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Research Article

Age-Group Differences in Human Papillomavirus Types and Cofactors for Cervical Intraepithelial Neoplasia 3 among Women Referred to Colposcopy

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Abstract

Background: Recommendations for high-risk human papillomavirus (HR-HPV) testing as an adjunct to cytology for cervical cancer screening differ by age group, because HR-HPV tests lack adequate specificity in women aged <30. Here, we assess age-group differences in HPV types and other risk factors for cervical intraepithelial neoplasia (CIN) grade 3 or worse (CIN3+) versus CIN0–2 in women from four colposcopy clinics.

Methods: Women ages 18 to 69 ($n = 1,658$) were enrolled and completed structured interviews to elicit data on behavioral risk factors prior to their examinations. HPV genotyping was done on exfoliated cervical cell samples. We estimated relative risks (RR) for HPV types and cofactors for CIN3+, overall and stratified by age group.

Results: After 2 years of follow-up, we identified 178 CIN3+, 1,305 CIN0−2, and 175 indeterminate outcomes. Nonvaccine HR-HPV types were only associated with CIN3+ among women ≥30 (RR = 2.3, 95% CI: 1.5–3.4; <30: RR = 0.9). Among all HR-HPV–positive women, adjusting for age, significant cofactors for CIN3+ included current smoking (RR = 1.5), former smoking (RR = 1.8), regular Pap screening (RR = 0.7), current regular condom use (RR = 0.5), and parity ≥5 (RR = 1.6, P_{trend} for increasing parity = 0.07). However, the parity association differed by age group (≥30: RR = 1.8, $P_{trend} = 0.008$; <30: RR = 0.9; $P_{trend} = .55$).

Conclusion: Subgroup variation by age in the risk of CIN3+ points to the importance of the timing of exposures in relation to CIN3+ detection.

Impact: Future screening strategies need to consider natural history and secular trends in cofactor prevalence in the pursuit of appropriately sensitive and specific screening tools applied to appropriate age groups. *Cancer Epidemiol Biomarkers Prev; 21(1); 111–21. ©2011 AACR.*

Introduction

Most high-risk human papillomavirus (HR-HPV) infections clear without treatment (1, 2) or are controlled immunologically and rendered undetectable (3) without clinical consequences. However, some infections persist, and a subset of persistent infections may progress to cervical intraepithelial neoplasia (CIN) or invasive cancer. Because neoplastic changes typically take years to occur, the prevalences of detectable HPV infections, high-grade CIN (e.g., CIN3), and invasive cervical cancer peak at different ages (4). HPV infection is most prevalent near the average age of sexual debut, when CIN3 and cancer are rare. CIN3 is most often detected by screening about a decade later, and invasive cancer is usually diagnosed in women who are 40 years or older. The different clinical implications of a positive HPV test over the life course have led to age differences in recommendations for application of HR-HPV testing in cervical cancer screening. Testing is currently recommended as an adjunct to cytology screening among women who are at least 30 years old; in younger women, it is only recommended for triage of atypical squamous cells of undetermined significance (ASCUS; ref. 5). Among women in their twenties and younger, the specificity and positive predictive value of HR-HPV testing are too low for the test to be clinically useful (4, 6).

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Consensus exists that CIN3 should be excised when detected at any age (7, 8). Although many CIN3 lesions might never lead to invasive cancer (9), no markers exist to distinguish those that will invade from those that will not. In practice, CIN2 is the threshold for treatment, even though this is an equivocal diagnosis and often signals active infection or lesions destined to regress (10). Excisional treatments may adversely impact future pregnancies (11, 12), a particular concern for younger patients who often have not completed childbearing. Thus, due to the risks of overtreatment, effectively distinguishing true cancer precursors from transient lesions is a priority, especially among young women. Presence of CIN3 or cervical cancer (CIN3+) in young women often signals an early age at HR-HPV infection (2). In addition, women who present with CIN3+ at young ages might be infected with more aggressively carcinogenic HPV types (e.g., HPV-16) or have more unfavorable HR-HPV cofactor profiles (i.e., more risk factors for progression to neoplasia from HR-HPV infection) than older women presenting with similar diagnoses. A greater understanding of age-group differences among women referred to a colposcopy clinic after abnormal cervical cancer screening could shed light on biological differences in earlier and later-onset cases of CIN3+, and thereby inform future efforts to implement risk stratification strategies aimed at reducing unnecessary referral and possible overtreatment of women without clinically important disease. Using data from women attending colposcopy clinics, we aimed to assess age-group differences in (1) the overall distribution of cervical cancer risk factors and CIN grades; (2) the association between HPV types and CIN3+; and (3) cofactors for CIN3+ among HR-HPV–positive women.

