Hongyu Xie, Xuan Rao, Junyan Li, Lifang Yao, Ying Ji, Juan Zhang, Hui Wang, Xinyu Wang* and Xiao Li*

Diagnostic accuracy of extended HPV DNA genotyping and its application for risk-based cervical cancer screening strategy

https://doi.org/10.1515/cclm-2023-0440
Received May 2, 2023; accepted June 22, 2023; published online July 13, 2023

Abstract

Objectives: To evaluate the consistency of 14 high-risk HPVs (hr-HPVs) detection between extended HPV DNA genotyping and a well-validated partial HPV genotyping kit, and to explore the diagnostic accuracy of risk stratification strategy based on extended HPV genotyping for cervical cancer (CC) screening.

Methods: Baseline data from a clinical trial of recombinant HPV 9-valent vaccine in China was analyzed. All enrolled women aged 20–45 years received cervical cytology, HPV detection by extended and partial HPV genotyping kits. Those who met the indications would further receive colposcopy. The primary endpoints were cervical intra-epithelial neoplasia 2/3 or worse (CIN2+/CIN3+).

Results: A total of 8,000 women were enrolled between April 2020 and July 2020 and 83/33 cases were diagnosed as CIN2+/CIN3+. The overall agreement between the extended and partial HPV genotyping was 92.66 %. And the agreement further increased with the progression of lesions, which lead to similarly high sensitivity and negative predictive value of these kits. A stratified triage strategy of CC screening was constructed based on the immediate CIN2+/CIN3+ risk of specific HPV. Compared with the conventional HPV primary CC screening strategy, the risk-based strategy had higher specificity for CIN (CIN2+: 94.84 vs. 92.46 %, CIN3+: 96.05 vs. 91.92 %), and needed fewer colposcopies for detecting one cervical disease.

Conclusions: Extended HPV genotyping had good agreement with a well-validated partial HPV genotyping CC primary screening kit in hr-HPV detection. Extended HPV genotyping could facilitate risk-based stratified management strategy and improve the diagnostic accuracy of primary CC screening.

Keywords: cervical cancer screening; cervical intra-epithelial neoplasia; extended HPV genotyping; risk-based stratified management strategy

Introduction

Cervical cancer (CC) is the fourth most common female malignancy worldwide [1]. Persistent high-risk human
papillomavirus (hr-HPV) genotypes infection is considered to be the main cause of high-grade squamous intraepithelial lesion and CC [2]. Although HPV vaccination is an effective primary prevention strategy for CC, CC screening remains an important approach for early detection of cervical precancerous lesions and cancers, given the short supply of vaccines in China. As a conventional CC screening strategy, HPV primary screening triaged by cervical cytology is recommended by multiple international guidelines [3–5].

Cobas 4,800 (Roche Molecular Systems Inc., Alameda, CA, USA) and Onclarity (BD Diagnostics, Sparks, MD, USA) HPV detection were the first two well-validated and approved partial HPV genotyping kits by the US Food and Drug Administration for CC primary screening [3]. Cobas 4,800 could distinguish 14 hr-HPVs into HPV16, HPV18, and other 12 hr-HPV genotypes (HPV31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68), while Onclarity could further classify other 12 hr-HPV genotypes into 7 groups (HPV31, 45, 51, 52, 33/58, 35/39/68, and 56/59/66). HPV 16/18 HPV were the most common carcinogenic genotypes [6]. However, only a small number of women with non-16/18 HPV positive eventually suffer from cervical cancer [7], and the carcinogenic hazard of specific non-16/18 hr-HPV genotype hasn’t been assessed precisely, which would result in excessive anxiety, over diagnosis and treatment for HPV positive women. Thus, it is urgently warranted for risk stratification of specific HPV genotype to provide precise risk evaluation for HPV infective women.

Extended HPV genotyping was thought as a highly promising method for risk assessment of specific HPV, since it could distinguish each specific HPV. But, there is no FDA-approved extended HPV genotyping kit for CC screening up to date. A number of clinical trials for varied extended HPV genotyping kits have been completed or are undergoing in China. Yaneng extended HPV DNA genotyping kit (BioScience, Shenzhen, China) could simultaneously detect 14 hr-HPVs (same as Cobas 4,800), 4 medium-risk HPVs (26, 53, 73, and 82), and 5 low-risk HPVs (6, 11, 42, 43, and 81) separately, which covered almost all oncogenic HPV genotypes [8]. Previous studies have suggested that Yaneng extended HPV DNA genotyping kit displayed good agreement with Cobas 4,800 and Cervista (Hologic, Madison, WI, USA) in the detection of 14 hr-HPVs, but the oncogenic risk of varied hr-HPV genotypes has not been further explored [9, 10]. Thus, present study tried to evaluate the consistency of 14 hr-HPVs detection between extended and partial HPV DNA genotyping and explore the CC screening performance of extended HPV DNA genotyping using risk stratification strategy of specific hr-HPV infection among women aged 20–45 through a large-scale population-based cross-sectional study.

