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Risk of precancer determined by HPV genotype combinations in women with minor cytologic abnormalities

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Abstract

Background—Studies suggest that testing for individual HPV genotypes can improve risk stratification in women with minor cytological abnormalities. We evaluated genotyping for HPV16, HPV16/18, and HPV16/18/45 in carcinogenic HPV-positive women with atypical squamous cells of undetermined significance (ASCUS) and low-grade squamous intraepithelial lesion (LSIL) cytology.

Methods—For women enrolled in the ASCUS-LSIL Triage Study (ALTS), we calculated the age-stratified (<30 and 30+ years) positivity, and cumulative risk over two years of cervical intraepithelial neoplasia grade 3 or worse (CIN3+) when testing positive or negative for three genotype combinations: HPV16, HPV16/18, and HPV16/18/45.

Results—Among women with ASCUS cytology, HPV16 positivity was 17.1% and increased to 22.0% ($P<.001$) for HPV16/18 and 25.6% ($P<.001$) for HPV16/18/45. Among women with LSIL cytology, HPV16 positivity was 21.1% and increased to 30.0% ($P<.001$) for HPV16/18 and 34.0% ($P=.017$) for HPV16/18/45. Regardless of cytology and age group, the greatest risk difference between test-positives and test-negatives was observed for HPV16 with decreasing risk stratification for HPV16/18 and HPV16/18/45. However, testing negative for any of the three combinations while being positive for another carcinogenic type still implied a 2-year risk of CIN3+ of 7.8% or greater.

Conclusions—Although genotyping for HPV16, 18, and 45 provided additional risk stratification in carcinogenic HPV-positive women with minor cytological abnormalities, the risk among genotype-negative women was still high enough to warrant immediate colposcopy referral.

Impact—HPV genotyping in HPV-positive women with minor cytological abnormalities will likely not alter clinical management. Adding HPV45 to genotyping assays is not warranted.

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Keywords

HPV testing; cervical intraepithelial neoplasia; cervical cytology; risk stratification

Introduction

Persistent infection with carcinogenic human papillomaviruses is a necessary cause of cervical cancer. It has been demonstrated that the risk of progression to cancer varies substantially between individual carcinogenic types and infections with HPV16 are associated with elevated risk of high grade lesions (cervical intraepithelial neoplasia grade 3) (1–5). In recently updated cervical cancer screening guidelines, testing for HPV16 and 18 (the second most important HPV type, and a major cause of adenocarcinoma) has been recommended as a triage for HPV-infected, cytology-negative women (6). It has also been suggested that HPV genotyping could be used for management of women with minor cytologic abnormalities (atypical squamous cells of undetermined significance (ASCUS) and low-grade squamous intraepithelial lesions (LSIL)), to decide who should be referred to immediate versus delayed colposcopy (5). Furthermore, it has been proposed that HPV45 be added to HPV genotyping assays. Following up on previous studies conducted in ALTS that showed the strong risk of precancer associated with HPV16 (5), we compared the risk stratification achieved with HPV genotyping combinations 16 vs. 16/18 vs. 16/18/45 in HPV-positive women with ASCUS and LSIL.

Methods

The ASCUS-LSIL Triage Study (ALTS) was a randomized trial directed by the National Cancer Institute (National Institutes of Health, Bethesda, MD, U.S.) that compared 3 triage strategies for women with ASCUS or LSIL. Details of the design, methods, and primary results of ALTS have been published extensively elsewhere (7–9). Briefly, women with ASCUS or LSIL cytology were recruited to participate in the study at four clinical centers. A total of 5,060 women enrolled in the study from January 1997 to December 1998. At enrollment, the ALTS participants were referred to colposcopy depending on study arm. In the immediate colposcopy arm, all women had colposcopy at, or soon after, the enrollment regardless of enrollment test results. In the HPV triage arm, women were referred to colposcopy if the enrollment HPV test was positive (56.4%) or missing (3.9%), or if the enrollment cytology was high-grade squamous intraepithelial lesion (HSIL), although cytology added almost no referrals. In the conservative management, women were referred to colposcopy if enrollment cytology was interpreted as HSIL. The ALTS participants were followed at six-month intervals for 2 years. At the semi-annual follow-up visits, regardless of randomization arm, colposcopic examinations were triggered only by HSIL cytology. At the exit visit, all women were scheduled for a colposcopic examination. Throughout the trial, women with histologic diagnosis of cervical intraepithelial neoplasia grade 2 or worse (CIN2+) as defined by the clinical center pathologists were treated by loop electrosurgical excision procedure (LEEP) or more extensive surgery if needed. At exit, women with persistent lower-grade lesions as well were offered LEEP to maximize safety after follow-up ended. NCI and local institutional review boards approved the study.

