Clinical Performance of Hybrid Capture 2 Human Papillomavirus Testing for Recurrent High-Grade Cervical/Vaginal Intraepithelial Neoplasm in Patients With an ASC-US Papanicolaou Test Result During Long-Term Posttherapy Follow-up Monitoring

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• ***Context.***—**Women who have been treated for high-grade cervical or vaginal intraepithelial neoplasia (CIN or VAIN) or invasive carcinoma are at risk for recurrent/persistent disease and require long-term monitoring. The role of human papillomavirus (HPV) testing in this setting is unclear.**

Objective.—**To evaluate the clinical performance of the Hybrid Capture 2 (HC2) HPV test for recurrent/residual high-grade CIN or VAIN in patients with a posttherapy abnormal squamous cells of undetermined significance (ASC-US) Papanicolaou test result.**

Design.—**We reviewed the follow-up data on 100 patients who had an ASC-US Papanicolaou test and HC2 HPV results after treatment for high-grade CIN/VAIN or carcinoma. Human papillomavirus genotyping was performed for women with a negative HC2 result whose follow-up biopsy revealed CIN/VAIN 2+.**

Results.—**The patients' mean age was 47 years. The HC2 test result was positive in 33% of the patients. Follow-up biopsy was available for 17 of these patients (52%) and for 25 of the 67 patients (37%) with a negative HC2 result. A total of 5 of the patients (29%) with a positive HC2 result and 2 of the patients (8%) with a negative HC2 result had CIN/VAIN 3 on follow-up biopsy, a statistically insignificant difference ($P = .10$). Human papillomavirus 16/18 genotypes were detected in the CIN/VAIN 2+ lesions of 5 patients with a negative HC2 result.**

Conclusions.—**HC2 yielded a false-negative rate of 8% for CIN 3. HC2 testing therefore may not be sufficient for triage of patients with an ASC-US Papanicolaou test result. Patients with ASC-US during long-term posttherapy follow-up need close monitoring, with colposcopic evaluation if clinically indicated.**

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Women who have been treated for high-grade cervical or vaginal intraepithelial neoplasia (CIN/VAIN 2 or 3, referred to here as CIN/VAIN 2+) or invasive carcinoma are at risk for recurrent/persistent lesions (5%–15%)[1–5] and at higher than normal risk for invasive carcinoma.[6,7] Posttherapy follow-up monitoring is critical for early detection of these recurrent/residual precancerous or cancerous lesions. According to the 2006 guidelines of the American Society for Colposcopy and Cervical Pathology (ASCCP), acceptable posttherapy management options for women with CIN 2+ include human papillomavirus (HPV) DNA testing or Papanicolaou test (Pap) cytologic testing with or without colposcopy at 6- to 12-month intervals.[8] The 2012 clinical management guidelines for screening for cervical cancer issued by the American College of Obstetricians and Gynecologists, as well as the 2012 clinical pathology screening guidelines for the prevention and early detection of cervical cancer, recommended screening for 20 years after initial posttherapy surveillance.[9,10] Recently, the 2012 updated ASCCP consensus guidelines for the management of abnormal cervical cancer screening test results and cancer precursors further recommended HPV and Pap cytology cotesting for posttherapy follow-up.[11]

DNA testing for high-risk HPV genotypes has been reported to be highly sensitive in detecting recurrent/residual CIN/VAIN 2+.[12–17] A commercial HPV testing assay, Hybrid Capture 2 (HC2; Qiagen, Valencia, California), which collectively detects 13 high-risk HPV genotypes, was reported to be more sensitive than Pap cytology in predicting CIN/VAIN 2+ in posttherapy follow-up.[18–20]

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Combined HC2 HPV DNA testing and Pap cytology has even greater sensitivity in detecting recurrent/residual CIN/VAIN 2+ lesions in this setting.[1,19–21]

Pap cytology is routinely used at our hospital for follow-up monitoring of women who have been treated for CIN/VAIN 2+ or cervical carcinoma. Colposcopic evaluation is usually required for patients who have Pap results of abnormal squamous cells of undetermined significance, cannot exclude high-grade squamous intraepithelial lesion (ASC-H); low-grade squamous intraepithelial lesion (LSIL); or high-grade squamous intraepithelial lesion (HSIL). However, management of cases of abnormal squamous cells of undetermined significance (ASC-US) during post-therapy follow-up can be challenging and complicated. Chemotherapy and/or radiation therapy can cause treatment effects in Pap specimen cells that mimic dysplastic cells and make interpretation difficult. Moreover, atrophy is frequent in the relatively older cancer patient population. Together, these changes may cause inadequate sampling, leading to an ASC-US Pap result. To better predict the risk of recurrent/residual CIN/VAIN 2+ lesions, the HC2 HPV DNA test has been used in many Pap test cases diagnosed as ASC-US in our hospital.

