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Association Between Common Vaginal Infections and Cervical Non–Human Papillomavirus (HPV) 16/18 Infection in HPV-Vaccinated Women

Shang-Ying Hu,[1,2,a] Sabrina H. Tsang,[2,a] Feng Chen,[1] Qin-Jing Pan,[3] Wen-Hua Zhang,[4] Ying Hong,[5] Joshua N. Sampson,[2] Allan Hildesheim,[2,©] Fang-Hui Zhao,[1,b,©] and Aimée R. Kreimer[2,b]

[1]Department of Cancer Epidemiology, National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China, [2]Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, USA, [3]Department of Pathology, National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China, [4]Department of Gynecologic Oncology, National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China, [5]Department of Obstetrics and Gynecology, Affiliated Drum Tower Hospital of Nanjing University Medical School, Nanjing, China

Background. How vaginal infections such as bacterial vaginosis, *Candida* spp, and *Trichomonas vaginalis* affect persistence of human papillomavirus (HPV) infection is not well established. Our study aimed to evaluate the association between common vaginal infections and cervical non-HPV16/18 infection, as risk factors associated with persistence of nonvaccine HPV types will become increasingly relevant in the setting of HPV vaccination.

Methods. We performed an analysis in 2039 AS04-HPV16/18–vaccinated women enrolled in a phase II/III trial in China, who were HPV DNA negative at month 0 and 6 and had at least 1 subsequent follow-up visit. Vaginal infections were detected in liquid-based cytology according to the diagnostic criteria of the Bethesda System. Associations between vaginal infections and incident and 6-month persistent non-HPV16/18 infections in the cervix were evaluated using generalized estimating equations, adjusting for the age at initial vaccination, as well as HPV types in the persistence analysis.

Results. Study visits with any vaginal infection had a statistically significant increased risk of incident non-HPV16/18 infection compared to those without vaginal infections (odds ratio [OR], 1.44 [95% confidence interval {CI}, 1.09–1.92]). However, vaginal infections were not associated with 6-month persistent non-HPV16/18 infection (OR, 1.02 [95% CI, .62–1.69]).

Conclusions. Our study suggests that common vaginal infections are not associated with persistence of non-HPV16/18 infection among HPV16/18-vaccinated women.

Keywords. human papillomavirus; bacterial vaginosis; *Candida* spp; *Trichomonas vaginalis*; vaccination.

Human papillomavirus (HPV) is one of the most common sexually transmitted infections (STIs) [1]. Persistent oncogenic HPV infection of the cervix may cause cervical intraepithelial neoplasia (CIN) and invasive cervical cancer [2]. Although most HPV infections are cleared within 2 years [3], differences in immune responses to HPV and other exogenous factors may increase the risk of HPV persistence and progression to cancer [1].

The role of vaginal infection (the most common types of which are bacterial vaginosis [BV], *Candida* spp, and *Trichomonas vaginalis* [TV]) has been investigated as a co-factor in cervical carcinogenesis [4–6]. The proposed mechanism supporting the association is that vaginal infections can induce substantial inflammatory responses that disrupt the integrity of the cervical epithelium, allowing for the penetrance and infection of HPV at the basement membrane, and can produce nonspecific antimicrobial oxidants, consequently causing DNA damage in the host [7]. Despite biological plausibility, existing epidemiological studies typically use cross-sectional or case-control study approaches and have shown inconsistent results regarding the association between vaginal infections and cervical HPV infection or CIN. To control for potential confounding, prospective studies that condition on HPV acquisition are required to define the effect of vaginal infections on subsequent HPV persistence, a precursor to HPV-driven cervical disease.

HPV16/18 vaccines have the potential to vastly reduce the incidence of HPV16/18 infections and related cervical cancers [8, 9]. Yet, among HPV16/18-vaccinated women, there is shifting concern about the burden of cervical cancer caused by nonvaccine HPV types that could eventually emerge after widespread HPV vaccination. Therefore, it is necessary to explore

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[a]S.-Y. H. and S. H. T. contributed equally to this work as first authors.

[b]F.-H. Z. and A. R. K. contributed equally to this work as last authors.

Correspondence: F.-H. Zhao, PhD, Department of Cancer Epidemiology, National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, 17 South Panjiayuan Lane, PO Box 2258, Beijing 100021, China (zhaofangh@cicams.ac.cn).

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the co-factors associated with persistent nonvaccine targeted HPV infections in the post–HPV vaccination era, as we hypothesize that other oncogenic HPV types may persist longer in the presence of contributing risk factors.

Here, we perform an analysis to assess the association between common vaginal infections and cervical non-HPV16/18 infection in women who had received the AS04-HPV16/18 vaccine and were HPV DNA negative at the time of vaccination, using data from a phase II/III double-blind, randomized clinical trial in China (NCT00779766).