Materials and Methods

Enrollment and baseline study protocol

Women attending colposcopy clinics affiliated with urban public hospitals in Southeastern Michigan (3 clinics) and Atlanta, Georgia (1 clinic) between December 2000 and December 2004 were approached, consented, and enrolled in the study prior to their examinations. These clinics primarily serve urban women who rely on public health clinics for their medical and gynecologic care. Women who were less than 18 years old or older than 69 years old, who had a history of hysterectomy or HIV, or who were pregnant at the time of the visit, were ineligible for the study. Of women approached for the study, 47% were eligible; of these, 69% agreed to participate. At the enrollment visit, women participated in a structured interview with a study nurse to ascertain information on demographics, reproductive history, health behaviors, screening history, health history, and cancer in first-degree relatives (13). During a pelvic examination, ecto- and endocervical cells were collected using a CytoBroom (Cytyc). A conventional Pap smear was prepared if clinically indicated, and the remaining cells were dislodged into PreservCyt collection medium (Cytyc), as previously described (14). During the colposcopy examinations, women underwent biopsy, endocervical curettage, conization, or loop electrosurgical excision procedure (LEEP) at the discretion of the treating physician.

CIN classification

Tissue specimens were submitted to the respective hospital pathology laboratories and subsequently reviewed by study pathologists. A consistent algorithm was used for combining data on histology, cytology, and colposcopic impression. As would be done clinically, histology findings were followed for classification of those enrolled as having cancer, CIN3, CIN2, CIN1, or no CIN. Women with no pathology results and cytology results within normal limits or indicating benign cellular changes and no lesions at colposcopy were classified as having no CIN. Otherwise, if only cytology results were available, or if findings were conflicting such that further testing would be required clinically to resolve (e.g., biopsy result no CIN and high-grade cytology), a classification of indeterminate was used; such cases were excluded from analyses.

Women were passively followed up using review of medical records for up to 2 years to ascertain disease that may have been missed at baseline. If a more severe diagnosis was identified on follow-up than was found at baseline, the patient was classified as having the more severe outcome.

HPV detection and typing

Details of HPV genotyping procedures were described previously (14). Briefly, a total nucleic acid extract for HPV genotyping was prepared from the PreservCyt cells. HPV detection and genotyping were conducted using the prototype Roche line blot assay (reagents provided as a gift from Roche Molecular Systems, Inc.). Samples were screened for the HPV amplicon using gel electrophoresis and positive samples were hybridized to the strips. Hybridized samples that did not yield a positive reaction on the strip were sequenced to determine the HPV type. The assay detected the 14 HR-HPV types targeted by commercial screening tests (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68), 16 low-risk (LR) HPV types (6, 11, 40, 42, 44, 54, 55, 61, 62, 64, 71, 72, 74, 81, 83, and 84), and 8 other types referred to here as possible HR types (26, 53, 67, 69, 70, 73, 82, and IS39). Sequencing detected the possible HR-HPV type 85 and LR-HPV types 32, 87, 89, 90, 91, and 89CP6108. Samples negative for HPV and the endogenous positive control (β-globin) were considered inadequate for evaluation and omitted ($n = 12$).

Analytic strategy

We calculated frequency distributions of demographic, reproductive and sexual history, and health behavior characteristics of all women, and evaluated age differences (<30, ≥30) in the distributions of these characteristics using χ^2 or Fisher's exact tests as appropriate. Risk factors were categorized consistently with previous literature when feasible, or using distribution-based cutpoints

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(e.g., quintiles). We also examined the distributions of age and years since sexual debut (i.e., age at first study visit minus age at sexual debut) among HR-HPV–positive women, and tested differences in these distributions by HPV-16 status among cases of CIN 3 and cancer (CIN3+) using the Wilcoxon test.

We calculated overall and age-stratified (<30, ≥30) risk ratios (RR) for the dichotomous outcome CIN3+ versus ≤CIN2 (i.e., no CIN, CIN1, and CIN2 combined; indeterminate diagnoses excluded) using log-binomial models (15). We regarded age-stratified models as potentially informative based on a priori clinical grounds and formally tested for effect measure modification using a likelihood ratio test for an age-group interaction term. We calculated crude (nonhierarchical) RRs for HPV16, HPV18, any vaccine HR-HPV type (16 or 18), nonvaccine HR-HPV types, any clinical HR-HPV type, and by species (i.e., α-9 and α-7 types; 16). We also calculated RRs for multiple genotypes among HPV-positive women, and recalculated all other RRs after excluding women with multiple HPV types.

Among HR-HPV–positive women, we identified demographic, reproductive history, sexual history, and health behavior-related cofactors for CIN3+ through multivariable models using backward selection ($P < 0.1$ in either age stratum to stay).

We repeated all regression analyses with age stratified at the median of HR-HPV–positive women (i.e., age <25 vs. ≥25 years). We also repeated analyses excluding CIN2 cases (i.e., comparing CIN3+ with ≤CIN1). All analyses were done using SAS v. 9.2 (Statistical Analysis Software).

Results

Study sample and outcomes

The flow diagram (Fig. 1) shows the derivation of the study sample, age groups, and diagnoses, and describes the distribution of outcomes at baseline and after incorporation of follow-up information. Of 1,658 women recruited into the study, 1,446 (87.2%) had a diagnosis of no CIN through cancer and 212 were indeterminate after their enrollment evaluations. The 6 cancers were included with CIN3 to form the group CIN 3+. There was a significant difference in the distribution of outcomes (i.e., CIN3+, CIN2, no CIN–CIN1) by age group ($P = .003$), driven by a higher prevalence of CIN2 in younger women (<30, 16.3%; ≥30, 10.2%); prevalence of CIN3+ was similar in both age groups (<30, 9.2%; ≥30, 9.5%).

