Journal of the American Society of Cytopathology (2019) 8, 149–156

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.jascyto.org/

ORIGINAL ARTICLE

HPV status in women with high-grade dysplasia on cervical biopsy and preceding negative HPV tests

Yimin Ge, MD[a,b,*], Roxanne R. Mody, MD[c], Randall J. Olsen, MD[a,b], Haijun Zhou, MD, PhD[a], Eric Luna, CT (ASCP)[d], Donna Armylagos, CT (ASCP)[d], Natu Puntachart, MB (ASCP) CM[a], Heather Hendrickson, MBA, MB (ASCP) CM[a], Mary R. Schwartz, MD[a], Dina R. Mody, MD[a,b]

[a] *Department of Pathology and Genomic Medicine, Houston Methodist Hospital, Houston, Texas*
[b] *Weill Medical College of Cornell University, New York, New York*
[c] *Department of Obstetrics and Gynecology, St. Joseph's Hospital, Denver, Colorado*
[d] *BioReference Laboratories, Houston, Texas*

Received 28 November 2018; received in revised form 6 January 2019; accepted 8 January 2019

KEYWORDS
Human papillomavirus;
HPV test;
Papanicolaou test;
HPV-cytology co-testing;
High-grade cervical lesion;
Cervical cancer

Introduction A considerable number of patients with high-grade cervical lesions have undergone preceding human papillomavirus (HPV) tests with negative results. In the present study, we attempted to elucidate the factors potentially contributing to the findings by testing biopsied samples from these patients.

Materials and methods Of the 1654 women with HPV testing and follow-up cervicovaginal biopsies from March 1, 2013 to June 30, 2014, 21 of 252 women (8.3%) with biopsy-confirmed high-grade squamous intraepithelial lesion (HSIL) or worse had had negative results from preceding high-risk (hr)HPV tests. The corresponding paraffin blocks were tested for HPV using the Cobas 4800 system, a DNA microarray against 40 HPV genotypes, and DNA sequencing.

Results HPV was detected in 20 (95%) of the 21 biopsies with HSIL or worse, including HPV16/18 in 4, non-16/18 hrHPV in 10, and non-hrHPV in 6. HPV59 and HPV45 were 2.2 times more frequently detected than HPV16/18 in these samples. One sample was negative for all 3 tests (5%).

Conclusions Our study has demonstrated that 8.3% of women with biopsy-confirmed HSIL or worse had preceding test results that were negative for hrHPV. The vast majority of the biopsied samples had detectable HPV, primarily hrHPV genotypes (67%) with HPV59 and HPV45 predominance. This genotypic prevalence pattern

*Corresponding author: Yimin Ge, MD, Department of Pathology and Genomic Medicine, Houston Methodist Hospital, Weill Cornell Medical College of Cornell University, 6565 Fannin Street, M227, Houston, TX 77030; Tel.: (713) 441-2283; Fax: (713) 793-1603.
E-mail address: yge@houstonmethodist.org (Y. Ge).

https://doi.org/10.1016/j.jasc.2019.01.001

was markedly different from those reported in the general population. Non-hrHPV genotypes contributed to 29% of the cases, and HPV-negative cases were rare. In addition to the limited Cobas testing panel and rare possible HPV-negative HSIL or worse, other possible contributing factors to the discrepancy include cytologic sampling, interference material, technical errors, and reduced L1 gene expression in high-grade lesions.

Introduction

Persistent infection with high-risk human papillomavirus (hrHPV) causes most cervical cancers and precancerous lesions.[1] In recent years, hrHPV testing has been increasingly used in clinical practice to triage equivocal cytology or as cotesting with cytology to maximize the detection rate of high-grade cervical lesions in women aged ≥30 years.[2] Recent studies conducted in Europe and Canada have demonstrated that primary hrHPV screening exhibits greater sensitivity in detecting precursor lesions of cervical cancer compared with cytology alone in single and multiple rounds of screening.[3-8] However, the efficiency of hrHPV testing alone as a primary screening method for cervical cancer has remained controversial.[9] After the Food and Drug Administration approved the Cobas HPV test in April 2014 as an option for primary cervical cancer screening in women aged ≥25 years, an interim clinical guideline for the test was reported.[10] Owing to the paucity of data, this interim guideline was largely based on studies performed in Europe rather than prospective US-based studies.[11,12]

Given that HPV cytology cotesting has been increasingly selected for women aged ≥30 years, cervical dysplasia and cancer with negative test results have been frequently encountered in clinical practice in the United States[13] and elsewhere.[14] In 2015, Blatt et al.[15] reported an extensive study involving 256,648 cases with cytologic and HPV cotesting. They demonstrated that 19% of women with cervical cancer could have had a misdiagnosis if only primary HPV cervical screening had been used.[15] It has been reported that 9% to 25% of patients with squamous cell carcinoma will have had negative HPV tests in the preceding 1 to 5 years.[16] A recent study suggested that more than one half of the cervical cancer cases with preceding negative HPV test results could be truly hrHPV-negative carcinoma.[17] In the present study, we attempted to elucidate the factors potentially contributing to the findings by performing HPV tests on biopsied samples from women who had biopsy-confirmed high-grade squamous intraepithelial lesions (HSILs) or worse and preceding HPV tests with negative results.