In most published studies using the HC2 assay, the sensitivities for CIN 2+ were compared between HC2 and Pap cytology for all cytologic diagnoses of ASC-US, LSIL, and HSIL.[1,19,20] The risk of CIN 2+ after colposcopic examination in women who had an ASC-US Pap result with HC2 result has been reported previously.[22] However, it remains unclear whether HC2 HPV testing is efficient in predicting CIN 2+ in women with ASC-US during posttherapy follow-up. The aim of the study was to determine the clinical performance of HC2 HPV testing for predicting recurrence/residual disease in women with an ASC-US Pap result following treatment for CIN/VAIN 2+ or invasive carcinoma. In this study, we retrospectively reviewed posttherapy follow-up data on women who had an ASC-US Pap result and underwent HC2 HPV DNA testing. All of the patients had been treated for a CIN/VAIN 2+ lesion or invasive carcinoma and monitored after therapy by the Department of Gynecologic Oncology and Reproductive Medicine at our institution.

MATERIALS AND METHODS

Patient Selection

We retrospectively reviewed data on 1308 patients who had an ASC-US Pap test result reviewed by the Department of Pathology at our institution during the period 2006–2009 and underwent reflex HC2 HPV DNA testing. Of these, a total of 100 women were qualified for the study on the basis of our selection criteria, which included the following: (1) a history of treatment for either CIN/VAIN 2+ or invasive carcinoma; (2) a posttherapy ASC-US Pap result and HC2 HPV DNA result; and (3) availability of follow-up test results, including biopsy findings or Pap cytology results with or without HC2 HPV DNA retest. Women without either a follow-up biopsy result or Pap cytology result were excluded from the study. The protocol for this study was approved by our center's Institutional Review Board.

Pap Cytology and Biopsy Specimens

SurePath Pap specimens (BD Diagnostics–TriPath Imaging, Burlington, North Carolina) were screened by cytotechnologists and verified by cytopathologists following the Bethesda System terminology for reporting Pap test results.[23] The follow-up biopsy specimens were processed and interpreted in the Department of Pathology at our institution.

HPV DNA Testing

Human papillomavirus DNA was analyzed by the Hybrid Capture 2 assay (Qiagen, Valencia, California), which collectively tests for 13 high-risk HPV DNA types. The residual SurePath specimen was sent to Quest Diagnostics (Houston, Texas) for HC2 HPV testing. For HPV genotyping, DNA was extracted from biopsy or SurePath Pap specimens using the DNeasy kit (catalog 69506, Qiagen) according to the manufacturer's instructions. Human papillomavirus genotyping was done with EasyChip HPV Blot (King Car Yuanshan Research Institute, I-Lan, Taiwan) as described previously.[24] The EasyChip HPV Blot was designed to detect 38 HPV types (HPV types 6, 11, 16, 18, 26, 31, 32, 33, 35, 37, 39, 42, 43, 44, 45, 51, 52, 53, 54, 55, 56, 58, 59, 61, 62, 66, 67, 68, 69, 70, 71, 72, 74, 81, 82, 83, 84, and 85, as well as 3 intrinsic controls).

Patient Follow-up

Follow-up data were collected at a mean interval of 30 months after the ASC-US Pap result and HC2 testing (range, 0–67 months) were obtained.

Statistical Analysis

Descriptive statistics were used for data analysis; the Fisher exact test was employed to assess the association between categoric variables. *P* values (2-sided test) less than .05 were considered significant. All computations were carried out using SAS version 8.0 (SAS Institute, Cary, North Carolina).

RESULTS

Patients and Clinical History

Patient ages at the time of the ASC-US Pap test result, during posttherapy follow-up, ranged from 15 to 82 years, with a mean of 47 years. Of the 100 patients, 56 were white, 22 were Hispanic, 17 were African American, and 5 were Asian. Their clinical histories included invasive squamous carcinoma (38%); cervical adenocarcinoma, including 2 cases of adenocarcinoma in situ (10%); CIN 2/3 (35%); VAIN 2/3 (6%); and unclassified high-grade dysplasia (11%). The patients underwent one or more treatments, including surgical treatment, such as cold-knife conization, loop electrosurgical excision procedures, or hysterectomy; ablation, such as cryoablation or laser ablation; and/or chemotherapy and/or radiation therapy (Table 1). Mean interval from initial diagnosis to ASC-US Pap test result and HC2 testing was 87 months (range, 5–420 months).

HC2 HPV DNA Testing Results and Patient Follow-up

Of the 100 ASC-US Pap test specimens, 56 were cervical and 44 were vaginal specimens. A total of 17 of 56 cervical specimens (30%) and 16 of 44 vaginal specimens (36%) were positive for HPV using the HC2 assay. A total of 25 of the 67 patients (37%) with a negative HC2 result and 17 of the 33 patients (52%) with a positive HC2 result underwent follow-up biopsy (Table 2). The mean interval between the Pap/HC2 testing and the follow-up biopsy was 5.1 months. Of these 42 biopsies, 27 were cervical and 15 were vaginal. Of the 27 cervical follow-up biopsies, 11 were from patients with a positive HC2 result and 16 were from patients with a negative HC2 result. A CIN 3 lesion was observed in 4 patients with a positive HC2 result and 1 patient with a negative HC2 result. The difference in rate of CIN 3 on follow-up biopsy between the two groups was not statistically significant ($P = .13$). Of the 15 vaginal follow-up biopsies, 6 were from patients with a positive HC2 result

