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The influence of type-specific human papillomavirus infections on the detection of cervical precancer and cancer: A population-based study of opportunistic cervical screening in the United States

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There are limited data on the prospective risks of detecting cervical precancer and cancer in United States (US) populations specifically where the delivery of opportunistic cervical screening takes place outside managed care and in the absence of organized national programs. Such data will inform the management of women with positive screening results before and after widespread human papillomavirus (HPV) vaccination and establishes a baseline preceding recent changes in US cervical cancer screening guidelines. Using data reported to the statewide passive surveillance systems of the New Mexico HPV Pap Registry, we measured the 3-year HPV type-specific cumulative incidence of cervical intraepithelial neoplasia grade 2 or more severe (CIN2+) and grade 3 or more severe (CIN3+) detected during real-world health care delivery across a diversity of organizations, payers, clinical settings, providers and patients. A stratified sample of 47,541 cervical cytology specimens from a screening population of 379,000 women underwent HPV genotyping. Three-year risks for different combinations of cytologic interpretation and HPV risk group ranged from <1% (for several combinations) to approximately 70% for CIN2+ and 55% for CIN3+ in women with high-grade (HSIL) cytology and HPV16 infection. A substantial proportion of CIN2+ (35.7%) and CIN3+ (30.9%) were diagnosed following negative cytology, of which 62.3 and 78.2%, respectively, were high-risk HPV positive. HPV16 had the greatest 3-year risks (10.9% for CIN2+,8.0% for CIN3+) followed by HPV33, HPV31, and HPV18. Positive results for high-risk HPV, especially HPV16, the severity of cytologic interpretation, and age contribute independently to the risks of CIN2+ and CIN3+.

It is now widely accepted that persistent cervical infection by high-risk types of human papillomavirus is the necessary but infrequent cause of cervical cancer.[1] This has led to development of vaccines that prevent HPV and to molecular tests

Key words: cervical intraepithelial neoplasia (CIN), cervical cancer, human papillomavirus (HPV), HPV vaccine effectiveness, cervical screening effectiveness, US opportunistic cervical screening, cytology, Pap test

Abbreviations: AGC: atypical glandular cells; AIS: adenocarcinoma *in situ*; ASC-H: atypical squamous cells cannot rule out HSIL; ASC-US: atypical squamous cells of undetermined significance; CIN: cervical intraepithelial neoplasia; CIN1: cervical intraepithelial neoplasia grade 1; CIN2: cervical intraepithelial neoplasia grade 2; CIN3: cervical intraepithelial neoplasia grade 3; CIS: carcinoma in situ; HPV: human papillomavirus; HPV LA: HPV LINEAR ARRAY; HSIL: high-grade squamous intraepithelial lesions; LSIL: low-grade grade squamous intraepithelial lesions; LSIL-H: LSIL cannot rule out HSIL; NMHPVPR: New Mexico HPV Pap Registry; PCR: polymerase chain reaction; TBS: the 2001 Bethesda System; US: United States

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What's new?
Age, cytologic diagnosis, and human papillomavirus (HPV) genotype are key factors in deciding how cervical precancer and cancer patients should be managed, but few studies have had sufficient case numbers to examine interplay among these factors. In this study, age, cytologic diagnosis, and HPV genotype were found to contribute independently to disease detection. A substantial proportion of disease occurred when women were cytology negative but high-risk HPV-positive. The data provide baseline measurements to judge HPV vaccination and cervical screening effectiveness in U.S. populations, where these interventions are delivered opportunistically.

that detect it and to better screening outcomes. In the recently updated US screening guidelines, HPV and cervical cytology cotesting every 5 years in women 30 and older has emerged as preferred[2] or acceptable[3] approach, and HPV genotyping can be helpful for management decisions among women who test HPV positive and have negative cytology.[2]

Cancer risk and harms associated with procedures should be the key metric for deciding how patients are managed. In cervical cancer screening, both cytologic grade and type-specific HPV infection influence risk. In the context of opportunistic cervical screening which spans the organizations, payers, providers and patients of New Mexico, we studied the impact of different combinations of the two on the ability to identify high grade cervical lesions. Of particular interest was the modifying effect of HPV positivity on women with different cytology results, especially those who were deemed negative (NILM) on cytology. Few studies have sufficient numbers of cases of cervical precancer and cancer and have representative screening populations to examine their interplay related to cervical cancer risk. We used data from the statewide New Mexico HPV Pap Registry (NMHPVPR), and conducted HPV genotyping on a stratified sample of cytology specimens to examine the risk with these two markers. To account for the insensitivities of, and differential referral to, colposcopy as routinely practiced,[4–6] we used the 3-year cumulative incidences of cervical intraepithelial neoplasia grade 2 or more severe (CIN2+) and grade 3 or more severe (CIN3+) as our measures of cervical cancer risk.