A total of 1,095 (66.0%) women had at least 1 follow-up visit. Follow-up was more common among women with more severe baseline diagnoses (CIN3+ 80%, CIN2 79%, no CIN–CIN1 62%, indeterminate 66%, $P < 0.0001$), but follow-up status did not differ by study site, race/ethnicity, age group, or smoking. Among women with follow-up, the median number of visits was 2 (range 1–9, IQR 2), and the median duration of follow-up was 341 days (range 1–730).

At follow-up, 43 cases of CIN3 and 0 cancers were diagnosed that were not evident at baseline (Fig. 1). More than half of these ($n = 23$) were upgraded from CIN2, 7 were upgraded from no CIN–CIN1, and 13 were resolutions of previously indeterminate outcomes. Among the 43 cases of CIN3 observed only through follow-up, 41 (95.4%) were positive for HR-HPV, and 19 (44.2%) were

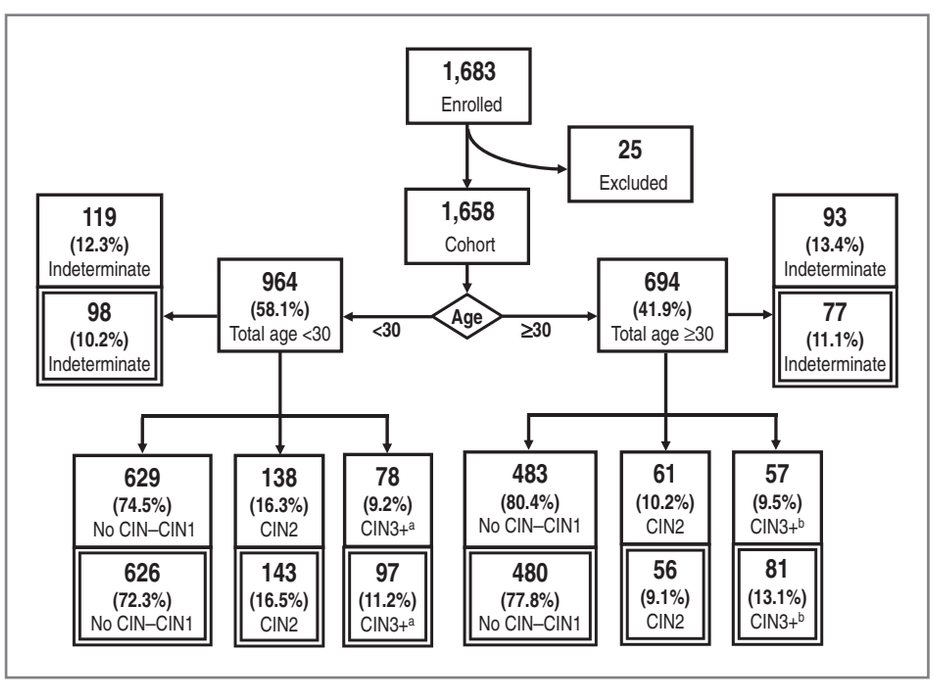

Figure 1. Flow diagram showing outcome at baseline (single border) and after 2 years of passive follow-up (double border). Reasons for exclusion from the cohort include missing HPV typing data ($n = 12$), missing diagnosis ($n = 5$), and biopsy of noncervix tissue ($n = 8$). [a]Includes 1 case of cancer. [b]Includes 5 cases of cancer.

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positive for HPV-16. Only 23 women were upgraded to CIN2 after follow-up, and the majority of these ($n = 15$) had previously been classified as no CIN–CIN1.

A total of 1,483 women with a diagnosis of no CIN through cancer (178 CIN3+, 199 CIN2, and 1,112 no. CIN–CIN1) were identified after 2-year follow-up information was incorporated. Similar to baseline, outcomes differed significantly by age group ($P = 0.0002$), driven by a higher prevalence of CIN2 in younger women (<30, 16.5%; ≥30, 9.1%); prevalence of CIN3+ remained similar in both age groups (<30, 11.2%; ≥30, 13.1%). The overall prevalence of CIN3+ increased from 9.3% to 12.0% after follow-up information was incorporated.

Distributions of most sociodemographic, health behavior, reproductive history, and sexual history characteristics differed significantly by age group (Table 1). The overall median age was 27 (range 18–69).

HPV types

Overall, 1,189 (71.7%) women tested positive for HPV, and 982 (59.2%) tested positive for HR-HPV. HPV and HR-HPV were more often detected in younger than older women (any HPV: <30, 81.6%, ≥30 57.6%, $P < 0.0001$; HR-HPV: <30, 70.3%, ≥30 43.8%, $P < 0.0001$). The 17 most prevalent HPV types had significantly higher prevalence in the younger age group (Fig. 1). A total of 596 women (36.0%) had multiple HPV types, and women aged <30 were more than twice as likely as women aged ≥30 to have multiple HPV types (47.6% vs. 19.7%, $P < 0.0001$). Stratified by outcome (CIN3+ and ≤CIN2), most HR-HPV types were still more common in the <30 age group, except for HPV-31, HPV18, and HPV45, which were nonsignificantly more common in older women among CIN3+ (Supplementary Fig. S1).

