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# Co-infections of HPV16/18 with other high-risk HPV types and the risk of cervical carcinogenesis: A large population-based study

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## HIGHLIGHTS

- 33.24% (2023/6086) HPV16/18 positive women co-infected with other high-risk HPV genotypes simultaneously.
- Co-infections of HPV16/18 with other high-risk HPV genotypes cause different changes in risk of cervical carcinogenesis.
- HPV16 co-infected with other high-risk HPV genotypes may reduce the risk of CIN3+ compared with single HPV16 infection.
- HPV18 co-infected with HPV16 increased the risk of CIN2 compared with single HPV18 infection.

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## ABSTRACT

*Objective:* Human papillomavirus (HPV) 16/18 genotyping is an effective method for triage of high-risk (hr) HPV-positive women in primary hrHPV screening for cervical cancer. The present study aimed to evaluate whether co-infected with other hrHPV types will affect the risk of cervical carcinogenesis in HPV16/18 positive women.

*Methods:* A total of 313,704 women aged ≥30 years were screened in China. Among them, 4,933 HPV16/18-positive participants underwent colposcopy-directed biopsy. The HPV genotypes were identified using the Cobas HPV genotyping system. Multinomial logistic regression was used to model different HPV16/18 infection patterns.

*Results:* The overall prevalence rates of hrHPV and HPV16/18 were 7.85% (24,456/311,382) and 1.95% (6,086/311,382) respectively. Among HPV16/18 positive individuals, 33.24% (2,023/6,086) were co-infection with multiple types. Of the 4933 women who underwent colposcopy, their HPV16/18 infection patterns were as follows: 52.38% (2,584/4,933) HVP16 only, 23.54% (1,161/4,933) HPV16 + other hrHPVs, 14.98% (739/4,933) HPV18 only, 6.83% (337/4,933) HPV18 + other hrHPVs, 1.13% (56/4,933) HPV16 + 18, 1.13% (56/4,933) HPV16 + 18+other hrHPVs. After adjusting for cofactors, compared with single HPV16 infection, the risk of developing cervical intraepithelial neoplasia (CIN) grade 3 or greater (CIN3+) was significantly lower in HPV16 + other hrHPVs group (odds ratio [OR] = 0.637, 95% confidence interval [CI] = 0.493-0.822).

*Conclusion:* HPV16/18 co-infection with other hrHPVs is a common phenomenon. Different HPV16/18 infection patterns may influence the risk of cervical carcinogenesis. HPV16 co-infected with other hrHPVs appears to have a lower associated risk of CIN3+ in ≥30 years old women.



*Abbreviations:* CI, confidence interval; CIN, cervical intraepithelial lesion; HPV, human papillomavirus; OR, odds ratio; hrHPV, high-risk human papillomavirus; BMI, body mass index.

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## 1. Introduction

Cervical cancer is a common malignant disease that threatens the health of women globally, causing 311,000 deaths worldwide in 2018 [1]. Human papillomavirus (HPV) infection is detected in almost 100% of cervical cancer samples, and it is considered to be the most necessary factor in the pathogenesis of cervical cancer [2,3]. Approximately 20.4%–56.3% of HPV infected women are simultaneously infected with multiple HPV types [4–8]. However, the effect of multiple-type HPV infections has been controversial. Some researchers have claimed that the combination of different HPV infections increased the incidence of high grade squamous intraepithelial lesions (HSIL) and/or invasive cervical cancer (ICC) [9]. However, some researchers have found that the effect of single and multiple HPV infections on severity of cervical neoplasia was not significant [6,8,10]. Additionally, a study has found that multiple HPV infections did not increase risk of HSIL and highlighted that the risk of HSIL was decreased in specific co-infections of varying HPV genotypes [11].

HPV16/18 are recognized to be the most common cervical cancer associated HPV genotypes [12,13], and HPV16 is noted to be the most prevalent hrHPV type with the highest risk of progression to CIN3+ among all hrHPVs [14,15]. However, whether co-infection with other hrHPVs will affect the risk of cervical carcinogenesis among HPV16/18 positive women is unclear. As more studies have recommended hrHPV testing as the primary screening method [16], the answer to this question may have high clinical significance. Sundstrom et al. have reported that co-infections of hrHPVs and low-risk (lr) HPVs reduced the risk of cervical cancer [17]. We speculated interactions might exist between HPV16/18 and other hrHPV genotypes, and such interactions could either increase or decrease the carcinogenic ability of HPV16/18.

The present study aimed to explore the proportion of individuals with HPV16/18 coinfection with other hrHPVs among a large population, and whether the interactions between HPV16/18 and other hrHPVs would influence the risk of cervical carcinogenesis.

## 2. Methods

### 2.1. Participant enrollment

From January 2017 to February 2018, a population-based, cross-sectional, multisite cervical cancer screening study was performed in Xiangyang, Hubei Province, China. The primary HPV screening algorithm was taken as recommended by Hub et al. [18]. The study population included women from urban areas (Xiangzhou, Xiangcheng, Fancheng districts), who were recruited mainly via media promotion, and from suburban and rural areas (Baokang, Nanzhang, Zaoyang, Yicheng, Gucheng, Laohekou), who were recruited via local health clinics and government notices. A total of 313,704 women were screened, and the selection process of those enrolled in this study is shown in Fig. 1. Among the participants, 6086 women were HPV16/18 positive. Of them, 4933 underwent colposcopy-directed biopsy. From these individuals. We collected information about HPV16/18 infection patterns, histological results, and epidemiological data.