METHODS

Study Design

The clinical trial was conducted in Jiangsu Province of China to assess the efficacy, immunogenicity, and safety of the AS04-HPV16/18 HPV vaccine (GlaxoSmithKline Biologicals, Belgium). Details of the study design and participant characteristics were described previously [10, 11]. This trial was approved by the ethics committees of the Center for Disease Control and Prevention of Jiangsu Province and the Cancer Foundation of China.

In brief, from October 2008 to February 2016, 6051 women aged 18–25 years were enrolled and followed up for 72 months. Sexually naive persons were not enrolled in the study due to cultural and ethical considerations. All participants provided written informed consent. Women were randomized to receive the HPV vaccine (n = 3026) or the aluminium hydroxide placebo control (n = 3025) at month 0, 1, and 6. Cervical samples were obtained every 6 months for HPV DNA testing. Cervical cytology was tested annually using the ThinPrep PapTest (Cytyc Corporation) and reported according to the Bethesda 2001 classification system by a senior cytologist in Cancer Hospital, Chinese Academy of Medical Sciences (CHCAMS). If the annual cytology test was missing or reported as unsatisfactory for evaluation, satisfactory but endocervical/transformation zone component absent, or abnormal (excluding atypical squamous cells of undetermined significance without oncogenic HPV infection), the woman received a cytology test at the next 6-month visit. Women with abnormal cytology were referred to colposcopy and received biopsy and treatment if necessary.

Assessment of Common Vaginal Infections and Cervical HPV Infection

Vaginal infections, including BV, *Candida* spp, and TV, were assessed at every study visit using liquid-based cytology (LBC) slides according to the criteria of the Bethesda System for Reporting Cervical Cytologic Diagnoses [12] and were diagnosed by the same senior cytologist in CHCAMS. The cytologist observed both the morphology of the microorganisms and specific changes of cervical cells induced by the microorganisms. BV was diagnosed in the presence of clue cells, along with the absence of *Lactobacillus*. *Candida* spp was diagnosed in the presence of budding yeast (3–7 μm) and/or pseudohyphae. Pseudohyphae can be quite long, spanning many cells, and are eosinophilic to gray brown on the Papanicolaou stain. Fragmented leukocyte nuclei and groups of squamous epithelial cells "speared" by pseudohyphae and held together in a rouleaux are often seen. The criteria for TV diagnosis were pear-shaped, oval, or round cyanophilic organism ranging in area from 15 to 30 μm^2 with pale, vesicular, and eccentrically located nucleus; eosinophilic cytoplasmic granules are often evident; flagella are sometimes observed; leptothrix may be seen in association with TV; and associated background changes include mature squamous cells with small perinuclear halos ("trich change") and 3-dimensional clusters of neutrophils ("polyballs").

A broad-spectrum polymerase chain reaction (PCR) assay, SPF10 DEIA-LiPA25 (version 1 based on licensed Innogenetics SPF10 technology; Labo Biomedical Products), and type-specific PCR for HPV16 and HPV18 DNA were used to test cervical samples for HPV DNA from 14 oncogenic HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) and 11 nononcogenic HPV types (6, 11, 34, 40, 42, 43, 44, 53, 54, 70, and 74). In this article, "non-HPV16/18 infection" referred to the presence of the other 12 oncogenic HPV types except HPV 16/18, and 11 nononcogenic HPV types.

Statistical Analysis

This analysis evaluated the association between common vaginal infections and both incidence and 6-month persistence of non-HPV16/18 infections in the cervix. First, we evaluated the relationship between vaginal infections (independent variable) and incident non-HPV16/18 infection (dependent variable). We considered the 10 766 postvaccination study visits from the 2039 women in the HPV-vaccinated arm who were HPV DNA-negative at month 0 and 6 (Figure 1). We compared the risk of incident HPV infection between visits with and without a vaginal infection. Specifically, we used generalized estimating equation (GEE) with a logit link, adjusting for age at the time of initial vaccination, to calculate the odds ratios (ORs) and the associated 95% confidence intervals (CIs) for having a cervical infection. Clusters were defined by woman. Second, we evaluated the relationship between vaginal infections and the 6-month persistence of an incident HPV infection. We considered the 595 visits (among 353 women) where a woman had an incident HPV infection and 1 study visit at least 6 months later; for any HPV type, we considered only the first incident HPV infection in a woman. We compared the risk of 6-month persistence of that HPV infection between visits with and without a vaginal infection. We again used a similar GEE to calculate ORs and 95% CIs, adjusting for age at the time of initial vaccination and HPV types. Statistical analyses were performed with SAS 9.2. All statistical tests were 2-sided, and a P value of $< .05$ was considered statistically significant.

