

## RESEARCH ARTICLE

# Associations of sexually transmitted infections and bacterial vaginosis with abnormal cervical cytology: A cross-sectional survey with 9090 community women in China

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## Abstract

### Background

Although it is well acknowledged that persistent infection with high-risk human papillomavirus types in genital sites plays a crucial role in the development of squamous cell cervical carcinoma, there is no unanimous consensus on the association between non-HPV sexually transmitted infections and abnormal cervical cytology.

### Methods

In the present study, we evaluated cervical cytology status, sexually transmitted infections and bacterial vaginosis status, and collected social-demographic information among recruited participants to explore the association of STIs and bacterial vaginosis with abnormal cervical cytology.

### Results

9,090 women's specimens were successfully tested, with a total of 8,733 (96.1%) women had normal cytology and 357 (3.9%) women exhibited abnormal cytology. The prevalence of HPV, *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and bacterial vaginosis was significantly higher in the  $\geq$ ASC-US group than the NILM group ( $P < 0.05$ ). Women with *Neisseria gonorrhoeae* infection (AOR = 5.30, 95% CIs = 1.30–21.51,  $P = 0.020$ ) or bacterial vaginosis (AOR = 1.94, 95% CIs = 1.08–3.47,  $P = 0.026$ ) exhibited an increased risk of abnormal cervical cytology after adjusted for carcinogenic HPV-positive status.

**Abbreviations:** STIs, Sexually transmitted infections; HPV, human papillomavirus; SCC, Squamous cell cervical carcinoma; CIN, Cervical intraepithelial neoplasia; SIL, Squamous intraepithelial lesion; ASC-US, Atypical squamous cells of undetermined significance; HSIL, high-grade squamous intraepithelial lesion; NILM, Negative for intraepithelial lesion or malignancy; AGC, Atypical glandular cells; CIs, confidence intervals; OR, Odds ratios; AOR, Adjusted odds ratios..

## Conclusions

Our results demonstrated that *Neisseria gonorrhoeae* infection in genital sites and/or bacterial vaginosis may independently increase the risk for cervical cytology abnormalities after adjusted for carcinogenic HPV-positive status. Besides, these results improved our understanding of the etiology of abnormal cervical cytology and may be useful for the management of women with ASC-US cytology.

## Background

Based on epidemiological and laboratory evidence, persistent infection with high-risk human papillomavirus (HPV) types in genital sites plays a crucial role in the development of squamous cell carcinoma (SCC) of the cervix [1, 2]. After the initial detection of high-risk HPV infection, the development of SCC of the cervix usually spanned decades and underwent a lengthy stage of squamous intraepithelial lesion (SIL) [2, 3]. Furthermore, most HPV infections are transient and only a small proportion of high-risk HPV infections persists and leads to high-grade squamous intraepithelial lesions or SCC [3], which suggests that other factors may facilitate the occurrence of SIL during this long transition period.

Previous studies reported that there are many other risk factors for SCC including long term use of oral contraceptives [4], high parity [5], cigarette smoking [6], co-infection with the human immunodeficiency virus [7], and sexually transmitted infections (STIs), such as *Chlamydia trachomatis* [8], *Neisseria gonorrhoeae* [9], herpes simplex virus type 2 [10, 11], *Trichomonas vaginalis* [12], *Mycoplasma* [13], *Ureaplasma* species [14, 15]. However, at present, there is no unanimous consensus on the impact of non-HPV STIs on abnormal cervical cytology. For example, as the most prevalent STI worldwide, *Chlamydia trachomatis* infection is considered as a risk factor for abnormal cervical cytology [16] or cervical intraepithelial neoplasia 2 (CIN-2) [17], but, Safaeian et al. [18] found no positive associations between *Chlamydia trachomatis* infection and cervical pre-malignancy after controlled for carcinogenic HPV-positive status.

Bacterial vaginosis relates to a remarkable shift in the vaginal microbiota to a dysbiotic state, marked by a diverseness of microorganism and increased loads of aerotolerant and strict anaerobes, including *Gardnerella vaginalis*, *Mobiluncus* and *Aptopobium vaginae*, and other fastidious bacteria such as *Megasphaera*, *Sneathia*, and *Clostridiales spp* [19]. Previous studies revealed that bacterial vaginosis is not only associated with reproductive and obstetric sequelae [20], but also with cervical pre-cancerous lesions [21]. Nevertheless, more studies are needed to verify the association and consolidate that evidence [17].

Hence, we investigated the association of STIs and bacterial vaginosis with abnormal cervical cytology among community women, which would improve our knowledge about the etiology of SCC and may be useful for disease control and prevention.

## Methods

### Study procedure and specimen collection

Our previous study [22] has described the study area and sample source in detail. From March to August 2017, we recruited participants from 9,249 women who met eligibility criteria and provided informed consent in our previous study [22], and all of the women signed an informed consent to this study. Women who met any of the following exclusion criteria were

not enrolled: pregnancy, without a history of sexual activity, sexual intercourse three days ago, menstrual period, previous hysterectomy, vaginal bleeding, vaginal douching or using a vaginal suppository, currently suffering from gynecological inflammation. The inclusion criteria were the same as inclusion criteria in our previous study [22]: being a female resident, aged 20–60 years and living locally in Shenzhen city Nanshan District during the past 3 months. All participants signed informed consent and were interviewed using a structured questionnaire to collect socio-demographic and clinical information before enrollment. All participants voluntarily agreed to provide a self-administrated 3–5 mL first-catch urine specimen (*Chlamydia trachomatis* and *Neisseria gonorrhoeae* tests), a cervical swab (HPV tests), two vaginal swabs (gynecological examinations), and an exfoliated cervical cells specimen (liquid-based cervical cytology test).

After allocating a unique identification number to each participant, two research nurses were assigned to check the integrity of questionnaire information and instruct participants in urine specimen collection, and guide participants to a specialized room for specimen collection. After collecting participant's specimens, laboratory testing procedures were started immediately. Laboratory staffs were blinded to the clinical findings.

### ***Chlamydia trachomatis* and *Neisseria gonorrhoeae* DNA test**

The method and procedures of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* DNA tests were described in our previous study [22].