The enrollment examination included a pelvic exam with the collection of cells for cytology and HPV DNA testing as well as high resolution photography of the cervix for visual screening (Cervicography, National Testing Laboratories, Fenton, MO). After liquid-based ThinPrep (Cytoc Corporation, Marlborough, MA) cytology slides were prepared and 4-ml aliquots of the residual PreservCyt samples were used for HPV DNA testing by Hybrid Capture 2 (HC2) (Qiagen Corporation, Gaithersburg, MD). As mentioned, clinical

management was based on the clinical center (CC) pathologists' cytologic and histologic diagnoses. In addition, all cytology and histology slides were sent to the Pathology Quality Control Group (Pathology QC) for independent review. Pathology QC histologic diagnoses were masked to cytology results and used in this data analysis to avoid center-specific variation.

Among women with ASCUS cytology, aliquots of the archived STM specimens were tested as previously described (10) using Linear Array (LA) (Roche Molecular Systems, Alameda, CA), a L1-based polymerase chain reaction (PCR) assay that employs a primer set designated PGMY09/11. Among women with LSIL, HPV genotyping on aliquots of the STM specimen was performed by Line Blot Assay (LBA) (Roche Molecular Systems, Alameda, CA), a research-use-only version of LA (11, 12). The two assays, LA and LBA have demonstrated comparable performance in these specimens (10). Women were considered infected with a carcinogenic HPV if they tested positive to one or more of the 13 carcinogenic HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68)(13) plus HPV66. Although HPV66 is classified as only "possibly carcinogenic," (13) historically, it has been combined with other carcinogenic HPV genotypes in pooled-probe HPV assays and is therefore included in this analysis.

We compared positivity and risk stratification among carcinogenic HPV-positive women for 3 combinations of genotypes: 1) HPV16, 2) HPV16 and HPV18, and 3) HPV 16, 18 and 45. For each group we calculated the cumulative risk given any positive vs. negative genotype results over two years follow-up of CIN2+ by CC diagnosis and cervical intraepithelial neoplasia grade 3 and cancer (CIN3+) by Pathology QC. Specifically, we compared HPV16 to the other 13 types as a group; HPV16 or HPV18 compared with the other 12 types as a group; and HPV16 or HPV18 or HPV45 compared with the other 11 types as a group. Results were stratified by enrollment cytology result (ASCUS or LSIL) and age (less than 30 vs. 30 or older). We examined whether findings differed by study arm. Also, because women aged 18–21 are no longer recommended for routine screening, we considered whether excluding them from the analysis would change our findings (6). Supplemental figures present ancillary analyses using the outcome of detection of CIN2+ by CC and CIN3+ by Pathology QC at enrollment as well as the risk for all women (HPV-positive and HPV-negative) enrolled in ALTS. Positivity was compared using probability tests of significance. Risks of precancer were compared using Chi-square test statistics.

Results

Overall, 55.6% of women with ASCUS cytology (Table 1) and 73.5% of women with LSIL cytology (Table 2) were positive for any carcinogenic HPV type. HPV16 positivity ranged from 8.0% among women 30 years and older with ASCUS cytology (Table 1) to 22.5% among women less than 30 years with LSIL cytology (Table 2). For women with ASCUS cytology, the addition of HPV18 compared with HPV16 alone resulted in almost one-third more referrals among both women younger than 30 (28.0% vs. 21.9%, $P<.01$) and women 30 or older (10.8% vs. 8.0%, $P=.01$) (Table 1). Adding HPV45 slightly increased the overall referral rate to 32.2% among women younger than 30 ($P<.01$) and 13.4% among women age 30 and older ($P=.02$). For women with LSIL cytology, the incremental positivity for adding HPV18 and HPV45 to HPV16 was similar to what was observed among women with ASCUS.

To show the extent of risk stratification provided by genotyping carcinogenic HPV-positive women, we plotted the absolute 2-year risk of CIN2+ and CIN3+ among women testing positive and negative for the different genotype combinations, stratified by referral cytology. Figure 1 shows women aged <30 while Figure 2 shows women aged 30 or older. In all

strata, HPV16 showed the greatest difference between risk in test-positives and test-negatives, a measure of risk stratification. Adding HPV18 and HPV45 decreased the risk in women testing positive, while the risk in test-negatives remained largely unchanged. Notably, for women age 30 or older and HPV16 positive the 2-year risk of a CIN3+ diagnosis was 48.6% (95% CI: 32.5–64.8), an elevated risk possibly warranting consideration of treatment in certain populations with limited access to follow-up.