Materials and methods

Study design

Totally 8,000 women were recruited for evaluating the efficacy of recombinant human papillomavirus 9-valent vaccine, in a multicenter phase III randomized clinical trial approved by NMPA and sponsored by Bovax Biotechnology (Shanghai, China, NCT04423360). The women received 3 doses of HPV vaccine at baseline, 2nd, and 6th month, and would be followed up with cytology and HPV detection every half year for 5 years. This trial was approved by the Ethics Committee of Guangxi Center for Disease Prevention and Control (approval number: GXIRB2019-0044-1). Eligible criteria included healthy women aged 20–45 years with sexual history, who did not report previous abnormal results on CC screening and were willing to provide written informed consent. Women who had a history of HPV infection, abnormal cervical cytology, cervical intraepithelial neoplasia (CIN) or worse, vulvar intraepithelial neoplasia or worse, vaginal intraepithelial neoplasia or worse, HPV vaccination, pregnant or lactating, or with other vaccine contraindications were excluded. Only baseline data were collected and analyzed in present study.

At baseline visit, two cervical samples were collected including one for cervical cytology (Hologic, USA) and Cobas 4,800 partial HPV genotyping detection, and another for Yaneng extended HPV genotyping test according to the manufacturer’s instructions. Cytology was reported using the classification of the Bethesda System [11]. Women who met the following criteria would receive colposcopy at baseline visit: (1) HPV 16/18 positive; (2) abnormal cytology worse than atypical lesion-free at baseline and would be followed up at the scheduled time. The primary endpoints were histological cervical intraepithelial neoplasia 2/3 or worse (CIN2+/CIN3+).

Statistical analysis

The consistency of both HPV detection methods was evaluated by Cohen’s kappa statistics [12]. Diagnostic accuracy was assessed by sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV), and 95% confidence intervals (95% CI) were computed using Wilson method. Chi-square test was used for pairwise comparison of two detection methods and p<0.05 was recognized as statistically significant.

The prevalence of hr-HPV genotypes by extended HPV genotyping and their immediate risk of CIN2+/CIN3+ were calculated by three previously published approaches [13–15], including minimum estimate (Min.) that was only for single HPV infection, any type estimate (Any.) was used for all HPV infection and repeated calculation for multiple infections, and hierarchical attribution estimate (Hier.) was to attribute the multiple HPV infections to a specific HPV genotype according to the HPV ranking of Any. Hier-HPV genotypes were further grouped into different risk groups by their immediate risk of CIN2+/CIN3+ based on extended HPV genotyping combined with cytology. All statistical analyses were conducted using SAS 9.4.
Results

Demographics and clinical information of study population

As shown in Supplemental Figure S1, 7,999 women aged 20–45 were successfully enrolled between April 2020 and July 2020. After excluding 3 unsatisfied cytology results and 9 loss of follow-up, a total of 7,987 were included for evaluating the efficacy of CC screening. The demographics and clinical information of study population were listed in Supplemental Table S1. In brief, partial HPV genotyping revealed 761 (9.51 %) women with hr-HPV positive, including 184 (2.3 %) HPV16/18 and 627 (7.84 %) other 12 hr-HPV genotypes. Extended HPV genotyping revealed 1,114 (13.93 %) women (9.51 %) women with hr-HPV positive, including 184 (2.3 %) HPV16, 99.41 % for HPV18, and 93.85 % for other 12 hr-HPVs (Table 1). These results indicated extended HPV genotyping in women with HPV infection and CIN2+/CIN3+ indicated that different HPV carried different cervical disease risks.