Table 1. Treatments for Invasive Carcinoma or Cervical Intraepithelial Neoplasia/Vaginal Intraepithelial Neoplasia 2+ (N = 100)

Treatment	No. of Patients
LEEP	21
Conization	11
LEEP + conization	6
Cryoablation/laser ablation	11
Hysterectomy	12
Radiotherapy	14
Chemotherapy	3
Chemotherapy + radiotherapy	14
Hysterectomy + radiotherapy	6
Hysterectomy + chemotherapy/radiotherapy	2
Total	**100**

Abbreviation: LEEP, loop electrosurgical excision procedure.

and 9 were from patients with a negative HC2 result. A VAIN 3 lesion was diagnosed in 1 patient with a positive HC2 result and 1 patient with a negative HC2 result. The difference in rate of CIN/VAIN 3 on follow-up biopsy between the two groups was not statistically significant ($P > .99$). The risk of CIN/VAIN 3 among patients with a negative HC2 result was 8% (2 of 25), and among patients with a positive HC2 result it was 29% (5 of 17; $P = .10$). The risk of CIN/VAIN 2+ was similar in women with a positive HC2 result (29%) and those with a negative HC2 result (28%).

Follow-up Pap cytology results for the 58 patients without biopsy follow-up are illustrated in Table 3. Of these 58 patients, none had an HSIL Pap result. A total of 42 patients had a negative HC2 result, and 3 of those (7%) had ASC-US/LSIL on follow-up Pap cytology. Of the 16 patients with a positive HC2 result, 7 (44%) had ASC-US/LSIL on follow-up Pap (Table 3). The difference between the two groups for the rates of ASC-US/LSIL on follow-up Pap was statistically significant ($P = .003$).

HC2 HPV DNA retest data were available for 29 patients with a baseline negative HC2 result and 17 patients with a baseline positive HC2 result. Of the 29 patients with a baseline negative HC2 result, 1 (4%) had a follow-up positive HC2 result. Of the 17 patients with a baseline positive HC2 result, 3 (18%) had a follow-up positive HC2 result.

HPV Genotyping

A total of 7 patients with a negative HC2 result had a follow-up biopsy showing CIN/VAIN 2+. Biopsy findings were CIN 2 in 2 patients, CIN 3 in 1 patient, VAIN 2 in 3 patients, and VAIN 3 in 1 patient. Human papillomavirus genotyping was done on 6 of the 7 biopsy specimens, 5 of which demonstrated high-risk HPV genotypes, including HPV 16/18 (Table 4). Of these 7 patients with an ASC-US Pap result and a negative HC2 result, only 1 patient (case number 3 in Table 4) underwent biopsy on the basis of ASC-US Pap result only. The other 6 patients underwent follow-up colposcopic evaluation and biopsy on the basis of previous cytology or biopsy results from outside hospitals/institutions, either abnormal Pap and HC2 results or abnormal biopsy results (Table 4).

Table 2. Comparison of Hybrid Capture 2 (HC2) and Follow-up Biopsy Results (N = 42)

Biopsy Findings	Negative HC2, No. (%)	Positive HC2, No. (%)	Total	*P* Value
Cervical				
Benign/CIN 1	13	7	20	
CIN 2	2	0	2	
CIN 3	1 (6)	4 (36)	5	.13
Vaginal				
Benign/VAIN 1	5	5	10	
VAIN 2	3	0	3	
VAIN 3	1 (11)	1 (17)	2	>.99
Total	**25**	**17**	**42**	

Abbreviations: CIN, cervical intraepithelial neoplasia; VAIN, vaginal intraepithelial neoplasia.

Table 3. Follow-up Papanicolaou (Pap) Cytology in Patients Without Follow-up Biopsy (N = 58)

Pap Cytology	Negative HC2, No. (%)	Positive HC2, No. (%)	*P* Value
Negative	39	9	
ASC-US/LSIL	3 (7)	7 (44)	.003
Total	**42**	**16**	

Abbreviations: ASC-US, abnormal squamous cells of undetermined significance; HC2, Hybrid Capture 2; LSIL, low-grade squamous intraepithelial lesion.

HC2 Testing Efficacy

Of 25 biopsies from patients with a negative HC2 result, 2 were CIN/VAIN 3, representing a false-negative rate of 8%. In predicting CIN/VAIN 3 lesions, HC2 showed 71.4% sensitivity, 65.7% specificity, 29.4% positive predictive value, and 92.0% negative predictive value.

COMMENT

In this retrospective study, we reviewed data on 100 patients who had an ASC-US Pap test result and underwent HC2 HPV DNA testing during long-term posttherapy follow-up monitoring. We found that the risk of CIN/VAIN 3 in women with a positive HC2 result (29%) was higher than that in women with a negative HC2 result (8%). However, the rates of recurrent/residual CIN/VAIN 3 between the two groups were not significantly different ($P = .10$). The risk of CIN 2+ was similar in women with a positive HC2 result (29%) and those with a negative HC2 result (28%). Human papillomavirus 16/18 genotypes were detected in 5 biopsies from patients with a negative HC2 result and recurrent/residual CIN/VAIN 2+, indicating false-negative HC2 results. Our findings demonstrate that the HC2 assay has relatively low sensitivity for triaging patients with an ASC-US Pap test result during long-term posttherapy follow-up. Consequently, patients with an ASC-US Pap test result and a negative HC2 result during posttherapy follow-up require further evaluation, such as colposcopic examination or repeat Pap/HPV testing, within a short interval of time.