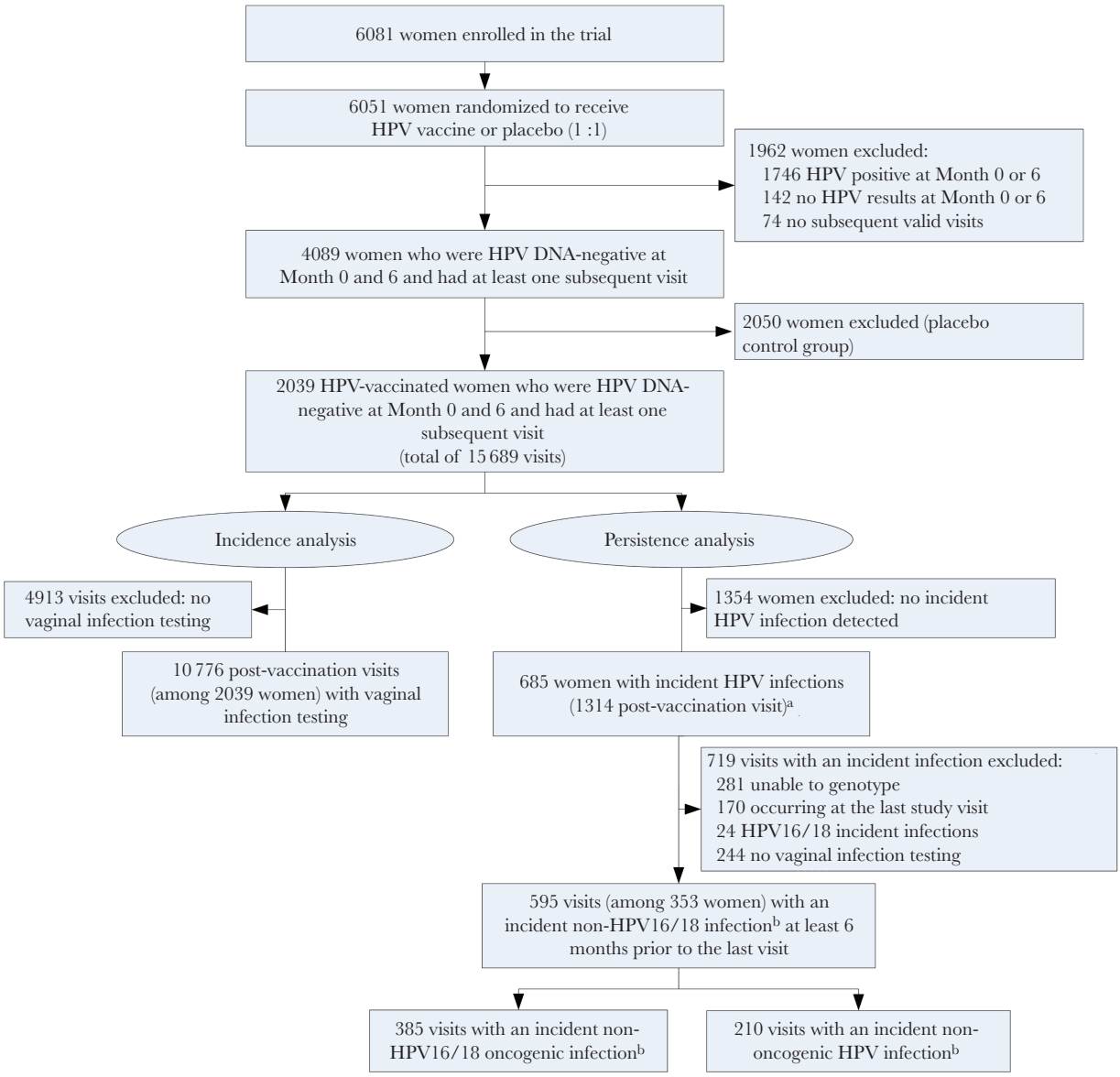

Figure 1. Selection of analytic cohorts. [a]Only the first incident infection for any given human papillomavirus (HPV) type was considered. [b]Non-HPV16/18 infection refers to the presence of the other 12 oncogenic HPV types (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) and 11 nononcogenic HPV types (6, 11, 34, 40, 42, 43, 44, 53, 54, 70, and 74).