### **HPV DNA test**

A skilled gynecologist collected the cervical swab for HPV tests. Swabs were taken and placed into a tube containing 3 mL cell preservation medium, and were stored at -20°C until testing. Fourteen high-risk HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68) and two low-risk HPV types (6, 11) in all cervical swabs were detected by using the PCR-based MALDI-TOF-MS assays [23, 24] in the Center of BGI Health clinical laboratory (BGI, Shenzhen, China). BGI Shenzhen designed the MALDI-TOF-MS-based HPV multiplex assay and HPV genotyping assay with excellent specificity [24]. China Food and Drug Administration approved the performance of the PCR-based multiplex genotyping and sequencing assays.

### **Gynecological examination**

Vaginal secretions swab specimens were collected by two skilled gynecologists and were rolled on to a glass slide for Gram staining immediately. Vaginal cleanliness, detection of hyphae and spores of *Candidiasis* and clue cells were confirmed by Gram staining of vaginal secretions. *Trichomonas Vaginalis* was diagnosed by microscopic examination of wet mounts immediately once the vaginal secretions swab collected. Amine test, pH of vaginal secretions and leukocytes were further confirmed within fifteen minutes. The diagnosis of bacterial vaginosis was based on Amsel's criteria [25], which was widely adopted to the clinical diagnosis of bacterial vaginosis. A positive diagnosis of bacterial vaginosis was made once three of the four following signs are present: the presence of clue cells, an adherent and homogenous grayish-white vaginal discharge, a vaginal pH exceeding a value of 4.5, a fishy or amine odor after the addition of a 10% potassium hydroxide solution.

### **Liquid-based cytology test**

Exfoliated cervical cells specimens were collected by two gynecologists with conical cytobrush and were placed into a tube with 4 mL preservation solution and were temporarily stored at

4°C condition until the required test. Cervical cytology samples were diagnosed by using liquid-based cytology technique (TriPath Imaging Inc., Burlington, USA) according to the manufacturer's instruction and were doubled-checked by cytotechnologists without information on STIs test results. The 2001 Bethesda system [26] was employed to classify the reported cytological results as following: negative for intraepithelial lesion or malignancy (NILM); Epithelial cell abnormalities including atypical squamous cells of undetermined significance (ASC-US), atypical squamous cells that cannot exclude HSIL (ASC-H), low-grade squamous intraepithelial lesion (LSIL), high-grade squamous intraepithelial lesion (HSIL), and atypical glandular cells (AGC). All abnormal epithelial cell specimens, as well as a randomly selected sample of normal cytology, were referred for a colposcopic examination and, if necessary, biopsy.

### Statistical analysis

Anonymous data collection and statistical analysis were performed by Microsoft Excel 2016 and R-statistics software (version 3.4.1) respectively. Abnormal cytology group ( $\geq$ ASC-US group) was defined as women who had a diagnosis of the following cytology findings: ASC-US, ASC-H, LSIL, HSIL or AGC. Variables were evaluated for comparisons between normal cytology (only NILM finding) group and  $\geq$ ASC-US group (include all abnormal cytological findings) or ASC-US group (only ASC-US finding) or LSIL+HSIL group (only LSIL finding and only HSIL finding) for analytical calculations. Prevalence of STIs and bacterial vaginosis were presented as positive rate and corresponding 95% confidential intervals (95% CIs). Chi-square test ( $\chi^2$ ), or two-sided Fisher exact test for 2x2 contingency table was used to evaluate the statistical significance of rates of variables between different groups. A univariate analysis was employed to estimate the associations of variables with different cytological findings. Variables with a significance level of  $p < 0.2$  were included in the multivariate logistic regression model adjusted by potential confounders. Crude odds ratios (OR), Adjusted odds ratio (AOR) and corresponding 95% CIs were calculated. A  $p < 0.05$  was considered significant. All statistical analyses were performed using SPSS 19.0 statistical software (SPSS Inc. Chicago, IL) and R-statistics 3.4.3 software.

### Ethics approval and consent to participate

The Ethical Review Committee of the Shenzhen Nanshan Center for Chronic Disease Control reviewed and approved the study (Approval No. LL20170017). A written informed consent was obtained from all participants. All experiments and procedures were performed in accordance with relevant guidelines and regulations of the People's Republic of China. Participants who tested positive for STIs or abnormal cervical cytology were contacted privately by a nurse for further treatment and other interventions.

## Results

### Overall prevalence

Out of the 9,249 women who met eligibility criteria and signed an informed consent, 156 (1.69%) women were excluded because of they met exclusion criteria, 9,093 (98.3%) women completed the questionnaire interview and provided all specimens, 3 urine specimens were invalid for *Chlamydia trachomatis* DNA and *Neisseria gonorrhoeae* DNA and failed to re-sample. 9,090 (98.3%) women's specimens were successfully tested, a total of 8,733 (96.1%) women had normal cytology (NILM) and 357 (3.9%) women exhibited abnormal cytology: 181 ASC-US (2.0%), 16 ASC-H (0.2%), 115 LSIL (1.3%), 40 HSIL (0.4%), 5 AGC (0.06%). High-risk HPV infection (6.77%, 95% CIs: 6.25%-7.29%) had the highest prevalence among detected

STIs. The prevalence of bacterial vaginosis and *Trichomonas vaginalis* was 2.27% (95% CIs: 1.96%-2.58%) and 0.35% (95% CIs: 0.23%-0.47%), respectively (Table 1).

### Analysis of prevalent HPV genotype and cytology status

As shown in Table 2, HPV-52 (1.8%) was the most prevalent genotype in studied population, followed by HPV-16 (1.1%), HPV-58 (1.0%), HPV-18 (0.5%). Among 357 women exhibited abnormal cytology, 192 women positive for high-risk HPV, with a prevalence of 52.1% (186/357, 95% CIs: 46.92%-57.28%). HPV-52 (12.9%) was the most prevalent genotype in  $\geq$ ASC-US group, followed by HPV-58 (10.4%), HPV-16 (8.4%), and HPV-51 (5.0%). The prevalence of high-risk HPV genotypes in abnormal groups ( $\geq$ ASC-US group: except for HPV-35, HPV-45; ASC-US group: except for HPV-18, HPV-31, HPV-56, HPV-66; LSIL+HSIL group: except for HPV-68) were significantly higher than NILM group (Table 2). No cases of AGC were positive for HPV.