Positivity tended to confer equal risk of high grade neoplasia across age strata (Figures 1 and 2). Although the absolute risk for CIN3+ did not differ by age for women with ASCUS, for women with LSIL the CIN3+ risks given HPV16 positivity was higher, albeit non-significantly) in older vs. younger women (48.6% in women 30 and over vs. 37.9% in women under 30, $P=.21$). The risks were similar by age when adding HPV18 ($P=.78$), HPV45 ($P=.94$) and all carcinogenic types ($P=.92$).

Analyses with the CIN2+ endpoint (Figures 1 and 2) and those restricting to endpoints detected at enrollment (Supplemental Figures 1 and 2) showed similar findings. In addition, results were similar across study arms and when limiting to women aged 21 and older. When carcinogenic HPV-negative women were included in the genotype-negative strata, the risk in genotype negative women was much lower (Supplemental Figures 3 and 4), potentially warranting delayed colposcopy. However, it is unlikely that HPV genotyping would be performed without prior carcinogenic HPV testing in clinical practice; therefore, we did not expand on this analysis here.

Discussion

Testing for HPV genotypes has recently been integrated in cervical cancer screening algorithms for HPV-positive/cytology-negative women. The approach has also been proposed for management of women with minor cytological abnormalities. In this study, we analyzed the risk stratification provided by HPV genotyping for three different type combinations HPV16, HPV16/18, and HPV16/18/45 in women with ASCUS and LSIL referral cytology. We observed substantial risk stratification for 2-year cumulative CIN3+ for all three genotype combinations that was highest for HPV16 alone and decreased with adding additional genotypes. For example, among women 30 years and older with HPV+ ASCUS, the risk difference between HPV16-positive and HPV16-negative women reached 21%, while the risk difference between HPV16/18/45-positive and HPV16/18/45-negative women was 13%.

To be useful for clinical management, the risk estimates provided by HPV genotyping need to lead to different management decisions, such as immediate referral to colposcopy vs. delayed colposcopy or repeat cytology/HPV testing. A previous analysis of ALTS data considered a 10% 2-year risk of CIN3+ to be a threshold for referral to colposcopy (14). As we show here, women with ASCUS cytology who tested negative for HPV16, HPV16/18, or HPV16/18/45 (but still positive for the remaining carcinogenic types) had a risk between 7.9 and 8.8, with the confidence intervals including the 10% threshold (Figures 1 and 2). Likewise, the risk in women with LSIL referral cytology was 10% or higher when they tested negative for the different genotyping combinations.

Despite the impressive risk stratification of genotyping in our study, the risk among women testing negative for the genotypes was not low enough to warrant delaying colposcopy evaluation, if currently accepted risk thresholds are applied (14). Thus, according to risk thresholds established in ALTS, both genotype positive and negative women would be referred to immediate colposcopy. While the absolute risk varies in other populations, it is likely that the relative differences would result in similar management recommendations.

Until recently, the only FDA-approved HPV test was Hybrid Capture 2 (HC2; Qiagen Corp., Gaithersburg, MD), a pooled-probe test that detects the presence of one or more of 13 HPV DNA genotypes. During the past years, the FDA approved additional HPV DNA tests that provide both testing for pooled carcinogenic types as well as individual detection of the two most carcinogenic HPV genotypes, HPV16 and HPV18 (cobas HPV test; Roche Molecular Systems, Pleasanton, CA (15) and Cervista; Hologic, Bedford, MA (16, 17)).