RESULTS

In the 2039 HPV-vaccinated women who were HPV DNA-negative at month 0 and 6, and had at least 1 subsequent follow-up visit, the mean age at the time of initial vaccination was 23.0 ± 1.7 years. The mean follow-up time for this study was 65.0 ± 14.3 months. Of these women, 58 (2.8%) were diagnosed with TV, 264 (12.9%) with *Candida* spp, 244 (12.0%) with BV, and 508 (24.9%) with any vaginal infection in at least 1 subsequent visit. About 26% (537/2039) of the women had at least 1 incident non-HPV16/18 infection during follow-up, of which 79.3% (426/537) of these had incident oncogenic HPV infections and 50.7% (272/537) had incident nononcogenic infections.

The 2039 women had 10 776 postvaccination study visits in which vaginal infections were evaluated. The odds of an incident non-16/18 HPV infection were higher at visits with a vaginal infection, as compared to visits without a vaginal infection (18.3% vs 8.5%; OR, 1.44 [95% CI, 1.09–1.92]) (Table 1). Vaginal infections were assessed individually: ORs were 1.96 for TV (95% CI, .74–5.15), 1.22 for *Candida* spp (95% CI, .86–1.73), and 1.42 for BV (95% CI, .89–2.26). When stratifying our analysis by oncogenic and nononcogenic HPV infections, the significant increase in risk of incident nononcogenic HPV infections among visits with any vaginal infection remained (9.1% vs 3.1%; OR, 2.21 [95% CI, 1.50–3.23]), as well as with BV (10.4% vs 3.3%; OR, 2.26 [95% CI, 1.29–3.96]). The increase

Table 1. Association Between Common Vaginal Infection and Incident Non–Human Papillomavirus (HPV) 16/18 Cervical Infection in HPV-Vaccinated Women Who Were HPV DNA Negative at Vaccination

Vaginal Infection	No. of Visits	No. of Visits With an Incident HPV Infection (%)	Odds Ratio (95% CI)
Non-HPV16/18 infection[a]			
Trichomonas vaginalis			
Yes	97	26 (26.8)	1.96 (.74–5.15)
No	10 679	963 (9.0)	Ref
Candida spp			
Yes	315	44 (14.0)	1.22 (.86–1.73)
No	10 461	945 (9.0)	Ref
Bacterial vaginosis			
Yes	309	62 (20.1)	1.42 (.89–2.26)
No	10 467	927 (8.9)	Ref
Any vaginal infection			
Yes	718	131 (18.3)	1.44 (1.09–1.92)
No	10 058	858 (8.5)	Ref
Non-HPV16/18 oncogenic HPV infection[a]			
Trichomonas vaginalis			
Yes	97	22 (22.7)	2.10 (.82–5.38)
No	10 679	731 (6.9)	Ref
Candida spp			
Yes	315	34 (10.8)	1.21 (.80–1.82)
No	10 461	719 (6.9)	Ref
Bacterial vaginosis			
Yes	309	43 (13.9)	1.19 (.67–2.11)
No	10 467	710 (6.8)	Ref
Any vaginal infection			
Yes	718	98 (13.7)	1.33 (.95–1.85)
No	10 058	655 (6.5)	Ref
Nononcogenic HPV infection[a]			
Trichomonas vaginalis			
Yes	97	15 (15.5)	3.03 (.79–11.57)
No	10 679	363 (3.4)	Ref
Candida spp			
Yes	315	19 (6.0)	1.60 (.95–2.68)
No	10 461	359 (3.4)	Ref
Bacterial vaginosis			
Yes	309	32 (10.4)	2.26 (1.29–3.96)
No	10 467	346 (3.3)	Ref
Any vaginal infection			
Yes	718	65 (9.1)	2.21 (1.50–3.23)
No	10 058	313 (3.1)	Ref

Abbreviations: CI, confidence interval; HPV, human papillomavirus; Ref, reference group.

[a]Non-HPV16/18 infection refers to the presence of the other 12 oncogenic HPV types (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) and 11 nononcogenic HPV types (6, 11, 34, 40, 42, 43, 44, 53, 54, 70, and 74).

in risk associated with vaginal infections and incident non-HPV16/18 oncogenic infections was not statistically significant.

In the persistence analysis where we considered only visits with an incident non-HPV16/18 infection, the association between vaginal infections and 6-month persistence of a non-HPV16/18 infection was null (OR, 1.02 [95% CI, .62–1.69]) (Table 2). For individual vaginal infection, the estimated OR ranged from 0.80 to 1.65 without any statistically significant associations. The analysis by oncogenic and nononcogenic HPV infections also did not show any statistically significant associations. The point estimates of the ORs were elevated for TV and 6-month persistence of non-HPV16/18 oncogenic infections (OR, 2.74 [95% CI, .89–8.41]), and for *Candida* spp and 6-month persistence of nononcogenic HPV infections (OR, 2.26 [95% CI, .61–8.31]), but their CIs were wide and included unity.