Material and Methods

Registry

The New Mexico HPV Pap Registry (NMHPVPR) acts as a designee of the New Mexico Department of Health and operates under New Mexico Administrative Code (NMAC) 7.4.3.12, which specifies the list of Notifiable Diseases and Conditions for the state of New Mexico. With the intention of monitoring the impact of HPV vaccination, in 2006, NMAC 7.4.3.12 specified under state regulation that laboratories must report to the NMHPVPR all results of cervical cytology, cervical pathology, and HPV tests performed on New Mexico residents. NMAC 7.4.3.12 was updated in 2009 to include vulvar and vaginal pathology (http://nmhealth.org/ERD/healthdata/documents/NotifiableDiseasesConditions022912final.pdf).

Study population and sample

During the 17-month period of December 2007 through April 2009 ~379,000 cervical cytology tests on 320,500 women were reported to the NMHPVPR by 9 in-state and 7 out-of-state clinical laboratories.[7] All available liquid cervical cytology specimens were collected from 7 of the 9 in-state laboratories, which accounted for 79% of all cervical cytology tests done during this period as previously described.[7] All specimens associated with a report of abnormal cytology and a random sample of those with negative cytology (45% of negative specimens in women aged ≤30 years, 8% of negative specimens in women aged >30 years) were collected. A larger proportion of young women with normal cytology were sampled in order to more accurately assess potential HPV vaccine impact in future studies. Prior to HPV genotyping, specimens were de-identified by the use of randomly assigned study-specific identifiers. Ultimately, a total of 59,644 specimens from 54,848 women were successfully genotyped for HPV. Only specimens from screening tests were used in this analysis (defined as a cervical cytology test with no previous cytology collected within 300 days), and for women with more than one cytology specimen in the sample, the chronologically earliest was selected. The final sample size was 47,541 women. The UNM Human Research Review Committee approved this study.

HPV genotyping

HPV genotyping was done as previously described.[7–9] Briefly, DNA was purified from 500-μL aliquots of vigorously mixed residual liquid cytologic specimens (SurePath™ (Beckton, Dickinson and Company, NJ) or ThinPrep® (Hologic, MA), using a Cobas X421 robot (Roche Molecular Systems (RMS), CA). Fifty microliters (50 μL) of purified DNA was transferred to a tube with 50 μL of HPV LINEAR ARRAY Genotyping Test (HPV LA; Roche Diagnostics, Indianapolis, IN) mastermix, and the mixture was amplified by polymerase chain reaction (PCR) as specified by the manufacturer. Controls for contamination and assay sensitivity were included in each 96-well assay.

The HPV LA is based on PGMY 09/11 consensus PCR primers and a prototype Line Blot assay, which have been previously reported in detail.[8–10] Using the Roche HPV LA detection kit, hybridizations were automated using Tecan ProfiBlot-48 robots (Tecan, Austria). The Roche HPV LA

Genotyping Test detects 13 high- and 24 low-risk HPV types. The presence of HPV 52 is not determined directly by a type-specific probe but through inference as previous described.[10] Two independent readers interpreted the presence of HPV types using a reference template provided by the manufacturer. Any discrepancies were identified by a custom computer application and were adjudicated by a third review.

HPV genotype classifications

HPV status was grouped into four mutually exclusive categories based on risk for cervical cancer: (*i*) positive for HPV16, (*ii*) negative for HPV16 but positive for one or more other high-risk HPV types (HPV18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 or 68), (*iii*) negative for any high-risk HPV type but positive for one or more low-risk HPV types (HPV6, 11, 26, 40, 42, 53, 54, 55, 61, 62, 64, 66, 67, 69, 70–73, 81–84, IS39 or 89), or (*iv*) negative for HPV.

Cytologic classifications

Cytologic results were classified according to the 2001 Bethesda System (TBS)[11]: high-grade squamous intraepithelial lesion (HSIL), atypical squamous cells cannot rule out HSIL (ASC-H), atypical glandular cells (AGC), low-grade squamous intraepithelial lesion (LSIL), atypical squamous cells of undetermined significance (ASC-US), and negative for intraepithelial lesion or malignancy. There were occasional uses of non-TBS terminology, which were classified as follows[7]: (*i*) LSIL cannot rule out HSIL (LSIL-H) as ASC-H; (*ii*) cervical intraepithelial neoplasia (CIN) grade 1 as LSIL; (*iii*) CIN2, CIN3, carcinoma *in situ* (CIS), and possible carcinoma as HSIL and (*iv*) atypical squamous and glandular cells of undetermined significance as AGC. Cytology was based on the local community reading and no attempt was made to review them centrally or undertake quality assurance activities.

Cervical precancer and cancer outcomes

Women were followed passively through electronic and paper medical records submitted to the NMHPVPR. Outcomes for cervical precancer and cancer were based on the results of all cervical biopsies, including excisional biopsies, and all endocervical curettages (ECC) from the date of the index cytology for a period of 3 years. Local community readings were used without central review. An outcome of CIN2+ was defined as a result of CIN2, CIN2–3, CIN3, CIS, AIS, carcinoma, or high-grade (not otherwise specified). An outcome of CIN3+ was defined as a result of CIN2–3, CIN3, CIS, AIS, or carcinoma. Adenocarcinomas that were identified as endometrial in origin ($n = 11$) were excluded.