Materials and methods

Patient population

We retrospectively reviewed 130,648 Papanicolaou (Pap) test results recorded in our Laboratory Information System database from March 1, 2013 to June 30, 2014 and identified 47,499 women who had undergone cytology and hrHPV cotesting with the Cobas 4800 system (Roche Molecular Diagnostics, Pleasanton, Calif). The Pap tests were performed using liquid-based methods with either the ThinPrep (Hologic, Madison, Wis) or SurePath (Becton Dickinson, Franklin Lakes, NJ) platform. Our study was conducted within a large general screening population in Texas with hrHPV prevalence and HSIL reporting rates similar to the general population reported in other established trials and surveys performed in the United States.[18] The cytology reporting performance in our laboratory benchmarked well with the national database reported by the College of American Pathologists.

Biopsy confirmation

The cases with follow-up biopsies performed within 1 year of the cytology−HPV cotesting were identified. Lesions equal to or worse than HSIL were considered high-grade lesions, including HSIL (cervical intraepithelial neoplasia [CIN]2, CIN3), adenocarcinoma in situ, and invasive carcinomas. Immunohistochemical staining for p16 was performed for all possible CIN2 lesions, and a diagnosis of CIN2 was rendered if a block-like immunostaining pattern was observed. Endometrial lesions were excluded from the present study.

HPV DNA extraction and genotyping

Cobas 4800 system

Paraffin blocks from the biopsies with HSIL or worse in women with negative hrHPV tests in the preceding cytology specimens were obtained. Ten unstained paraffin sections (4 μm each) from each case were collected with extreme precaution. The microtome blade and water bath were changed between cases to avoid potential cross-contamination. The sections were kept in closed containers before DNA extraction. The lesional tissue was microdissected from the paraffin sections and eluted with SurePath preservative fluid (Becton Dickinson). The Cobas 4800 HPV test uses primers and probes designed to detect 14 hrHPV genotypes. An additional primer pair and probe were used to detect the human β-globin gene as a control. HPV DNA was isolated on the Cobas 4800. Subsequently, a real-time polymerase chain reaction was performed on the Cobas 4800, using the

Table 1 Human papillomavirus test results on biopsy samples from women with preceding negative test result.

Age (y)	Previous cytology and HPV test results		Diagnosis and HPV genotyping results from biopsy specimens			
	Cytology diagnosis	Negative HPV tests	Biopsy diagnosis	Cobas 4800	DNA microarray	DNA sequencing
32	ASC-H	1	CIN3	16	16, 18,67, 83	16, 18
23	ASC-US	2	CIN2	Non-16/18	39, 59. 66. 69	66
22	ASC-US	1	CIN2	Non-16/18	59, 66	66
22	LSIL	2	CIN2	Non-16/18	58, 59, 66, 91	66
28	ASC-US	1	CIN3	Non-16/18	45	45
43	ASC-H	1	CIN2	Non-16/18	53, 59, 90	53
37	ASC-H	1	CIN3	Non-16/18	11, 59, 91	59
36	NILM	3	CIN3	Non-16/18	6, 16(W)	16
25	ASC-H	1	CIN3	Non-16/18	6, 83, 84	84
21	HSIL	1	CIN3	Non-16/18	61	61
27	ASC-US	1	CIN2	Non-16/18	62	62
26	ASC-H	1	CIN3	Negative	11(W), 45	45
29	HSIL	3	CIN3	Negative	16(W), 45, 84	45
19	ASC-US	1	CIN2	Negative	68A(W), 69	69
44	HSIL	2	CIN3	Negative	81	81
49	LSIL	2	CIN2	Negative	55	55
35	NILM	1	CIN2	Negative	Negative	Negative
32	ASC-H	1	CIN2	Invalid	18	18
43	HSIL	1	CIN3	Invalid	11, 45, 59	11
46	HSIL	2	CIN3	Invalid	59, 69	69
25	ASC-H	1	CIN3	Invalid	83	83

Abbreviations: HPV, human papillomavirus; ASC-H, atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion; ASC-US, atypical squamous cells of undetermined significance; CIN2, cervical intraepithelial neoplasia, grade 2; CIN3, cervical intraepithelial neoplasia, grade 3; LSIL, low-grade squamous intraepithelial lesion; NILM, negative for intraepithelial lesion or malignancy; HSIL, high-grade squamous intraepithelial lesion; Non-16/18, high-risk HPV excluding HPV16/18; W, weakly positive.

primer pairs and probes to detect and genotype HPV16/18 and other hrHPV genotypes.