DISCUSSION

To our knowledge, this is the first analysis evaluating common vaginal infections (ie, BV, *Candida* spp, and TV) as a co-factor for non-HPV16/18 infection in an HPV16/18-vaccinated population. Research on the risk factors for persistent nonvaccine type HPV infections is increasingly relevant for the prevention of related cervical lesions in the postvaccination era. Using persistent HPV infection as an endpoint, our study did not show a statistically significant association with vaginal infections, despite women with these vaginal infections being more likely to have a concurrent non-HPV16/18 infection in the incidence analysis. The current report focuses on the novel research question in vaccinated women. We had also performed the same analysis in the unvaccinated arm, as well as an analysis combining the vaccinated and unvaccinated arms, and obtained similar findings (data not shown).

Most of the existing studies support a positive association between vaginal infection and HPV acquisition or concurrent HPV infection [4, 13–16], but a few studies showed no association [17, 18]. Furthermore, it is reported that vaginal infection was also correlated with specific HPV types. A study in rural Tanzania showed TV was significantly associated with oncogenic HPV infection (OR, 4.1), specifically HPV16 [19]. Another study in Kenya displayed significant associations between BV and HPV type 58, and between *Candida* spp and HPV types 16 and 53 [20]. In our analytical cohort of HPV-vaccinated women, visits with any vaginal infection were more likely to coincide with the detection of an incident non-HPV16/18 infection, particularly for incident nononcogenic HPV infection, where the risk was doubled. Of course, the correlation could be explained by a shared route of sexual transmission for vaginal infections and HPV. Using the same cohort, we also compared the incidence of non-HPV16/18 infections among those with or without vaginal infections at baseline. An increased, but not statistically significant, trend for incident non-HPV16/18 infection was indicated in those with vaginal infections at baseline compared to those without (TV: 33.3% vs 26.3%; *Candida*: 28.8% vs 26.3%; BV: 35.2% vs 26.1%; any vaginal infection: 32.3% vs 26.0%; all P values > .05).

Several studies reported an increased risk of cervical precancerous or cancerous lesions in women infected with BV [6, 21, 22] or TV [23]. However, none of these studies adjusted for HPV infection status or sexual behavior. When conditioning on HPV-positive women, or adjusting for HPV status or sexual

Table 2. Association Between Common Vaginal Infection and Persistent Non–Human Papillomavirus (HPV) 16/18 Cervical Infection in HPV-Vaccinated Women With Incident Non-HPV16/18 Infections

Vaginal Infection	No. of Visits With an Incident HPV Infection	No. of 6-mo Persistent HPV Infection (%)	Odds Ratio (95% CI)
Non-HPV16/18 infection[a]			
Trichomonas vaginalis			
Yes	16	9 (56.3)	1.65 (.75–3.64)
No	579	249 (43.0)	Ref
Candida spp			
Yes	23	9 (39.1)	1.09 (.42–2.81)
No	572	249 (43.5)	Ref
Bacterial vaginosis			
Yes	47	17 (36.2)	0.80 (.40–1.60)
No	548	241 (44.0)	Ref
Any vaginal infection			
Yes	85	35 (41.2)	1.02 (.62–1.69)
No	510	223 (43.7)	Ref
Non-HPV16/18 oncogenic HPV infection[a]			
Trichomonas vaginalis			
Yes	10	7 (70.0)	2.74 (.89–8.41)
No	375	181 (48.3)	Ref
Candida spp			
Yes	11	4 (36.4)	0.55 (.17–1.81)
No	374	184 (49.2)	Ref
Bacterial vaginosis			
Yes	27	10 (37.0)	0.60 (.27–1.37)
No	358	178 (49.7)	Ref
Any vaginal infection			
Yes	48	21 (43.8)	0.79 (.42–1.49)
No	337	167 (49.6)	Ref
Nononcogenic HPV infection[a]			
Trichomonas vaginalis			
Yes	6	2 (33.3)	0.79 (.24–2.55)
No	204	68 (33.3)	Ref
Candida spp			
Yes	12	5 (41.7)	2.26 (.61–8.31)
No	198	65 (32.8)	Ref
Bacterial vaginosis			
Yes	20	7 (35.0)	1.11 (.40–3.02)
No	190	63 (33.2)	Ref
Any vaginal infection			
Yes	37	14 (37.8)	1.44 (.68–3.08)
No	173	56 (32.4)	Ref

Abbreviations: CI, confidence interval; HPV, human papillomavirus; Ref, reference group.