Statistical methods

Follow up was complete up to 1 July, 2012 at the time of this analysis, which provided 3 years of follow-up for all index cytology specimens. The intent of this work is to report the ascertainment of disease under routine clinical practice occurring under conditions of opportunistic cervical screening and diagnostic follow-up. Therefore, no attempt was made to adjust estimates of cumulative incidence of cervical precancer and cancer for loss to follow-up. Cumulative incidence was computed as the percent of all women within each age, cytology, and HPV category with a diagnosis of CIN2+ or CIN3+ over the 3-year follow-up period. All confidence intervals and p values are exact and were computed using the SAS v9.3 procedure FREQ, except those reported for combined sampling strata. In these cases, the cumulative incidence, confidence intervals and p values were computed using sample survey techniques appropriate for a stratified random sample with unequal sampling fractions (SAS procedure SURVEYFREQ). Sample weights were computed as the inverse of the sampling fraction and variances were computed by the Taylor series linearization method. Weighted confidence intervals were based on normal approximations.

Results

Diagnoses of cervical precancer over the 3 years following screening cytology were based only on cervical biopsy and endocervical curettage (ECC) records in the passive surveillance systems of the NMHPVPR. Diagnostic follow-up with cervical biopsy or ECC increased with increasing severity of the cytologic result (Table 1). While <5% of women with negative cytology had diagnostic follow-up over the 3-year period, 29% of women with ASC-US, 61–68% of women with LSIL, ASC-H or AGC, and 83% of women with HSIL had diagnostic follow-up.

Three-year risks of detecting CIN2+ and CIN3+ outcomes increased with increasing severity of squamous cell abnormalities for both younger and older women (Table 1). However, risks of detecting CIN2+ were significantly greater for younger than older women following negative ($p < 0.0001$), ASC-US ($p < 0.001$), LSIL ($p = 0.02$) and AGC ($p < 0.0001$) cytology, whereas risk of detecting CIN3+ was greater in older women compared to younger women following HSIL cytology ($p = 0.02$).

Three-year risks of detecting CIN2+ and CIN3+ stratified by index cytology and HPV status for both age groups and for all ages combined are shown in Table 2. Within a given HPV risk category the risk of detecting CIN2+ and CIN3+ increased with increasing severity of cytologic interpretation. Likewise, within a given cytologic interpretation, the risk increased with higher HPV risk category. The 3-year risks of detecting either CIN2+ or CIN3+ using different combinations of cytologic interpretation and HPV risk group ranged from <1% (for several combinations) to 70.8% for CIN2+ and 54.5% for CIN3+ in women with HSIL cytology and HPV16 infection respectively. In general, the 3-year risks were similar for the two age groups with somewhat higher risks for older women with ASC-H or HSIL cytology and HPV16 or other high-risk HPV types. Notably, the risk of detecting CIN3+ for ASC-H and HPV16 was 28.6% (95%CI:

Table 1. Risk of detecting CIN2+ and CIN3+ within 3 years of screening cytology, stratified by age and cytologic result

Age at screening cytology	Cytologic result	Women screened	Diagnostic follow-up	CIN2+[1]		CIN3+[2]	
		N	%	*N*	%	*N*	%
≤ 30 years	Negative	23,030	4.9	218	0.9	80	0.3
	ASC-US	4,066	35.4	286	7.0	98	2.4
	LSIL	2,502	58.4	291	11.6	91	3.6
	AGC	102	67.6	23	22.5	11	10.8
	ASC-H	334	64.7	99	29.6	59	17.7
	HSIL	308	84.1	175	56.8	105	34.1
	All*	30,342	9.8	1,092	2.2	444	0.9
> 30 years	Negative	10,539	4.1	18	0.2	7	0.1
	ASC-US	4,664	23.5	116	2.5	52	1.1
	LSIL	1,027	67.7	92	9.0	26	2.5
	AGC	445	63.4	34	7.6	27	6.1
	ASC-H	315	70.8	82	26.0	54	17.1
	HSIL	209	80.4	126	60.3	93	44.5
	All*	17,199	5.6	468	0.5	259	0.2
All ages[3]	Negative	33,569	4.4	236	0.4	87	0.2
	ASC-US	8,730	29.3	402	4.7	150	1.7
	LSIL	3,529	61.0	383	10.9	117	3.3
	AGC	547	64.2	57	10.6	38	7.0
	ASC-H	649	67.5	181	28.0	113	17.4
	HSIL	517	82.7	301	58.2	198	38.1
	All	47,541	7.0	1,560	1.0	703	0.4

[1]CIN2+ includes CIN2, high-grade (NOS), CIN2–3, CIN3, CIS, AIS, and cancer.
[2]CIN3+ includes CIN2–3, CIN3, CIS, AIS, and cancer.
[3]Because of sample weights, percentages for all ages combined and all cytologic results combined cannot be calculated from the number of women and number of events.

21.3%, 36.8) for women aged ≤30 years compared to 54.9% (95%CI: 40.3%, 68.9%) for women aged >30 years. Of particular interest was high-grade disease in women with negative index cytology. Based on predicted numbers for the entire population, this comprised an estimated 35.7% of all CIN2+ and 30.9% of all CIN3+ for women of all ages, of which 62.3% (CIN2+) and 78.2% (CIN3+) was high-risk HPV positive.