Among HR-HPV—positive women, the median age was 25 (IQR 21–32). In women with CIN3+, those positive for HPV-16 had a slightly younger median age than those positive only for other types [median (IQR) 28 (24–34) vs. 30 (25–40), $P = 0.097$]. The median number of years since first sexual intercourse among HR-HPV–positive women was 9 (IQR 5–15). In the subset with CIN3+, those positive for HPV-16 had fewer years since first sexual intercourse than those positive only for other types [median (IQR) 11 (7–17)] vs. 14 (8–22), $P = 0.044$].

RR of CIN3+ by HPV types

Among all study participants, significantly increased risk of CIN3+ was observed for women with any HR-HPV, HPV-16, and any α-9 genotype (Table 2). There were significant interactions with age group ($P < 0.05$) for nonvaccine HR-HPV types and for α-7 HPV, and a nearly significant interaction for HPV-18 ($P = 0.06$): in stratified analyses, these types or groups were only associated with CIN3+ among women aged ≥30. Excluding women with multiple HPV types increased several RRs, and suggested that the HPV16 association was stronger in younger versus older women, although CIs were wide ($P_{interaction} = 0.32$).

Stratifying age at 25 did not meaningfully change RRs for most HPV types (not shown), except to suggest an age-group interaction for multiple types (≥25: RR = 1.4, 95% CI: 1.0–1.8; <25 (RR = 0.8, 95% CI: 0.5–3.1; $P_{interaction} = 0.09$). Excluding CIN2 cases resulted in slightly stronger associations (e.g., RR for HR-HPV increased from 24.2 to 29.0).

HPV cofactors

Adjusted RRs of CIN3+ among HR-HPV–positive women are shown in Table 3 (unadjusted RRs and absolute numbers shown in Supplementary Table S1). Tests of age interactions with dichotomous forms of all variables (smoking ever vs. never, current condom use ever vs. never, annual screening yes vs. no, parity 3+ vs. 0–2), revealed a statistically significant interaction with parity ($P = 0.048$). Increasing parity was only associated with CIN3+ among women ≥30 ($P_{trend} = 0.008$). Additional adjustment for race/ethnicity did meaningfully alter the cofactors models (not shown).

Stratifying age at 25 did not change the cofactors analysis much, although the RR for parity ≥ 5 in the older group declined from 1.8 to 1.4 and an interaction with screening history was suggested (≥ 25: RR = 0.6, 95% CI: 0.5–0.9; <25: RR = 1.1, 95% CI: 0.6–1.9; $P_{interaction} = 0.08$). Excluding CIN2 did not meaningfully change RRs for cofactors (not shown).

Discussion

In this study of colsposcopy clinic patients, women in the 2 age strata defined by clinical guidelines for use of HR-HPV testing in cervical cancer screening differed in their distribution of cervical cancer risk factors, type-specific HPV associations with CIN3+, and cofactors for CIN3+. Subgroup variation by age in the risk of CIN3+ indicates that adjusting for age as a confounder may not be adequate to control for the relations among age, HPV types, cofactors, and CIN3+.

Consistent with the hypothesis that HR-HPV types other than HPV-16 require more time to progress to clinically significant disease, we found that nonvaccine HR-HPV types and α-7 (HPV-18 related) types were only associated with CIN3+ in women aged ≥30. However, the RR for CIN3+ by HPV16 status were very high in all age groups, suggesting that among women with abnormal screening, a finding of HPV16 is important regardless of age; a sensitivity analysis excluding women with multiple type infections suggested that the association with HPV16 might be stronger for younger women. We also noted that 59% of CIN3+ cases were HPV-16 positive (i.e., 105/178, see Table 2), and HPV-positive CIN3+ had a slightly younger age at diagnosis and shorter interval since first intercourse than HPV-negative CIN3+ cases, consistent with results from a large U.S. screening trial (17) and supporting the hypothesis that HPV-16 infections can lead to

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Table 1. Description of study sample, overall, and stratified by age

	Total		Age <30		Age ≥30		
	n	%	*n*	%	*n*	%	*P*
Total	1,658		964		694		
Sociodemographics							
Race/ethnicity ($n = 1,654$)							
Non-Hispanic Black	1,216	73.5	739	76.7	477	69.0	0.0002
Non-Hispanic White	205	12.4	99	10.3	106	15.3	
Hispanic	196	11.9	111	11.5	85	12.3	
Other	37	2.2	14	1.5	23	3.3	
Highest education completed ($n = 1,644$)							
Less than high school	386	23.5	232	24.3	154	22.4	0.0087
High school	581	35.3	339	35.5	242	35.2	
Some college	418	25.4	258	27.0	160	23.3	
Completed college	259	15.8	127	13.3	132	19.2	
Yearly household income ($n = 1,530$)							
< $20,000	1,095	71.6	659	75.7	436	66.2	<0.0001
$20,000–40,000	256	16.7	137	15.7	119	18.1	
> $40,000	179	11.7	75	8.6	104	15.8	
Health behaviors and general health status							
Smoking ($n = 1,653$)							
Current	318	19.2	147	15.3	171	24.7	<0.0001
Former	210	12.7	67	7.0	143	20.6	
Never	1,125	68.1	746	77.7	379	54.7	
Alcohol, average in last 5 years ($n = 1,654$)							
Rarely/never	764	46.2	507	52.8	257	37.1	<0.0001
At least once per month but less than once per week	454	27.5	269	28.0	185	26.7	
At least once per week	436	26.4	185	19.3	251	36.2	
Screening history ($n = 1,571$)							
5 or more pap tests in last 5 years	986	62.8	566	62.1	420	63.7	0.5
Less than 5 pap tests in last 5 years	585	37.2	346	37.9	239	36.3	
Currently use condoms							
No	988	60.8	486	51.2	502	74.3	<0.0001
Sometimes	236	14.5	172	18.1	64	9.5	
Regularly	402	24.7	292	30.7	110	16.3	
BMI (kg/m^2; $n = 1,407$[a])							
Underweight (<18.5)	37	2.6	27	3.3	10	1.7	<0.0001
Normal weight (18.5–24.9)	455	32.3	311	38.0	144	24.5	
Overweight (25.0–29.9)	369	26.2	208	25.4	161	27.4	
Obese (≥30)	546	38.8	273	33.3	273	46.4	
Reproductive history							
Gravidity ($n = 1,655$)							
0	254	15.4	189	19.7	65	9.4	<0.0001
1–2	686	41.5	492	51.2	194	28.0	