### Prevalence of STIs and bacterial vaginosis by cytology status

As shown in Table 3, compared to the NILM group, the prevalence of *Chlamydia trachomatis* infection, *Neisseria gonorrhoeae* infection, and bacterial vaginosis were significantly higher in  $\geq$ ASC-US group and ASC-US group. In the LSIL+HSIL group, the prevalence of *Chlamydia trachomatis* infection, *Neisseria gonorrhoeae* infection, and Vulvovaginal candidiasis were significantly higher than the NILM group.

### Factor associated with abnormal cytological findings

As shown in Table 4, after adjusted for potential confounders, compared to the NILM group, high-risk HPV infection (AOR = 20.49, 95% CIs = 16.25–25.83,  $P < 0.001$ ), *Neisseria gonorrhoeae* infection (AOR = 5.30, 95% CIs = 1.30–21.51,  $P = 0.020$ ), bacterial vaginosis (AOR = 1.94, 95% CIs = 1.08–3.47,  $P = 0.026$ ) and 40–46 years old (compared to 20–39 years, AOR = 1.32, 95% CIs = 1.03–1.68,  $P = 0.027$ ) significantly increased the risk of  $\geq$ ASC-US cytology. Compared to the NILM group, high-risk HPV infection (AOR = 7.66, 95%

**Table 1. Prevalence of STIs and bacterial vaginosis among participants.**

Variables <sup>a</sup>	Test Results	No.	Prevalence (95% CIs)*
High-risk human papillomavirus	Negative	8475	6.77% (6.25%-7.29%)
	Positive	615	
<i>Chlamydia trachomatis</i>	Negative	8715	4.12% (3.71%-4.53%)
	Positive	375	
<i>Neisseria gonorrhoeae</i>	Negative	9074	0.18% (0.09%-0.27%)
	Positive	16	
<i>Trichomonas vaginalis</i>	Negative	9058	0.35% (0.23%-0.47%)
	Positive	32	
Vulvovaginal candidiasis	Negative	8561	5.82% (5.34%-6.30%)
	Positive	529	
Bacterial vaginosis	Negative	8884	2.27% (1.96%-2.58%)
	Positive	206	

<sup>a</sup> Positive refers to women not only positive for corresponding variables but also may co-infection with other sexually transmitted pathogens as well and/or positive for bacterial vaginosis at the same time

\* 95% CIs, 95% confidence intervals;  $P$  value  $< 0.05$  was considered significant.

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CI = 5.45–10.75,  $P < 0.001$ ), *Neisseria gonorrhoeae* infection (AOR = 5.72, 95% CI = 1.12–29.10,  $P = 0.036$ ) and bacterial vaginosis (AOR = 2.64, 95% CI = 1.38–5.04,  $P = 0.003$ ) significantly increased the risk of ASC-US cytology, and only high-risk HPV infection

**Table 2. The prevalence of HPV genotypes by cytology status.**

HPV infection	Overall (n = 9090)	NILM (n = 8733)	≥ASC-US (n = 357)			ASC-US (n = 181)			LSIL+HSIL (n = 155)		
	n (%)	n (%)	n (%)	P value*	OR (95% CIs)**	n (%)	P value*	OR (95% CIs)**	n (%)	P value*	OR (95% CIs)**
HPV-6	19 (0.2)	14 (0.2)	5 (1.4)	<b>&lt;0.001</b>	8.8 (3.2–24.7)	4 (2.2)	<b>&lt;0.001</b>	14.1 (4.6–43.2)	1 (0.7)	0.178	4.0 (0.5–30.9)
HPV-11	5 (0.1)	4 (0.1)	1 (0.3)	0.105	6.1 (0.7–55.0)	1 (0.6)	0.026	12.1 (1.3–109.0)	0	—	—
HPV-16	100 (1.1)	70 (0.8)	30 (8.4)	<b>&lt;0.001</b>	11.4 (7.3–17.7)	4 (2.2)	0.048	2.8 (1.0–7.7)	21 (13.6)	<b>&lt;0.001</b>	19.4 (11.6–32.5)
HPV-18	47 (0.5)	39 (0.5)	8 (2.2)	<b>&lt;0.001</b>	5.1 (2.4–11.0)	2 (1.1)	0.211	2.5 (0.6–10.4)	6 (3.9)	<b>&lt;0.001</b>	9.0 (3.7–21.5)
HPV-31	21 (0.2)	13 (0.2)	8 (2.2)	<b>&lt;0.001</b>	15.4 (6.3–37.3)	1 (0.6)	0.206	3.7 (0.5–28.6)	6 (3.9)	<b>&lt;0.001</b>	27 (10.1–72.0)
HPV-33	25 (0.3)	16 (0.2)	9 (2.5)	<b>&lt;0.001</b>	14.1 (6.2–32.1)	2 (1.1)	0.017	6.1 (1.4–26.7)	6 (3.9)	<b>&lt;0.001</b>	21.9 (8.5–56.8)
HPV-35	13 (0.1)	11 (0.1)	2 (0.6)	0.052	4.5 (1.0–20.2)	0	—	—	2 (1.3)	0.002	10.4 (2.3–47.2)
HPV-39	36 (0.4)	27 (0.3)	9 (2.5)	<b>&lt;0.001</b>	8.3 (3.9–17.9)	3 (1.7)	0.006	5.4 (1.6–18.1)	6 (3.9)	<b>&lt;0.001</b>	13.0 (5.3–31.9)
HPV-45	14 (0.2)	12 (0.1)	2 (0.6)	0.066	4.1 (0.9–18.4)	0	—	—	2 (1.3)	0.003	9.5 (2.1–42.8)
HPV-51	40 (0.4)	22 (0.3)	18 (5.0)	<b>&lt;0.001</b>	21.0 (11.2–39.6)	8 (4.4)	<b>&lt;0.001</b>	18.3 (8.0–41.7)	10 (6.5)	<b>&lt;0.001</b>	27.3 (12.7–58.7)
HPV-52	163 (1.8)	117 (1.3)	46 (12.9)	<b>&lt;0.001</b>	10.9 (7.6–15.6)	20 (11.1)	<b>&lt;0.001</b>	9.1 (5.6–15.1)	23 (14.8)	<b>&lt;0.001</b>	12.8 (7.9–20.7)
HPV-56	17 (0.2)	11 (0.1)	6 (1.7)	<b>&lt;0.001</b>	13.6 (5.0–36.9)	1 (0.6)	0.157	4.4 (0.6–34.3)	5 (3.2)	<b>&lt;0.001</b>	26.4 (9.1–77.0)
HPV-58	86 (1.0)	49 (0.6)	37 (10.4)	<b>&lt;0.001</b>	20.5 (13.2–31.9)	6 (3.3)	<b>&lt;0.001</b>	6.1 (2.6–14.4)	28 (18.1)	<b>&lt;0.001</b>	39.1 (23.8–64.2)
HPV-59	11 (0.1)	9 (0.1)	2 (0.6)	0.030	5.5 (1.2–25.4)	0	—	—	2 (1.3)	<b>&lt;0.001</b>	12.7 (2.7–59.1)
HPV-66	25 (0.3)	13 (0.2)	12 (3.4)	<b>&lt;0.001</b>	23.3 (10.6–51.5)	1 (0.6)	0.206	3.7 (0.5–28.6)	11 (7.1)	<b>&lt;0.001</b>	51.2 (22.6–116.3)
HPV-68	45 (0.5)	36 (0.4)	9 (2.5)	<b>&lt;0.001</b>	6.2 (3.0–13.1)	7 (3.9)	<b>&lt;0.001</b>	9.7 (4.3–22.1)	2 (1.3)	0.116	3.2 (0.8–13.2)
HPV	638 (7.0)	446 (5.1)	192 (53.8)	<b>&lt;0.001</b>	21.6 (17.2–27.2)	58 (32.0)	<b>&lt;0.001</b>	8.8 (6.3–12.1)	122 (78.7)	<b>&lt;0.001</b>	68.7 (46.2–102.1)
High-risk HPV	616 (6.8)	430 (4.9)	186 (52.1)	<b>&lt;0.001</b>	21 (16.7–26.4)	53 (29.3)	<b>&lt;0.001</b>	8.0 (5.7–11.2)	121 (78.1)	<b>&lt;0.001</b>	68.7 (46.4–101.8)
Single HPV	608 (6.7)	428 (4.9)	180 (50.4)	<b>&lt;0.001</b>	19.7 (15.7–24.8)	56 (30.9)	<b>&lt;0.001</b>	8.7 (6.3–12.1)	113 (72.9)	<b>&lt;0.001</b>	52.2 (36.2–75.4)
Multiple HPV	30 (0.3)	18 (0.2)	12 (3.4)	<b>&lt;0.001</b>	16.8 (8.0–35.2)	2 (1.1)	0.024	5.4 (1.2–23.5)	9 (5.8)	<b>&lt;0.001</b>	29.8 (13.2–67.5)

