Type-Specific Human Papillomavirus in Endocervical and Vaginal Self-Collected Specimens: Implications for Vaginal Self-Collection

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Abstract

Introduction

To elucidate why vaginal self-collection and high-risk human papillomavirus (HR-HPV) testing with Hybrid Capture® (hc2) has a lower sensitivity and specificity for cervical intraepithelial neoplasia grade 2 or worse (cCIN 2) [1], we determined the specific HPV types present in the endocervical and vaginal self-collected specimens in a cohort of women being screened for cervical cancer.

Methods

Between 5/06 and 4/07, a multi-center, population-based cross-sectional study was conducted in three rural sites in China to evaluate the association of specific HPV type with cCIN 2. Eligible women were age 16 to 54 years, non-pregnant, had no history of pelvic radiation, hysterectomy, previous treatment for cervical cancer, or seropositivity for HIV. Performing women performed vaginal self-collection (insert conical-shaped brush high into the vagina, rotate three times, and withdraw) then self-practitioner-collected specimens for HPV from the perineum, lower and upper vagina, and endocervix and cervical specimens for liquid-based cytology.

Endocervical and vaginal self-collected specimens were tested for HR-HPV with hc2. Linear Array® (Roche, Inc.), a PCR-based HPV genotype test, was performed on the brush specimens obtained from the endocervical, upper vaginal, lower vaginal, perineal, and vaginal self-collected specimens for women whose endocervical or vaginal self-collected specimens tested hc2 positive, on women with cCIN 2, and for a 3.4% random sample of the women whose endocervical and vaginal self-collected specimens tested hc2 negative.

Results

397 of 2,625 participating women had positive HR-HPV by hc2 in endocervical or vaginal self-collected specimens. Linear Array tests were obtained for these 397, for the single woman with negative HR-HPV by hc2 in endocervical and vaginal self-collected specimens but with cCIN 2, and for a random sample of 71 of the remaining 2,228 women. Colposcopy and biopsy was performed on 395 of the 405 eligible women. 47 of 2,625 women had a cCIN 2.

When tested with Linear Array, the 98 women that were HR-HPV by hc2 positive in the vaginal self-collected specimens and negative in the endocervical specimens, none of whom had a cCIN 2 (these 98 women result in the lower specificity of the vaginal self-collected specimens) have a lower prevalence of HR-HPV (8.2% vs. 49.0%) in the endocervical specimens and a lower prevalence of LR-HPV (11.2% vs. 32.7%) in the endocervical specimens. The 65 women that were HR-HPV by hc2 negative in the vaginal self-collected specimens and positive in the endocervical specimens, 8 of whom had a cCIN 2 (these 65 women result in the lower sensitivity of the vaginal self-collected specimens) have a higher prevalence of HR-HPV in the endocervical specimens (58.5% vs. 40.0%) and a similar prevalence of LR-HPV in the endocervical specimens (16.9% vs. 27.7%).

Of the 98 women that were HR-HPV by hc2 positive in the vaginal self-collected specimens but negative in the endocervical specimens, 48 (40.0%) had vaginal self-collected specimens that were positive for HR-HPV by Linear Array. Forty-one of these 48 women had negative HR-HPV by Linear Array in their endocervical specimens. These 41 women are a subset of the 59 women with positive HR-HPV by Linear Array in the vaginal self-collected specimens but negative in the endocervical specimens. None of these 59 women had a cCIN 2. The mean self-collected specimen hC2 signal strength, a semiquantitative measure of viral load, for these 59 women (19.8 RLU/CO) was lower than the mean self-collected specimen signal strength of the 225 women with HR-HPV by Linear Array in both the self-collected and endocervical specimens (286.1 pg RLU/CO, p < .001, t-test).

The proportion of type 16 or 18 HPV in women HR-HPV positive by Linear Array in the endocervical specimens (39.6%, 99/250) did not differ from that in the self-collected vaginal specimens (38.0%, 108/284, p=.71, Chi-Square). Among the 38 women with a cCIN 2 with paired endocervical and vaginal self-collected specimens that both tested hc2 positive, the mean signal strength of the endocervical specimens (650.2 RLU/CO) was greater than that of the self-collected specimens (264.7 RLU/CO, paired t-test, p = .003).