HPV DNA microarray (GoodGene 40 HPV genotyping chip)

HPV DNA was extracted from the paraffin sections, as previously reported,[19] and the L1 region of the HPV genome was amplified by polymerase chain reaction. The amplified HPV DNA was then labeled with Cy5 and hybridized with an HPV DNA microarray chip with 40 HPV genotypes, including 14 hrHPV and 26 non-hrHPV genotypes (GG HPV DNA Genotyping Chip Kit; GoodGene Inc, Seoul, Korea). The signal was visualized using a GenePix 4000B Microarray Scanner (Molecular Devices, Inc, Sunnyvale, Calif).

Direct HPV DNA sequencing

Conventional direct DNA sequencing methods were used to further confirm the HPV DNA microarray data for the biopsy samples from women with preceding negative HPV tests, as previously described.[19] The sequence data obtained by automated DNA sequencing were analyzed by the Basic Local Alignment Search Tool search tool (available at: http://www.ncbi.nlm.nih.gov/BLAST/) for HPV genotypes. DNA sequencing identified the most dominant genotype in a given specimen and served as a confirmatory assay.

Categorization of HPV genotype and statistical analysis

The 2009 recommendations of the expert working group at the International Agency for Research on Cancer (IARC) categorized HPV into 4 groups: carcinogenic (group 1), probably carcinogenic (group 2A), possibly carcinogenic (group 2B), and not classifiable (group 3).[20] The HPV genotypes in IARC groups 1 and 2A are commonly referred to as hrHPV. These genotypes include 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68A/68B. In the present study, IARC groups 2B and 3 have been referred to as non-hrHPV. All statistical analyses were performed with STATA version 15 (StataCorp LP, College Station, TX). Significance was defined as two-tailed $P < 0.05$.

Institutional review board approval

The institutional review board of Houston Methodist Hospital Research Institute approved the present study.

Results

Interpretable follow-up biopsy specimens were obtained from 1654 women with a mean age of 37.9 years (range, 15.6-94.5). The corresponding Pap tests were performed on

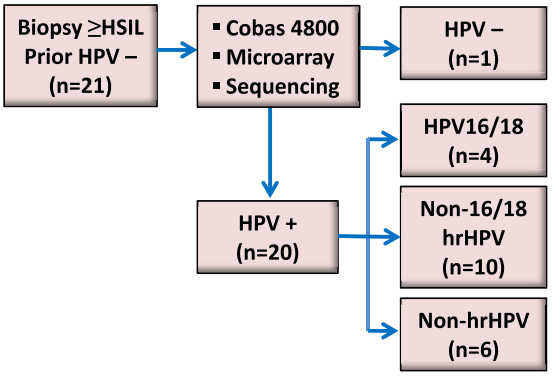

Figure 1 Human papillomavirus (HPV) testing results from biopsy samples with high-grade squamous intraepithelial lesions (HSILs) from women with preceding negative HPV test results. hrHPV, high-risk HPV.

either ThinPrep (n = 867; 52.4%) or SurePath (n = 787; 47.6%) platforms. Among the 1652 follow-up biopsy specimens from women with cytology−HPV cotesting, 252 were high-grade cervical lesions (HSIL or worse) by histologic evaluation. The preceding Pap tests had detected 230 of the 252 cases, with a sensitivity of 91.3% (95% confidence interval [CI], 86.7%-94.1%). In contrast, the HPV tests were positive in 231 of the 252 cases, with a sensitivity of 91.7% (95% CI, 87.1%-94.5%). No statistically significant difference (P = 1.0) was found between the sensitivities of the Pap and HPV tests in detecting biopsy-confirmed high-grade cervical lesions.

Of the 252 women with biopsy-confirmed HSIL or worse, 21 (8.3%; CIN2 in 9 and CIN3 in 12) had negative Cobas HPV test results in the preceding cervical samples (Table 1). The preceding Pap test results for the 21 women included negative for intraepithelial lesions or malignancy (n = 2); atypical squamous cells of undetermined significance (ASC-US; n = 5); low-grade squamous intraepithelial lesion (LSIL; n = 2); atypical squamous cells, cannot rule out high-grade squamous intraepithelial lesion

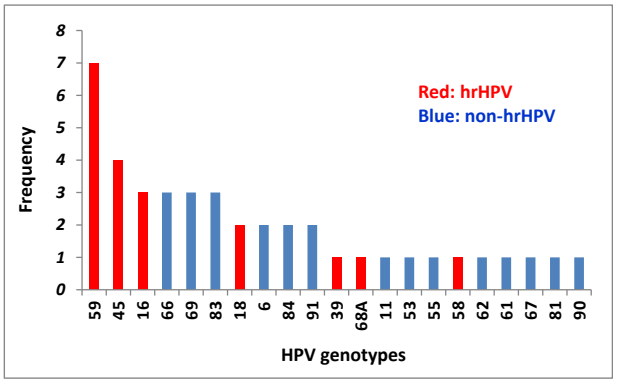

Figure 2 Human papillomavirus (HPV) genotype-specific prevalence in biopsy-confirmed high-grade squamous intraepithelial lesions from women with preceding negative HPV test results. hrHPV, high-risk HPV.