All recruited women completed our validated questionnaire. Before conducting HPV testing, a trained medical staff administered a questionnaire to each participant and obtained informed consent. To ensure the rationality and validity of the questionnaire, it was designed by gynecologic oncologists. The personnel who collected the questionnaire underwent rigorous professional training, and a double-entry method was adopted to ensure the accuracy and credibility of the data obtained using the questionnaire. The inclusion criteria were as follows: ≥30 years old; non-pregnant; sexually active for more than 1 year; no previously HPV vaccinated. The exclusion criteria were as follows: total hysterectomy and history of pelvic radiotherapy. Informed and written consent was obtained from all recruited women. The ethics committee of Xiangyang Central Hospital approved the protocol, and all procedures were in accordance with the ethical standards specified by the institution.

### 2.2. HPV genotyping

Cervical specimens were collected from the respective collection hospitals, and all specimens were tested at the Department of Obstetrics and Gynecology of the Xiangyang Central Hospital. The Cobas 4800 (Roche Molecular Systems, Alameda, CA) technology was used for hrHPV genotyping according to the manufacturer's recommendations. Cobas 4800 classifies positive test results as HPV16, HPV18, and "other hrHPVs" combined (HPV31/33/35/39/45/51/52/56/58/59/66/68).

### 2.3. Colposcopy-directed biopsy and histological diagnosis

According to Huh et al. recommended for primary HPV testing for cervical cancer screening [18], HPV16/18-positive women should undergo colposcopy and biopsy directly without cytological examination. Only women who are positive for other hrHPVs should undergo liquid-based cytology examination, among them, whose cytologic result ≥ atypical squamous cells of undetermined significance (ASC-US+) should undergo colposcopy and biopsy. For HPV16/18 infected participants plus other hrHPV positive women with abnormal cervical cytology (ASCUS+), colposcopy and biopsy were performed by two skilled gynecologic oncologists. All detected abnormalities under coloscopy were biopsied. If there were no visible abnormalities, a random biopsy was performed at 2, 4, 8, and 10 points of the cervix. Endocervical curettage (ECC) was be performed when the cervical squamous column junction was not satisfied. All histological diagnoses were obtained based on a consensus decision by two expert gynecological pathologists who reviewed each biopsy. The pathologist was unaware of the results of the screening test results. The diagnosis was based on the worst biopsy result obtained (colposcopy orientation, random, or ECC). Pathology results were divided into normal (including cervicitis), CIN1, CIN2, CIN3 (including carcinoma in situ), and cervical cancer (including cervical adenocarcinoma, cervical squamous cell cancer, cervical adenosquamous carcinoma).

### 2.4. Statistical analyses

For statistical purposes, histological results were further categorized as < CIN2 (including normal and CIN1), CIN2, CIN3+ (including CIN3 and cervical cancer). The associations between HPV16/18 infection patterns and the histological results were analyzed.

The Kruskal-Wallis test was adopted to compare the observed histological results (three categories: <CIN2, CIN2, and CIN3+) in different HPV16/18 infection patterns. The demographic characteristics included education, post-coital bleeding, menopause or not, menopause age, ever pregnant, gravidity, ever live births, parity, family history of cancer, vaginal cleanliness, trichomonas infection, abnormal leucorrhea, the frequency of cytology test, and body mass index (BMI). The reasons for the inclusion of these epidemiological factors are described in the Supplemental Material. The association between histological results (three categories: <CIN2, CIN2, and CIN3+ and dichotomy: <CIN2 and CIN2+) and different demographic characteristics was assessed via multinomial logistic regression analyses to calculate for adjusted odds ratios (ORs) with 95% confidence intervals (CIs). Adjustment was made for