The test performance for HPV16 and 18 utilizing enrollment colposcopically-guided biopsy results mirrors that of the recent ATHENA trial. In the ATHENA trial where women with an ASCUS cytology were enrolled, the absolute risk of CIN2+ and CIN3+ at enrollment was 24.4% (95% CI: 18.7%–31.3%) and 15.9% (95% CI: 11.2%–22.0%), respectively (15). The absolute risks of CIN2+ and CIN3+ among women testing positive for carcinogenic HPV but negative for both HPV16 and HPV18 were 8.6% (95% CI: 6.0%–12.1%) and 4.4% (2.7%–7.2%), respectively. These risks are similar to the those observed among women in ALTS with ASCUS cytology and enrolled in the immediate colposcopy or HPV triage arms: testing HPV16 or 18 positive conferred a 25.8% (95% CI: 22.0%–29.9%) and 15.1% (95% CI: 12.1%–18.6%) risk of CIN2+ and CIN3+, respectively at enrollment while testing carcinogenic HPV-positive but negative for both HPV16 and HPV18 conferred a 10.6% (95% CI: 8.5%–13.0%) and 3.8% (95% CI: 2.6%–5.5%) risk for CIN2+ and CIN3+, respectively at enrollment. The risks for precancer at enrollment reported in a study of the Cervista HPV16/18 test were lower among women testing HPV16 or 18 positive at 17.1% (95% CI: 13.0%–22.2%) and 6.6% (95% CI: 4.2%–10.3%) risk of CIN2+ and CIN3+, respectively (16). Similarly, the risks when testing negative for HPV16 or HPV18, but positive for carcinogenic HPV by Cervista were lower at 4.0% (95% CI: 2.5%–6.1%) and 1.0% (95% CI: 0.3%–2.3%) of CIN2+ and CIN3+, respectively. Both, overall differences in the population, or different HPV genotyping test characteristics could explain the lower absolute risk observed in the Cervista trial.

The addition of carcinogenic types HPV 18 and HPV45 to HPV16 as a possible triage test for ASCUS and LSIL cytology results resulted in higher positivity and reduced risk stratification. In general, our analysis did not suggest a benefit of adding HPV45 to genotyping assays, because the risk among test-positives was reduced compared with HPV16 and HPV16/18 without providing greater reassurance among test-negatives. While the incremental benefit of adding HPV18 was also limited in our study, HPV18 is the second most common genotype in cervical cancers and therefore warrants inclusion in genotyping assays, despite the noted deficit of cervical precancers associated with this type.

We found that changes in positivity and predictive value were not uniform across age groups. Women younger than 30 years with ASCUS were more likely to test positive across all HPV genotype combinations although the age-stratified absolute risk for CIN3+ was statistically similar. The noted exception was in women with LSIL cytology where the risk associated with HPV16 alone was non-significantly higher in older vs. younger women (age less than 30).

We demonstrated that HPV genotyping provides substantial additional risk stratification among HPV-positive women with mild cytological abnormalities. However, in ALTS, the risk in women testing negative for the HPV genotypes was close to or above the threshold that is widely considered for referral to colposcopy. We confirmed that HPV16 provides the biggest risk stratification, while the additional benefit of HPV18 and HPV45 is limited. Although the high attribution of HPV18 to invasive cancers warrants inclusion of that type in typing assays, there does not seem to be benefit of including HPV45 in genotyping assays. It is possible that testing for HPV45 offers some additional sensitivity to detect

adenocarcinomas, although this study was not sufficiently powered to address this question and the tradeoff is a much higher referral rate and lower positive predictive value.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Among women aged <30 with carcinogenic HPV infection, absolute risks of CIN2+ (left panel) and CIN3+ (right panel) in 2 years given category of HPV genotyping results by referral cytology

“Any HPV” refers to 13 carcinogenic genotypes (16,18,31,33,35,39,45,51,52,56,58,59,68) plus HPV66.

Positive: Test positive for one or more genotypes in category. Negative: Tests negative for all genotypes in category.

Endpoints are cervical intraepithelial neoplasia (CIN) grade 2 or more severe (CIN2+; clinical center diagnosis) and CIN grade 3 or more severe (CIN3+; Pathology Quality Control Group diagnosis). Diagnoses of CIN2+ and CIN3+ were made independently by clinical center and pathology quality control group, respectively.

Figure 2.

Among women aged 30+ with carcinogenic HPV infection, absolute risks of CIN2+ (left panel) and CIN3+ (right panel) in 2 years given category of HPV genotyping results by referral cytology

“Any HPV” refers to 13 carcinogenic genotypes (16,18,31,33,35,39,45,51,52,56,58,59,68) plus HPV66.

Positive: Test positive for one or more genotypes in category. Negative: Tests negative for all genotypes in category.

Endpoints are cervical intraepithelial neoplasia (CIN) grade 2 or more severe (CIN2+; clinical center diagnosis) and CIN grade 3 or more severe (CIN3+; Pathology Quality Control Group diagnosis). Diagnoses of CIN2+ and CIN3+ were made independently by clinical center and pathology quality control group, respectively.