[a] Non-HPV16/18 infection refers to the presence of the other 12 oncogenic HPV types (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) and 11 nononcogenic HPV types (6, 11, 34, 40, 42, 43, 44, 53, 54, 70, and 74).

behavior, most studies did not demonstrate that vaginal infections had a role in cervical precancerous lesions [4, 5, 18] or in HPV persistence [16], which is consistent with our findings in this study. In contrast, the study by Watts et al suggested that TV infection may shorten the duration of HPV infection [16], further documenting the inconsistency in this field and highlighting the importance of considering methodologic differences. Recent studies have demonstrated the higher diversity of vaginal microbiota and decreased relative abundance of *Lactobacillus* spp, which is frequently associated with BV, in HPV-positive women [24, 25]. However, their ability to derive a causal link is limited by the cross-sectional nature of most studies. Large, adequately powered, longitudinal studies are needed to confirm whether an association between the vaginal microbiota and persistent HPV exists. Meanwhile, many studies reported that *Chlamydia trachomatis* (CT) was another infectious co-factor associated with HPV acquisition and cervical cancer [26–29], but CT was not assessed in our analyses because it was not tested in our trial. Published CT studies did not show consistent results. Especially when controlling for HPV DNA status, some studies demonstrated no association between CT and CIN2 and/or CIN3+ [30–34]. This suggests that careful design/adjustment, ruling out confounding by HPV status or by an increased susceptibility to HPV infection, is crucial when investigating the role of STIs (other than HPV) in HPV-driven cervical carcinogenesis because of the strong confounding effects such as smoking and sexual behaviors between HPV infections with other STIs. Additionally, despite nonsignificant associations between TV and 6-month persistence of non-HPV16/18 oncogenic infections in our study, as well as *Candida* spp and 6-month persistence of nononcogenic HPV infections, additional data are needed for further evaluation.

The major strength of our analysis is the prospective study design, which allowed for the evaluation of persistent HPV infections, a prerequisite for progression to high-grade cervical precancerous or cancerous lesions. Most of the existing reports are cross-sectional or case-control studies and could not assess the association between vaginal infections and HPV persistence. Our prospective HPV vaccine trial, which has a long follow-up time (~6 years), with regular and frequent clinic visits, and well-established HPV genotyping assessment, offers an invaluable opportunity to address the impact of vaginal infections on non-HPV16/18 cervical infections.

Our study had limitations. First, since this study was a secondary analysis of the initial clinical trial, it was not powered to detect the role of vaginal infections as a co-factor of persistent non-HPV16/18 infection. Thus, larger cohort studies are still needed. Second, we did not adjust for sexual behavior and other risk factors because these covariates were not collected during the trial. Nevertheless, we minimized potential confounding by conditioning on visits with incident HPV infections. Third, vaginal infections were detected by LBC instead of the ideal gold standards because they were not the main endpoints in the clinical trial. Currently, Gram stain for BV, culture for *Candida* spp, and nucleic acid amplification (PCR) testing for TV are considered the diagnostic standard [35]. Nonetheless, cervical cytology has relatively high specificity for most of the microorganisms [12]. Compared to Gram stain, Amsel criteria, the traditional method for BV diagnosis, is highly accurate (both

sensitivity and specificity: 91%) [36]; the accuracy of Pap smears for BV diagnosis was comparable to Amsel criteria, with a sensitivity of 85% and a specificity of 92% [37]. Also, LBC for TV diagnosis was sensitive (96.2%) and specific (99.5%) compared to PCR testing [38]. There is limited literature on the accuracy of LBC compared with culture for *Candida* diagnosis. It is worth mentioning that high diagnostic accuracy for cervical cytology was provided by an expert cytologist in CHCAMS. Last, we could not distinguish between symptomatic and asymptomatic vaginal infections to evaluate the impact of the severity of inflammation induced by vaginal infection on non-HPV16/18 acquisition and persistence. Moreover, no information on vaginitis treatment and other STIs was recorded. However, the women in this study were recruited from the general population, and the risk of being infected with other STIs was relatively low, as inferred by the prevalence of oncogenic HPV among these women (15.3% [10], comparable to the average of 17.7% [39] across the mainland of China).

Overall, our prospective analysis did not support the association between common vaginal infections and persistent non-HPV16/18 infection. Non-HPV16/18 incidence with coinfection of TV had a statistically nonsignificant elevated risk of persistence that warrants further investigation. More prospective investigation into the interaction between vaginal coinfections, inflammation, and the risk of non-HPV16/18 persistence and its associated carcinogenesis is needed.

Notes

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Author contributions. S.-Y. H., A. R. K., A. H., and F.-H. Z. were the study conception initiators and designed the study. F.-H. Z., S.-Y. H., F. C., Q.-J. P., W.-H. Z., and Y. H. were involved in data collection and assembly. S.-Y. H., S. H. T., and A. R. K. wrote the manuscript. S.-Y. H. and J. N. S. did the data analysis. S.-Y. H., S. H. T., J. N. S., A. H., and A. R. K. participated in data interpretation. F.-H. Z. and Q.-J. P. provided constructive comments and revisions on the manuscript. All authors have reviewed and approved the final draft.