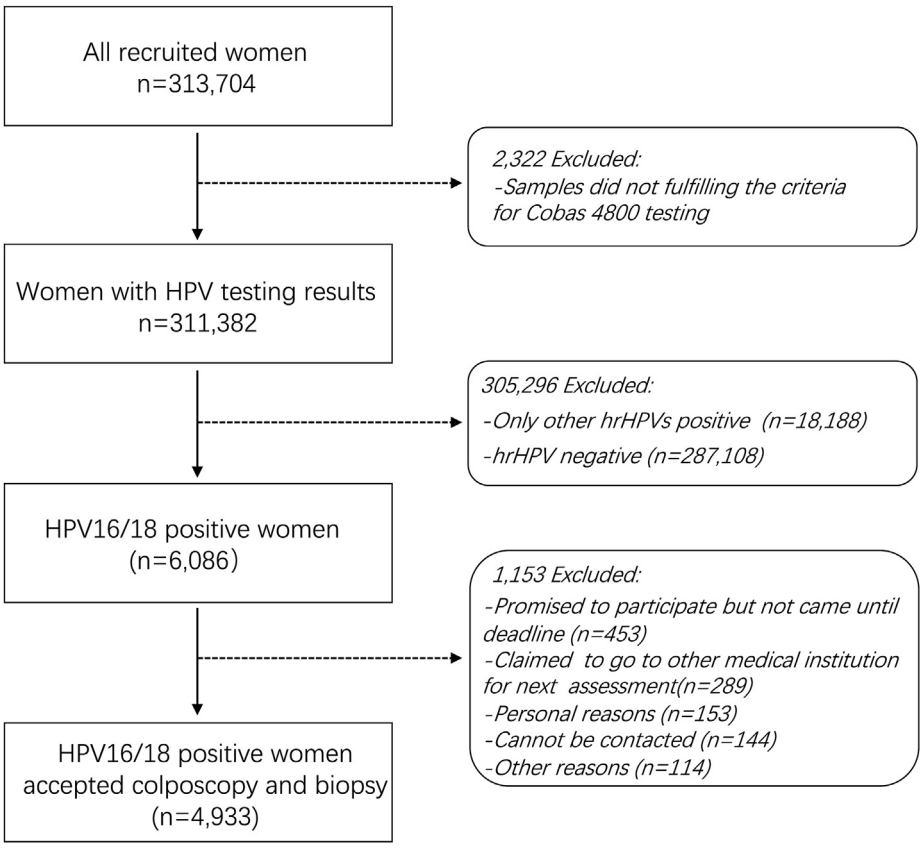


**Fig. 1.** Flowchart of participant identification and enrollment.
Abbreviations: HPV, human papillomavirus; other hrHPVs, high-risk HPV genotypes HPV31/33/35/39/45/51/52/56/58/59/66/68.

age (dichotomy). Subsequently, the significant demographic characteristics which can maximally explain the risk of developing the disease were identified. The ORs for CIN3+ and CIN2 were calculated using a single HPV infection (including HPV16 or 18) as the reference category. Multinomial logistic regression analysis was conducted to explore the association between histological results (three categories: <CIN2, CIN2, and CIN3+ and dichotomy: <CIN2 and CIN2+) and HPV16/18 infection patterns and significant demographic characteristics. The database was established with EpiData 3.1, and the statistical analyses were performed using the SAS statistical analysis package 9.4 version and software PASS version 15.0.

## 3. Results

In this study, 7.85% (24,456/311,382) and 1.95% (6086/311,382) women were detected to be hrHPV positive and HPV16/18 positive respectively, and 18,188 women were “only other hrHPVs” infection. The age distribution of the participants as shown in Supplementary Fig. 1. Among the HPV16/18 positive individuals, 33.24% (2,023/6,086) were co-infection with multiple HPV types. A total of 1,153 HPV16/18-positive women did not undergo colposcopy, and the main reasons were as follows: 39.29% (453/1,153), promised to participate but did not come until the deadline; 25.07% (289/1,153), claimed to go to other medical institutions for the next assessment; 13.27 (153/1,153), personal reasons; 12.49% (144/1,153), cannot be contacted; and 9.89% (114/1,153), other reasons. Here, the HPV16/18 infection patterns of the 4,933 women who underwent colposcopy their HPV16/18 infection patterns were divided into six groups mainly based on the presence or absence of other hrHPVs. These groups were as follows: 52.38% (2,584/4,933), HVP16 only; 23.54% (1,161/4,933), HPV16 + other hrHPVs; 14.98% (739/4,933), HPV18 only; 6.83% (337/4,933), HPV18 + other hrHPVs; 1.13% (56/4,933), HPV16 + 18; 1.13% (56/4,933), HPV16 + 18+other hrHPVs. The distribution of histological results (five categories: normal, CIN1, CIN2, CIN3 and cervical cancer) between different HPV16/18 infection patterns is shown in Table 1. The results of the Kruskal-Wallis test used for comparing the histological results (three categories: <CIN2, CIN2, and CIN3+) in different HPV16/18 infection patterns were shown in Table 2 (power = 0.9056, calculating by software PASS 15.0). The results revealed a statistically significant difference among the HPV16 ($p = 0.0073$) or HPV18 infection groups ($p < 0.0001$) and histological results.

The analysis results of the association between different HPV16/18 infection patterns and the histological results (three categories: <CIN2, CIN2, and CIN3+) are depicted in Fig. 2. In the HPV16-infected groups (<CIN2 vs. CIN3+), the incidence of CIN3+ in HPV16 + other hrHPVs group was significantly lower than that in the HPV16 only group (OR = 0.594, 95% CI = 0.464-0.762). In the HPV18 infection groups (<CIN2 vs. CIN2), the incidence of CIN2 in women infected with HPV16 + 18 (OR = 4.661, 95% CI = 1.636-13.276) or HPV16 + 18+other hrHPVs (OR = 6.667, 95% CI = 2.612-17.016) was significantly higher than that of women infected with HPV18 only.

To identify potential HPV cofactors, women diagnosed with CIN2 and CIN3+ were compared with those diagnosed with <CIN2 (Table 3). Among all the potential HPV cofactors, significant differences were observed in five variables including education (OR = 1.587, 95% CI = 1.266-1.990), post-coital bleeding (OR = 2.579, 95% CI = 1.356-4.903), menopause or not (OR = 0.542, 95% CI = 0.439-0.670), family history of cancer (OR = 2.334, 95% CI = 1.292-4.217),