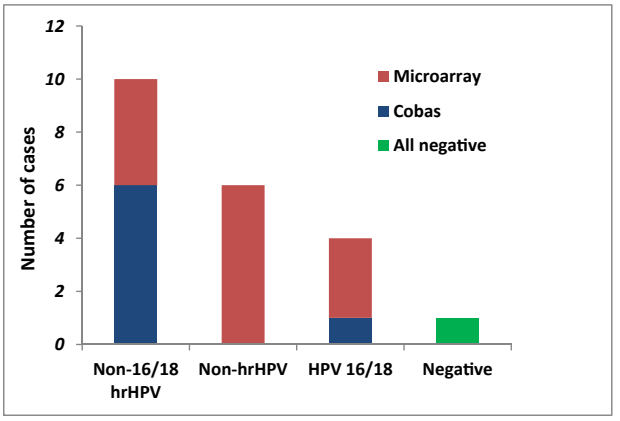

Figure 3 Detection of human papillomavirus (HPV) genotypes in biopsy samples using different testing methods. hrHPV, high-risk HPV.

(ASC-H, n = 7); and HSIL (n = 5). The age of the HPV-negative group of women ranged from 19 to 49 years, with an average age of 32 years, which was younger than the average of 38 years for this screening population.

The HPV test results from the biopsy samples from the 21 women in the HPV-negative group are summarized in Table 1 and Fig. 1. The Cobas HPV test results were positive in 11 biopsies (52%) with HPV16 in 1 and non-16/18 hrHPV in 10 cases. Of the 11 cases with positive Cobas genotyping results, 7, including 1 cases of HPV16 and 6 cases of non-6/18 hrHPV, were confirmed by DNA microarray or DNA sequencing assays. Genotyping discrepancies were found in the 4 remaining cases with non-16/18 hrHPV found on Cobas but non-hrHPV genotypes (n = 3) or HPV16 (n = 1) found on DNA microarray or sequencing assays.

Of the remaining 10 biopsies that were negative or inadequate on Cobas testing, the DNA microarray test detected HPV16 or HPV18 in 2, non-16/18 hrHPV in 4, and non-hrHPV in 3 cases, findings that were consistent with the results of the DNA sequencing assay. The concordance rate between Cobas and microarray/sequencing assays was 64% for those positive on Cobas test. The sensitivity of the Cobas HPV test for hrHPV in the biopsy tissue was 60% (9 of 15). Only 1 biopsy was negative for all 3 tests (1 of 21; 5%). A block-like p16 immunostaining pattern was observed in all cases of CIN2.

Overall, HPV genotypes were detected in 20 (95%) of 21 biopsies with a diagnosis of HSIL or worse in women with preceding negative Cobas HPV test results. Of the 20 biopsy specimens with positive HPV tests, 14 (70%) had hrHPV genotypes. Collectively, non-16/18 hrHPV genotypes (10 of 20; 50%) were more commonly identified than HPV16/18 (4 of 20; 20%) and non-hrHPV (6 of 20; 30%). Of the 14 cases that were positive for hrHPV, HPV59 was the most common genotype detected (n = 7), followed by HPV45 (n = 4), HPV16 (n = 3), HPV18 (n = 2), HPV39 (n = 1), HPV68A (n = 1), and HPV58 (n = 1; Fig. 2). HPV59/45 were 2.2 times more frequently detected than HPV16/18 in these samples. Multiple genotypic HPV infections were

common (13 of 20, 65%) in this cohort and primarily included non-16/18 hrHPV genotypes (Table 1). Of the 6 cases with non-hrHPV infection, single HPV genotypic infection was detected in 5 of the cases (83%), including HPV genotypes 55, 61, 62, 81, and 83. One of the 6 cases had multiple non-hrHPV genotypes, including HPV6, HPV83, and HPV84.

