Distribution of high-risk human r slloanvsrmsr e oiy e

## Introduction

Cervical cancer is the 4th most commonly diagnosed cancer in women globally, and it is the 2nd most diagnosed in women living in less-developed regions (1). GLOBOCAN 2018 estimates that there were an estimated 569,847 new cases and approximately 311,365 deaths from cervical cancer worldwide (1). A large majority of the cervical cancer burden occurs in less-developed regions (2). In mainland China, the incidence rate of cervical cancer is estimated to be about 15.4/100,000 and cervical cancer is the 8th leading cause of cancer deaths in Chinese women based on 2018 data, with the mortality rate being as high as 6.9/100,000 (3).

Persistent high-risk human papillomavirus (HR-HPV) infection is the most important cause of the progression of cervical cancer and its precursors. There are more than 150 HPV types being identified and at least 13 of them are regarded as “high risk” contributing to the development of cervical cancer including HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68 (4); it has been suggested that their relative carcin ʿ arci ל Σ ʿ er ci ng ל haH خ ל

*Detection and genotyping of HPV*

***Care***HPV testing (Qiagen, Gaithersburg, MD, USA)
*Care*HPV testing is based on nucleic acid hybridization detection, targeting 14 high-risk HPV types (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68). However, *care*HPV testing cannot determine the specific HPV genotype. The test measures the ratio of relative light units emitted by microplate reader (RLU) to cutoff (CO). If the RLU/CO value ≥1.0, the participant is considered to be high-risk HPV positive; otherwise, considered to be high-risk HPV negative.

PCR-based HPV testing without genotyping (Sansure, Changsha, China)
Sansure PCR HPV testing employs the One-Step Fast Release technology, targeting 15 HR-HPV types (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66 and 68), and has been approved by a European Union Certificate (CE). By applying real-time fluorescent quantitative PCR, the testing utilizes pairs of specific primers and specific probes accompanied with other reagents in the PCR mix to achieve rapid detection of HR-HPV DNA. The manual sample processing requires about 45 min by minimally trained personnel prior to an automated detection procedure, which can be completed within 1 h and 20 min. HPV positivity is measured by the cycle numbers observed (Ct) when the fluorescent signal reaches the set threshold. A Ct ≤39 is considered HPV positive and a Ct >39 is considered negative.

HPV test with genotyping (Sansure, Changsha, China)
Subsequent genotyping for HR-HPV types (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66 and 68) was performed according to the above-mentioned PCR test principles and workflow but with detection of the exact types.

*Confirmation of disease status*

Pathology was the gold-standard endpoint measure. Experienced pathologists at CICAMS reviewed every histology slide and classified each finding as negative, CIN grade 1/2/3, squamous cell carcinoma (SCC), adenocarcinoma *in situ* (AIS), or adenocarcinoma (ADC). For women initially without pathological results, final classification was based on the overall combination of testing measures. To exclude verification bias, we applied the following criterion for final analysis: women without biopsies were classified as normal if they either had a negative colposcopy impression or if they had negative results for the HR-HPV test both in *care*HPV and PCR HPV tests (*Figure 1*).

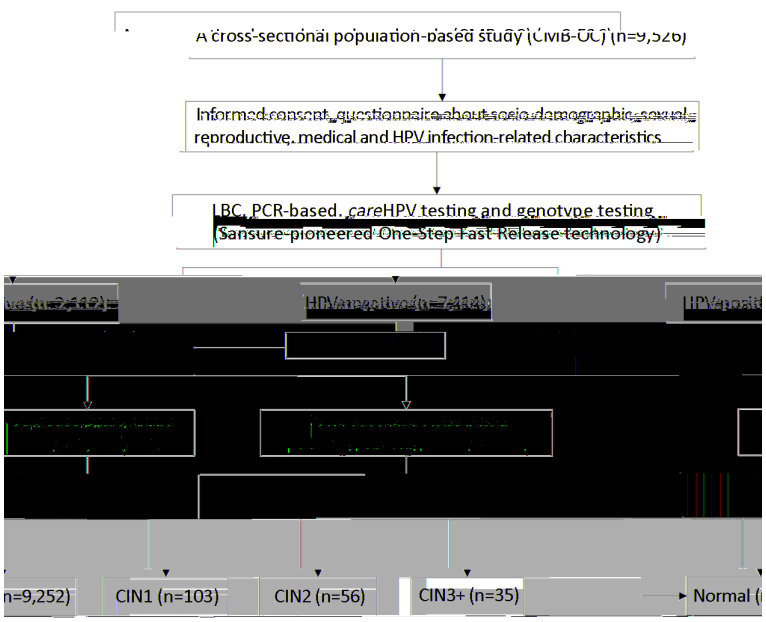


Figure 1 Screening flowchart. CMB-OC, Point of Care (POC) for the Cervical Cancer Screening and Management in Low-resource Settings in China study; HPV, human papillomavirus; LBC, liquid-based cytology; PCR, polymerase chain reaction; CIN1, cervical intraepithelial neoplasia grade 1; CIN2, cervical intraepithelial neoplasia grade 2; CIN3+, cervical intraepithelial neoplasia grade 3 or worse.