Single HPV refers to women infection with only one of HPV genotype; Multiple HPV refers to women infection with at least two kinds of HPV genotype; NILM, negative for intraepithelial lesion or malignancy; ≥ASC-US, include atypical squamous cells of undetermined significance (ASC-US), low-grade squamous intraepithelial lesion (LSIL), atypical squamous cells that cannot exclude HSIL (ASC-H), high-grade squamous intraepithelial lesion (HSIL), atypical glandular cells (AGC). Bold type indicates statistically significant values

\*P value, compare the prevalence of HPV genotypes between NILM and abnormal cytological findings using  $\chi^2$  test or Fisher exact test

\*\* OR (95% CIs), Odd Ratio (95% Confidence Intervals).

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**Table 3. The prevalence of STIs, bacterial vaginosis and social-demographic factors by cytology status.**

Variables	Overall (n = 9090)	NILM (n = 8733)	≥ASC-US (n = 357)			ASC-US (n = 181)			LSIL +HSIL (n = 155)		
	n (%)	n (%)	n (%)	P value*	OR (95% CIs)**	n (%)	P value*	OR (95% CIs)**	n (%)	P value*	OR (95% CIs)**
<i>Chlamydia trachomatis</i> (+) <sup>a</sup>	375 (4.1)	346 (4.0)	29 (8.1)	<0.001	2.1 (1.4–3.2)	16 (8.8)	0.001	2.4 (1.4–4.0)	12 (7.7)	0.020	2.0 (1.1–3.7)
<i>Neisseria gonorrhoeae</i> (+) <sup>b</sup>	16 (0.2)	12 (0.1)	4 (1.1)	<0.001	8.2 (2.6–25.7)	2 (1.1)	0.006	8.1 (1.8–36.5)	2 (1.3)	0.003	9.5 (2.1–42.8)
<i>Trichomonas vaginalis</i> (+) <sup>c</sup>	32 (0.4)	30 (0.3)	2 (0.6)	0.502	1.6 (0.4–6.9)	1 (0.6)	0.640	1.6 (0.2–11.9)	0	—	—
Vulvovaginal candidiasis (+) <sup>d</sup>	529 (5.82)	516 (5.9)	13 (3.6)	0.076	0.6 (0.3–1.1)	8 (4.4)	0.401	0.7 (0.4–1.5)	3 (1.9)	0.048	3.2 (1.0–10)
Bacterial Vaginosis (+) <sup>e</sup>	206 (2.3)	190 (2.2)	16 (4.5)	0.005	2.1 (1.3–3.6)	11 (6.1)	0.001	2.9 (1.6–5.4)	5 (3.2)	0.380	1.5 (0.6–3.7)
Age (40–60) <sup>f</sup>	4857 (53.4)	4643 (53.2)	214 (59.9)	0.012	1.3 (1.1–1.6)	108 (59.7)	0.083	1.3 (1–1.8)	90 (58.1)	0.226	1.2 (0.9–1.7)
Contraception (Condom) <sup>g</sup>	5379 (59.2)	5151 (59.0)	228 (63.9)	0.066	1.2 (1.0–1.5)	117 (64.6)	0.126	1.3 (0.9–1.7)	96 (61.9)	0.459	1.1 (0.8–1.6)
APOs (Yes) <sup>h</sup>	1619 (17.8)	1559 (17.9)	60 (16.8)	0.613	0.9 (0.7–1.2)	27 (14.9)	0.308	0.8 (0.5–1.2)	28 (18.0)	0.945	1 (0.7–1.5)
Ethnicity (Minority) <sup>i</sup>	274 (3.0)	266 (3.1)	8 (2.2)	0.385	0.7 (0.4–1.5)	6 (3.3)	0.835	1.1 (0.5–2.5)	1 (0.7)	0.117	0.2 (0–1.5)
Marital status (Married) <sup>j</sup>	9029 (99.3)	8677 (99.4)	352 (98.6)	0.093	0.5 (0.2–1.1)	179 (98.9)	0.448	0.6 (0.1–2.4)	152 (98.1)	0.062	0.3 (0.1–1.1)