(Continued on the following page)

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Table 1. Description of study sample, overall, and stratified by age (Cont'd)

	Total		Age <30		Age ≥30		
	n	%	*n*	%	*n*	%	*P*
3–4	438	26.5	205	21.3	233	33.6	
5–6	191	11.5	60	6.2	131	18.9	
≥7	86	5.2	15	1.6	71	10.2	
Parity ($n = 1,654$)							
0	390	23.6	290	30.2	100	14.4	<0.0001
1–2	791	47.8	529	55.1	262	37.8	
3–4	354	21.4	122	12.7	232	33.5	
≥5	119	14.3	20	2.1	99	14.3	
Age at menarche ($n = 1,633$)							
7–10	170	10.4	106	11.1	64	9.4	0.19
11–12	677	41.5	406	42.7	271	39.8	
13–14	553	33.9	316	33.2	237	34.8	
≥15	233	14.3	124	16.0	109	16.0	
Age at first pregnancy ($n = 1,631$)							
Never pregnant	254	15.6	189	19.9	65	9.6	<0.0001
11–16	369	22.6	236	24.8	133	19.6	
17–19	564	34.6	342	35.9	222	32.7	
20–24	322	19.7	169	17.8	153	22.5	
25+	122	7.5	16	1.7	106	15.6	
Lifetime oral contraceptive use ($n = 1,647$)							
Never	436	26.5	283	29.6	153	22.2	<0.0001
<3 mo	283	17.2	186	19.4	97	14.1	
3–11 mo	221	13.4	137	14.3	84	12.2	
12–35 mo	305	18.5	184	19.2	121	17.5	
At least 36 mo	235	34.1	167	17.5	235	34.1	
Injectable contraceptive use (ever, $n = 1,642$)							
Yes, regularly	517	31.5	408	42.7	1099	15.9	<0.0001
Yes, sometimes	213	13.0	145	15.2	68	9.9	
Never	912	55.5	403	42.2	509	74.2	
Any hormonal contraceptive use							
Yes	1,407	84.9	839	87.0	568	81.8	0.004
No	251	15.1	125	13.0	126	18.2	
Periods stopped ($n = 1,652$)							
Yes, natural menopause	99	6.0	0	0.0	99	14.4	<0.0001[b]
Yes, other reason	30	1.8	4	0.4	26	3.8	
No	1,523	92.2	960	99.6	563	81.8	
Sexual history							
Lifetime number of male sexual partners ($n = 1,567$)							
0–1 (1 woman claims 0)	158	10.1	98	10.5	60	9.4	0.15
2–5	802	51.2	490	52.7	312	49.0	
≥6	607	38.7	342	36.8	365	41.6	
Age at first sexual intercourse							
5–14	339	21	220	23.1	119	17.9	<0.0001
15–16	614	38	414	43.5	200	30.1	
17–18	418	25.9	225	23.7	193	29.0	
19–20	143	8.9	66	6.9	77	11.6	
21 or older	102	6.3	26	2.7	76	11.4	

[a]231 women enrolled prior to 08/27/2009 were not asked about height and weight, and 20 other women did not answer.
[b]*P*-value for comparison between Yes and No.

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Table 2. Relative risk (RR) of CIN3+ versus ≤CIN2 by HPV type categories, overall, and stratified by age group