Figure 1 shows the cumulative incidence of CIN2+ over the 3-year follow-up period by HPV risk status, stratified by cytologic result (normal *vs.* abnormal), for the two age groups. In these plots, the much greater risk of detecting CIN2+ for women infected with HPV16 is evident. HPV33 was the next most risky HPV genotype. Women with other high-risk HPV types including HPV18 had an intermediate risk, while those with only low-risk HPV types had a risk of CIN2+ which was only slightly greater than that for HPV negative. This was observed in both age groups and for both abnormal and normal cytology.

Figure 2 shows the cumulative incidence of CIN2+ by cytologic result for women infected with HPV16 and with other high-risk HPV types. In both age groups the risk of detecting CIN2+ increased dramatically with increasing severity of squamous abnormality. However, the increase in risk for LSIL compared to ASC-US was modest for HPV16 and negligible for other high-risk types. The risk for AGC was generally comparable to that of ASC-H except in women >30 with HPV16, in which it was similar to that for LSIL.

Shown in Table 3 are the risks of detecting CIN2+ and CIN3+ for each high-risk HPV type in women of all ages with a single HPV type infection, stratified by cytologic interpretation. Women with single infections of HPV16 had greater risks of detecting CIN2+ and CIN3+ than women with single infections of other high-risk HPV types for every cytologic interpretation with few exceptions. Among HPV16-positive women, the risk of detecting CIN2+ and CIN3+ ranged from 3.6 to 2.8%, respectively, for women with

Table 2. Risk of detecting CIN2+ and CIN3+ within 3 years of screening cytology, stratified by age, HPV status, and cytologic result

		≤ 30 years					> 30 years					All ages[2]				
			CIN2+		CIN3+			CIN2+		CIN3+			CIN2+		CIN3+	
HPV[1]	Cytologic result	Women screened	*n*	(%)	*N*	(%)	Women screened	*n*	(%)	*n*	(%)	Women screened	*n*	(%)	*n*	(%)
HPV16	Negative	1,214	67	(5.5)	39	(3.2)	150	1	(0.7)	1	(0.7)	1,364	68	(3.7)	40	(2.2)
	ASC-US	676	137	(20.3)	55	(8.1)	220	45	(20.5)	31	(14.1)	896	182	(20.3)	86	(9.5)
	LSIL	565	143	(25.3)	63	(11.2)	109	33	(30.3)	15	(13.8)	674	176	(26.1)	78	(11.5)
	AGC	20	11	(55.0)	5	(25.0)	23	7	(30.4)	7	(30.4)	43	18	(42.4)	12	(27.8)
	ASC-H	140	61	(43.6)	40	(28.6)	51	36	(70.6)	28	(54.9)	191	97	(50.4)	68	(35.2)
	HSIL	157	107	(68.2)	77	(49.0)	84	64	(76.2)	55	(65.5)	241	171	(70.8)	132	(54.5)
Other high-risk	Negative	4,004	74	(1.8)	26	(0.6)	699	5	(0.7)	2	(0.3)	4,703	79	(1.3)	28	(0.5)
	ASC-US	1,800	135	(7.5)	41	(2.3)	869	59	(6.8)	19	(2.2)	2,669	194	(7.3)	60	(2.2)
	LSIL	1,417	127	(9.0)	27	(1.9)	523	45	(8.6)	9	(1.7)	1,940	172	(8.9)	36	(1.9)
	AGC	30	10	(33.3)	5	(16.7)	68	22	(32.4)	15	(22.1)	98	32	(32.7)	20	(20.3)
	ASC-H	140	35	(25.0	17	(12.1)	121	44	(36.4)	25	(20.7)	261	79	(30.0)	42	(15.9)
	HSIL	137	63	(46.0)	28	(20.4)	91	53	(58.2)	33	(36.3)	228	116	(50.6)	61	(26.5)
Low-risk only	Negative	3,329	24	(0.7)	2	(0.1)	1,111	5	(0.5)	1	(0.1)	4,440	29	(0.6)	3	(0.1)
	ASC-US	638	7	(1.1)	1	(0.2)	781	8	(1.0)	1	(0.1)	1,419	15	(1.1)	2	(0.1)
	LSIL	436	16	(3.7)	1	(0.2)	284	13	(4.6)	1	(0.4)	720	29	(4.0)	2	(0.3)
	AGC	10	0	(0.0)	0	(0.0)	46	0	(0.0)	0	(0.0)	56	0	(0.0)	0	(0.0)
	ASC-H	21	3	(14.3)	2	(9.5)	31	2	(6.5)	1	(3.2)	52	5	(9.8)	3	(5.9)
	HSIL	11	4	(36.4)	0	(0.0)	13	7	(53.8)	3	(23.1)	24	11	(45.5)	3	(12.0)
HPV negative	Negative	14,483	53	(0.4)	13	(0.1)	8,579	7	(0.1)	3	(0.0)	23,062	60	(0.2)	16	(0.0)
	ASC-US	952	7	(0.7)	1	(0.1)	2,794	4	(0.1)	1	(0.0)	3,746	11	(0.3)	2	(0.1)
	LSIL	84	5	(6.0)	0	(0.0)	111	1	(0.9)	1	(0.9)	195	6	(3.2)	1	(0.5)
	AGC	42	2	(4.8)	1	(2.4)	308	5	(1.6)	5	(1.6)	350	7	(2.0)	6	(1.7)
	ASC-H	33	0	(0.0)	0	(0.0)	112	0	(0.0)	0	(0.0)	145	0	(0.0)	0	(0.0)
	HSIL	3	1	(33.3)	0	(0.0)	21	2	(9.5)	2	(9.5)	24	3	(12.7)	2	(8.2)