<sup>a b c d e</sup> (+) refers to women not only positive for corresponding variables but also may co-infection with other sexually transmitted pathogens as well and/or positive for bacterial vaginosis at the same time

<sup>f</sup> Age (40–60), compared to 20–30 age group

<sup>g</sup> Contraception (condom), compared to those participants who adopted contraception methods other than condom, including oral pills, intrauterine devices (IUD), injectables, and vaginal ring

<sup>h</sup> APOs, adverse pregnancy outcomes include having any lifelong experience of abortion, premature delivery, ectopic pregnancy or infertility

<sup>i</sup> Ethnicity group (Minority), compared to Han ethnicity

<sup>j</sup> Marital status (Married), compared to single or others status, include single, separated, divorced or widowed status

\*P value, compare the prevalence of variables between NILM and abnormal cytological findings using  $\chi^2$  test or Fisher exact test

\*\*OR (95% CI), Odd Ratio (95% Confidence Intervals).  $\geq$ ASC-US, include atypical squamous cells of undetermined significance (ASC-US), low-grade squamous intraepithelial lesion (LSIL), atypical squamous cells that cannot exclude HSIL (ASC-H), high-grade squamous intraepithelial lesion (HSIL), atypical glandular cells (AGC). Bold type indicates statistically significant values.

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(AOR = 68.96, 95% CIs = 46.45–102.39,  $P < 0.001$ ) significantly increased the risk of LSIL +HSIL cytology.

## Discussion

In the present study, we investigated the association of STIs and bacterial vaginosis with cervical cytology abnormalities in the general population. Our main findings were women with *Neisseria gonorrhoeae* infection or bacterial vaginosis exhibited an increased risk of  $\geq$ ASC-US and ASC-US cytology after adjusted for carcinogenic HPV-positive status, suggesting *Neisseria gonorrhoeae* infection or bacterial vaginosis may act as an independent risk factor for atypical squamous cells formation, predominantly for ASC-US cytology.

In the 2001 Bethesda system, atypical squamous cells not rule out the diagnosis of abnormal cytology and all atypical squamous cells is considered to be suggestive of squamous

**Table 4. Independent factors associated with abnormal cytological findings.**

Variables	≥ASC-US		ASC-US		LSIL+HSIL	
	Adjusted OR (95% CIs)*	P value**	Adjusted OR (95% CIs)*	P value**	Adjusted OR (95% CIs)*	P value**
High-risk HPV (+)	20.49 (16.25–25.83)	<0.001	7.66 (5.45–10.75)	<0.001	68.96 (46.45–102.39)	<0.001
<i>Chlamydia trachomatis</i> (+)	1.27 (0.81–1.99)	0.300	1.66 (0.96–2.87)	0.071	0.97 (0.49–1.91)	0.927
<i>Neisseria gonorrhoeae</i> (+)	5.30 (1.30–21.51)	0.020	5.72 (1.12–29.10)	0.036	3.73 (0.55–25.21)	0.177
Vulvovaginal candidiasis (+)	1.56 (0.86–2.84)	0.146	—	—	2.83 (0.85–9.46)	0.090
Bacterial Vaginosis (+)	1.94 (1.08–3.47)	0.026	2.64 (1.38–5.04)	0.003	—	—
Age (40–60) <sup>a</sup>	1.32 (1.03–1.68)	0.027	1.26 (0.91–1.73)	0.162	—	—
Contraception (condom) <sup>b</sup>	1.05 (0.82–1.35)	0.705	1.13 (0.81–1.56)	0.474	—	—
Ethnicity group (Minority) <sup>c</sup>	—	—	—	—	0.15 (0.02–1.10)	0.061
Marital status (Married) <sup>d</sup>	0.68 (0.24–1.92)	0.463	—	—	2.73 (0.50–14.75)	0.244

<sup>a</sup> Age (40–60), compared to 20–30 age group

<sup>b</sup> Contraception (condom), compared to other contraception methods refer to those other than condom, including oral pills, intrauterine devices (IUD), injectables, and vaginal ring

<sup>c</sup> Ethnicity group (Minority), compared to Han ethnicity

<sup>d</sup> Marital status (Married), compared to single or others status, include single, separated, divorced or widowed status; ≥ASC-US, include atypical squamous cells of undetermined significance (ASC-US), low-grade squamous intraepithelial lesion (LSIL), atypical squamous cells that cannot exclude HSIL (ASC-H), high-grade squamous intraepithelial lesion (HSIL), atypical glandular cells (AGC).

\* Adjusted OR (95% CIs), Adjusted Odd Ratio (95% Confidence Intervals)

\*\* P value, Wald test for AOR value.

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intraepithelial lesions (SIL), and atypical squamous cells are qualified as “of undetermined significance” (ASC-US) or “cannot exclude HSIL” (ASC-H) [26]. In the present study, ASC-US cytology (2.0%) was the most prevalent abnormal cytological findings among participants and accounts for ninety-two percent of atypical squamous cells. Although ASC-US cytology was defined as low-grade epithelial cell abnormality [26], about 3.5% of cervical cancers are from the follow-up of ASC-US cytology [27]. ASC-US cytology consists of a wide variety of cervical cytological lesions, such as, CIN-2 or CIN-3 [28], and women with ASC-US are at an increased risk of developing SCC. Therefore, we inferred that *Neisseria gonorrhoeae* infection in genital sites and/or bacterial vaginosis may increase the risk of cervical epithelial cell abnormalities. A previous study concluded that bacterial vaginosis [29] was significantly correlated with the presence of cervical intraepithelial neoplasia such as CIN-1 or CIN-2/3. In line with previous reports, we found that bacterial vaginosis was associated with an increased risk for ASC-US cytology, which implies that evaluation of bacterial vaginosis will be helpful to the management and treatment of patients with abnormal cytology.