We observed a relatively high rate of recurrent/residual CIN/VAIN 2+ in these 100 women (12%), indicating the considerable risk for recurrent/residual CIN/VAIN 2+ in patients with ASC-US during long-term posttherapy follow-up. In short-term follow-up, rates of recurrent/residual CIN/VAIN 2+ ranged from 7% to 17.9%.[18–20,25–27] There are very few reports of long-term follow-up monitoring studies. In a large cohort of patients treated for CIN 3, Melnikow et al[5] reported a rate of recurrent/

Table 4. High-Risk Human Papillomavirus (HPV) Genotypes in Cervical Intraepithelial Neoplasia/Vaginal Intraepithelial Neoplasia 2+ (CIN/VAIN 2+) Follow-up Biopsy Specimens From Patients With Abnormal Squamous Cells of Undetermined Significance (ASC-US) Papanicolaou (Pap) and Negative Hybrid Capture 2 (HC2) Results (N = 7)

Age, y	Pap	Primary Diagnosis	Treatment	Follow-up Biopsy	HPV Type	Time, mo/Reason for Biopsy
48	Cervical	CIN 2	LEEP	CIN 2	N/A	0/previous HSIL
19	Cervical	CIN 3	LEEP	CIN 2	18/35	2/previous ASC-H
32	Cervical	CIN 3	LEEP	CIN 3	–	0/ASC-US
43	Vaginal	Carcinoma	TAH	VAIN 2	16/18/52	2/previous NILM
52	Vaginal	VAIN 2	Ablation	VAIN 2	18/58	6/previous ASC-US/HC2+
55	Vaginal	VAIN 3	Excision	VAIN 2	16/18	1/previous LSIL
52	Vaginal	CIN/VAIN 3	TAH	VAIN 3	18/33	1/previous HSIL

Abbreviations: ASC-H, abnormal squamous cells of undetermined significance, cannot exclude HSIL; HSIL, high-grade squamous intraepithelial lesion; LEEP, loop electrosurgical excision procedure; LSIL, low-grade squamous intraepithelial lesion; N/A, not available; NILM, negative for intraepithelial lesion or malignancy; TAH, total abdominal hysterectomy; –, negative result.

residual CIN 2+ of 14%. Kreimer et al[17] reported a 1.7% rate of recurrent/residual CIN 2+ in a long-term follow-up study.

To the best of our knowledge, our study is the first report on HC2 efficacy for women with an ASC-US Pap result during long-term posttherapy monitoring. In most published studies of posttherapy follow-up monitoring, authors compare the efficacy of HC2 testing with that of Pap test cytology only for all cases diagnosed as ASC-US, LSIL, and HSIL. Most of these studies reported only short-term posttherapy follow-up intervals (<24 months).[13] The pooled sensitivity of HC2 for CIN 2+ in these studies was 79% to 90.7%.[13,14] It has been well documented that women with an ASC-H, LSIL, or HSIL Pap test result have a higher risk of CIN 2+ and a higher positive rate of high-risk HPV than those with an ASC-US Pap result.[28] Therefore, the relatively high sensitivity of HC2 in these reported studies may represent the sensitivity of HC2 in all patients with ASC-US, ASC-H, LSIL, or HSIL.

Human papillomavirus testing is more clinically relevant in women with an ASC-US Pap test result during posttherapy follow-up monitoring than in individuals with an ASC-H, LSIL, or HSIL Pap test result during that period. In a screening population (ASCUS-LSIL Triage Study [ALTS]), Safaeian et al[29] reported the risk of CIN 3 associated with HC2 status in women with ASC-US. They observed a lower risk of CIN 3 in women with an ASC-US Pap test result and a negative HC2 result (1.4%) than in those with an ASC-US Pap test result and a positive HC2 result (15.2%). As part of the ALTS study, Walker et al[22] reported a higher risk of CIN 3 in women who had an ASC-US Pap test result and a positive HC2 result (10.8%) while being monitored after colposcopy than in those individuals who had an ASC-US Pap test result and a negative HC2 result (5%). In our study of posttherapy follow-up monitoring, risk of CIN/VAIN 3 was higher in women with ASC-US/HC2–positive results (29%) than in women with ASC-US/HC2–negative results (8%). The high risk of CIN/VAIN 3 in our cohort is mainly due to the higher risk in patients with a significant history of cervical/vaginal dysplasia or carcinoma than in a screening population. Although we observed an HC2 sensitivity for CIN 3 (5 of 7; 71%) comparable with that reported by Safaeian et al[29] (5 of 7; 71.4%) in the screening population, our patients had a much higher risk level (29.4%) than the ALTS screening population (3.8%). However, the sensitivity of HC2 for CIN 2+ in our study was lower (41%) than that in the ALTS study (67%). Notably, unlike the reflex HPV testing for women with ASC-US Pap results, or Pap and HPV coscreening for women 30 years and older in a screening population, HPV testing for posttherapy follow-up has been considered an off-label application of the Food and Drug Administration–approved test, mainly due to lack of clinical trial studies. Recently, Katki et al,[30] in a follow-up study of 3273 women who were treated for CIN 2+ or adenocarcinoma in situ at the Kaiser Permanente Medical Care Program (Oakland, California), reported a lower 5-year CIN 2+ risk (1.5%) after 2 negative cotest results with HPV and Pap cytology than after 2 negative HPV (2.7%) or Pap cytology (2.7%) tests, indicating that HPV and Pap cotesting is more efficient than HPV testing or Pap cytology alone for predicting CIN 2+. These findings and our observation support HPV and Pap cytology cotesting during posttherapy follow-up, which was recently recommended by the ASCCP.[11]