	All ages				Age <30				Age ≥30			
	CIN3+	≤CIN2	RR	95% CI	CIN3+	≤CIN2	RR	95% CI	CIN3+	≤CIN2	RR	95% CI
All women (n = 1,483)	178	1,305			97	769			83	534		
HR-HPV[a]	173	700	24.2	10.0–58.5	95	506	20.9	5.2–84.3	78	194	32.9	10.5–103.3
HPV-16	105	164	6.5	5.0–8.5	61	125	6.2	4.2–9.0	44	39	7.7	5.3–11.1
HPV-18	10	73	1.0	0.6–1.8	4	54	0.6	0.2–1.6	6	19	1.9	0.9–3.9
Vaccine HR-HPV types[b]	114	222	6.1	4.6–8.1	64	166	5.4	3.6–7.9	50	53	7.8	5.2–11.6
Non-vaccine HR-HPV types[c]	95	595	1.3	1.0–1.7	53	439	0.9	0.6–1.3	42	156	2.3	1.5–3.4
Any α-9 HPV type[d]	155	476	9.1	5.9–13.9	89	349	10.9	5.3–22.1	66	127	9.7	5.7–16.5
Any α-7 HPV type[e]	34	144	0.9	0.69–1.2	17	213	0.6	0.4–1.0	17	73	1.6	1.0–2.5
Multiple (vs. single) HPV types[f]	175	881	0.9	0.7–1.1	52	356	0.9	0.6–1.2	30	93	1.1	0.8–1.7
Women without multiple HPV types (n = 952)												
Total	96	856			45	413			51	443		
HR-HPV[a]	91	276	29.0	11.9–70.7	43	170	24.7	6.1–100.9	48	106	35.3	11.2–111.6
HPV-16	61	57	12.3	8.5–17.8	32	30	15.7	8.7–28.3	29	27	10.3	6.4–16.6
HPV-18	4	19	1.8	0.7–4.4	1	12	0.8	0.1–5.2	3	7	3.0	1.1–8.1
Vaccine HR-HPV types[b]	65	76	12.1	8.2–17.8	33	42	14.0	7.6–25.9	32	34	10.9	6.6–18.1
Nonvaccine HR-HPV types[c]	26	200	1.2	0.8–1.8	10	128	0.7	0.3–1.3	16	72	2.1	1.2–3.6
Any α-9 HPV type[d]	80	161	14.8	8.8–24.7	40	349	19.8	8.0–49.0	40	69	12.8	6.8–24.2
Any α-7 HPV type[e]	8	72	1.0	0.5–2.0	1	213	0.2	0.03–1.4	7	26	2.2	1.1–4.5

[a]Includes types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68.
[b]Includes types 16 and 18.
[c]Includes types 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68.
[d]Includes types 16, 31, 33, 35, 52, 58, and 67.
[e]Includes types 18, 39, 45, 59, 68, 70, and 85.
[f]Excludes 427 HPV-negative women.

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Table 3. Overall and age-stratified relative risk (RR) for CIN3+ versus ≤CIN2 by HPV cofactors, among HR-HPV–positive women, from adjusted log-binomial regression models

	All ages ($n = 812$)		Age <30 ($n = 556$)		Age ≥30 ($n = 256$)	
	RR	95% CI	RR	95% CI	RR	95% CI
Age						
18–21	1.0		1.0			
22–24	1.3	0.8–2.4	1.4	0.8–2.5		
25–29	2.1	1.3–3.4	2.4	1.4–4.0		
30–39	2.3	1.4–3.8			1.0	
≥ 40	1.7	1.0–3.0			0.7	0.5–1.7
Smoking						
Current	1.5	1.1–2.1	1.6	1.0–2.5	1.5	1.0–2.4
Former	1.8	1.2–2.5	1.3	0.7–2.5	1.9	1.3–3.0
Never	1.0		1.0		1.0	
Screening history						
5 or more pap tests in last 5 years	0.7	0.6–0.9	0.8	0.5–1.1	0.7	0.5–1.0
Less than 5 pap tests in last 5 years	1.0		1.0		1.0	
Parity						
0	1.0		1.0		1.0	
1–2	1.0	0.7–1.5	1.1	0.7–1.7	0.9	0.5–1.7
3–4	1.2	0.8–1.8	0.7	0.3–1.5	1.4	0.8–2.5
≥5	1.6	1.0–2.5	0.9	0.3–1.5	1.8	1.0–3.3
P_{trend}	$P = 0.07$		$P = 0.55$		$P = 0.008$	
Currently use condoms						
Never	1.0		1.0		1.0	
Sometimes	0.8	0.6–1.1	0.9	0.6–1.4	0.6	0.2–1.4
Regularly	0.5	0.3–0.9	0.6	0.3–1.1	0.6	0.3–1.1

significant abnormalities more quickly than other HR-HPV types. Previous studies have suggested an over-representation of HPV-16 or HPV-16 and HPV-18 in neoplasia affecting younger women in case series of CIN3+, CIN2, or CIN2+ (10, 17–20). HPV-16 is the most oncogenic type and clearly an important contributor to high-grade cervical neoplasia at all ages. Our findings suggest that other HR-HPV types may become more important to risk of CIN3+ as women age, although the magnitude of their risk remains very low compared with that of HPV-16.