In the present study, the prevalence of high-risk HPV in women with abnormal cytology was 52.1%, which is consistent with Northwest China (53.3%) [30] but there was a big gap with Northeast China (62.8%) [31]. In agreement with the previous study [1], women with high-risk HPV infection exhibited a high risk of cervical cytology abnormalities (Odd Ratio values range from 7.66 to 68.96). Meanwhile, we found that the prevalence of HPV genotypes (except for HPV-18, HPV-31, HPV-56, HPV-66) was significantly different between the NILM group and ASC-US group, which means that the surveillance of HPV genotypes is an important tool in the triage of ASC-US cytology [32, 33]. HPV-52 was the most prevalent genotype in the studied population (1.8%), followed by HPV-16 (1.1%), HPV-58 (1.0%), HPV-51 (0.4%), which is consistent with previous reports conducted in the same areas of China [34].

At present, it is unclear whether a single *Chlamydia trachomatis* infection could trigger the formation of cervical lesion, because *Chlamydia trachomatis* is independently associated with



persistence of high-risk HPV [35]. Although the prevalence of *Chlamydia trachomatis* in ASC-US group or LSIL+HSIL group was significantly higher than NILM group, a multivariate logistic regression analysis revealed that *Chlamydia trachomatis* infection may not act as an independent risk factor for atypical squamous cells formation after potential confounders controlled, which reinforces the conclusion of Safaeian et al.'s study [18]. Theoretically speaking, *Chlamydia trachomatis* infection triggers the formation of oxidative reactive species (ROS), induces genetic instability, and inhibits damaged DNA repair pathways in the endocervical epithelial cells [36, 37], which may facilitate the entries of HPV and cervical lesions. We deduced that the *Chlamydia trachomatis* infection may aggravate cervical lesions triggered by high-risk HPV infection and play a possible synergistic action with high-risk HPV in cervical lesion progression [9].

Furthermore, management of women with ASC-US cytology confused clinicians in the past decades [28, 38, 39], until recently, a systematic review concluded that HPV DNA test shows higher accuracy and sensitivity than repeat cytology tests in triage women with ASC-US cytology [33]. The association between ASC-US cytology and *Neisseria gonorrhoeae* infection or bacterial vaginosis in our study provided a clue that evaluation of *Neisseria gonorrhoeae* DNA and/or bacterial vaginosis may be a supplementary tool to triage women with ASC-US cytology. Therefore, it is necessary to evaluate the risk of SIL in women with co-presence of ASC-US and *Neisseria gonorrhoeae* infection or bacterial vaginosis in the future study, which may improve our management of women with ASC-US cervical cytology.

The current study has several limitations. Firstly, the methodology used for the identification of *Trichomonas Vaginalis* and Vulvovaginal candidiasis may limit the strengthening of our results. For example, wet mount microscopy of *Trichomonas Vaginalis* has low sensitivity, which ranges from 44% to 68%, though with 100% specificity [40], but microscopic examination of vaginal wet mounts has the advantage of providing instant results as a point-of-care test and is widely available and relatively inexpensive. Therefore, a highly sensitive detection method such as nucleic acid amplification test is needed in the further study to explore the relationship between *Trichomonas Vaginalis* or Vulvovaginal candidiasis and abnormal cytology. Secondly, without identification and characterization of bacterial vaginosis-associated pathogens in the present study limited the analysis of the association between bacterial vaginosis-associated pathogens and abnormal cervical cytology. Consolaro MEL et al.'s [41] study revealed that coinfection between bacterial vaginosis (include Gardnerella vaginalis or Megaspheera type I) with HPV was associated with an increased risk for LSIL or HSIL. Hence, it is necessary to explore the association between bacterial vaginosis-associated pathogens and abnormal cervical cytology after adjusted for carcinogenic HPV-positive status in future studies. Thirdly, because of the standard practice of management for ASC-US cytology is colposcopy or follow-up, information about histological confirmation of the cytological outcomes in ASC-US was missing, which limits the analysis of the association between STIs and histological status.

## Conclusions

In conclusion, this study provided an opportunity to determine the prevalence of STIs and bacterial vaginosis in different cervical cytological findings. We found that *Neisseria gonorrhoeae* infection in genital sites and/or bacterial vaginosis may independently increase the risk for cervical cytology abnormalities, which suggests that performing non-HPV STIs tests will be helpful to diagnosis and treatment of patients in routine cervical cancer screening. Meanwhile, these findings enhance our understanding of the etiology of abnormal cervical cytology, and may also be useful for the management of women with ASC-US cervical cytology.

## Supporting information

**S1 File. STROBE Statement—checklist of items that should be included in reports of observational studies.**

(DOCX)

**S1 Dataset.**

(XLSX)

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## References

1. Walboomers JM, Jacobs MV, Manos MM, Bosch FX, Kummer JA, Shah KV, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. *The Journal of pathology*. 1999; 189(1):12–9. [https://doi.org/10.1002/\(SICI\)1096-9896\(199909\)189:1<12::AID-PATH431>3.0.CO;2-F](https://doi.org/10.1002/(SICI)1096-9896(199909)189:1<12::AID-PATH431>3.0.CO;2-F) PMID: 10451482
2. Schiffman M, Wentzensen N, Wacholder S, Kinney W, Gage JC, Castle PE. Human papillomavirus testing in the prevention of cervical cancer. *Journal of the National Cancer Institute*. 2011; 103(5):368–83. <https://doi.org/10.1093/jnci/djq562> PMID: 21282563
3. Rodriguez AC, Schiffman M, Herrero R, Hildesheim A, Bratti C, Sherman ME, et al. Longitudinal study of human papillomavirus persistence and cervical intraepithelial neoplasia grade 2/3: critical role of duration of infection. *Journal of the National Cancer Institute*. 2010; 102(5):315–24. <https://doi.org/10.1093/jnci/djq001> PMID: 20157096
4. La Vecchia C, Boccia S. Oral contraceptives, human papillomavirus and cervical cancer. *European journal of cancer prevention: the official journal of the European Cancer Prevention Organisation (ECP)*. 2014; 23(2):110–2. <https://doi.org/10.1097/cej.0000000000000000> PMID: 24469243