The reasons for the HC2 false-negative results in our study are unclear. Several factors may affect the sensitivity of the HC2 assay in posttherapy follow-up monitoring. The major factor may be the older mean age of the patient population in our hospital. Stoler et al,[31] in a screening population of women with ASC-US, reported positivity for high-risk HPV decreased from 54.1% in women ages 21 to 29 years to 14.7% in women ages 40 to 49 years. HC2 sensitivity also declined with age, from 93.3% in women ages 21 to 29 years to 67.7% in women 40 years and older.[32] Einstein et al,[33] using the Cervista HPV HR testing assay, reported similar findings, that sensitivity declined with age. In our cohort, the mean age was 47 years, older than the mean ages of patient cohorts in previously published reports. Park et al[34] also reported a relatively low HC2 sensitivity in predicting CIN 2+ in a cohort with a mean age of 41 years. Although the cause of the lower sensitivity of HPV testing for CIN 2+ in older women compared with younger women is still unknown, it might be associated with a lower viral load in the former.[35] We speculated that the reduced sensitivity of HC2 seen in our study could be due to the presence of scant dysplastic cells, leading to a low viral load that is suboptimal for HC2 testing. This reduced tissue acquisition could be related to the atrophy in older patients or therapy effects.

Reduced sensitivity in our group may also be related to the degree of analytic sensitivity of the HC2 HPV DNA assay. Unlike polymerase chain reaction (PCR)–based HPV assays, the HC2 assay does not rely on target amplification, and thus its analytic sensitivity is lower than that of the PCR-based assays. In a study with the largest reported cohort of patients in posttherapy follow-up, Kreimer et al[20] compared the HC2 assay with a PCR assay in predicting recurrent/residual CIN 2+. The results showed that the sensitivity of the PCR assay (97%) was higher than that of

the HC2 assay (91%). High sensitivity of PCR-based HPV DNA testing for posttherapy follow-up was also documented by other investigators.[13,36] Therefore, we speculate that the low sensitivity of HC2 testing in predicting CIN/VAIN 2+ in our study might be attributed in part to the relatively low analytic sensitivity of the HC2 assay. We used a PCR-based assay to test 3 residual Pap test specimens from patients with a negative HC2 result but CIN/VAIN 2+ on follow-up biopsy, and all 3 samples were positive for high-risk HPV types (data not shown). The tissue specimens showing CIN/VAIN 2+ from patients with a negative HC2 result exhibited high-risk HPV types, including HPV 16/18. These findings may reflect the differential sensitivities of HC2 and PCR-based HPV assays.

Another possible factor in the high HC2 false-negative rate is the off-label use of the HC2 assay with SurePath specimens. The SurePath collecting medium is an alcohol-based preservative containing a trace amount of formalin. It has been recognized that formalin-containing fixatives can cause cross-linking of nucleic acid to protein, which interferes with enzymatic lysis of fixed cells during DNA extraction for molecular testing, resulting in a low yield of DNA extraction. A study comparing recovery of nucleic acids in cell lines stored in SurePath or ThinPrep medium showed a low yield of DNA extracted from cells in SurePath medium without an appropriate enzyme treatment. The yield of DNA was significantly improved with proteinase K treatment.[37] Another recently published study showed that SurePath specimens treated with combined proteinase K and heat to reverse formalin-induced cross-linking were not statistically different in the reproducibility than ThinPrep specimens for HPV DNA testing using a PCR assay.[38] In addition, because the use of HC2 HPV testing in SurePath Pap specimens is off-label, there is no official protocol recommended by the manufacturer. Instead, laboratories in which SurePath Pap specimens are used for HC2 HPV testing usually validate HC2 testing in their own laboratory. These laboratory validations are rarely published. Consequently, there is no standardized HC2 HPV testing protocol for SurePath Pap specimens, which may explain the relatively lower accuracy of HC2 testing in cell lines fixed in SurePath medium (96.2%) than in ThinPrep medium (98%), as seen in the HPV proficiency testing performed by the College of American Pathologists.[39]

Although the off-label use of the HC2 assay in SurePath specimens may have intrinsic weaknesses for HPV detection, the availability of up-to-date published clinical studies comparing the clinical performance of the HC2 assay in SurePath and ThinPrep specimens is limited. Therefore, there is little convincing evidence that the clinical performance of HC2 in SurePath specimens is significantly lower than that seen in ThinPrep specimens. In a prospective clinical study evaluating the clinical performance of HC2 HPV testing in SurePath and ThinPrep Pap specimens, Zhao et al[40] reported comparable HC2 clinical sensitivities in paired sampling of SurePath (91%) and ThinPrep (89%) specimens. Ko et al[41] also reported that HC2 had comparable sensitivity in SurePath and ThinPrep specimens. Siddiqi et al[42] reported an indirect comparison of HC2 HPV testing in SurePath and ThinPrep specimens in a screening population and found no statistical difference in age-adjusted HPV positivity or in the rate of CIN 2/3 positivity on biopsy follow-up between the two liquid-based Pap specimens.