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Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. de Sanjosé S, Brotons M, Pavón MA. The natural history of human papillomavirus infection. Best Pract Res Clin Obstet Gynaecol **2018**; 47:2–13.
2. Marks MA, Castle PE, Schiffman M, Gravitt PE. Evaluation of any or type-specific persistence of high-risk human papillomavirus for detecting cervical precancer. J Clin Microbiol **2012**; 50:300–6.
3. Winer RL, Hughes JP, Feng Q, et al. Early natural history of incident, type-specific human papillomavirus infections in newly sexually active young women. Cancer Epidemiol Biomarkers Prev **2011**; 20:699–707.
4. Feng R-M, Wang MZ, Smith JS, et al. Risk of high-risk human papillomavirus infection and cervical precancerous lesions with past or current trichomonas infection: a pooled analysis of 25 054 women in rural China. J Clin Virol **2018**; 99–100:84–90.
5. Ghosh I, Muwonge R, Mittal S, et al. Association between high risk human papillomavirus infection and co-infection with *Candida* spp. and *Trichomonas vaginalis* in women with cervical premalignant and malignant lesions. J Clin Virol **2017**; 87:43–8.
6. Gillet E, Meys JF, Verstraelen H, et al. Association between bacterial vaginosis and cervical intraepithelial neoplasia: systematic review and meta-analysis. PLoS One **2012**; 7:e45201.
7. Ames BN, Gold LS, Willett WC. The causes and prevention of cancer. Proc Natl Acad Sci U S A **1995**; 92:5258–65.
8. Arbyn M, Xu L, Simoens C, Martin-Hirsch PP. Prophylactic vaccination against human papillomaviruses to prevent cervical cancer and its precursors. Cochrane Database System Rev **2018**; 5:CD009069.
9. Kreimer AR, Sampson JN, Porras C, et al. Evaluation of durability of a single-dose of the bivalent HPV vaccine: the CVT Trial [manuscript published online ahead of print 24 February 2020]. J Natl Cancer Inst **2020**. doi:10.1093/jnci/djaa011.
10. Zhao FH, Zhu FC, Chen W, et al; HPV-039 Study Group. Baseline prevalence and type distribution of human papillomavirus in healthy Chinese women aged 18-25 years enrolled in a clinical trial. Int J Cancer **2014**; 135:2604–11.
11. Zhu FC, Hu SY, Hong Y, et al. Efficacy, immunogenicity and safety of the AS04-HPV-16/18 vaccine in Chinese women aged 18-25 years: End-of-study results from a phase II/III, randomised, controlled trial. Cancer Med **2019**; 8:6195–211.
12. Nayar R, Wilbur DC, eds. The Bethesda system for reporting cervical cytology. Definitions, criteria, and explanatory notes. 3rd ed. New York, NY: Springer International Publishing, **2015**.
13. Depuydt CE, Leuridan E, Van Damme P, Bogers J, Vereecken AJ, Donders GG. Epidemiology of *Trichomonas*