Risk stratification based on different hr-HPV genotypes combined with cervical cytology

As shown in Figure 1G and Supplemental Table S5, the top 6 ranking HPVs (33, 16, 58, 31, 52, 18) carried higher immediate risk for CIN2+/CIN3+ when using Min. method. And similar results were revealed when HPV ranking for CIN2+ and CIN3+ risks by Any. and Hier., with the reversed order of HPV31/58 and HPV18/52 when calculating CIN2+ risk (Figure 1H and I and Supplemental Table S5). Considering the influence of different cervical cytology results (Supplemental Table S6), the immediate CIN2+/CIN3+ risk for each hr-HPV by Hier. were further evaluated combined with cervical cytology (Supplemental Table S7). Since HPV 16/18 have been widely recognized as the most common carcinogenic genotypes, only other 12 hr-HPVs were further divided into 3 groups. Group A (high-risk) carried immediate CIN2+ (Hier.1)/CIN3+ (Hier.2) risk ≥ 4 % when combined with ASC-US. Group B (medium-risk) carried immediate CIN2+ /CIN3+ risk ≥ 4 % when combined with low-grade squamous intraepithelial lesion (LSIL), but < 4 % when combined with ASC-US. Group C (low-risk) carried immediate CIN2+/CIN3+ risk < 4 % when combined with LSIL (Figure 2).

Comparison of conventional and risk-based primary screening strategies by extended HPV genotyping

Risk-based management strategy was further constructed, and women who met the following criteria should receive colposcopy: (1) HPV 16/18 positive; (2) Group An HPV positive with cytology ASC-US or worse; (3) Group B HPV positive with cytology LSIL or worse; (4) Group C HPV positive and cytology High-grade (Table 3). The risk-based management strategy demonstrated significantly higher specificity in...
Current study demonstrated that extended HPV genotyping exhibited good agreement with the partial HPV genotyping for detecting 14 hr-HPV genotypes, and the agreement could be further improved with the progression of cervical lesions. Moreover, there was an excellent consistency between extended and partial HPV genotyping for diagnostic accuracy using conventional HPV primary screening strategy. A risk-based precision CC screening strategy was further constructed based on the immediate CIN2+/CIN3+ risks of specific HPV, which improved the efficacy of CC screening significantly. And the number of recompositories performed to detect one CIN2+/CIN3+ lesion was also lower compared to the conventional primary screening strategy.

The overall prevalence of 14 hr-HPV infection in present study was slightly lower than previously reported (ranging from 9.9 to 23.84 %) in China [16–19], which might attribute to the fact that women with HPV infection history were excluded at baseline. Due to the excellent consistency of extended and partial HPV genotyping for HPV detection and diagnostic accuracy using conventional HPV primary screening strategy, extended HPV genotyping was further used for assessing the distribution and the risk of carcinogenesis for specific HPV genotype. Yu et al. reported that the prevalence of hr-HPV with descending order was HPV 16, 52,
58, 18, 33, 51, 68, 39, 31, 56, 66, 59, 35 and 45 and the top 6 ranking HPV in CIN2 were HPV 16, 58, 52, 33, 31, and 18 after systematically evaluated in 856,535 Chinese women [20]. Our data revealed generally similar results. Moreover, the prevalence of HPV31 and HPV33 was ranked tied for 10th in the population, but the cases of CIN2+/CIN3+ for HPV 31 and HPV 33 were ranked the 6th and the 3rd by Min. estimate. This discordance of the HPV ranking between the population and the patients with CIN2+/CIN3+ suggested that the infectivity and carcinogenicity was discordant for specific HPV genotype.

HPV16 and 18 were universally known as the most and second common types in cervical cancer. However, it was debated for non-16/18 hr-HPV that whether the genotypes should be further distinguished for primary CC screening [3, 21, 22]. Although Monsonego et al. [22] supported that pooled non-16/18 hr-HPV could provide sufficient information for screening, their results showed HPV31, 33, 52 and 45 carried high CIN3+ risk (7.9, 5.4, 4.4 and 4.3 % successively) in aged ≥30. These results suggested that a detailed genotype analysis should be essential for clinical purpose, since 2019 ASCCP guideline had suggested 4 % risks for CIN3+ should be
Figure 2: Risk stratification based on different hr-HPV genotypes combined with cervical cytology. (A) HPV genotyping grouping for CIN2+. Group A: HPV31, 33, 39, and 68; Group B: HPV51, 52, and 58; Group C: HPV35, 45, 56, 59, and 66; (B) HPV genotyping grouping for CIN3+. Group A: HPV31; Group C: HPV33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68.

Table 3: Risk-based management strategy according to different hr-HPV genotypes grouping.

