we hypothesized that a higher number of integrated cells more robustly supports the abnormal proliferation of the same clone. In summary, HIVID is promising in the clinical stratification and clinical management of high-risk CIN lesions and is affordable for the majority of patients in our country.

There were also some limitations to this study. First of all, the follow-up period was short. Considering only 5 women progressed into CIN2 + and 12 women had persistent integration, it was hard to calculate the statistical power. Only a small proportion of HPV integration was productive integration, increasing the risk of transformation from cervical precancerous lesions to cancer. In addition, HPV can be integrated into different human chromosomes and different gene loci (Table S3). The integration reads and the persistence of HPV integration are the results of the natural selection of different integration sites. Many studies have shown that the frequency of distinct integration sites is different and the ability to promote cancer development may also depend on the integration site [48]. These findings should be verified in a bigger population. Finally, the sample size was relatively small and a larger sample size is needed to investigate whether HPV integration test can safely stratify follow-up intervals and prevent excessive treatment. For example, women with HPV integration reads more than 40 integration reads need shorter follow-up intervals if diagnostic cervical conization is not conducted. A multicenter cohort study based on a large population with longitudinal follow-up led by our hospital is being conducted.

Conclusions

This study was the first to explore risk stratification based on HPV integration levels and HPV integration status conversion among HPV integration-positive women. The CIN2 + risk increased with the number of HPV integration reads. After 1 year of follow-up, the majority of women with more than 40 integration reads experienced persistent integration at the same breakpoint. Women with more than 40 integration reads or persistent integration at the same site need to be vigilantly monitored in clinical practice. In conclusion, the number of HPV integration reads may be a potential biomarker for early warning and precise identification of high-risk CIN lesions.

Abbreviations

95% CI	95% confidence interval
AIS	Adenocarcinoma in situ
CIN	Cervical intraepithelial neoplasia
CIN1	Cervical intraepithelial neoplasia grade 1
CIN2	Cervical intraepithelial neoplasia grade 2
CIN2 +	Cervical intraepithelial neoplasia grade 2 or worse
CIN3	Cervical intraepithelial neoplasia grade 3
HIVID	High-throughput viral integration detection
HPV	Human papillomavirus
NGS	Next-generation sequencing
OR	Odds ratio
WGS	Whole genome sequencing

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12885-025-14138-4.

Supplementary Material 1

Acknowledgements

The authors would like to express their gratitude to EditSprings (https://www.editsprings.cn) for the expert linguistic services provided.

Author contributions

Kexin Li: Conceptualization, Formal analysis, Validation, Writing—original draft and Writing—review and editing.Ting Hu: Conceptualization, Formal analysis, Validation, Supervision, Writing—original draft and Writing—review and editing. Fanwei Huang: Formal analysis, Validation and Writing—review and editing. Tao Zhang: Validation and Writing—review and editing. Fan Yang: Data curation and Validation. Weitao Duan: Data curation and Validation. Shimin Chen: Data curation and Validation. Xiaoyuan Huang: Supervision, Validation and Writing—review and editing.

Funding

This work received funding from the National Key R&D Program of China (2021YFC2701201).

Data availability

No datasets were generated or analysed during the current study.

Declarations

Patient consent for publication
All authors are in agreement with the content of the manuscript.

Informed consent
Patient consent was waived by the Ethics Committee of Tongji Hospital of Huazhong University of Science and Technology.

Competing interests
The authors declare no competing interests.

Author details
[1]Department of Obstetrics and Gynecology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei 430030, China
[2]New Technology Platform, Wuhan KDWS Biological Technology Co., Ltd., Wuhan, Hubei 430074, China
[3]Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, 1095 Jiefang Anv, Wuhan 430030, China