Discussion

It is commonly perceived that hrHPV testing has greater sensitivity than cytomorphology in detecting cervicovaginal dysplasia based on data from several large trials.[3-8,11] However, our earlier study[13] from our clinical quality assurance database demonstrated that the sensitivities of hrHPV and Pap tests in predicting high-grade lesions or any dysplastic lesions on follow-up biopsy samples were statistically similar (91.3% versus 90.9% for high-grade lesions, $P = 1.0$; and 80.8% versus 81.2% for any dysplastic lesions, $P = 0.86$). In the present study, we found that 8.3% (21 of 252) of the biopsy-confirmed high-grade cervical lesions had preceding negative hrHPV tests. This finding was not surprising because HPV-negative HSIL or cervical cancer cases have been reported in earlier studies. For example, a study involving 256,648 cases with cytologic and HPV cotesting demonstrated that 19% of the women with cervical cancer had preceding negative hrHPV test results.[15] Preceding negative HPV test results have also been reported in up to 25% of women with cervical cancer.[16]

We further analyzed the 21 biopsy samples with preceding negative hrHPV test results using multiple sensitive assays, including Cobas 4800 system, HPV DNA microarray, and direct DNA sequencing. Our data showed that various HPV genotypes were detected by at least one of the assays in most (95%) of the biopsy samples, despite previous negative HPV test results on cytology samples (Table 1, Fig. 1). In the biopsy samples with hrHPV infection, the Cobas test identified more than one half (57%) but missed the remaining cases (43%). Cobas testing was also negative in 6 samples (29%) with non-hrHPV genotypes (Fig. 3). Only 1 sample was negative using all three HPV assays (5%). These findings have demonstrated that HPV infection is associated with the vast majority of biopsy-confirmed HSIL or worse lesions despite preceding negative HPV test results, and hrHPV genotypes were the most common causative agents (70%) of these high-grade cervical lesions.

The underlying reasons for the discrepancy between positive hrHPV test results in biopsy samples and previous negative hrHPV test results in cytology samples are not known. Several factors could potentially contribute to the negative HPV test results in cytology samples, including a low viral load, inadequate sampling, and interference material or technical errors. We found that approximately one third of the biopsy samples with hrHPV genotypes were negative or inadequate on Cobas testing, and many of those samples were also weakly positive on the DNA microarray assay. The targeted viral DNA in these samples could have been present in amounts less than the threshold of the Cobas test.

In addition to possible inadequate sampling, high-grade cervical lesions usually occur in women with persistent HPV infection characterized by overexpression of E6/E7 oncogenes after viral DNA integration into the host genome. Compared with the early phase of productive infection, the viron production and L1 gene expression might be significantly lower in women with persistent HPV infection when the incidence of high-grade lesions increases. In support of this notion, recent studies have found that HPV E6/E7 mRNA testing has improved the specificity for CIN2-positive cervical lesions compared with HPV DNA assays.[21-23] We recently studied the performance of Cobas HPV DNA test and Aptima E6/E7 mRNA HPV test among 4562 women with cotesting and follow-up biopsies. We found that Aptima E6/E7 mRNA testing had significantly greater specificity and positive predictive values than Cobas testing for biopsy-confirmed HSILs or worse.[24] Using immunohistochemistry, Grapsa et al[25] demonstrated that the p16-positive/L1-negative pattern was significantly more common in HSILs than in LSILs. Given that a sufficient amount of the HPV L1 gene is required for most of the HPV DNA assays, including the Cobas test, reduced L1 expression could contribute to false-negative HPV test results in a number of women with high-grade cervical lesions.

The HPV16/18 genotypes were less commonly detected than non-16/18 hrHPV genotypes among women with positive hrHPV findings in the biopsy samples (4 of 14 [29%] versus 10 of 14 [71%]; Fig. 3). This pattern of genotypic prevalence markedly differed from that observed in women in the same study population with biopsy-confirmed HSILs or worse and previous positive HPV test results. In the latter group, the prevalence for HPV16/18 and non-16/18 HPV was about equal. HPV59 and HPV45 were the 2 leading genotypes detected in the cohort with previous negative HPV test results and accounted for 90% of those infected with non-16/18 hrHPV (Fig. 2). This is an unusual pattern compared with the reported data on genotypic composition in women with HSILs in the United States (in the decreasing prevalence of HPV16, HPV18, HPV31, HPV58, HPV35, HPV56, HPV52, HPV33, HPV66, and HPV51).[26-28] The genotypic prevalence pattern we detected was also clearly inconsistent with the results from our earlier study, which showed that HPV45 and HPV59 ranked 7th and 14th, respectively, in a local cohort of women with cervical dysplasia.[29] This suggests that HPV59 and HPV45 might have lower expression levels of the L1 gene compared with other hrHPV genotypes, especially in high-grade lesions with HPV DNA integration. Another possibility is that the Cobas test might have relatively lower sensitivity in detecting non-16/18 hrHPV genotypes, especially HPV59 and HPV45.

