Method for Testing for Human Papillomavirus Infection in Patients with Cervical Intraepithelial Disease

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Cervical cancer is the fifth-leading cause of death by cancer of women worldwide, with about 258,000 patients dying in 2001 (18). The current screening method is based on the cytological classification of cervical smears according to the Bethesda system (11), leading to colposcopy and histological sampling. However, this test is not perfect, and false-negative rates of 5 to 50% have been reported (5, 16).

Human papillomavirus (HPV) infection has been shown to be the cause of greater than 99% of all cases of cervical cancer (17). More than 80 types of HPV have been identified; about 40 infect the genital tract, but only 10 to 15 types cause cancer (1, 6). To meet the increasing demand for testing, the Hybrid Capture II (HC II) (Digene, Inc., Gaithersburg, Md.) assay is being used on unamplified tissue DNA, and it allows for a degree of quantitation of the viral DNA in specimens (4). We evaluated the performance of HC II in detecting high-grade squamous intraepithelial lesions (HSILs) in patients with high-risk HPV (HR-HPV), estimated the optimal cutoff value for Taiwanese patients, and investigated its utility in follow-up testing of patients with cervical intraepithelial disease.

Eight hundred two women who underwent cervical cancer screening at the Department of Obstetrics and Gynecology and Health Center of the Cathay General Hospital, Taipei, Taiwan, were recruited for the study between June 2001 and January 2003. The age range was 18 to 85 years. Seventy-seven women who had histologically confirmed high-grade cervical intraepithelial diseases or cancer were enrolled for receiver operating characteristic (ROC) analysis. Forty-eight patients with a history of SILs who had been treated with cervical conization were monitored by serial HR-HPV DNA testing.

Cervical scrapes for HR-HPV DNA testing were collected with the Digene Cervical Sampler. All scrapes were analyzed for the presence of HR-HPV (types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) by enzyme-linked immunosorbent assay. Samples were classified as positive if the relative light unit reading obtained from the luminesmeter was equal to or greater than the mean of the three positive control values supplied by the kit. Histological results from loop electrosurgical excision procedures or endocervical curettage were included in the disease diagnosis.

The HR-HPV viral load related to the stage of the disease was compared by analysis of variance. ROC analysis for detecting high-grade cervical intraepithelial lesions and cancer by HC II assay was based on different cutoff values. Sensitivity and specificity were determined by comparing the results to those of histology. Ninety-five percent confidence intervals (CIs) for these values were assessed using either binomial or normal distribution, according to the data. The differences in HR-HPV DNA data at various conditions were compared using chi-square statistics, with the P value set to 5%.

Table 1 presents the prevalence, according to age, of HR-HPV infection in 802 women. One hundred forty-six (18.2%) out of 802 women were positive for HR-HPV DNA. There was a peak in the rate of infection in women under 30 years of age (29.2% of women), with a decrease in the rate of infection up to 60 years of age. Thirty-nine (47.0%) out of the 83 women with a history of low- or high-grade lesions were infected with HR-HPV; however, only 107 (14.9%) out of 719 women without a medical history of SIL (P < 10−11) were infected.

The diagnoses as shown in Table 2 were based on the results of pathological biopsy from colposcopy. HR-HPV positivity among the participants was 60 of 77 (77.9%) at a cutoff value of 1.0 pg/ml. Sensitivity was 94.7% (95% CI, 73.9 to 99.1), and specificity was 27.6% (95% CI, 16.7 to 40.9) (P < 0.05). The viral load related to the stage of the disease confirmed by colposcopic examination does not have significance (P = 0.37).

ROC analysis was subsequently used to estimate the best sensitivity and specificity for detecting HSIL and cancer. Patients with normal, atypical squamous cells of undetermined significance (ASCUS) and low-grade squamous intraepithelial lesions (LSIL) were combined into one category and analyzed versus the group of patients with cancers and high-grade precancerous lesions. At the 1.86 pg/ml cutoff value, the test was associated mostly with the detection of high-grade lesions and cancers in our cohort (Table 2). Sensitivity was 94.7% (95% CI, 73.9 to 99.1), and specificity ascended to 36.2% (95% CI, 24.0 to 49.9) (P < 0.01). We repeated the ROC analysis for patients older than 30 years. The test performance is optimal where **Corresponding author. Mailing address: Graduate Institute of Medical Technology, College of Medicine, National Taiwan University, No. 7, Chung-Shan South Road, Taipei 100, Taiwan, ROC. Phone: (886) 2-23123456x6094. Fax: (886) 2-23711574. E-mail: jtkao@ha.mc.ntu.edu.tw.
sensitivity is sustained (94.4% [95% CI, 72.6 to 99.1] versus 94.7%) but specificity is increased (40.4% [95% CI, 27.0 to 54.9]) versus 36.2%) (P < 0.01).

Finally, we evaluated the utility of the HC II assay in determining the prognosis of patients who were monitored for treatment of cervical intraepithelial disease. Among the 48 patients with HSIL or cervical cancer who were monitored, 10 patients developed recurrent disease. Eight out of 10 patients with a posttherapeutic appearance of HR-HPV DNA had recurrent symptoms such as metaplasia, LSIL, HSIL, or recurrent cancer. Five patients had another surgery within 17 months (range, 12 to 17 months) after the conization therapy. HR-HPV DNA was detected at 1.62 and 1.89 pg/ml in 2 of the 38 patients without recurrences 15 and 21 months, respectively, after conization treatment. There was no clinically abnormal finding by cytological or histological exam. Three patients temporarily displayed decreasing values after surgical treatment (range, 3 to 10 months), and the HR-HPV DNA disappeared in the subsequent follow-up period (range, 4 to 8 months). Persistent appearance of HR-HPV DNA after surgical treatment was a high-risk factor for recurrence of cervical intraepithelial disease (P < 0.001).