**Table 1**
Distribution of HPV16/18 infection patterns among the study population.

| HPV16/18 infection patterns | Normal | CIN1 | CIN2 | CIN3 | Cancer | Total |
|---|---|---|---|---|---|---|
| | n(%) | n(%) | n(%) | n(%) | n(%) | n |
| HPV16 only | 1872 (72.45) | 236 (9.13) | 163 (6.31) | 261 (10.10) | 52 (2.01) | 2584 |
| HPV16 + other hrHPVs | 877 (75.54) | 120 (10.34) | 76 (6.55) | 76 (6.55) | 12 (1.03) | 1161 |
| HPV18 only | 618 (83.63) | 83 (11.23) | 16 (2.17) | 11 (1.49) | 11 (1.49) | 739 |
| HPV18 + other hrHPVs | 276 (81.90) | 51 (15.13) | 6 (1.78) | 4 (1.19) | 0 (0.00) | 337 |
| HPV16 + 18 | 37 (66.07) | 10 (17.86) | 5 (8.93) | 4 (7.14) | 0 (0.00) | 56 |
| HPV16 + 18+ other hrHPVs | 40 (71.43) | 6 (10.71) | 7 (12.50) | 2 (3.57) | 1 (1.79) | 56 |
| Total | 3720 (75.41) | 506 (10.26) | 273 (5.53) | 358 (7.26) | 76 (1.54) | 4933 |

Abbreviations: CIN, cervical intraepithelial neoplasia; HPV, human papillomavirus; other hrHPVs, high-risk HPV genotypes HPV31/33/35/39/45/51/52/56/58/59/66/68.

and vaginal cleanliness (OR = 1.656, 95% CI = 1.352-2.027) between women with < CIN2 and CIN3+. Menopause or not (OR = 0.745, 95% CI = 0.572-0.971) and menopause age (OR = 1.934, 95% CI = 1.055-3.544) were statistically significant between women with <CIN2 and CIN2. Thus, we included these statistically significant variables in next the multivariate regression analysis.

Based on the abovementioned results, we did not include the menopause age as variable due to the fact that a high percentage of subjects did not reached menopausal age. To further explore the association between histological results and HPV16/18 infection patterns, The variables education, post-coital bleeding, menopause or not, family history of cancer and vaginal cleanliness as well as with age were included in the four multivariate logistic regression models for adjustment. Two multinomial models (histological results for trichotomy: <CIN2, CIN2, and CIN3+) were established, which included HPV16 only infection (Table 4) and HPV18 only infection (Supplemental Table 1) as the reference of the dummy variable respectively. In the model using HPV16 only infection as reference, a significant difference was observed in HPV16/18 infection patterns and menopause or not between women with CIN2 and <CIN2. However, no significant difference was showed between HPV16 only infection with HPV16 + HPV18 or HPV16 + HPV18 + other hrHPVs infection in either CIN2 versus < CIN2 or CIN3+ versus < CIN2. Women infected with HPV18 only (OR = 0.288, 95% CI = 0.168-0.493) or HPV18 + other hrHPVs (OR = 0.254, 95% CI = 0.111-0.581) and women who were menopause (OR = 0.733, 95% CI = 0.554-0.971) had a lower risk of CIN2. When CIN3+ was compared with <CIN2, the risk of CIN3+ decreased in women infected with HPV16 + other hrHPVs (OR = 0.636, 95% CI = 0.493-0.821), HPV18 only (OR = 0.200, 95% CI = 0.127-0.315), or HPV18 + other hrHPVs (OR = 0.085, 95% CI = 0.031-0.229) and women who were menopause (OR = 0.590, 95% CI = 0.472-0.737). Moreover, women aged ≥50 years old (OR = 1.945, 95% CI = 1.374-2.754), those with post-coital bleeding (OR = 2.293, 95% CI = 1.162-4.525), those who attended high school or above (OR = 1.488, 95% CI = 1.173-1.888), those with vaginal cleanliness III/IV (OR = 1.769, 95% CI = 1.437-2.176), and those with a family history of cancer (OR = 2.386, 95% CI = 1.291-4.411) were at a higher risk of CIN3+. In the model with HPV18 only infection as reference (Supplemental Table 1), the results were consistent with the first model (Table 4) except for HPV16/18 infection patterns. When CIN2 was compared with <CIN2, women infected with HPV16 only (OR = 3.476, 95% CI = 2.030-5.950), HPV16 + other hrHPVs (OR = 3.565, 95% CI = 2.021-6.288), HPV 16 + 18 (OR = 4.822, 95% CI = 1.676-13.872) or HPV16 + 18+other hrHPVs (OR = 5.559, 95% CI = 1.924-16.060) had a statistically significant higher risk of CIN2. Moreover, a significant difference was observed in HPV16/18 infection patterns when comparing CIN3+ versus < CIN2, with HPV16 only (OR = 4.997, 95% CI = 3.176-7.862) and HPV16 + other hrHPVs (OR = 3.179, 95% CI = 1.946-5.192) were at a higher risk of CIN3+. In addition, another two outcome dichotomous (histological results for dichotomy: <CIN2+ and CIN2+) logistic regression analyses were performed (Supplemental Table 1). Women infected with HPV16 + other hrHPVs were at a lower risk of CIN2+ (OR = 0.770, 95% CI = 0.630-0.940) than those infected with HPV16 only, which has the same trend as results in CIN3+. Meanwhile, women infected with HPV18 only had a lower risk of CIN2+ than women infected with HPV16 only (OR = 4.351, 95CI = 3.060-6.186), women infected with HPV16 + HPV18 (OR = 3.571, 95CI = 1.614-7.902), women infected with HPV16 + other hrHPVs (OR = 3.349, 95CI = 2.295-4.886) and women infected with HPV16 + 18+other hrHPVs (OR = 3.411, 95CI = 1.427-8.149).