Non-hrHPV genotypes were less commonly detected in biopsy samples with HSIL or worse and preceding negative HPV test results (6 of 21; 29%). Three of the 6 biopsy samples tested positive for non-16/18 hrHPV on the Cobas test; however, non-hrHPV genotypes were confirmed by DNA microarray or sequencing assays, suggesting possible cross-reactions among the genotypes on the Cobas test. The non-hrHPV genotypes detected in the 6 samples included HPV genotypes 6, 55, 61, 62, 83, and 84 (Fig. 2). Understandably, the preceding Cobas HPV test results from the cytology specimens were negative because the Cobas test panel is limited to hrHPV. Abnormal cytomorphology was detected in previous Pap tests in all 6 cases and included ASC-US (n = 1), LSILs (n = 1), ASC-H (n = 2), and HSIL (n = 2). Testing of follow-up biopsy specimens showed CIN2 in 2 cases and CIN3 in 4 cases. Of the 6 biopsy samples, 5 (83%) had a single HPV infection with genotypes 55, 61, 62, 81, or 83. In the absence of other HPV genotypes, these non-hrHPV genotypes were most likely the causative agents for high-grade cervical lesions in these women.

Using the IARC classification, these non-hrHPV genotypes belong to group 3 and are commonly referred to as "non-hrHPV" owing to insufficient data on their oncogenic potential. We believe that some of these genotypes might not be as benign as commonly perceived and might potetially cause cervical dysplasia or even cancer, especially in women with "negative" hrHPV test results. In an earlier study, we first reported that HPV90, one of the "non-hrHPV," was associated with cervical dysplasia, including HSILs, in a North American population.[30] In a Western blot assay, Fu et al.[31] demonstrated that the K16N mutation of HPV90 E6 enabled it to fully degrade p53 as effectively as HPV16 E6 in a single-transfected cell assay. In the present study, we identified the association of additional "non-hrHPV" genotypes (HPV55, HPV61, HPV62, HPV81, and HPV83) with HSILs or worse as a single genotypic infection. These nonconventional HPV genotypes have often been overlooked because they are undetectable by most HPV assays commonly used in practice. In addition, these genotypes have generally been perceived as low risk or even nononcogenic. However, our data have indicated that at least several of those "non-hrHPV" genotypes likely are the genuine causative agents for high-grade cervical lesions and might play increasingly important roles in the development of cervical cancer and precancerous lesions in the post-HPV vaccination era. Further studies are needed to elucidate the oncogenic potential of these uncommon HPV genotypes.

Our study also demonstrated a high rate of multiple genotypic HPV infection (62%) in women with biopsy-confirmed HSIL or worse and previous negative HPV test results. The rate was considerably higher than the 20% to 40% in most previously reported studies, with variations dependent on the age and severity of the cervical lesions.[29,32-34] In this general population, women with mixed HPV16/18 and non-16/18 hrHPV found on preceding HPV tests accounted for only 17%. Several studies, including ours,[35,36] have indicated that the HPV genotypes might have competitive interactions in women with multiple genotypic infections. However, it is unknown whether such interactions among HPV genotypes are significant enough to affect HPV detection. Further studies are necessary.

One of the 21 biopsied samples with HSIL or worse tested negative with all 3 HPV assays, even with multiple repeated attempts. It is unknown whether the negative HPV assay findings represented a genuine HPV-negative HSIL or false-negative test results. Although persistent HPV infection has been considered the cause of most cervical cancers, HPV is not always detected before the cancer diagnosis. One study indicated that only 91% of cervical squamous cell carcinomas were HPV positive using the linear array HPV genotyping test.[37] The data from the Kaiser Permanente study showed that 37% of women with cervical squamous cell carcinoma were HPV-negative 5 years before the histologic diagnosis of cervical cancer.[38] However, multiple factors should be considered before considering a case is genuine HPV-negative HSIL or cancer. False-negative test results can be caused by a low viral load, small lesion size, interfering material, technical errors, a limited testing panel, or insufficient sensitivity of the assays.

Several factors could have affected the interpretation of our results. First, ours was a retrospective study rather than a controlled trial. Although patient selection bias was unavoidable, the present study more likely reflects real-world clinical practice than would a controlled trial. Second, the study analyzed HPV tests performed on SurePath and ThinPrep samples together, and HPV-negative cases with biopsy-confirmed HSIL or worse lesions were detected in both preparations. It has been suggested that some liquid-based preparations can interfere with HPV testing sensitivity and, thus, might have affected the interpretation of our data. Third, the study cohort was relatively small and lacked patients with glandular lesions or carcinoma. In addition, the lesions in the biopsy samples were generally small and could have affected the HPV assays, despite careful tissue dissection. Large prospective studies are necessary to further validate the findings.