Comparison of HC2 false-negative rates in SurePath and ThinPrep specimens can also provide indirect evidence of the clinical performance of HC2 HPV testing. In a 3-year follow-up study at our institution for patients with negative SurePath Pap and negative HC2 results, we observed a low follow-up rate for CIN 3 (0.17%; M.G., unpublished data). Zhao et al[43] reported a similar CIN 3 rate (0.17%) with a mean follow-up period of 44 months for women with negative ThinPrep Pap and negative HC2 HPV testing results. Nevertheless, current guidelines for cervical cancer screening issued by the ASCCP and American College of Obstetricians and Gynecologists advise against unapproved off-label use of HPV testing.[9,10] Studies directly comparing the two liquid-based cytology collecting media for HPV testing or large-scale follow-up studies to compare age-adjusted clinical sensitivities of HC2 HPV testing assays in SurePath and ThinPrep specimens are necessary to clarify the issue of whether the false-negative rate for HC2 HPV testing in SurePath specimens is higher than that in ThinPrep specimens.

In our cohort of 100 Pap specimens, 44 were vaginal Pap specimens. Published data on HC2 testing in vaginal Pap test specimens are scant. Castle et al[44] compared HPV testing (PCR and type-specific dot blot hybridization) in cervical and vaginal Pap specimens and reported a similar prevalence of carcinogenic HPV types in both types of specimens. Bansal et al[45] reported a higher rate of vaginal squamous intraepithelial lesions in women with ASC-US/HC2–positive results (47.8%) than in women with ASC-US/HC2–negative results (4.7%), indicating the usefulness of HC2 testing in triage of women with ASC-US. In our cohort, it appeared that HC2 efficacy was lower in vaginal Pap test specimens than in cervical Pap test specimens. However, the small size of our cohort precluded any valid comparison.

Our findings suggest that, for long-term follow-up monitoring in a relatively older patient population, HC2 testing may not be as effective as it is in a screening population for triaging patients with ASC-US for colposcopic evaluation. Because of a relatively high false-negative rate, HC2 HPV testing alone may not be sufficient for posttherapy follow-up. Furthermore, an ASC-US Pap result may be sufficient to warrant colposcopic evaluation during posttherapy follow up, depending on the specific clinical setting. For instance, in the 7 patients in our cohort with ASC-US/HC2–negative results and CIN/VAIN 2+ follow-up biopsy results, 6 had previous abnormal Pap or biopsy results (Table 4), the Pap result of ASC-US triggered colposcopic evaluation even though HC2 test was negative. Persistence of HPV infection or HPV 16/18 genotyping may be a better indicator of recurrent CIN 2+ during long-term posttherapy follow-up.[17,46,47] However, the cost-effectiveness of these strategies as triage tools to predict recurrent/residual CIN/VAIN 2+ in patients with an ASC-US Pap result in the long-term follow-up setting needs further evaluation.

The main limitation of our study is that it is retrospective, with patient heterogeneity that included different types of cervical/vaginal precancerous lesions/carcinoma and a variety of therapies. Furthermore, our study cohort was relatively small. Finally, for most patients, residual Pap test specimens were not available to verify HPV status, which is necessary to confirm false-negative HC2 results. Further studies are necessary to determine the efficacy of HPV and

Pap cytology cotesting for patients during long-term posttherapy follow-up monitoring.

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References

1. Sarian LO, Derchain SF, Andrade LA, et al. HPV DNA test and Pap smear in detection of residual and recurrent disease following loop electrosurgical excision procedure of high-grade cervical intraepithelial neoplasia. *Gynecol Oncol*. 2004;94(1):181–186.

2. van Hamont D, van Ham MA, Struik-van der Zanden PH, et al. Long-term follow-up after large-loop excision of the transformation zone: evaluation of 22 years treatment of high-grade cervical intraepithelial neoplasia. *Int J Gynecol Cancer*. 2006;16(2):615–619.

3. Mitchell MF, Tortolero-Luna G, Cook E, et al. A randomized clinical trial of cryotherapy, laser vaporization, and loop electrosurgical excision for treatment of squamous intraepithelial lesions of the cervix. *Obstet Gynecol*. 1998;92(5):737–744.

4. Persad VL, Pierotic MA, Guijon FB. Management of cervical neoplasia: a 13-year experience with cryotherapy and laser. *J Low Genit Tract Dis*. 2001;5(4):199–203.