[1]HPV groups are hierarchical: HPV16, else other high-risk, else low-risk, else HPV negative.
[2]Because of sample weights, percentages for all ages combined cannot be calculated from the number of women and number of events.

negative cytology to 63.6 and 49.6% for women with high-grade cytology. Women with single infections of HPV33 and HPV31 showed the second and third greatest risks. Notably, risks for HPV18-positive women, an HPV type that causes the second highest proportion of cervical cancers, were lesser than those for HPV33 and HPV31, except when HSIL was present and much less than the risks for HPV16 for all cytology categories other than high-grade. However, the risk of CIN2+ following AGC cytology was greater, but not significant, for HPV18 (57.7%) than for HPV16 (41.3%) ($p = 0.4$) and was significantly greater than the risk for other high-risk HPV types combined (23.2%) ($p = 0.02$) (Table 4). Of the women with AGC cytology, 4 of the 7 HPV18 positive CIN2+ were AIS/Adenocarcinoma compared to 3 of 9 HPV16 positive CIN2+ ($p = 0.1$) and 0 of 9 other high-risk HPV positive CIN2+ ($p = 0.02$).

Although our primary estimates of the 3-year cumulative incidence of CIN2+ and CIN3+ made no adjustment for lack of diagnostic follow-up, as a supplemental analysis we restricted our estimates to women with adequate follow-up (Supporting Information Tables 1 and 2). We considered two scenarios: minimally adequate follow-up and fully adequate follow-up. Minimally adequate follow-up was defined as cervical biopsy, ECC, or repeat cytology for all baseline cytology groups. Fully adequate follow-up varied by baseline cytology and was defined as (*i*) cervical biopsy or ECC for baseline cytology results of HSIL, ASC-H or AGC, (*ii*) cervical biopsy, ECC, or repeat negative cytology that was negative for high-risk HPV for baseline cytology of LSIL or high-risk HPV positive ASC-US, and (*iii*) cervical biopsy, ECC, or repeat negative cytology for baseline cytology of negative or high-risk HPV negative ASC-US. For negative baseline cytology, the cumulative incidence of CIN2+ and CIN3+ increased by about 40% for women with minimally adequate follow-up and also for women with fully adequate follow-up. For abnormal baseline cytology, the cumulative incidence increased by about 20% for women with minimally adequate follow-up and 30% for women with fully adequate follow-up.