5. Jensen KE, Schmiedel S, Norrild B, Frederiksen K, Iftner T, Kjaer SK. Parity as a cofactor for high-grade cervical disease among women with persistent human papillomavirus infection: a 13-year follow-up. *British journal of cancer*. 2013; 108(1):234–9. <https://doi.org/10.1038/bjc.2012.513> PMID: 23169283
6. Plummer M, Herrero R, Franceschi S, Meijer CJ, Snijders P, Bosch FX, et al. Smoking and cervical cancer: pooled analysis of the IARC multi-centric case—control study. *Cancer causes & control: CCC*. 2003; 14(9):805–14.
7. Kadhel P, Multigner L, Bardinet F, Goerger-Sow M, Janky E. Cervical intraepithelial neoplasia and invasive cancer risks in women infected with HIV in the French West Indies. *HIV medicine*. 2012; 13(1):79–82. <https://doi.org/10.1111/j.1468-1293.2011.00939.x> PMID: 21819528
8. Jensen KE, Thomsen LT, Schmiedel S, Frederiksen K, Norrild B, van den Brule A, et al. Chlamydia trachomatis and risk of cervical intraepithelial neoplasia grade 3 or worse in women with persistent human papillomavirus infection: a cohort study. *Sexually transmitted infections*. 2014; 90(7):550–5. <https://doi.org/10.1136/sextrans-2013-051431> PMID: 24728044
9. de Abreu AL, Malaguti N, Souza RP, Uchimura NS, Ferreira EC, Pereira MW, et al. Association of human papillomavirus, *Neisseria gonorrhoeae* and *Chlamydia trachomatis* co-infections on the risk of high-grade squamous intraepithelial cervical lesion. *American journal of cancer research*. 2016; 6(6):1371–83. PMID: 27429850
10. Smith JS, Herrero R, Bosetti C, Munoz N, Bosch FX, Eluf-Neto J, et al. Herpes simplex virus-2 as a human papillomavirus cofactor in the etiology of invasive cervical cancer. *Journal of the National Cancer Institute*. 2002; 94(21):1604–13. <https://doi.org/10.1093/jnci/94.21.1604> PMID: 12419786
11. Cao S, Gan Y, Dong X, Lu Z. Herpes simplex virus type 2 and the risk of cervical cancer: a meta-analysis of observational studies. *Archives of gynecology and obstetrics*. 2014; 290(6):1059–66. <https://doi.org/10.1007/s00404-014-3365-7> PMID: 25030659
12. 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(12):e86266. <https://doi.org/10.1371/journal.pone.0086266> PMID: 24386492
13. Biernat-Sudolska M, Szostek S, Rojek-Zakrzewska D, Klimek M, Kosz-Vnenchak M. Concomitant infections with human papillomavirus and various mycoplasma and ureaplasma species in women with abnormal cervical cytology. *Advances in medical sciences*. 2011; 56(2):299–303. <https://doi.org/10.2478/v10039-011-0028-9> PMID: 21940266
14. Lukic A, Canzio C, Patella A, Giovagnoli M, Cipriani P, Frega A, et al. Determination of cervicovaginal microorganisms in women with abnormal cervical cytology: the role of *Ureaplasma urealyticum*. *Anti-cancer research*. 2006; 26(6C):4843–9. PMID: 17214350
15. Parthenis C, Panagopoulos P, Margari N, Kottaridi C, Spathis A, Pouliakis A, et al. The association between sexually transmitted infections, human papillomavirus, and cervical cytology abnormalities among women in Greece. *International journal of infectious diseases: IJID: official publication of the International Society for Infectious Diseases*. 2018; 73:72–7. <https://doi.org/10.1016/j.ijid.2018.06.001> PMID: 29902519
16. Kim HS, Kim TJ, Lee IH, Hong SR. Associations between sexually transmitted infections, high-risk human papillomavirus infection, and abnormal cervical Pap smear results in OB/GYN outpatients. *Journal of gynecologic oncology*. 2016; 27(5):e49. <https://doi.org/10.3802/jgo.2016.27.e49> PMID: 27329197
17. Lehtinen M, Ault KA, Lyytikainen E, Dillner J, Garland SM, Ferris DG, et al. *Chlamydia trachomatis* infection and risk of cervical intraepithelial neoplasia. *Sexually transmitted infections*. 2011; 87(5):372–6. <https://doi.org/10.1136/sti.2010.044354> PMID: 21471141
18. Safaeian M, Quint K, Schiffman M, Rodriguez AC, Wacholder S, Herrero R, et al. *Chlamydia trachomatis* and risk of prevalent and incident cervical premalignancy in a population-based cohort. *Journal of the National Cancer Institute*. 2010; 102(23):1794–804. <https://doi.org/10.1093/jnci/djq436> PMID: 21098758
19. Fredricks DN, Fiedler TL, Marrazzo JM. Molecular identification of bacteria associated with bacterial vaginosis. *The New England journal of medicine*. 2005; 353(18):1899–911. <https://doi.org/10.1056/NEJMoa043802> PMID: 16267321
20. Unemo M, Bradshaw CS, Hocking JS, de Vries HJC, Francis SC, Mabey D, et al. Sexually transmitted infections: challenges ahead. *The Lancet Infectious diseases*. 2017; 17(8):e235–e79. [https://doi.org/10.1016/S1473-3099\(17\)30310-9](https://doi.org/10.1016/S1473-3099(17)30310-9) PMID: 28701272
21. Gillet E, Meys JF, Verstraelen H, Verhelst R, De Sutter P, Temmerman M, et al. Association between bacterial vaginosis and cervical intraepithelial neoplasia: systematic review and meta-analysis. *PloS one*. 2012; 7(10):e45201. <https://doi.org/10.1371/journal.pone.0045201> PMID: 23056195
22. Luo ZZ, Li W, Wu QH, Zhang L, Tian LS, Liu LL, et al. Population-based study of chlamydial and gonococcal infections among women in Shenzhen, China: Implications for programme planning. *PloS one*. 2018; 13(5):e0196516. <https://doi.org/10.1371/journal.pone.0196516> PMID: 29715319