**Table 2**
Kruskal-Wallis Test for comparing the distribution of histologic results (tri categorical) in different HPV16/18 infection patterns.

| HPV16/18 infection patterns | <CIN2 | CIN2 | CIN3+ | P |
|---|---|---|---|---|
| | N (%) | N (%) | N (%) | |
| HPV16 only | 2108(81.58) | 163(6.31) | 313(12.11) | 0.0073 |
| HPV16 + other hrHPVs | 997(85.87) | 76(6.55) | 88(7.58) | |
| HPV16 + 18 | 47(83.93) | 5(8.93) | 4(7.14) | |
| HPV16 + 18+other hrHPVs | 46(82.14) | 7(12.50) | 3(5.36) | |
| HPV18 only | 701(94.86) | 16(2.17) | 22(2.98) | <0.0001 |
| HPV18 + other hrHPVs | 327(97.03) | 6(1.78) | 4(1.19) | |
| HPV16 + 18 | 47(83.93) | 5(8.93) | 4(7.14) | |
| HPV16 + 18+other hrHPVs | 46(82.14) | 7(12.50) | 3(5.36) | |

Abbreviations: CIN, cervical intraepithelial neoplasia; HPV, human papillomavirus; other hrHPVs, high-risk HPV genotypes HPV31/33/35/39/45/51/52/56/58/59/66/68.

## 4. Discussion

We defined “multiple HPV infections” as the simultaneous infection of at least two HPV types, and it was commonly observed in HPV positive individuals [5,11,19,20]. Several studies have shown the possible association between different HPV types. However, the effects of these interactions on cervical carcinogenesis are unclear [17,19]. Analysis of HPV type interactions could help to improve clinical surveillance of women with cervical precancerous lesions or cervical cancer and predict disease progression in infected women.

The Cobas 4800 system has four kinds of probes with different reporter dyes. Dyes 1–4 target other hrHPVs, HPV16, and HPV18, and β-globin (control), respectively [21]. Linear Array HPV genotyping assay is a well-validated HPV testing method that generates individual qualitative results for the 14 types of hrHPVs [22–24]. A previous study has shown that, for detecting the minimum number of hrHPV types present (1, 2, or 3) (e.g., if positive for HPV18 and for the pool of other hrHPVs simultaneously, a “2” was assigned), the results obtained using the Cobas 4800 are in good agreement with Linear Array HPV genotyping assay which have proven its high sensitivity of detecting HPV co-infection [21]. In addition, Cobas 4800 system has also been used in some large population HPV screening studies and has shown a sensitive HPV detection effect compared to other HPV genotyping technologies [25–27].

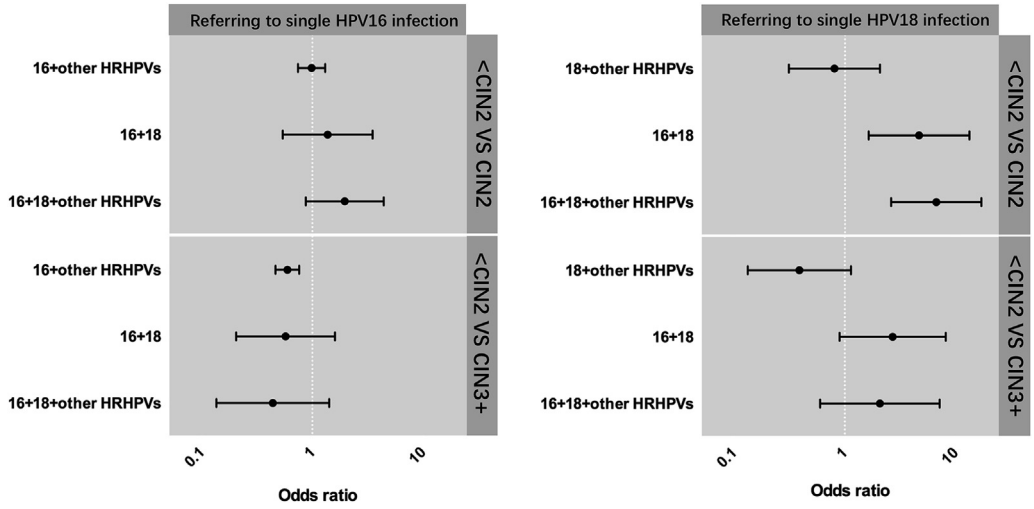


**Fig. 2.** Relative risks for CIN3+ and CIN2 versus < CIN2 in different HPV16/18 infection patterns. All statistical tests were two-sided.
Abbreviations: CIN, cervical intraepithelial neoplasia; other hrHPVs, high-risk HPV genotypes HPV31/33/35/39/45/51/52/56/58/59/66/68.

Therefore, we believe that the results obtained by using the Cobas 4800 for hrHPV coinfection are reliable.