Our study partially supports the existing literature on cofactors for cervical neoplasia among HR-HPV–positive women, although the racial make-up of the study population is quite different from most previous studies. Among primarily white women referred to 1 colposcopy center, cofactors for CIN3 (vs. <CIN2) included increasing parity and current smoking, in agreement with our study, and also included higher income (i.e., ≥40,000 vs. <10,000), and extremes of body mass index (i.e., ≥30 and <20), after adjustment for age quintiles (21). Another U.S. study investigated reproductive cofactors for CIN3 among women with LSIL or ASCUS cytology (ALTS; ref. 22). In contrast with our findings, the authors identified increased risk of CIN3 (vs. CIN<2) with current injectable contraceptive use, but not OC, Norplant, parity, gravidity, or age at first pregnancy, after adjusting for HPV-16 DNA, education, age, and smoking status. A large data pooling study identified a graded increase in CIN3/carcinoma *in situ* with increasing parity and age at first full-term pregnancy, although when these risk factors were combined in a model, age at first full-term pregnancy was the stronger risk factor (23). The study did not report on whether the association remained after restricting the analysis to HR-HPV–positive women (23). None of these previous studies reported on whether the observed associations were uniform across age groups (21, 22). A pooled analysis of international case–control studies found that smoking was associated with similar 2-fold greater odds of both carcinoma *in situ* and invasive cervical cancer among HPV-positive women, and found no evidence of age-group differences in the effects of smoking with invasive and *in situ* cases combined (24). Our selection of candidate cofactors for CIN3+ was informed by the

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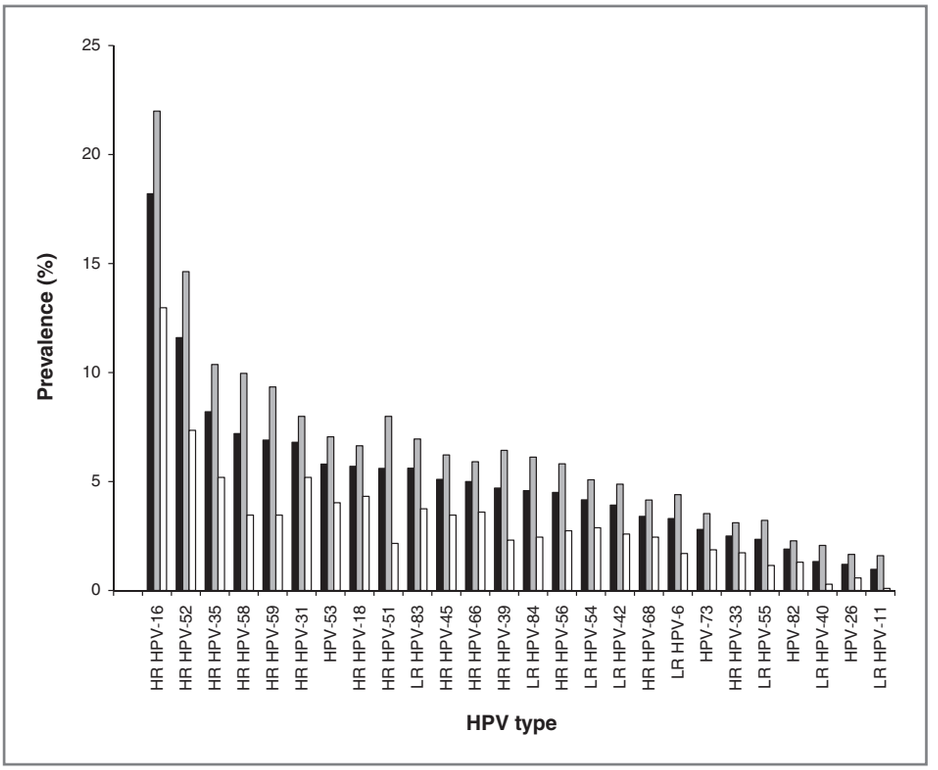

Figure 2. Prevalence of the most common HPV types in colposcopy referral study population ($n = 1{,}658$) overall (black bars), among women aged younger than 30 (grey bars) and among women 30 or older (white bars). Figure includes the 26 types found in at least 1% of the study population (i.e., at least as common as HPV-11, the least common vaccine type). Age-group comparisons for all types at least as common as HPV-42 were statistically significant (P value from χ^2 test < 0.05). HR, high-risk (i.e., detected by clinical HR-HPV tests); LR, low-risk. Other types are possibly high-risk but not detected by clinical HR-HPV tests. Not shown in figure: HPV-70 (0.5%), HPV-67 (0.1%), HPV-69 (0.1%), HPV-IS39 (0.1%), and HPV-85 (0%).

larger body of literature on invasive cervical cancer, which has identified associations with age at first full-term pregnancy and number of full-term pregnancies (23), lifetime number of sexual partners (25), and hormonal contraceptive use (26), and smoking (24, 27). It is important to consider cofactors for CIN3+ because precancerous lesions are the target of screening activities, and cofactors for this endpoint might differ from those for invasive cancers because of the timing of exposures, the portion of the pathophysiologic process affected by the risk factor, the age of cases, or differences in control selection.

Several mechanisms for cofactors for cervical cancer and CIN3 have been suggested. Smoking by-products are found in cervical mucus (28), and could induce immune suppression against active HPV infection or direct oncogenic effects (29). Reproductive factors, including parity, are related to sexual activity, raising concern that such variables are proxies for HPV exposure (23). However, associations with parity and other reproductive history and sexual behavior variables have remained after restricting cases and controls to HR-HPV–positive women in this and many other studies. Multiparity might promote neoplastic changes through hormonal influences or local tissue changes during the vaginal birth process that expose the transformation zone to carcinogenic agents (23, 30). Our finding of a protective effect of current condom use echoes previous findings of protective effects of barrier contraceptives for invasive cervical cancer among HPV-positive women (31, 32). Although this association might be attributable to residual confounding by HPV exposure, condoms also protect against other sexually transmitted infections, such as Chlamydia trachomatis, which could act as cofactors for CIN3+, perhaps by contributing to HPV persistence (31).