vaginalis and human papillomavirus infection detected by real-time PCR in Flanders. Gynecol Obstet Invest **2010**; 70:273–80.
14. Donders GG, Depuydt CE, Bogers JP, Vereecken AJ. Association of *Trichomonas vaginalis* and cytological abnormalities of the cervix in low risk women. PLoS One **2013**; 8:e86266.
15. Zhang D, Li T, Chen L, Zhang X, Zhao G, Liu Z. Epidemiological investigation of the relationship between common lower genital tract infections and high-risk human papillomavirus infections among women in Beijing, China. PLoS One **2017**; 12:e0178033.
16. Watts DH, Fazzari M, Fazarri M, et al. Effects of bacterial vaginosis and other genital infections on the natural history of human papillomavirus infection in HIV-1-infected and high-risk HIV-1-uninfected women. J Infect Dis **2005**; 191:1129–39.
17. Verteramo R, Pierangeli A, Mancini E, et al. Human papillomaviruses and genital co-infections in gynaecological outpatients. BMC Infect Dis **2009**; 9:16.
18. Castle PE, Hillier SL, Rabe LK, et al. An association of cervical inflammation with high-grade cervical neoplasia in women infected with oncogenic human papillomavirus (HPV). Cancer Epidemiol Biomarkers Prev **2001**; 10:1021–7.
19. Lazenby GB, Taylor PT, Badman BS, et al. An association between *Trichomonas vaginalis* and high-risk human papillomavirus in rural Tanzanian women undergoing cervical cancer screening. Clin Ther **2014**; 36:38–45.
20. Menon S, Broeck DV, Rossi R, Ogbe E, Harmon S, Mabeya H. Associations between vaginal infections and potential high-risk and high-risk human papillomavirus genotypes in female sex workers in western Kenya. Clin Ther **2016**; 38:2567–77.
21. Engberts MK, Verbruggen BS, Boon ME, van Haaften M, Heintz AP. *Candida* and dysbacteriosis: a cytologic, population-based study of 100 605 asymptomatic women concerning cervical carcinogenesis. Cancer **2007**; 111:269–74.
22. Sodhani P, Gupta S, Gupta R, Mehrotra R. Bacterial vaginosis and cervical intraepithelial neoplasia: is there an association or is co-existence incidental? Asian Pac J Cancer Prev **2017**; 18:1289–92.
23. Viikki M, Pukkala E, Nieminen P, Hakama M. Gynaecological infections as risk determinants of subsequent cervical neoplasia. Acta Oncol **2000**; 39:71–5.
24. Mitra A, MacIntyre DA, Marchesi JR, Lee YS, Bennett PR, Kyrgiou M. The vaginal microbiota, human papillomavirus infection and cervical intraepithelial neoplasia: what do we know and where are we going next? Microbiome **2016**; 4:58.
25. Di Paola M, Sani C, Clemente AM, et al. Characterization of cervico-vaginal microbiota in women developing persistent high-risk human papillomavirus infection. Sci Rep **2017**; 7:10200.
26. Smith JS, Bosetti C, Muñoz N, et al; IARC Multicentric Case-Control Study. Chlamydia trachomatis and invasive cervical cancer: a pooled analysis of the IARC multicentric case-control study. Int J Cancer **2004**; 111:431–9.
27. Silins I, Ryd W, Strand A, et al. *Chlamydia trachomatis* infection and persistence of human papillomavirus. Int J Cancer **2005**; 116:110–5.
28. Samoff E, Koumans EH, Markowitz LE, et al. Association of *Chlamydia trachomatis* with persistence of high-risk types of human papillomavirus in a cohort of female adolescents. Am J Epidemiol **2005**; 162:668–75.
29. Naldini G, Grisci C, Chiavarini M, Fabiani R. Association between human papillomavirus and *Chlamydia trachomatis* infection risk in women: a systematic review and meta-analysis. Int J Public Health **2019**; 64:943–55.
30. Miller WC, Ko EM. Does chlamydial infection increase the risk of cervical dysplasia? Sex Transm Infect **2011**; 87:366–7.
31. Castle PE, Escoffery C, Schachter J, et al. *Chlamydia trachomatis*, herpes simplex virus 2, and human T-cell lymphotrophic virus type 1 are not associated with grade of cervical neoplasia in Jamaican colposcopy patients. Sex Transm Dis **2003**; 30:575–80.
32. Safaeian M, Quint K, Schiffman M, et al. *Chlamydia trachomatis* and risk of prevalent and incident cervical premalignancy in a population-based cohort. J Natl Cancer Inst **2010**; 102:1794–804.
33. Jensen KE, Thomsen LT, Schmiedel S, et al. *Chlamydia trachomatis* and risk of cervical intraepithelial neoplasia grade 3 or worse in women with persistent human papillomavirus infection: a cohort study. Sex Transm Infect **2014**; 90:550–5.
34. Lehtinen M, Ault KA, Lyytikainen E, et al; FUTURE I and II Study Group. *Chlamydia trachomatis* infection and risk of cervical intraepithelial neoplasia. Sex Transm Infect **2011**; 87:372–6.
35. Paladine HL, Desai UA. Vaginitis: diagnosis and treatment. Am Fam Physician **2018**; 97:321–9.
36. Mohammadzadeh F, Dolatian M, Jorjani M, Alavi Majd H. Diagnostic value of Amsel's clinical criteria for diagnosis of bacterial vaginosis. Glob J Health Sci **2014**; 7:8–14.
37. Eriksson K, Forsum U, Bjørnerem A, Platz-Christensen JJ, Larsson PG. Validation of the use of Pap-stained vaginal smears for diagnosis of bacterial vaginosis. APMIS **2007**; 115:809–13.
38. Aslan DL, Gulbahce HE, Stelow EB, et al. The diagnosis of *Trichomonas vaginalis* in liquid-based Pap tests: correlation with PCR. Diagn Cytopathol **2005**; 32:341–4.
39. Liao G, Jiang X, She B, et al. Multi-infection patterns and co-infection preference of 27 human papillomavirus types among 137 943 gynecological outpatients across China. Front Oncol **2020**; 10:449.