In this study, the ratio of hrHPV positive women (7.85%) was similar but slightly higher than another large population study in which 6.1% of recruited women aged ≥30 years were infected with hrHPV [28]. Compared to CIN2, CIN3 is more reproducible, and is a well-defined precancerous lesion in histology while CIN2 is not [29–31]. Untreated CIN2 is less likely to develop cervical cancer especially among young women, however, the risk of untreated CIN3 progressing to cervical cancer is very high [32,33]. CIN3+ is also a reasonable agent for the risk of cervical cancer and has high consistency between independent review diagnoses by different pathologists [34,35]. Besides, BMJ has recently reported that hrHPV testing as a primary cervical cancer screening tool could significantly improve the detection rate of CIN3+ [36], and HPV16 has been proved to be the dominant HPV type in CIN3+ progression [14]. Therefore, in our primary classification, we defined CIN3+ as a pathological outcome and kept CIN2 as a separate histologic stage in order to clearly maintain the difference between CIN3+ and <CIN2. We identified five other significant HPV cofactors, which included education, post-coital bleeding, menopause or not, vaginal cleanliness, and family history of cancer. Interestingly, the ORs in Fig. 2 indicated that women with single HPV16 infection had a higher risk of CIN3+ than those who were positive for HPV16 + other hrHPVs (excluding HPV18). This trend was confirmed in the logistic regression models incorporating statistically significant HPV cofactors. Hence, a negative interaction effect was observed between HPV16 and other hrHPVs. When focusing on HPV18 infections, the distribution of histological diagnostics of single HPV18 infection and HPV18 + other hrHPVs (excluding HPV16) was not significantly different. However, women with HPV16 + 18 and HPV16 + 18+other hrHPVs positive were at a higher risk of CIN2 than single HPV18 infection (Fig. 2).

A previous study has found that HPV16 was the most predominant causal hrHPV genotype, and a similar phenomenon was also found in our study [37]. The risk of developing CIN2 in HPV18 positive patients with co-infected with HPV16 was higher than that in patients with a single HPV18 infection. When the risk of cervical carcinogenesis from single HPV16/18 infections to multiple HPV16/18 infections was compared in some studies based on cytological results [19,38], the cytological outcomes were not completely equivalent to the histological outcomes, which might have caused a difference in the results with us; or the number of participants positive for HPV16/18 may limited. Based on the histopathology results in our study, co-infections of HPV16 and other hrHPVs reduced the incidence of CIN3+, a phenomenon that has not been previously reported. The mechanism of this antagonism is unclear, and it is possible that hrHPV infections may interfere with the carcinogenic effects of HPV16.

Although a current opinion is that different HPV types independently cause cervical lesions in multiple infections [37,39], antagonistic interactions may still be observed between the different HPV types. A previous prospective study observed that a combination of high-risk and low-risk HPV infections reduces the risk of developing cervical cancer [17]. Additionally, previous serological-based studies found that the low-risk HPV6 may have an antagonistic effect on the HPV16-associated cervical carcinogenesis [40,41]. However, the synergy of some kinds of hrHPVs with HPV16 could not be excluded in our study because we were unable to distinguish the specific genotypes of other hrHPVs and the HPV16 + other hrHPVs group represented a mixture of different HPV16 infection patterns. In general, our study provided evidence of the possible negative interactions between different types of hrHPV.

A previous article has shown that multiple HPV infections may increase the risk of CIN2+, which seemed inconsistent with our conclusion [7]. The following are the possible reasons for this phenomenon: Firstly, the increased risk is primarily within the α9 species (HPV16/31/33/35/52/58) in this article. In our study, “other hrHPVs” included HPV31/33/35/39/45/51/52/56/58/59/66/68, therefore, in this study, the HPV interactions we studied were not limited to the α9 species. The patterns of hrHPV co-infection were not exactly the same in two articles. Our conclusion may not be precisely the opposite of what this study reported. In addition, for HPV18 infection, we also found that the combination of HPV16 infection can increase the risk of cervical lesions. Hence, the two studies have some similarities in terms of the increased risk of multiple hrHPV infections. We believe that the result of multiple infections is a complicated problem. There may be some differences between different HPV subtypes. Secondly, no overlap was observed between the age of the participants in the two studies. Our study focused on women ≥30 years. However, the age of the women in this study ranged from 18 to 25 years. As the author mentioned in this article, the patterns of HPV coinfections might be different in older women. Thirdly, our study recruited a higher