Conclusions

The vaginal self-collection specimen’s lower sensitivity for cCIN 2 of HR-HPV testing by hc2 is secondary to the self-collection specimen obtaining fewer cells. The lower specificity is secondary to the presence of HR-HPV solely in the vagina which is not associated with a cCIN 2 and to a higher prevalence of LR-HPV in the vaginal self-collection which cross-reacts with hc2 [2]; it is not related to a predilection of type 16 or 18 HPV for the endocervix. One possible explanation for the HR-HPV present solely in the 59 vaginal self-collected specimens is that incident HPV infections may ascend from the vulvo-vaginal area to the cervix [3].

The sensitivity of the vaginal self-collected specimen might be increased to that of the practitioner-collected endocervical specimen either by changing the collection device so it is easier for women to obtain a larger specimen or using an HR-HPV assay with a lower cut-point (similar to Linear Array). As there is HR-HPV present solely in the vaginal self-test which is not associated with a cCIN 2, regardless of the assay used, the specificity of the vaginal self-collected specimen for HR-HPV will likely remain lower than that of the endocervical specimen.

In collaboration with the N.I.H. we are evaluating a vaginal self-collecting device with a smooth sleeve and a larger swab designed to obtain a larger specimen. As it is difficult to mail liquids, the ideal vaginal self-collection HR-HPV test would not require a liquid transport medium.

BIBLIOGRAPHY


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Introduction

When compared with high-risk human papillomavirus (HR-HPV) testing with Hybrid Capture® (hc2) of practitioner-collected endocervical specimens, vaginal self-collected specimens have a lower sensitivity and specificity for cervical intraepithelial neoplasia grade 2 or worse (≥CIN 2) [1]. The lower sensitivity of the vaginal self-collected specimens could be secondary to a lower prevalence of HR-HPV and specifically, type 16 HPV in the vagina [2]. The lower specificity of the vaginal self-collected specimens could be secondary to cross-reaction of hc2 with LR-HPV [3], LR-HPV having been reported to have a higher prevalence in the vagina than in the endocervix [4]. To further our understanding of vaginal self-collection and HR-HPV testing, we determined the specific HPV types present in the endocervix, upper and lower vagina, perineum, and vaginal self-collected specimens in a cohort of women being screened for cervical cancer.

Methods

Between 5/06 and 4/07, a multi-center, population-based cross-sectional study was conducted in three rural sites in China to evaluate the association of specific HPV type with ≥CIN 2. Eligible women were age 16 to 54 years, non-pregnant, had no history of pelvic infection, hysterectomy, previous treatment for cervical cancer, or seropositivity for HIV. Participating women performed vaginal self-collection (insert conical-shaped brush high into the vagina, rotate three times, and withdraw) then had practitioner-collected specimens for HPV from the perineum, lower and upper vagina, and endocervix and cervical specimens for liquid–based cytology.

Endocervical and vaginal self-collected specimens were tested by hc2. Linear Array® (Roche, Inc.), a PCR-based HPV genotype test, was performed on the brush specimens obtained from the endocervical, upper vaginal, lower vaginal, perineal, and vaginal self-collected specimens for women whose endocervical or vaginal self-collected specimens tested hc2 positive, on women with ≥CIN 2, and for a 3.4% random sample of the women whose endocervical and vaginal self-collected specimens were hc2 negative.

Women whose endocervical or vaginal self-collected specimens were hc2 positive or had cervical cytology other than normal or ASC-US underwent colposcopy with the Preventive Oncology International, Inc. (PO.I.) five-microbiopsy protocol. [1]

Results

397 of 2,625 participating women had positive HR-HPV by hc2 in endocervical or vaginal self-collected specimens. Linear Array tests were obtained for these 397, for the single woman with negative HR-HPV by hc2 in endocervical and vaginal self-collected specimens but with ≥CIN 2, and for a random sample of 71 of the remaining 2,228 women. Colposcopy and biopsy was performed on 395 of the 405 eligible women. 47 of 2,625 women had ≥CIN 2.

The sensitivity for ≥CIN 2 of HR-HPV testing with hc2 in the endocervix (97.9%) is similar to that of HR-HPV testing with hc2 in the upper vagina (91.5%), but higher than that of HR-HPV testing with hc2 in the lower vagina (85.1%), perineum (46.8%), or vaginal self-collected specimens (80.9%). With Linear Array testing for HR-HPV, the sensitivities for ≥CIN 2 of the five anogenital sites are similar.