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It should be noted that local laboratory technicians and physicians involved in this study were trained uniformly by CICAMS and WHO/IARC experts in preparation for this study.

*Statistical analysis*

Table 1 Demographic characteristics of study population correlated to cervical lesion severity

| Characteristics | Normal | CIN1 | CIN2 | CIN3+ | $\chi^2$ | P |
|---|---|---|---|---|---|---|
| Age (year) | | | | | | |
| ≤47 | 4,885 | 51 | 27 | 14 | 3.172 | 0.366 |
| >47 | 4,367 | 52 | 29 | 21 | | |
| Education level | | | | | | |
| No education | 607 | 12 | 5 | 2 | 4.777* | 0.171 |
| Educated | | | | | | |

HR-HPV-positive cervical lesions, along with an estimate of the attributable fraction of HPV (defined as an estimate of the proportion of lesions caused by a given HPV type), was shown in *Table 2*.

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Table 2 Distribution and attributable proportion of HR-HPV genotypes among different grades of cervical lesions

| HR-HPV genotype | Normal pathology (n=9,252) | | | CIN1 (n=103) | | | CIN2+ (n=91) | | | Total population (n=9,526)*** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | n (%)* | Single infection (n) | Attributable proportion(%)** | n (%)* | Single infection (n) | Attributable proportion (%)** | n (%)* | Single infection (n) | Attributable proportion (%)** | n (%)* | Single infection (n) | Attributable proportion (%)** |
| Any type | 1,611 | 951 | 100.00 | 98 | 50 | 100.00 | 90 | 49 | 100.00 | 1,874 | 1,089 | 100.00 |
| 16 | 187 (11.61) | 84 | 8.09 | 36 (36.73) | 13 | 30.68 | 68 (75.56) | 37 | 73.53 | 303 (16.17) | 140 | 12.09 |
| 18 | 83 (5.15) | 26 | 2.27 | 12 (12.24) | 3 | 5.64 | 6 (6.67) | 0 | 0.37 | 105 (5.60) | 31 | 2.41 |
| 31 | 113 (7.01) | 45 | 4.02 | 8 (8.16) | 2 | 3.40 | 8 (8.89) | 1 | 1.68 | 137 (7.31) | 49 | 3.91 |
| 33 | 141 (8.75) | 53 | 5.22 | 8 (8.16) | 1 | 2.01 | 9 (10.00) | 1 | 3.42 | 164 (8.75) | 56 | 4.71 |
| 35 | 66 (4.10) | 16 | 1.45 | 3 (3.06) | 1 | 1.13 | 3 (3.33) | 1 | 1.17 | 75 (4.00) | 19 | 1.45 |
| 39 | 152 (9.44) | 27 | | | | | | | | | | |

HPV16 was attributed to 30.68% of CIN1, 73.53% of CIN2+; it was the genotype with the highest attributable proportion observed in CIN1 and CIN2+ (*Figure 3*).

In total, HPV16, 56, 58, 53, 52, 59, 68, and 18 combined were attributed to 84.17% of all CIN1 lesions, and HPV16, 58, and 52 combined were attributed to 86.98% of all CIN2+ lesions (*Figure 3*).

## Discussion

This cross-sectional population-based study describes the distribution of high-risk HPV and their respective attribution proportions to cervical precancerous lesions among women from rural areas in North China. Overall, HR-HPV prevalence was 22.2% (2,112/9,526), which is higher than the pooled analysis from 17 population-based studies throughout China which indicated HR-HPV prevalence was 18.0% in rural women (12); this is attributed to prevalence results arising from positive for pre HPV or PCR HPV testing without genotyping. In our study, HPV52 was the most commonly detected HR-HPV types in l eS ihich ih i ns strn ihth i hei

knowledge of the distribution of HPV types in different cervical lesions will benefit to estimate the potential protection provided by current HPV vaccines. And the determination of the most common HPV types in cervical lesions can influence the development of new polyvalent HPV vaccines. Our study suggests that HPV16, HPV52, and HPV58 play important roles in the development of cervical lesions in rural North China, and these particular HPV carcinogenic types should be given priority in the development of new polyvalent HPV vaccines. Moreover, based on the ecological principles, there exists competitive

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## Footnote

*Conflicts of Interest*: The authors have no conflicts of interest to declare.

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