**Table 3**
Univariate analyses (adjusted for age in dichotomy) for HPV cofactors in <CIN2 compared with CIN2 and CIN3+.

| Characteristics | <CIN2 | CIN2 | CIN3+ | <CIN2 vs. CIN2 | <CIN2 vs. CIN3+ |
|---|---|---|---|---|---|
| | N | N | N | OR (95%CI) | OR (95%CI) |
| **Education** | | | | | |
| Primary & Junior | 3354 | 211 | 312 | 1.000 (ref) | 1.000 (ref) |
| ≥High School | 872 | 62 | 122 | 1.119 (0.830-1.508) | **1.587 (1.266 - 1.990)** |
| **Post-coital bleeding** | | | | | |
| No | 4179 | 271 | 422 | 1.000 (ref) | 1.000 (ref) |
| Yes | 47 | 2 | 12 | 0.652 (0.157 - 2.699) | **2.579 (1.356 - 4.903)** |
| **Menopause or not** | | | | | |
| No | 2049 | 151 | 262 | 1.000 (ref) | 1.000 (ref) |
| Yes | 2177 | 122 | 172 | **0.745 (0.572 - 0.971)** | **0.542 (0.439 - 0.670)** |
| **Menopause age** | | | | | |
| <44 | 1842 | 104 | 140 | 0.410 (0.165 - 1.016) | 1.157 (0.702 - 1.906) |
| 44-54 | 216 | 5 | 19 | 1.000 (ref) | 1.000 (ref) |
| >54 | 119 | 13 | 13 | **1.934 (1.055 - 3.544)** | 1.437 (0.790 - 2.612) |
| **Ever pregnant** | | | | | |
| No | 112 | 7 | 7 | 1.000 (ref) | 1.000 (ref) |
| Yes | 4114 | 266 | 427 | 1.035 (0.477 - 2.243) | 1.659 (0.768 - 3.584) |
| **Gravidity** | | | | | |
| 0 | 112 | 7 | 7 | 1.000 (ref) | 1.000 (ref) |
| 1-2 | 2003 | 129 | 195 | 1.029 (0.470 - 2.255) | 1.562 (0.718 - 3.401) |
| >=3 | 2111 | 137 | 232 | 1.040 (0.475 - 2.276) | 1.751 (0.806 - 3.802) |
| **Ever live births** | | | | | |
| No | 118 | 8 | 8 | 1.000 (ref) | 1.000 (ref) |
| Yes | 4108 | 265 | 426 | 0.953 (0.461 - 1.971) | 1.523 (0.739 - 3.138) |
| **Parity** | | | | | |
| 0 | 118 | 8 | 8 | 1.000 (ref) | 1.000 (ref) |
| 1–2 | 3425 | 226 | 365 | 0.973 (0.470 - 2.017) | 1.572 (0.762 - 3.244) |
| ≥3 | 683 | 39 | 61 | 0.849 (0.387 - 1.865) | 1.278 (0.596 - 2.741) |
| **Family history of cancer** | | | | | |
| No | 4167 | 271 | 420 | 1.000 (ref) | 1.000 (ref) |
| Yes | 59 | 2 | 14 | 0.523 (0.127 - 2.152) | **2.334 (1.292 - 4.217)** |
| **Vaginal cleanliness**[a] | | | | | |
| I/II | 2677 | 159 | 225 | 1.000 (ref) | 1.000 (ref) |
| III/IV | 1394 | 98 | 195 | 1.186 (0.915 - 1.538) | **1.656 (1.352 - 2.027)** |
| **Trichomonas infection**[a] | | | | | |
| No | 3980 | 250 | 409 | 1.000 (ref) | 1.000 (ref) |
| Yes | 126 | 9 | 14 | 1.135 (0.570 - 2.259) | 1.085 (0.619 - 1.903) |
| **Abnormal Leucorrhea** | | | | | |
| No | 3965 | 254 | 408 | 1.000 (ref) | 1.000 (ref) |
| Yes | 261 | 19 | 26 | 1.133 (0.699 - 1.836) | 0.978 (0.645 - 1.482) |
| **Cytology test (frequency)**[a] | | | | | |
| Never | 3343 | 226 | 332 | 1.000 (ref) | 1.000 (ref) |
| Nearest test <3y | 687 | 39 | 74 | 0.839 (0.591 - 1.190) | 1.089 (0.835 - 1.419) |
| Nearest test≥3y | 194 | 7 | 28 | 0.536 (0.249 - 1.153) | 1.437 (0.951 - 2.170) |
| **BMI**[a] | | | | | |
| 18.5–25 | 3022 | 201 | 312 | 1.000 (ref) | 1.000 (ref) |
| <18.5 | 156 | 12 | 19 | 1.152 (0.629 - 2.109) | 1.205 (0.737 - 1.969) |
| ≥25 | 981 | 56 | 95 | 0.861 (0.634 - 1.169) | 0.924 (0.726 - 1.176) |

Abbreviations: CIN, cervical intraepithelial neoplasia; BMI, body mass index; CI, confidence interval; OR, odds ratio.
[a] Do not add up to the whole number because of missing values.

number of participants. Increasing the number of participants may also improve the accuracy of the conclusions.

The mechanism of HPV co-infection maybe complicated, and it is considered to be unclear [17,19]. A previous report has shown that multiple HPV infections may impair the local immune system [42]. Moreover, cross-protection was observed between the different types of HPV. In multiple HPV infections [43], perhaps the sequence of different HPV infections causes the body's immune response to HPV to reduce the pathogenicity of subsequent HPV types. In addition, viral copies and the expression of E6 and E7 have also been proven to be associated with cervical carcinogenesis [43,44]. In particular, Schmitt et al. have reported that multiple HPV infections with high viral loads were associated with precancerous lesions [45]. Further research into these issues will help explain the mechanisms involved in HPV co-infection.

To the best of our knowledge, this population-based study has the largest number of HPV16/18 positive women with corresponding histologic results. Histologic results are generally acknowledged as the "gold standard" for cervical carcinogenesis diagnosis [46], thus using it as an endpoint could have more clinical significance. We collected all the questionnaires of the participants, which contained detailed epidemiological data. In our screening process, we used fresh specimens for hrHPV genotyping to prevent degradation of the HPV gene in paraffin-embedded specimens as in retrospective studies.