The signal strength as measured by hc2 in the endocervical specimens exceeds that in the vaginal specimens. The signal strength in the upper vaginal specimens exceeds that in the lower vaginal specimens. None of the women with positive Linear Array tests for HR-HPV in the upper vagina (N=65), lower vagina (N=66), perineum (N=52), or vaginal self-collected specimens (N=59) but negative Linear Array tests for HR-HPV in the endocervix had ≥CIN 2.

Conclusions

The lower sensitivity of the vaginal self-collected specimens with HR-HPV testing by hc2 is related to their lower HR-HPV viral loads. If hc2 is the test for HR-HPV, obtaining a larger specimen from the upper vagina should result in a higher sensitivity for ≥CIN 2. We, in collaboration with the N.I.H., are evaluating a vaginal self-collecting device with a smooth sleeve and a larger swab that helps women obtain a larger specimen from the upper vagina. An alternative way to increase the sensitivity of the vaginal self-collection is to use a more sensitive HR-HPV assay (similar to Linear Array). The lower specificity of the vaginal self-collected specimens with HR-HPV testing by hc2 is secondary to HR-HPV present solely in the vagina and perineum that is not associated with ≥CIN 2 and to cross-reaction of LR-HPV found in excess in the vagina.

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Prevalence of Type-Specific Human Papillomavirus (HPV) in the Endocervix, Upper Vagina, Lower Vagina and Perineum; Implications For Vaginal Self-Collection

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Table 1: Weighted prevalence of HR-HPV and LR-HPV in five anogenital sites

<table>
<thead>
<tr>
<th>Anogenital Site</th>
<th>HR-HPV</th>
<th>LR-HPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endocervix</td>
<td>9.5%</td>
<td>5.1%</td>
</tr>
<tr>
<td>Upper Vagina</td>
<td>14.2%</td>
<td>12.9%</td>
</tr>
<tr>
<td>Lower Vagina</td>
<td>14.0%</td>
<td>19.8%</td>
</tr>
<tr>
<td>Perineum</td>
<td>14.8%</td>
<td>14.3%</td>
</tr>
<tr>
<td>Vaginal Self-collection</td>
<td>12.0%</td>
<td>7.6%</td>
</tr>
</tbody>
</table>

Prevalence of LR-HPV in the lower vagina (19.8%) differed significantly from LR-HPV in endocervix (5.1%), p<.0004. Other differences in HPV prevalence were not significant.

Table 2: Sensitivity for ≥CIN 2 of HR-HPV by hc2 and HR-HPV by Linear Array for five anogenital sites

<table>
<thead>
<tr>
<th>Anogenital Site</th>
<th>Sensitivity for ≥CIN 2 of HR-HPV by hc2</th>
<th>Sensitivity for ≥CIN 2 of HR-HPV by Linear Array</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endocervix</td>
<td>97.9%</td>
<td>100.0%</td>
</tr>
<tr>
<td>Upper Vagina</td>
<td>91.5%</td>
<td>97.9%</td>
</tr>
<tr>
<td>Lower Vagina</td>
<td>85.1%</td>
<td>95.7%</td>
</tr>
<tr>
<td>Perineum</td>
<td>46.8%</td>
<td>91.5%</td>
</tr>
<tr>
<td>Vaginal Self-collection</td>
<td>80.9%</td>
<td>96.7%</td>
</tr>
</tbody>
</table>

Table 3: Mean signal strength (RLU/CO) as measured by hc2 in 34 women with ≥CIN 2 that have positive HR-HPV by hc2 in the endocervix, upper and lower vaginal, and vaginal self-collected specimens

<table>
<thead>
<tr>
<th>Anogenital Site</th>
<th>Endocervix</th>
<th>Upper Vagina</th>
<th>Lower Vagina</th>
<th>Vaginal Self-collection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean RLU/CO</td>
<td>688.2 ± 2.2 1</td>
<td>118.0 ± 1.1 1</td>
<td>51.2 ± 2.1 1</td>
<td>273.5 ± 4.9 1</td>
</tr>
</tbody>
</table>

1688.2 vs. 118.0, p<.001; 688.2 vs. 51.1, p<.001; 688.2 vs. 273.5, p<.004; 118.0 vs. 51.2, p<.05 (all paired t-test with two degrees of freedom)