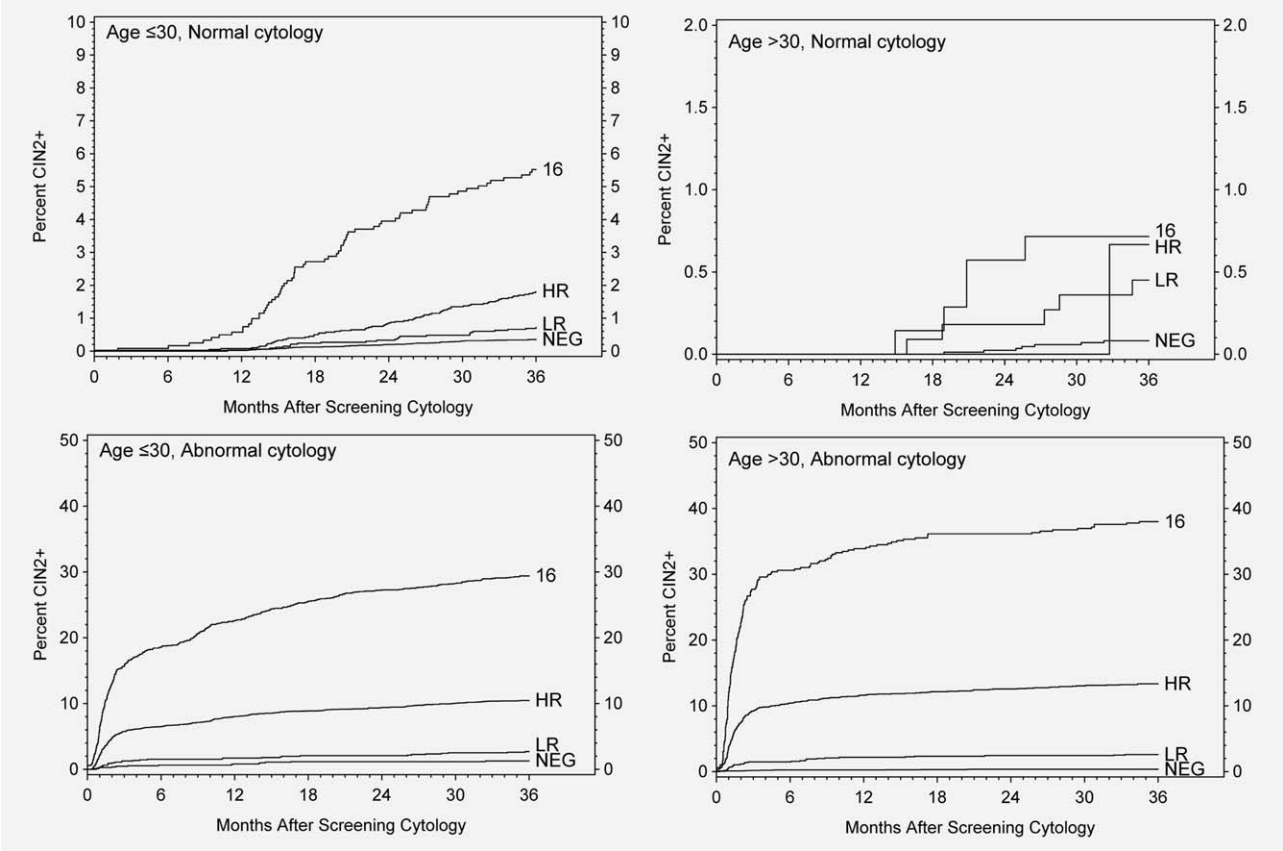

Figure 1. Cumulative incidence of cervical intraepithelial neoplasia grade 2 or more severe (CIN2+) for each HPV classification, stratified by age (≤30 and >30 years old) and by cytology (normal *vs*. abnormal). HPV classification was hierarchical: HPV16 positive (16), else HPV16 negative and other high-risk HPV positive (HR) else high-risk HPV negative and low-risk HPV positive (LR) else HPV negative (NEG).

Discussion

In our large population-based study of opportunistic cervical screening, we demonstrated large variation in risk for detecting CIN2+ and CIN3+ based on two risk markers, cytologic interpretation and detection of individual high-risk HPV genotypes. Previous studies have shown one or the other with precision but not both because of small numbers. Here, we observed a range of 3-year risk of detecting CIN2+ from ≤1% to more than 70% and for CIN3+ from <1 to 55%. The greatest risks for detecting CIN2+ and CIN3+ were observed among women with HPV16-positive high-grade cytology. The risks for detecting CIN2+ and CIN3+ increased ~1.5 to 4.0 fold among women with HPV16 compared to those with other high-risk HPV types, depending on the severity of the cervical cytology.

The lowest risks were combinations of women without any detectable high-risk HPV (negative or low-risk HPV) and with either negative or equivocal (ASC-US) cytologic results. Notably, there was no difference in the risk of detecting CIN3+ for any combination of HPV negative or low-risk HPV positive and cytology negative or ASC-US, suggesting that (*i*) HPV negative and either cytologic-negative or ASC-US can be considered as cotest negative and (*ii*) there is no patient benefit for testing for low-risk HPV.

The observed risks following an HPV16-positive, negative cytology were lower than previously reported.[12,13] We offer two possible explanations. First, the analytic sensitivity of HPV16 detection in this study might be greater than in other studies. As a result, women with very low-viral load HPV16 infections that are not as predictive of CIN2+ (*vs*. high-viral load HPV16) would have been called HPV16 positive, inflating the denominator (the number of HPV16-positive women) for the cumulative incidence calculation. Consistent with this explanation, the percent HPV16 positive among high-risk HPV-positive, cytologic-negative women >30 years of age was much higher in this study (17.7%) than observed in the study by Khan *et al.* (10.4%),[12] and slightly more than Wright *et al.* (14.9%).[13]

Second, in this real-world setting with only passive follow-up, there is bias by indication and women with negative cytology, regardless of HPV status, underwent less diagnostic evaluation than those with positive cytology. Therefore, risks for all subgroups of cytologic-negative women were underestimated to an unknown degree since none went immediately to colposcopy. Nevertheless, over the 3-year follow-up period, an estimated 22% of CIN2+ and 24% of CIN3+ in the population occurred in women who were high-risk HPV positive at the initial screen and who might have had their disease detected