The present study had some limitations. Due to this study's cross-sectional design, the dynamic progression of cervical lesions in infected individuals could not be observed. The Cobas HPV test produces comparable results with other HPV tests [47], and it is an effective primary screening method for cervical cancer [48]. It has the ability to detect 14 kinds of hrHPVs, however, it can only clearly distinguish HPV16/18. Therefore, it is impossible to explore the influence of all types of lrHPV and specific hrHPV except for HPV16/18. There were no records of using hormone, birth control, smoking history, and number of sexual partners in the epidemiological data collected, and there may be recall bias in the included

**Table 4**
Multivariate logistic regression model (polytomous) of the HPV cofactors and HPV16/18 infection patterns (referring to single HPV16 infection).

| Characteristics | Referring to single HPV16 infection | |
|---|---|---|
| | <CIN2 vs. CIN2 | <CIN2 vs. CIN3+ |
| | OR (95%CI) | OR (95%CI) |
| **Age** | | |
| 30-49 | 1.000 (ref) | 1.000 (ref) |
| ≥50 | 1.115 (0.752 - 1.653) | **1.945 (1.374 - 2.754)** |
| **HPV16/18 infection patterns** | | |
| HPV16 only | 1.000 (ref) | 1.000 (ref) |
| HPV16 + other hrHPVs | 1.026 (0.765 - 1.375) | **0.636 (0.493 - 0.821)** |
| HPV16 + 18 | 1.387 (0.543 - 3.547) | 0.539 (0.191 - 1.519) |
| HPV16 + 18+other hrHPVs | 1.600 (0.623 - 4.107) | 0.345 (0.083 - 1.438) |
| HPV18 only | **0.288 (0.168–0.493)** | **0.200 (0.127–0.315)** |
| HPV18 + other hrHPVs | **0.254 (0.111–0.581)** | **0.085 (0.031–0.229)** |
| **Post-coital bleeding** | | |
| No | 1.000 (ref) | 1.000 (ref) |
| Yes | 0.670 (0.160 - 2.803) | **2.293 (1.162–4.525)** |
| **Education** | | |
| Primary & Junior | 1.000(ref) | 1.000(ref) |
| ≥High School | 1.110 (0.812 - 1.518) | **1.488 (1.173–1.888)** |
| **Menopause or not** | | |
| No | 1.000(ref) | 1.000(ref) |
| Yes | **0.733 (0.554–0.971)** | **0.590 (0.472–0.737)** |
| **Vaginal cleanliness** | | |
| I/II | 1.000(ref) | 1.000(ref) |
| III/IV | 1.222 (0.941 - 1.589) | **1.769 (1.437 - 2.176)** |
| **Family history of cancer** | | |
| No | 1.000(ref) | 1.000(ref) |
| Yes | 0.533 (0.129 2.209) | **2.386 (1.291–4.411)** |

Abbreviations: CIN, cervical intraepithelial neoplasia; HPV, human papillomavirus; other hrHPVs, high-risk HPV genotypes HPV31/33/35/39/45/51/52/56/58/59/66/68; CI, confidence interval; OR, odds ratio.

epidemiological data. For the further classification of our pathological outcomes, the previous study has reported that the conception of CIN2 may not be well-defined [30]. Immunohistochemical staining of p16 on the paraffin sections of the biopsy specimens could further classify CIN2 and improve the reliability and clinical value of the results [49]. In addition, the cytological results of HPV16/18-infected patients have not been obtained due to screening procedures; thus, it was challenging to validate whether similar conclusions can be obtained in cytology. Due to the fact that only some women infected with other hrHPVs underwent colposcopy and biopsy, and these individuals were selected based on cytologic results, not randomly, the pathological outcomes of these individuals were not incorporated into the data analysis. We did not have the cytological samples preserved in RNA later. Thus, it was difficult to assess the expression of E6/E7 in the cytological samples. Our findings may need further evaluation to investigate these potential mechanisms.

A growing number of studies have shown that primary hrHPV screening can be used as an alternative to cytology-based cervical cancer screening methods, which included both cytology co-testing and alone [18,48]. For primary hrHPV screening, HPV16/18 positivity can be used to identify women who are at higher risk of CIN3+ as HPV16 and HPV18 are more serious types than other non-HPV16/18 hrHPVs. Among the HPV16/18 positive women, whether the risk of cervical carcinogenesis could be classified based on co-infected with other hrHPVs or not is unclear. If our conclusion is correct, then the different HPV16/18 infection patterns may be classified into subgroups and more detailed interventions can be provided at an earlier time for groups who are at higher risk (such as the single HPV16 infection subgroup). However, because the mechanism of HPV coinfection is generally considered unclear [17,19], then the verification of our conclusion is based on more prospective studies. Our study may provide evidence of risk stratification in HPV16/18 positive women.

## 5. Conclusion

This study is one of the largest population-based studies in this area of research, and results showed that HPV16/18 co-infected with other hrHPVs is a common phenomenon. Moreover, we also observed that the single HPV16 infection are at a higher risk for the progression to CIN3+ than those with simultaneous infections of HPV16 and other hrHPVs (excluding HPV18). Thus, an antagonistic interaction may exist between the HPV16 and other hrHPVs. However, the findings of this study must be further assessed via prospective studies.

## Author Contributions

Hui Xing, Ping Yin, Jia Liu, Ping Wu designed and conceived the study; Huangguo Xiong and Ping Wu performed the statistical analysis and drafted the article; Mei Yang, Lin Li, Peng Wu, Cordelle Lazare, Canhui Cao, PeiPei Gao, Yifan Meng, Wenhua Zhi, Shitong Lin, Junbo Hu, Juncheng Wei, Ding Ma contributed to the quality control and data collection; Hui Xing, Ping Yin, Jia Liu made critical revisions to the manuscript. All authors revised and commented on the article and approved the final version before submission.

## Declaration of competing interest

The authors have declared there is no competing interest exists.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ygyno.2019.10.003.

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