Conclusions

Our study has demonstrated that 8.3% of women with biopsy-confirmed HSIL or worse had negative hrHPV test results in the preceding cytology samples. Despite the previous negative HPV test results, a wide range of HPV genotypes were detected in the vast majority (95%) of biopsy samples. These women were often infected by hrHPV (67%) with HPV59 and HPV45 predominance, a genotypic prevalence pattern markedly different from that encountered in the general population. Less commonly, non-hrHPV genotypes were associated with 29% of HSIL or worse lesions in the cohort, primarily with a single genotype infection. No detectable HPV was found in 1 of the biopsy samples after

analysis using all 3 HPV assays. The latter results might be attributable to a genuine non−HPV-driven lesion. In addition to infection by non-hrHPV genotypes or possible non−HPV-related dysplasia, multiple other factors could have contributed to the false-negative hrHPV test results in preceding cytology samples, including inadequate sampling, interfering material, technical errors, and reduced L1 gene expression in high-grade lesions. Additional studies are necessary to validate the findings and elucidate the contributing factors and underlying mechanisms.

Acknowledgments

The authors thank Woo-Chul Moon, MD, PhD, for assisting the human papillomavirus genotyping tests, and Helen Chifotides, PhD, for editing the manuscript.

Funding sources

The present study was funded by a microgrant from the Department of Pathology and Genomic Medicine, Houston Methodist Hospital, Houston, Texas.

Conflict of interest disclosures

The authors made no disclosures.

References

1. Schiffman M, Castle PE. The promise of global cervical-cancer prevention. *N Engl J Med*. 2005;353:2101−2104.
2. Moyer VA. Screening for cervical cancer: U.S. Preventive Services Task Force recommendation statement. *Ann Intern Med*. 2012;156:880−891.
3. Gyllensten U, Gustavsson I, Lindell M, Wilander E. Primary high-risk HPV screening for cervical cancer in post-menopausal women. *Gynecol Oncol*. 2012;125:343−345.
4. Leinonen MK, Nieminen P, Lönnberg S, et al. Detection rates of precancerous and cancerous cervical lesions within one screening round of primary human papillomavirus DNA testing: prospective randomised trial in Finland. *BMJ*. 2012;345:e7789.
5. Malila N, Leinonen M, Kotaniemi-Talonen L, et al. The HPV test has similar sensitivity but more overdiagnosis than the Pap test—a randomised health services study on cervical cancer screening in Finland. *Int J Cancer*. 2013;132:2141−2147.
6. Ogilvie GS, Krajden M, van Niekirk DJ, et al. Primary cervical cancer screening with HPV testing compared with liquid-based cytology: results of round 1 of a randomised controlled trial—the HPV FOCAL study. *Br J Cancer*. 2012;107:1917−1924.
7. Rijkaart DC, Berkhof J, van Kemenade FJ, et al. HPV DNA testing in population-based cervical screening (VUSA-Screen study): results and implications. *Br J Cancer*. 2012;106:975−981.
8. Ronco G, Dillner J, Elfström KM, et al. Efficacy of HPV-based screening for prevention of invasive cervical cancer: follow-up of four European randomised controlled trials. *Lancet*. 2014;383:524−532.
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. *Am J Clin Pathol*. 2012;137:516−542.
10. Huh WK, Ault KA, Chelmow D, et al. Use of primary high-risk human papillomavirus testing for cervical cancer screening: interim clinical guidance. *Obstet Gynecol*. 2015;125:330−337.
11. Wright Jr TC, Stoler MH, Behrens CM, et al. The ATHENA human papillomavirus study: design, methods, and baseline results. *Am J Obstet Gynecol*. 2012;206:46.e1−46.e11.
12. Austin RM, Zhao C. Is 58% sensitivity for detection of cervical intraepithelial neoplasia 3 and invasive cervical cancer optimal for cervical screening? *CytoJournal*. 2014;11:14.
13. Zhou H, Mody RR, Luna E, et al. Clinical performance of the Food and Drug Administration-approved high-risk HPV test for the detection of high-grade cervicovaginal lesions. *Cancer Cytopathol*. 2016;124:317−323.
14. Vassilakos P, Tran PL, Sahli R, Low N, Petignat P. HPV-negative CIN3 and cervical cancer in Switzerland: any evidence of impact on screening policies? *Swiss Med Wkly*. 2017;147:w14559.
15. Blatt AJ, Kennedy R, Luff RD, Austin RM, Rabin DS. Comparison of cervical cancer screening results among 256,648 women in multiple clinical practices. *Cancer Cytopathol*. 2015;123:282−288.
16. Zhao C, Li Z, Nayar R, et al. Prior high-risk human papillomavirus testing and Papanicolaou test results of 70 invasive cervical carcinomas diagnosed in 2012: results of a retrospective multicenter study. *Arch Pathol Lab Med*. 2015;139:184−188.
17. Tao X, Zheng B, Yin F, et al. Polymerase chain reaction human papillomavirus (HPV) detection and HPV genotyping in invasive cervical cancers with prior negative HC2 test results. *Am J Clin Pathol*. 2017;147:477−483.
18. Mody DR, Krishnamurthy S, Anton R, Thrall M. *Diagnostic Pathology: Cytopathology*. Philadelphia, PA: Lippincott, Williams & Wilkins; 2014.
19. Zhou H, Schwartz MR, Coffey D, et al. Should LSIL-H be a distinct cytology category? A study on the frequency and distribution of 40 human papillomavirus genotypes in 808 women. *Cancer Cytopathol*. 2012;120:373−379.
20. International Agency for Research on Cancer. *International Agency for Research on Cancer Monographs: Human Papillomaviruses*. **100B. Lyon, France: International Agency for Research on Cancer; 2009.**
21. Szarewski A, Mesher D, Cadman L, et al. Comparison of seven tests for high-grade cervical intraepithelial neoplasia in women with abnormal smears: the Predictors 2 study. *J Clin Microbiol*. 2012;50:1867−1873.
22. Castle PE, Eaton B, Reid J, Getman D, Dockter J. Comparison of human papillomavirus detection by Aptima HPV and Cobas HPV tests in a population of women referred for colposcopy following detection of atypical squamous cells of undetermined significance by Pap cytology. *J Clin Microbiol*. 2015;53:1277−1281.
23. Iftner T, Becker S, Neis KJ, et al. Head-to-head comparison of the RNA-based Aptima human papillomavirus (HPV) assay and the DNA-based hybrid Capture 2 HPV test in a routine screening population of women aged 30 to 60 years in Germany. *J Clin Microbiol*. 2015;53:2509−2516.
24. Ge Y, Christensen P, Luna E, et al. Performance of Aptima and Cobas HPV testing platforms in detecting high-grade cervical dysplasia and cancer. *Cancer Cytopathol*. 2017;125:652−657.
25. Grapsa D, Frangou-Plemenou M, Kondi-Pafiti A, et al. Immunocytochemical expression of P53, PTEN, FAS (CD95), P16INK4A and HPV L1 major capsid proteins in ThinPrep cervical samples with squamous intraepithelial lesions. *Diagn Cytopathol*. 2014;42:465−475.
26. Castellsagué X, de Sanjosé S, Aguado T, et al. HPV and cervical cancer in the world: 2007 report. *Vaccine*. 2007;25(suppl 3):C1−C230.
27. Clifford GM, Smith JS, Aguado T, Franceschi S. Comparison of HPV type distribution in high-grade cervical lesions and cervical cancer: a meta-analysis. *Br J Cancer*. 2003;89:101−105.