<table>
<thead>
<tr>
<th>Grouping</th>
<th>n=831 CIN2+ (n=40)</th>
<th>Immediate risk</th>
<th>Management</th>
<th>n=823 CIN3+ (n=13)</th>
<th>Immediate risk</th>
<th>Management</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NILM</td>
<td>88</td>
<td>0</td>
<td>0.00 %</td>
<td>Follow-up</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td>ASC-US</td>
<td>59</td>
<td>6</td>
<td>10.17 %</td>
<td>Colposcopy</td>
<td>15</td>
<td>1</td>
</tr>
<tr>
<td>LSIL</td>
<td>36</td>
<td>2</td>
<td>5.56 %</td>
<td>Colposcopy</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>High-grade</td>
<td>12</td>
<td>7</td>
<td>58.33 %</td>
<td>Colposcopy or treatment</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NILM</td>
<td>253</td>
<td>0</td>
<td>0.00 %</td>
<td>Follow-up</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ASC-US</td>
<td>102</td>
<td>2</td>
<td>1.96 %</td>
<td>Follow-up</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>LSIL</td>
<td>71</td>
<td>5</td>
<td>7.04 %</td>
<td>Colposcopy</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>High-grade</td>
<td>31</td>
<td>16</td>
<td>51.61 %</td>
<td>Colposcopy or treatment</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NILM</td>
<td>98</td>
<td>0</td>
<td>0.00 %</td>
<td>Follow-up</td>
<td>421</td>
<td>0</td>
</tr>
<tr>
<td>ASC-US</td>
<td>48</td>
<td>0</td>
<td>0.00 %</td>
<td>Follow-up</td>
<td>193</td>
<td>0</td>
</tr>
<tr>
<td>LSIL</td>
<td>31</td>
<td>0</td>
<td>0.00 %</td>
<td>Follow-up</td>
<td>132</td>
<td>0</td>
</tr>
<tr>
<td>High-grade</td>
<td>2</td>
<td>2</td>
<td>100.00 %</td>
<td>Colposcopy or treatment</td>
<td>44</td>
<td>12</td>
</tr>
</tbody>
</table>

CIN2+: Group A: HPV31, 33, 39, and 68; Group B: HPV51, 52, and 58; Group C: HPV35, 45, 56, 59, and 66; CIN3+: Group A: HPV31; Group B: No; Group C: HPV33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68; *we could not provide a definite follow-up interval in current management strategy because no follow-up data was available for cumulative risk at 3 or 5 years.

Table 4: The efficacy of conventional and risk-based HPV primary screening strategies for identifying CIN2+/CIN3+ by extended HPV genotyping.

<table>
<thead>
<tr>
<th>Strategies</th>
<th>Sensitivity (95 % CI)</th>
<th>Specificity (95 % CI)</th>
<th>PPV (95 % CI)</th>
<th>NPV (95 % CI)</th>
<th>Colposcopy/CIN</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIN2+</td>
<td>Extended (conventional)</td>
<td>95.18 (87.45–98.44)</td>
<td>92.46 (91.85–93.03)</td>
<td>11.70 (9.43–14.43)</td>
<td>99.95 (99.85–99.98)</td>
</tr>
<tr>
<td></td>
<td>Extended (risk-based)</td>
<td>92.77 (84.35–97.03)</td>
<td>94.84 (94.32–95.31)</td>
<td>15.88 (12.80–19.51)</td>
<td>99.92 (99.82–99.97)</td>
</tr>
<tr>
<td>CIN3+</td>
<td>Extended (conventional)</td>
<td>96.97 (82.49–99.84)</td>
<td>91.92 (91.29–92.50)</td>
<td>4.74 (3.32–6.70)</td>
<td>99.99 (99.91–100.00)</td>
</tr>
<tr>
<td></td>
<td>Extended (risk-based)</td>
<td>96.97 (82.49–99.84)</td>
<td>96.05 (95.60–96.46)</td>
<td>9.25 (6.51–12.93)</td>
<td>99.99 (99.92–100.00)</td>
</tr>
</tbody>
</table>
referral to colposcopy [4]. Similarly, Rohner et al. found that HPV16, 31, 33/58, and 52 had a high risk for CIN2+ [23]. Our result also observed that HPV33, 16, 58, and 31 carried high risk of CIN2+/CIN3+ (>4%). Surprisingly, we even found that HPV33 carried a higher CIN3+ risk than HPV16, which was consistent with Monsonego's report, they found that HPV33 carried the highest CIN3+ risk in women aged 25-29 [22]. In addition, Schiffman et al. reported that HPV45 ranking 7th for the risk of CIN3+ and 9th for CIN2+ in women aged ≥30 with negative cytology and positive hr-HPVs after a 3-year follow-up [24]. But in current study, HPV45 showed low oncogenic risk for both CIN2+ and CIN3+, which might attribute to the small sample size and lack of follow-up. So a larger population-based study focused on HPV33 and HPV45 should be conducted to investigate their pathogenicity in the future.