HPV DNA genotyping result at enrollment visit among women with ASCUS cytology and sensitivity and specificity to detect CIN2+/CIN3+ over 2 year follow-up

Table 1

	Referral rate		CIN2+ over 2 year follow-up		CIN3+ over 2 year follow-up				
	Total N	% positive	95% CI	CIN2+ n	Sensitivity	Specificity	CIN3+ n	Sensitivity	Specificity
Age 18-88									
HPV16	590	17.1	15.8-18.3	240	45.4		171	57.2	86.7
HPV16,18	760	22.0	20.6-23.4	272	51.4		186	62.2	81.8
HPV16,18,45	886	25.6	24.2-27.1	290	54.8		195	65.2	78.1
Any carcinogenic type ,66	1920	55.6	53.9-57.2	474	89.6		277	92.6	48.0
Total	3456	100.0		529			299		
Age 18-29 (mean=23.0)									
HPV16	493	21.9	20.2-23.7	206	47.5		143	58.1	82.5
HPV16,18	630	28.0	26.2-29.9	228	52.5		155	63.0	76.3
HPV16,18,45	724	32.2	30.2-34.2	246	56.7		164	66.7	72.1
Any carcinogenic type ,66	1508	67.0	65.0-69.0	395	91.0		230	93.5	36.2
Total	2250	100.0		434			246		
Age 30-88 (mean=39.5)									
HPV16	97	8.0	6.6-9.7	34	35.8		28	52.8	94.0
HPV16,18	130	10.8	9.1-12.7	44	46.3		31	58.5	91.4
HPV16,18,45	162	13.4	11.6-15.5	44	46.3		31	58.5	88.6
Any carcinogenic type ,66	412	34.2	31.5-36.9	79	83.2		47	88.7	68.3
Total	1206	100.0		95			53		

Endpoint is cervical intraepithelial neoplasia (CIN) grade 2 or more severe (CIN2+; clinical center diagnosis) and cervical intraepithelial neoplasia (CIN) grade 3 or worse (CIN3+; Pathology Quality Control Group diagnosis). Diagnoses of CIN2+ and CIN3+ were made independently by clinical center and pathology quality control group, respectively.

HPV DNA genotyping result at enrollment visit among women with LSIL cytology and sensitivity and specificity to detect CIN2+/CIN3+ over 2 year follow-up

Table 2

	Referral rate		CIN2+over 2 year follow-up			CIN3+over 2 year follow-up			
	Total N	% positive	95% CI	CIN2+ n	Sensitivity	Specificity	CIN3+ n	Sensitivity	Specificity
Age 18-88									
HPV16	327	21.1	19.0-23.1	167	42.5	86.2	128	54.9	84.9
HPV16,18	465	30.0	27.7-32.2	205	52.2	77.6	148	63.5	76.0
HPV16,18,45	527	34.0	31.6-36.3	222	56.5	73.7	156	67.0	71.9
Any carcinogenic type ,66	1140	73.5	71.3-75.7	349	88.8	31.8	214	91.8	29.8
Total	1552	100.0		393			233		
Age 18-29 (mean=23.0)									
HPV16	290	22.5	20.2-24.8	146	42.9	84.9	110	55.0	83.5
HPV16,18	402	31.1	28.6-33.7	180	52.9	76.7	127	63.5	74.8
HPV16,18,45	457	35.4	32.8-38.1	195	57.4	72.5	135	67.5	70.5
Any carcinogenic type ,66	983	76.1	73.7-78.4	302	88.8	28.4	185	92.5	26.9
Total	1291	100.0		340			200		
Age 30-88 (mean=39.5)									
HPV16	37	14.2	10.2-19.0	21	39.6	92.3	18	54.5	91.7
HPV16,18	63	24.1	19.1-29.8	25	47.2	81.7	21	63.6	81.6
HPV16,18,45	70	26.8	21.5-32.6	27	50.9	79.3	21	63.6	78.5
Any carcinogenic type ,66	157	60.2	53.9-66.1	47	88.7	47.1	29	87.9	43.9
Total	261	100.0		53			33		

Endpoint is cervical intraepithelial neoplasia (CIN) grade 2 or more severe (CIN2+; clinical center diagnosis) and cervical intraepithelial neoplasia (CIN) grade 3 or worse (CIN3+; Pathology Quality Control Group diagnosis). Diagnoses of CIN2+ and CIN3+ were made independently by clinical center and pathology quality control group, respectively.