23. Yi X, Li J, Yu S, Zhang A, Xu J, Yi J, et al. A new PCR-based mass spectrometry system for high-risk HPV, part I: methods. *American journal of clinical pathology*. 2011; 136(6):913–9. <https://doi.org/10.1309/AJCPWTZDT0Q7DOVI> PMID: 22095377
24. Yi X, Zou J, Xu J, Liu T, Liu T, Hua S, et al. Development and validation of a new HPV genotyping assay based on next-generation sequencing. *American journal of clinical pathology*. 2014; 141(6):796–804. <https://doi.org/10.1309/AJCP9P2KJSXEKCB> PMID: 24838323
25. Verstraelen H, Verhelst R. Bacterial vaginosis: an update on diagnosis and treatment. *Expert review of anti-infective therapy*. 2009; 7(9):1109–24. <https://doi.org/10.1586/eri.09.87> PMID: 19883331
26. Solomon D, Davey D, Kurman R, Moriarty A, O'Connor D, Prey M, et al. The 2001 Bethesda System: terminology for reporting results of cervical cytology. *Jama*. 2002; 287(16):2114–9. <https://doi.org/10.1001/jama.287.16.2114> PMID: 11966386
27. Landy R, Castanon A, Hamilton W, Lim AW, Dudding N, Hollingworth A, et al. Evaluating cytology for the detection of invasive cervical cancer. *Cytopathology: official journal of the British Society for Clinical Cytology*. 2016; 27(3):201–9. <https://doi.org/10.1111/cyt.12259> PMID: 26126636
28. Kinney WK, Manos MM, Hurley LB, Ransley JE. Where's the high-grade cervical neoplasia? The importance of minimally abnormal Papanicolaou diagnoses. *Obstetrics and gynecology*. 1998; 91(6):973–6. [https://doi.org/10.1016/s0029-7844\(98\)00080-5](https://doi.org/10.1016/s0029-7844(98)00080-5) PMID: 9611007
29. Nam KH, Kim YT, Kim SR, Kim SW, Kim JW, Lee MK, et al. Association between bacterial vaginosis and cervical intraepithelial neoplasia. *Journal of gynecologic oncology*. 2009; 20(1):39–43. <https://doi.org/10.3802/jgo.2009.20.1.39> PMID: 19471662
30. Wang J, Tang D, Wang J, Zhang Z, Chen Y, Wang K, et al. Genotype distribution and prevalence of human papillomavirus among women with cervical cytological abnormalities in Xinjiang, China. *Human vaccines & immunotherapeutics*. 2019; 15(7–8):1889–96. <https://doi.org/10.1080/21645515.2019.1578598> PMID: 30735478
31. You W, Li S, Du R, Zheng J, Shen A. Epidemiological study of high-risk human papillomavirus infection in subjects with abnormal cytological findings in cervical cancer screening. *Experimental and therapeutic medicine*. 2018; 15(1):412–8. <https://doi.org/10.3892/etm.2017.5357> PMID: 29375696
32. Aoyama-Kikawa S, Fujita H, Hanley SJB, Kasamo M, Kikuchi K, Toriogoe T, et al. Comparison of human papillomavirus genotyping and cytology triage, COMPACT Study: Design, methods and baseline results in 14 642 women. *Cancer science*. 2018; 109(6):2003–12. <https://doi.org/10.1111/cas.13608> PMID: 29660849
33. Arbyn M, Roelens J, Simoons C, Buntinx F, Paraskevaidis E, Martin-Hirsch PP, et al. Human papillomavirus testing versus repeat cytology for triage of minor cytological cervical lesions. *The Cochrane database of systematic reviews*. 2013;(3):CD008054. <https://doi.org/10.1002/14651858.CD008054.pub2> PMID: 23543559
34. Zhang Y, Wang Y, Liu L, Guo C, Liu Z, Nie S. Prevalence of human papillomavirus infection and genotyping for population-based cervical screening in developed regions in China. *Oncotarget*. 2016; 7(38):62411–24. <https://doi.org/10.18632/oncotarget.11498> PMID: 27566561
35. Samoff E, Koumans EH, Markowitz LE, Sternberg M, Sawyer MK, Swan D, et al. Association of Chlamydia trachomatis with persistence of high-risk types of human papillomavirus in a cohort of female adolescents. *American journal of epidemiology*. 2005; 162(7):668–75. <https://doi.org/10.1093/aje/kwi262> PMID: 16120706
36. O'Connell CM, Ferone ME. Chlamydia trachomatis Genital Infections. *Microbial cell (Graz, Austria)*. 2016; 3(9):390–403. <https://doi.org/10.15698/mic2016.09.525> PMID: 28357377
37. Chumduri C, Gurumurthy RK, Zadora PK, Mi Y, Meyer TF. Chlamydia infection promotes host DNA damage and proliferation but impairs the DNA damage response. *Cell host & microbe*. 2013; 13(6):746–58. <https://doi.org/10.1016/j.chom.2013.05.010> PMID: 23768498
38. Cox JT. Management of women with cervical cytology interpreted as ASC-US or as ASC-H. *Clinical obstetrics and gynecology*. 2005; 48(1):160–77. <https://doi.org/10.1097/01.grf.0000151571.91814.f3> PMID: 15725868
39. Sawaya GF. A 21-year-old woman with atypical squamous cells of undetermined significance. *Jama*. 2005; 294(17):2210–8. <https://doi.org/10.1001/jama.294.17.2210> PMID: 16264163
40. Hobbs MM, Sena AC. Modern diagnosis of Trichomonas vaginalis infection. *Sexually transmitted infections*. 2013; 89(6):434–8. <https://doi.org/10.1136/sextrans-2013-051057> PMID: 23633669
41. TT S, N M, E D, Uchimura NS, Gimenes F, Souza RP, et al. Association of human papillomavirus and bacterial vaginosis with increased risk of high-grade squamous intraepithelial cervical lesions. *International journal of gynecological cancer: official journal of the International Gynecological Cancer Society*. 2019. <https://doi.org/10.1136/ijgc-2018-000076> PMID: 30630884