To construct the stratified management strategy based on CIN2+/CIN3+ risk, we further divided other 12 hr-HPV genotypes into high-risk, medium-risk, and low-risk groups based on their oncogenic risk. There were significant differences in other 12 hr-HPV risk classification between CIN2+ and CIN3+ groups, CIN2+ group had more high- and medium-risk HPV genotypes than CIN3+ group, which might be that some CIN2 lesions were not the true precursor stage of CC [25]. While CIN3+, the immediate precursor lesion of CC, was a better predictor of CC compared with CIN2+ [26, 27]. Our results suggested that the risk-based HPV genotyping screening strategy could effectively improve the specificity in the detection of CIN2+ and/or CIN3+ compared to conventional HPV primary screening strategy with Yaneng extended or Cobas partial HPV genotyping and maintain high sensitivity, which would decrease unnecessary referrals for colposcopy in a screening setting for women aged 20–45 with hr-HPV infection. Previous studies have revealed that the number of colposcopies required to detect one case of CIN2+ by conventional primary screening strategy ranged from 6 to 8, and CIN3+ were 11–13 [28, 29], and the number of current study was slightly higher than previous study for detecting one CIN3+. The possible reason was that the participants in current analysis were aged 20–45 and the younger women would be more likely to clear the HPV virus due to their immunocompetence.

Our study adopted the international recognized method of HPV detection combined with cervical cytology for CC screening, which could fully assess the cervical diseases. In addition, we focused to evaluate the women aged 20–45 who had no previous abnormal results for CC screening, which could reduce the impact of past history and provide more accurate guidance for clinical application. There were also several limitations. Firstly, present study was lack of follow-up data on the natural history of different HPV genotypes infection as a cross-sectional study. Secondly, the sample size of current study was not large enough to accurately assess the oncogenic risk of rare HPV genotype.

Conclusions

We showed that extended HPV genotyping had good agreement with a well-validated partial HPV genotyping kit for hr-HPV detection consistency and primary CC screening accuracy. In addition, our new risk stratification strategy based on extended HPV genotyping not only retains the sensitivity but also greatly improves the specificity of CC primary screening. Which could reduce the unnecessary colposcopies and guide the clinical stratification management for HPV positive population. Taken all together, current study provided a new triage strategy for CC screening, but a larger sample study is still needed for evaluating the diagnostic accuracy of our risk-based screening strategy.

Acknowledgments: We thank Guangxi Center for Disease Prevention and Control, Hebei Provincial Center for Disease Control and Prevention, Zhejiang Provincial Center for Disease Control and Prevention, and Sichuan Center for Disease Control and Prevention for their hard work in a clinical trial of 9-valent vaccine.

Research funding: This study was provided by the National Key R&D Program of China (2021YFC2701204), Key Research and Development Program of Zhejiang Province, China (2023C03169, 2020C03025), the National Natural Science Foundation of China (Grant number 82003551), Zhejiang Province Medical and Health Technology Projects (Grant number WKJ-ZJ-2113).

Author contributions: X.H.Y: conceptualization, data curation, formal analysis, writing-original draft, writing-review and editing. R.X: data curation, writing-original draft, writing-review and editing. L.J.Y: data curation, writing-review. Y.L.F: data curation, writing-review. J.Y: data curation, writing-review and editing. L.J.Y: data curation, writing-review and editing. R.X: data curation, writing-original draft, writing-review & editing. W.X.Y: conceptualization, formal analysis, writing-original draft, writing-review & editing. W.H: conceptualization, formal analysis, writing-original draft, writing-review & editing. L.X: conceptualization, formal analysis, writing-original draft, writing-review & editing. W.X.Y: conceptualization, formal analysis, writing-original draft, writing-review & editing. Z.J: data curation, writing-review. W.H: conceptualization, formal analysis, writing-original draft, writing-review & editing. L.X: conceptualization, data curation, formal analysis, writing-original draft, writing-review & editing. All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Competing interests: Authors state no conflict of interest.

Informed consent: Informed consent was obtained from all individuals included in this study.
Ethical approval: This trial was approved by the Ethics Committee of Guangxi Center for Disease Prevention and Control (approval number: GXIRB2019-0044-1).

References


Supplementary Material: This article contains supplementary material (https://doi.org/10.1515/cclm-2023-0440).