Received: 1 January 2024 / Accepted: 11 April 2025
Published online: 19 May 2025

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. Cancer J Clin. 2018;68(6):394–424.
2. Chen W, Zheng R, Baade PD, et al. Cancer statistics in China, 2015. Cancer J Clin. 2016;66(2):115–32.
3. Spence AR, Franco EL, Ferenczy A. The role of human papillomaviruses in cancer. Am J Cancer. 2005;4(1):49–64.
4. Walboomers JM, Jacobs MV, Manos MM, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol. 1999;189(1):12–9.
5. Bosch FX, Manos MM, Muñoz N, et al. Prevalence of human papillomavirus in cervical cancer: a worldwide perspective. International biological study on cervical cancer (IBSCC) study group. J Natl Cancer Inst. 1995;87(11):796–802.
6. Li Y, Xiang Y, Zhang RF, et al. [Viral load, genomic integration frequency of human papillomavirus 16 in cervical cancer and precancerous lesions]. Zhonghua Yi Xue Za Zhi. 2011;91(13):906–10.
7. Rusan M, Li YY, Hammerman PS. Genomic landscape of human papillomavirus-associated cancers. Clin cancer Research: Official J Am Association Cancer Res. 2015;21(9):2009–19.
8. Akagi K, Li J, Broutian TR, et al. Genome-wide analysis of HPV integration in human cancers reveals recurrent, focal genomic instability. Genome Res. 2014;24(2):185–99.
9. Oyervides-Muñoz MA, Pérez-Maya AA, Rodríguez-Gutiérrez HF, et al. Understanding the HPV integration and its progression to cervical cancer. Infect Genet Evolution: J Mol Epidemiol Evolutionary Genet Infect Dis. 2018;61:134–44.
10. el Awady MK, Kaplan JB, O'Brien SJ, Burk RD. Molecular analysis of integrated human papillomavirus 16 sequences in the cervical cancer cell line SiHa. Virology. 1987;159(2):389–98.
11. Carmody MW, Jones M, Tarraza H, Vary CP. Use of the polymerase chain reaction to specifically amplify integrated HPV-16 DNA by virtue of its linkage to interspersed repetitive DNA. Mol Cell Probes. 1996;10(2):107–16.
12. Donaldson YK, Arends MJ, Duvall E, Bird CC. A PCR approach to discriminate between integrated and episomal HPV DNA in small clinical specimens. Mol Cell Probes. 1993;7(4):285–92.
13. Zheng Y, Peng ZL, Lou JY, Wang H. [Detection of physical status of human papillomavirus 16 in cervical cancer tissue and SiHa cell line by multiplex real-time polymerase chain reaction]. Ai zheng = Aizheng = Chin J cancer. 2006;25(3):373–7.
14. Holmes A, Lameiras S, Jeannot E, et al. Mechanistic signatures of HPV insertions in cervical carcinomas. NPJ Genom Med. 2016;1:16004.
15. Groves IJ, Coleman N. Human papillomavirus genome integration in squamous carcinogenesis: what have next-generation sequencing studies taught Us?? J Pathol. 2018;245(1):9–18.
16. Andersen K, Holm K, Tranberg M et al. Targeted next generation sequencing for human papillomavirus genotyping in cervical Liquid-Based cytology samples. Cancers 2022;14(3).
17. Arias-Pulido H, Peyton CL, Joste NE, Vargas H, Wheeler CM. Human papillomavirus type 16 integration in cervical carcinoma in situ and in invasive cervical cancer. J Clin Microbiol. 2006;44(5):1755–62.
18. Hudelist G, Manavi M, Pischinger KI, et al. Physical state and expression of HPV DNA in benign and dysplastic cervical tissue: different levels of viral integration are correlated with lesion grade. Gynecol Oncol. 2004;92(3):873–80.
19. Hu Z, Zhu D, Wang W, et al. Genome-wide profiling of HPV integration in cervical cancer identifies clustered genomic hot spots and a potential microhomology-mediated integration mechanism. Nat Genet. 2015;47(2):158–63.
20. Bouchilloux S, Fer F, Lemée F, et al. Correlation between integration of high-risk HPV genome into human DNA detected by molecular combing and the severity of cervical lesions: first results of the EXPL-HPV-002 study. Ceska Gynekol. 2019;84(2):84–92.
21. Huang J, Qian Z, Gong Y, et al. Comprehensive genomic variation profiling of cervical intraepithelial neoplasia and cervical cancer identifies potential targets for cervical cancer early warning. J Med Genet. 2019;56(3):186–94.
22. Hopman AH, Smedts F, Dignef W, et al. Transition of high-grade cervical intraepithelial neoplasia to micro-invasive carcinoma is characterized by integration of HPV 16/18 and numerical chromosome abnormalities. J Pathol. 2004;202(1):23–33.
23. Van Tine BA, Kappes JC, Banerjee NS, et al. Clonal selection for transcriptionally active viral oncogenes during progression to cancer. J Virol. 2004;78(20):11172–86.
24. Integrated genomic and. Molecular characterization of cervical cancer. Nature. 2017;543(7645):378–84.
25. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinf (Oxford England). 2009;25(14):1754–60.
26. Steenbergen RDM, Snijders PJF, Heideman DAM, Meijer CJLM. Clinical implications of (epi)genetic changes in HPV-induced cervical precancerous lesions. Nat Rev Cancer. 2014;14(6):395–405.
27. Pett M, Coleman N. Integration of high-risk human papillomavirus: a key event in cervical carcinogenesis? J Pathol. 2007;212(4):356–67.
28. Duensing S, Münger K. The human papillomavirus type 16 E6 and E7 oncoproteins independently induce numerical and structural chromosome instability. Cancer Res. 2002;62(23):7075–82.
29. Adey A, Burton JN, Kitzman JO, et al. The haplotype-resolved genome and epigenome of the aneuploid HeLa cancer cell line. Nature. 2013;500(7461):207–11.
30. Kadaja M, Sumerina A, Verst T, Ojarand M, Ustav E, Ustav M. Genomic instability of the host cell induced by the human papillomavirus replication machinery. EMBO J. 2007;26(8):2180–91.
31. Dall KL, Scarpini CG, Roberts I, et al. Characterization of naturally occurring HPV16 integration sites isolated from cervical keratinocytes under noncompetitive conditions. Cancer Res. 2008;68(20):8249–59.
32. Shukla S, Mahata S, Shishodia G, et al. Physical state & copy number of high risk human papillomavirus type 16 DNA in progression of cervical cancer. Indian J Med Res. 2014;139(4):531–43.
33. Wentzensen N, Vinokurova S, von Knebel Doeberitz M. Systematic review of genomic integration sites of human papillomavirus genomes in epithelial dysplasia and invasive cancer of the female lower genital tract. Cancer Res. 2004;64(11):3878–84.
34. Kalantari M, Chase DM, Tewari KS, Bernard HU. Recombination of human papillomavirus-16 and host DNA in exfoliated cervical cells: a pilot study of L1 gene methylation and chromosomal integration as biomarkers of carcinogenic progression. J Med Virol. 2010;82(2):311–20.
35. Li W, Tian S, Wang P, et al. The characteristics of HPV integration in cervical intraepithelial cells. J Cancer. 2019;10(12):2783–7.
36. Vinokurova S, Wentzensen N, Kraus I, et al. Type-dependent integration frequency of human papillomavirus genomes in cervical lesions. Cancer Res. 2008;68(1):307–13.
37. Ho CM, Chien TY, Huang SH, Lee BH, Chang SF. Integrated human papillomavirus types 52 and 58 are infrequently found in cervical cancer, and high viral loads predict risk of cervical cancer. Gynecol Oncol. 2006;102(1):54–60.
38. Perkins RB, Guido RS, Castle PE, et al. 2019 ASCCP Risk-Based management consensus guidelines for abnormal cervical cancer screening tests and cancer precursors. J Lower Genit Tract Dis. 2020;24(2):102–31.
39. Fan J, Fu Y, Peng W, et al. Multi-omics characterization of silent and productive HPV integration in cervical cancer. Cell Genom. 2023;3(1):100211.