28. Smith JS, Lindsay L, Hoots B, et al. Human papillomavirus type distribution in invasive cervical cancer and high-grade cervical lesions: a meta-analysis update. *Int J Cancer*. 2007;121:621−632.
29. Zhou H, Mody D, Schwartz MR, Schwartz DM. Genotype-specific prevalence and distribution of human papillomavirus genotypes in underserved Latino women with abnormal Papanicolaou tests. *J Am Soc Cytopathol*. 2014;3:42−48.
30. Quiroga-Garza G, Zhou H, Mody DR, Schwartz MR, Ge Y. Unexpected high prevalence of HPV 90 infection in an underserved population: is it really a low-risk genotype? *Arch Pathol Lab Med*. 2013;137:1569−1573.
31. Fu L, Van Doorslaer K, Chen Z, et al. Degradation of p53 by human Alphapapillomavirus E6 proteins shows a stronger correlation with phylogeny than oncogenicity. *PLoS One*. 2010;5.
32. Cuschieri KS, Cubie HA, Whitley MW, et al. Multiple high risk HPV infections are common in cervical neoplasia and young women in a cervical screening population. *J Clin Pathol*. 2004;57:68−72.
33. Vaccarella S, Francheschi S, Snijders PJ, et al. Concurrent infection with multiple human papillomavirus types: pooled analysis of the IARC HPV Prevalence Surveys. *Cancer Epidemiol Biomarkers Prev*. 2010;19:503−510.
34. Beca F, Pinheiro J, Rios E, Pontes P, Amendoeira I. Genotypes and prevalence of HPV single and multiple concurrent infections in women with HSIL. *Diagn Cytopathol*. 2014;42:919−923.
35. Salazar KL, Zhou HS, Xu J, et al. Multiple human papilloma virus infections and their impact on the development of high-risk cervical lesions. *Acta Cytol*. 2015;59:391−398.
36. Chaturvedi AK, Myers L, Hammons AF, et al. Prevalence and clustering patterns of human papillomavirus genotypes in multiple infections. *Cancer Epidemiol Biomarkers Prev*. 2005;14:2439−2445.
37. Hopenhayn C, Christian A, Christian WJ, et al. Prevalence of human papillomavirus types in invasive cervical cancers from 7 US cancer registries before vaccine introduction. *J Low Genit Tract Dis*. 2014;18:182−189.
38. Katki HA, Kinney WK, Fetterman B, et al. Cervical cancer risk for women undergoing concurrent testing for human papillomavirus and cervical cytology: a population-based study in routine clinical practice. *Lancet Oncol*. 2011;12:663−672.