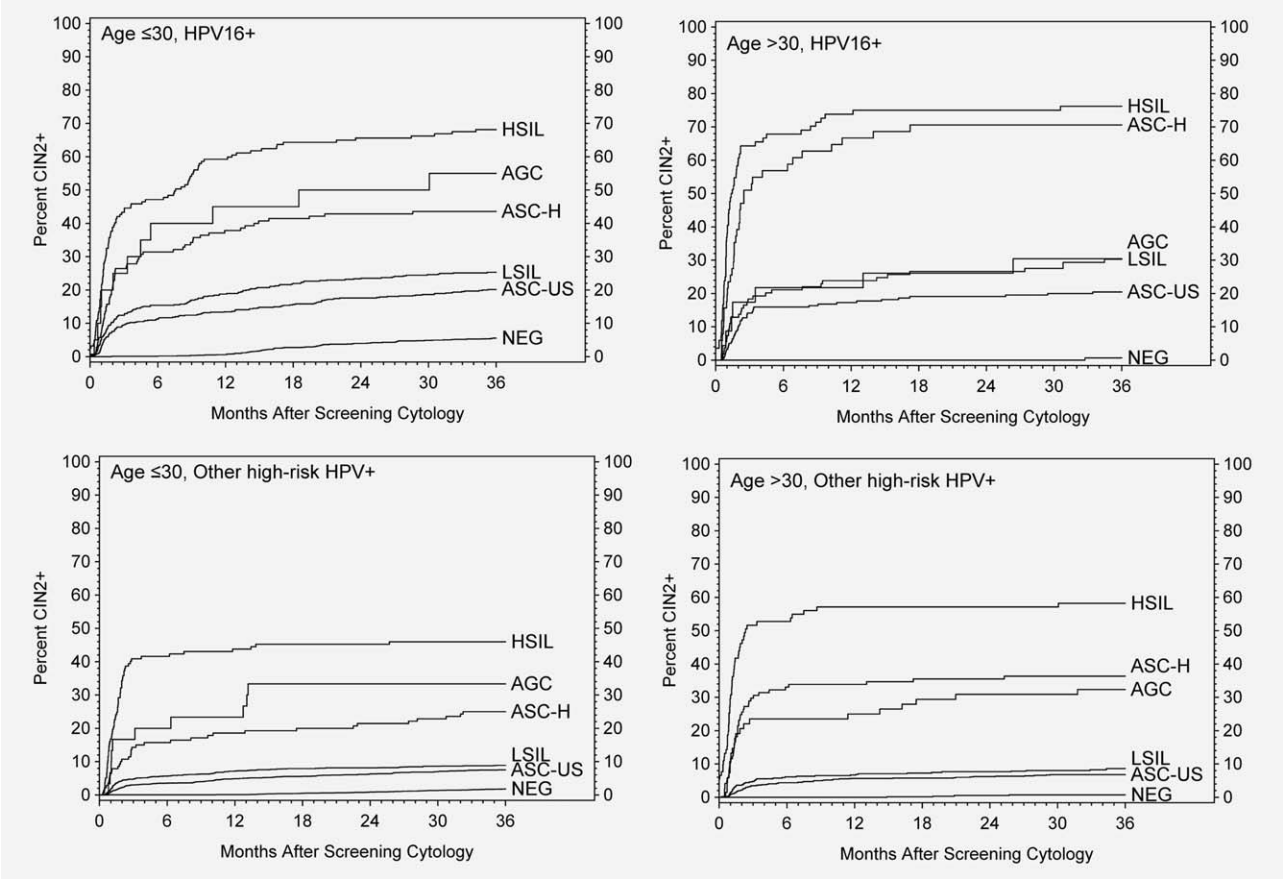

Figure 2. Cumulative incidence of cervical intraepithelial neoplasia grade 2 or more severe (CIN2+) for each cytologic interpretation, stratified by age (≤30 and >30 years old) by HPV status (HPV16 *vs*. other high-risk HPV types). Abbreviations: HSIL, high-grade squamous intraepithelial lesion; ASC-H, atypical squamous cells cannot rule out HSIL; AGC, atypical glandular cells; LSIL, low-grade squamous intraepithelial lesion (LSIL); ASC-US, atypical squamous cells of undetermined significance; NEG, negative for intraepithelial lesion or malignancy.

earlier had cotesting been employed. As there has been a shift from annual to biennial screening in the New Mexico population in the last 5 years, most cytologic-negative women in this population had only one follow-up cytology test and <5% had any diagnostic evaluation (cervical biopsy or ECC). In the study by Khan *et al.*,[12] women were screened every year. In the study by Wright *et al.*,[13] women who were HPV and/or cytologic-positive were referred to colposcopy. Together, the combination of higher HPV16 positivity and poorer disease ascertainment in the cytologic-negative population in this study compared to these other studies may explain the observed differences in risk.

It should be emphasized that HPV testing for our study was performed retrospectively and was not used for patient care. Women in this population were not screened for high-risk HPV with the exception of those undergoing ASC-US reflex HPV triage and a relatively small proportion in which HPV cotesting was performed. Data from the NMHPVPR suggests that HPV co-testing has low uptake in routine clinical practice (data not shown). Thus, the risks of detecting CIN2+ and CIN3+ for high-risk HPV-positive, cytologic-negative women in our study were less than the risks reported for Kaiser Permanente Northern California,[14] where HPV cotesting is the standard of practice in women ≥30 years of age, and where women who repeatedly test HPV-positive, cytologic-negative are referred for colposcopic evaluation.