5. Melnikow J, McGahan C, Sawaya GF, et al. Cervical intraepithelial neoplasia outcomes after treatment: long-term follow-up from the British Columbia Cohort Study. *J Natl Cancer Inst*. 2009;101(10):721–728.

6. Soutter WP, Sasieni P, Panoskaltsis T. Long-term risk of invasive cervical cancer after treatment of squamous cervical intraepithelial neoplasia. *Int J Cancer*. 2006;118(8):2048–2055.

7. Kalliala I, Anttila A, Pukkala E, et al. Risk of cervical and other cancers after treatment of cervical intraepithelial neoplasia: retrospective cohort study. *BMJ*. 2005;331(7526):1183–1185.

8. Wright TC Jr, Massad LS, Dunton CJ, et al. 2006 consensus guidelines for the management of women with cervical intraepithelial neoplasia or adenocarcinoma in situ. *Am J Obstet Gynecol*. 2007;197(4):340–345.

9. Saslow D, Solomon D, Lawson HW, et al. American Cancer Society, American Society for Colposcopy and Cervical Pathology, and American Society for Clinical Pathology screening guidelines for the prevention and early detection of cervical cancer. *CA Cancer J Clin*. 2012;62(3):147–172.

10. Practice Bulletin No. 131: clinical management guidelines for obstetrician-gynecologists. *Obstet Gynecol*. 2012;120(5):1514–2121.

11. Massad LS, Einstein MH, Huh WK, et al. 2012 updated consensus guidelines for the management of abnormal cervical cancer screening tests and cancer precursors. *J Low Genit Tract Dis*. 2013;17(5)(suppl 1):S1–S27.

12. Arbyn M, Paraskevaidis E, Martin-Hirsch P, et al. Clinical utility of HPV-DNA detection: triage of minor cervical lesions, follow-up of women treated for high-grade CIN: an update of pooled evidence. *Gynecol Oncol*. 2005;99(suppl 1):S7–S11.

13. Kocken M, Uijterwaal MH, de Vries AL, et al. High-risk human papillomavirus testing versus cytology in predicting post-treatment disease in women treated for high-grade cervical disease: a systematic review and meta-analysis. *Gynecol Oncol*. 2012;125(2):500–507.

14. Chan BK, Melnikow J, Slee CA, et al. Posttreatment human papillomavirus testing for recurrent cervical intraepithelial neoplasia: a systematic review. *Am J Obstet Gynecol*. 2009;200(4):422.e1–9.

15. Zielinski GD, Bais AG, Helmerhorst TJ, et al. HPV testing and monitoring of women after treatment of CIN 3: review of the literature and meta-analysis. *Obstet Gynecol Surv*. 2004;59(7):543–553.

16. Paraskevaidis E, Arbyn M, Sotiriadis A, et al. The role of HPV DNA testing in the follow-up period after treatment for CIN: a systematic review of the literature. *Cancer Treat Rev*. 2004;30(2):205–211.

17. Kreimer AR, Schiffman M, Herrero R, et al. Long-term risk of recurrent cervical human papillomavirus infection and precancer and cancer following excisional treatment. *Int J Cancer*. 2012;131(1):211–218.

18. Verguts J, Bronselaer B, Donders G, et al. Prediction of recurrence after treatment for high-grade cervical intraepithelial neoplasia: the role of human papillomavirus testing and age at conisation. *BJOG*. 2006;113(11):1303–1307.

19. Alonso I, Torne A, Puig-Tintore LM, et al. Pre- and post-conization high-risk HPV testing predicts residual/recurrent disease in patients treated for CIN 2–3. *Gynecol Oncol*. 2006;103(2):631–636.

20. Kreimer AR, Guido RS, Solomon D, et al. Human papillomavirus testing following loop electrosurgical excision procedure identifies women at risk for posttreatment cervical intraepithelial neoplasia grade 2 or 3 disease. *Cancer Epidemiol Biomarkers Prev*. 2006;15(5):908–914.

21. Smart OC, Sykes P, Macnab H, et al. Testing for high risk human papilloma virus in the initial follow-up of women treated for high-grade squamous intraepithelial lesions. *Aust N Z J Obstet Gynaecol*. 2010;50(2):164–167.

22. Walker JL, Wang SS, Schiffman M, et al. Predicting absolute risk of CIN3 during post-colposcopic follow-up: results from the ASCUS-LSIL Triage Study (ALTS). *Am J Obstet Gynecol*. 2006;195(2):341–348.

23. Solomon D, Davey D, Kurman R, et al. The 2001 Bethesda System: terminology for reporting results of cervical cytology. *JAMA*. 2002;287(16):2114–2119.

24. Lin CY, Chen HC, Lin RW, et al. Quality assurance of genotyping array for detection and typing of human papillomavirus. *J Virol Methods*. 2007;140(1–2):1–9.

25. Acladious NN, Sutton C, Mandal D, et al. Persistent human papillomavirus infection and smoking increase risk of failure of treatment of cervical intraepithelial neoplasia (CIN). *Int J Cancer*. 2002;98(3):435–439.