40. Costa S, Negri G, Sideri M, et al. Human papillomavirus (HPV) test and PAP smear as predictors of outcome in conservatively treated adenocarcinoma in situ (AIS) of the uterine cervix. Gynecol Oncol. 2007;106(1):170–6.
41. Teoh D, Musa F, Salani R, Huh W, Jimenez E. Diagnosis and management of adenocarcinoma in situ: A society of gynecologic oncology Evidence-Based review and recommendations. Obstet Gynecol. 2020;135(4):869–78.
42. Ronco G, Giorgi-Rossi P, Carozzi F, et al. Efficacy of human papillomavirus testing for the detection of invasive cervical cancers and cervical intraepithelial neoplasia: a randomised controlled trial. Lancet Oncol. 2010;11(3):249–57.
43. Li J, Zhang X, Wang P, Li W. The help of HPV integration testing to avoid the misdiagnosis of a patient with stage Ia1 cervical cancer: A case report and literature review. Pharmgenomics Pers Med. 2021;14:1457–61.
44. Egemen D, Cheung LC, Chen X, et al. Risk estimates supporting the 2019 ASCCP Risk-Based management consensus guidelines. J Lower Genit Tract Dis. 2020;24(2):132–43.
45. Gao G, Johnson SH, Vasmatzis G, et al. Common fragile sites (CFS) and extremely large CFS genes are targets for human papillomavirus integrations and chromosome rearrangements in oropharyngeal squamous cell carcinoma. Genes Chromosomes Cancer. 2017;56(1):59–74.
46. Parfenov M, Pedamallu CS, Gehlenborg N, et al. Characterization of HPV and host genome interactions in primary head and neck cancers. Proc Natl Acad Sci USA. 2014;111(43):15544–9.
47. Michelini M, Rosellini A, Simoncini T, Papini S, Revoltella RP. A three-dimensional organotypic culture of the human uterine exocervix for studying mucosal epithelial differentiation and migrating leukocytes. Differ Res Biol Divers. 2004;72(4):138–49.
48. Yang-Chun F, Sen-Yu W, Yuan Z, Yan-Chun H. Genome-Wide profiling of human papillomavirus DNA integration into human genome and its influence on PD-L1 expression in Chinese Uygur cervical cancer women. J Immunol Res. 2020;2020:6284960.

Publisher's note