In this study, we used the HC II assay on 802 randomly selected women, which clearly underlines the significance of the prevalence and/or persistence of HR-HPV infection in the women presenting precancerous and malignant cervical lesions. Its sensitivity was quite high and was similar to that found for HCII assays in other studies (2, 14) and to PCR with consensus primers (1), but the specificity was not as good as in other studies. The prevalence of HR-HPV infection in women under 30 years old (29.2%) is also similar to that reported previously (3, 8). The high frequency of HR-HPV infection in women older than 60 years was due to sixteen (29.1%) out of fifty-five women having a history of cervical lesions. The high positive rate may be due to genital HPV infections being the most prevalent sexually transmitted infections (15), but it is also partly due to the technique used, with the possibility of cross-hybridization with other HPV types not included in the HC II assay probe cocktails (3), which may increase the number of positive results.

In this study, when the positivity threshold increased to 1.86 pg/ml, it did not change the sensitivity but became more specific (36.2 versus 27.6%) for colposcopy referral. It is approximate to that reported by Schiffman et al. (14) at 1.0 pg/ml and Cuzick et al. (5) at 2.0 pg/ml. Semi-quantitation provided by the HC II assay relates to the concentration of viral DNA per milliliter of specimen transport medium but does not control for variability in lesion size, specimen adequacy, or viral copy per infected cell. Lorincz et al. (10) reported that although the presence of HPV strongly increased the risk of cervical cancer, high viral load did not predict a further risk of cancer. As we observed in our study, the quantitative approach for the assessment of the viral load could not clearly distinguish between patients with or without high-grade lesions. In the future, combinations of techniques and more-focused screening strategies hold the promise of making screening more effective, safer, and less costly.

HR-HPV DNA testing uses DNA molecular technology to detect the presence of HR-HPV even before visible cell changes occur. The test is currently approved for use following an equivocal cervical cytologic result with ASCUS (4), Rozendaal et al. (12) have emphasized that women with normal smears and HPV genotypes are 116 times more at risk of developing HSIL than women without HPV. However, most cervical precancers grow slowly and can be removed or treated successfully; some women seem to be overscreened and overtreated. New guidelines on cervical cancer screening have been issued by the American Cancer Society (13). The guidelines mention that HPV positivity might be useful in detecting early cervical cancer in women over 30 years of age. Because a very high percentage of women with cytologic evidence of normal to low-grade SIL are positive for HR-HPV DNA and because numerous HPV infections are known to regress spontaneously (7, 9), there is limited potential for HR-HPV testing to direct decisions about the clinical management of these women.

Our results indicate that the appearance of HR-HPV DNA after conization therapy is a high-risk factor for recurrence of cervical intraepithelial disease (P < 0.001). Statistically, HR-HPV DNA testing has better performance as a follow-up method than as a screening method for this disease. In conclusion, HR-HPV DNA testing is not only a good screening method but is also a useful follow-up method for the monitoring of HR-HPV DNA in patients with cervical intraepithelial disease.

We thank Pei-Ling Yeh for the data collection.

### TABLE 1. Prevalence of HR-HPV infections according to age

<table>
<thead>
<tr>
<th>Age group (yr)</th>
<th>Women</th>
<th>HR-HPV positive women</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. (%)</td>
<td>History</td>
</tr>
<tr>
<td>≤30</td>
<td>72 (9.0)</td>
<td>5 (6.9)</td>
</tr>
<tr>
<td>31–40</td>
<td>274 (34.1)</td>
<td>24 (8.8)</td>
</tr>
<tr>
<td>41–50</td>
<td>283 (35.3)</td>
<td>28 (9.9)</td>
</tr>
<tr>
<td>51–60</td>
<td>118 (14.7)</td>
<td>10 (8.5)</td>
</tr>
<tr>
<td>&gt;60</td>
<td>55 (6.9)</td>
<td>16 (29.1)</td>
</tr>
<tr>
<td>Total</td>
<td>802 (100)</td>
<td>83 (10.4)</td>
</tr>
</tbody>
</table>

* History of low- or high-grade lesions.
* Percentage of total number of women in study.
* Percentage of women in age group.
* Percentage of number of HR-HPV-positive women in age group.

### TABLE 2. Evaluation of HR-HPV testing at cutoff points 1 and 1.86 as a screening method for cervical intraepithelial disease as confirmed by histological diagnosis

<table>
<thead>
<tr>
<th>Histological diagnosis</th>
<th>No. diagnosed</th>
<th>No. of HPV-positive diagnoses at indicated cutoff point</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Normal</td>
<td>15</td>
<td>9 (60.0)</td>
</tr>
<tr>
<td>ASCUS</td>
<td>8</td>
<td>5 (62.5)</td>
</tr>
<tr>
<td>LSIL</td>
<td>35</td>
<td>28 (80.0)</td>
</tr>
<tr>
<td>HSIL&lt;sup&gt;b&lt;/sup&gt;</td>
<td>19</td>
<td>18 (94.7)</td>
</tr>
<tr>
<td>Total</td>
<td>77</td>
<td>60 (77.9)</td>
</tr>
</tbody>
</table>

* ASCUS indicates atypical squamous cells of undetermined significance; LSIL indicates a low-grade squamous intraepithelial lesion; and HSIL indicates a high-grade squamous intraepithelial lesion.
<sup>b</sup> HSIL included a histologically defined high-grade squamous intraepithelial lesion and cervical cancer.
REFERENCES