26. Fuste P, Bellosillo B, Santamaria X, et al. HPV determination in the control after LEEP due to CIN II-III: prospective study and predictive model. *Int J Gynecol Pathol*. 2009;28(2):120–126.

27. Jeong NH, Lee NW, Kim HJ, et al. High-risk human papillomavirus testing for monitoring patients treated for high-grade cervical intraepithelial neoplasia. *J Obstet Gynaecol Res*. 2009;35(4):706–711.

28. Castle PE, Fetterman B, Thomas Cox J, et al. The age-specific relationships of abnormal cytology and human papillomavirus DNA results to the risk of cervical precancer and cancer. *Obstet Gynecol*. 2010;116(1):76–84.

29. Safaeian M, Solomon D, Wacholder S, et al. Risk of precancer and follow-up management strategies for women with human papillomavirus-negative atypical squamous cells of undetermined significance. *Obstet Gynecol*. 2007;109(6):1325–1331.

30. Katki HA, Schiffman M, Castle PE, et al. Five-year risk of recurrence after treatment of CIN 2, CIN 3, or AIS: performance of HPV and Pap cotesting in posttreatment management. *J Low Genit Tract Dis*. 2013;17(5)(suppl 1):S78–S84.

31. Stoler MH, Wright TC Jr, Sharma A, et al. High-risk human papillomavirus testing in women with ASC-US cytology: results from the ATHENA HPV study. *Am J Clin Pathol*. 2011;135(3):468–475.

32. Stoler MH, Wright TC Jr, Sharma A, et al. The interplay of age stratification and HPV testing on the predictive value of ASC-US cytology: results from the ATHENA HPV study. *Am J Clin Pathol*. 2012;137(2):295–303.

33. Einstein MH, Martens MG, Garcia FA, et al. Clinical validation of the Cervista HPV HR and 16/18 genotyping tests for use in women with ASC-US cytology. *Gynecol Oncol*. 2010;118(2):116–122.

34. Park JY, Bae J, Lim MC, et al. Role of high risk-human papilloma virus test in the follow-up of patients who underwent conization of the cervix for cervical intraepithelial neoplasia. *J Gynecol Oncol*. 2009;20(2):86–90.

35. Kovacic MB, Castle PE, Herrero R, et al. Relationships of human papillomavirus type, qualitative viral load, and age with cytologic abnormality. *Cancer Res*. 2006;66(20):10112–10119.

36. Bais AG, Eijkemans MJ, Rebolj M, et al. Post-treatment CIN: randomised clinical trial using hrHPV testing for prediction of residual/recurrent disease. *Int J Cancer*. 2009;124(4):889–895.

37. Powell N, Smith K, Fiander A. Recovery of human papillomavirus nucleic acids from liquid-based cytology media. *J Virol Methods*. 2006;137(1):58–62.

38. Agreda PM, Beitman GH, Gutierrez EC, et al. Long-term stability of human genomic and human papillomavirus DNA stored in BD SurePath and Hologic PreservCyt liquid-based cytology media. *J Clin Microbiol*. 2013;51(8):2702–2706.

39. Moriarty AT, Bentz JS, Winkler B, et al. The College of American Pathologists' first 3 years' experience with high-risk human papillomavirus proficiency testing for cytology and other laboratories. *Arch Pathol Lab Med*. 2013;137(5):606–609.

40. Zhao FH, Hu SY, Bian JJ, et al. Comparison of ThinPrep and SurePath liquid-based cytology and subsequent human papillomavirus DNA testing in China. *Cancer Cytopathol*. 2011;119(6):387–394.

41. Ko V, Tambouret RH, Kuebler DL, et al. Human papillomavirus testing using hybrid capture II with SurePath collection: initial evaluation and longitudinal data provide clinical validation for this method. *Cancer*. 2006;108(6):468–474.

42. Siddiqi A, Spataro M, McIntire H, et al. Hybrid capture 2 human papillomavirus DNA testing for women with atypical squamous cells of undetermined significance Papanicolaou results in SurePath and ThinPrep specimens. *Cancer*. 2009;117(5):318–325.

43. Zhao C, Weng B, Li Z, et al. Follow-up outcomes of a large cohort of low-risk women with negative imaged liquid-based cytology and negative HPV test results. *Am J Clin Pathol*. 2013;139(1):32–38.

44. Castle PE, Rodriguez AC, Porras C, et al. A comparison of cervical and vaginal human papillomavirus. *Sex Transm Dis*. 2007;34(11):849–855.

45. Bansal M, Li Z, Zhao C. Correlation of histopathologic/cytologic follow-up findings with vaginal ASC-US and ASC-H Papanicolaou test and HPV test results. *Am J Clin Pathol*. 2012;137(3):437–443.

46. Kang WD, Oh MJ, Kim SM, et al. Significance of human papillomavirus genotyping with high-grade cervical intraepithelial neoplasia treated by a loop electrosurgical excision procedure. *Am J Obstet Gynecol*. 2013;203(1):72.e1–6.

47. Gage JC, Schiffman M, Solomon D, et al. Comparison of measurements of human papillomavirus persistence for postcolposcopic surveillance for cervical precancerous lesions. *Cancer Epidemiol Biomarkers Prev*. 2010;19(7):1668–1674.