Age group differences in cofactors suggest that the timing of exposures or elapsed time between exposure and detection of precancerous lesions are important. We speculate that the lack of association between multiparity and CIN3+ among women <30 in this study could be attributable to relatively low parity in this age group and insufficient follow-up time between multiple pregnancies and future development of disease. The distributions of nearly all the classical cervical cancer risk factors we examined differed between the 2 age groups. Several of these characteristics, such as education, income, body mass index (BMI), gravidity, parity, and menopausal status are truly age related—that is, with the passage of time, women have more opportunities to acquire these risk factors. Other age group variation, especially in behaviors like smoking, alcohol, condom, or other contraceptive use, may instead represent cohort differences. For example, the prevalence of smoking declined from 1978 to 2002 (33), thus the lower rates of current smoking noted among younger women in this study might be attributable to birth cohort differences rather than true age differences. Although population trends suggest

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declining age at onset of sexual activity (34), the younger onset of sexual activity in women under 30 in this colposcopy cohort might be a proxy for age at first exposure to HPV, and reflect a selection effect rather than a cohort effect. As screening practices and prevention strategies change (e.g., introduction of HR-HPV cotesting and HPV vaccine), the descriptive epidemiology of colposcopy patients will also change. When researchers adjust for age in regression models of cervical cancer outcomes, part of the intent is to adjust for the time needed for cervical cancer to develop; however, adjusting for age also adjusts for other behavioral and clinical characteristics representing a mixture of age and cohort effects.

Several limitations should be considered when interpreting this study; most of these are related to the referral population. The study design assumed that all women entering care at the colposcopy clinic had a legitimate reason for referral. Consequently, relatively few exclusion criteria were applied, and information on referral indications was not collected. On the basis of the study years (2000–2004), most women would have been referred following an abnormal Pap test (HR-HPV testing had not yet been approved for screening); however, some women may have been referred for additional evaluation and treatment following a biopsy in another setting. Thus, some women may have had their most severe lesion removed during a prior biopsy, which would lead to outcome misclassification (e.g., classified as CIN0 when a CIN3 lesion had been removed). We would expect this to have the effect of reducing differences between outcome groups. Women were recruited from urban public hospital-affiliated clinics which serve large inner city minority populations of relatively disadvantaged women who depend on public health services. Although this has the benefit of providing more information on this population which has been relatively underrepresented in other similar studies (21, 22), generalizability to nonurban or relatively advantaged populations is unknown. All associations are conditional on women having abnormal screening tests, and cannot be generalized to women with normal cytology. Finally, despite a substantial number of CIN3+ cases, statistical power to detect interactions was limited.

This study's internal validity is supported by efforts to reduce misclassification of both the exposures and outcomes. The HPV typing was conducted in an experienced laboratory with extensive quality control procedures. The highly sensitive genotyping assay mitigates concern that some cofactors are markers for undetected HR-HPV. The pathology findings were subject to an expert review process, and follow-up information was available on 2/3 of participants to enhance detection of CIN3 missed at baseline, including clarifying a portion of previously indeterminate outcomes. CIN3 cases identified during follow-up were interpreted as prevalent cases having previous false negative results or lack of biopsy at baseline colposcopy rather than as cases that developed during the follow-up period (35). Although these follow-up cases did not undergo the same rigorous review process as the baseline outcomes, we feel confident that the CIN3 cases ascertained at follow-up are valid, because this diagnosis is not subject to as much interpretation as CIN2 (36, 37). In addition, the observed associations were similar when done using only the baseline outcomes (data not shown). Our decision to include CIN2 with CIN0-1 in the reference category in our models was based on the goal of avoiding spectrum bias and the related potential for generating overly optimistic estimates of effects when intermediate outcome categories are excluded from analyses.

The age distribution of the study population may impact study findings regarding relations among HPV types, cofactors, and CIN3+ because of age group differences in risk factor prevalence, in the time since exposure to HR-HPV or other risk factors, or to differential impact of specific HPV types. Additional research on type-specific risk may eventually support a clinical application of HPV genotyping in certain situations. Because HR-HPV cofactors may inform disease risk without invasive procedures or additional expense, future efforts to incorporate clinical and demographic data into screening algorithms might improve the efficiency of existing markers. As cervical cancer screening strategies for vaccinated cohorts of women are developed and clinical HR-HPV testing is incorporated, it will be important to consider the natural history of HPV and cervical neoplasia and secular trends in cofactors in the pursuit of appropriately sensitive and specific screening tools applied to the appropriate age groups or other subpopulations.

Disclosure of Potential Conflicts of Interest

The findings and conclusions in this report are those of the author(s) and do not necessarily represent the official position of the Centers for Disease Control and Prevention. The views expressed in this publication are the views of the authors and do not necessarily reflect the views of the Ontario Ministry of Health and Long-Term Care.

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Age-Group Differences in Human Papillomavirus Types and Cofactors for Cervical Intraepithelial Neoplasia 3 among Women Referred to Colposcopy

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