Unlike previous studies with longer follow-up,[12] we did not find that HPV18 infection conveyed a higher risk of CIN2+ and CIN3+ compared to the other non-HPV16 high-risk HPV infections. Its unremarkable short-term predictive value for CIN2+ belies its importance as the second leading cause of cervical cancer after HPV16.[15] It is well known that HPV18-related lesions tend to be under-represented in cross-sectional studies. In prospective studies, there appears to be a significant time delay in finding HPV18-related disease so that HPV18 is under-represented in precancerous lesions for several years,[12,16] even following 1-year HPV18 persistence,[17] which may also explain our observations. The causes of this type-specific phenotype are uncertain but there is some evidence to suggest that HPV18-related lesions are harder to find by cytology and colposcopy.[15,18] With more follow-up time of this cohort, HPV18-related risks may rise disproportionately to the risks related

Table 3. Risk of detecting CIN2+ and CIN3+ within 3 years of screening cytology that is positive for a single HPV type, stratified by cytologic result; all ages combined

Cytologic result	HPV	Women	CIN2+			CIN3+		
			n	%[3]	(95% CI)[2]	*n*	%[3]	(95% CI)[2]
All results	16	1,055	249	10.9	(9.1, 12.7)	181	8.0	(6.5, 9.6)
	33	124	25	10.9	(5.8, 16.0)	12	5.2	(1.8, 8.6)
	31	468	64	7.3	(4.7, 9.9)	31	3.1	(1.9, 4.4)
	18	246	32	5.7	(3.4, 7.9)	19	2.9	(1.5, 4.3)
	58	277	34	5.4	(3.3, 7.4)	12	1.7	(0.7, 2.8)
	35	207	21	3.6	(1.8, 5.3)	5	0.8	(0.1, 1.5)
	45	188	13	2.5	(1.0, 4.1)	7	1.3	(0.2, 2.4)
	51	521	26	2.3	(1.3, 3.3)	4	0.4	(0.0, 0.9)
	52	407	22	2.2	(1.2, 3.2)	12	1.1	(0.4, 1.7)
	39	467	20	1.6	(0.9, 2.4)	6	0.4	(0.1, 0.8)
	59	422	12	1.3	(0.5, 2.1)	5	0.6	(0.0, 1.1)
	56	221	3	0.6	(0.0, 1.2)	1	0.2	(0.0, 0.6)
	68	128	2	0.4	(0.0, 0.9)	1	0.2	(0.0, 0.6)
Negative	16	505	28	3.6	(2.0, 5.2)	21	2.8	(1.4, 4.3)
	33	54	4	4.7	(0.0, 9.3)	2	2.3	(0.0, 5.6)
	31	242	10	3.5	(0.7, 6.3)	5	1.3	(0.1, 2.4)
	18	121	3	1.5	(0.0, 3.1)	0	0.0	n/a
	58	137	2	0.9	(0.0, 2.2)	0	0.0	n/a
	35	103	1	0.5	(0.0, 1.4)	0	0.0	n/a
	45	130	2	0.9	(0.0, 2.1)	1	0.4	(0.0, 1.3)
	51	263	3	0.7	(0.0, 1.5)	1	0.2	(0.0, 0.7)
	52	263	4	0.9	(0.0, 1.8)	1	0.2	(0.0, 0.7)
	39	281	2	0.4	(0.0, 1.0)	0	0.0	n/a
	59	308	4	0.8	(0.0, 1.5)	2	0.4	(0.0, 0.9)
	56	86	0	0.0	n/a	0	0.0	n/a
	68	83	0	0.0	n/a	0	0.0	n/a
ASC-US	16	229	53	23.3	(17.8, 28.8)	37	16.2	(11.4, 20.9)
	33	27	2	7.7	(0.0, 17.9)	0	0.0	n/a
	31	125	21	16.9	(10.3, 23.5)	10	8.1	(3.3, 12.9)
	18	57	1	1.7	(0.0, 5.0)	1	1.7	(0.0, 5.0)
	58	71	8	11.2	(3.9, 18.6)	2	2.9	(0.0, 6.8)
	35	62	9	14.4	(5.7, 23.1)	1	1.5	(0.0, 4.5)
	45	32	5	15.4	(2.9, 27.8)	2	6.0	(0.0, 14.2)
	51	105	3	2.9	(0.0, 6.0)	0	0.0	n/a
	52	95	10	10.6	(4.4, 16.7)	3	3.2	(0.0, 6.7)
	39	110	7	6.4	(1.8, 11.0)	2	1.9	(0.0, 4.4)
	59	84	4	4.7	(0.2, 9.1)	2	2.4	(0.0, 5.6)
	56	59	1	1.6	(0.0, 4.8)	0	0.0	n/a
	68	28	0	0.0	n/a	0	0.0	n/a
LSIL	16	119	39	32.5	(24.1, 40.9)	22	18.5	(11.5, 25.5)
	33	24	7	29.4	(11.1, 47.6)	3	12.6	(0.0, 25.9)
	31	47	10	21.1	(9.5, 32.8)	3	6.4	(0.0, 13.4)

Table 3. Risk of detecting CIN2+ and CIN3+ within 3 years of screening cytology that is positive for a single HPV type, stratified by cytologic result; all ages combined (Continued)

Cytologic result	HPV	Women	CIN2+			CIN3+		
			n	%[3]	(95% CI)[2]	*n*	%[3]	(95% CI)[2]
	18	31	6	19.6	(5.6, 33.7)	3	9.7	(0.0, 20.1)
	58	41	9	21.7	(9.1, 34.2)	2	4.6	(0.0, 10.9)
	35	25	3	11.8	(0.0, 24.4)	0	0.0	n/a
	45	12	1	8.7	(0.0, 25.0)	1	8.7	(0.0, 25.0)
	51	123	12	9.8	(4.5, 15.0)	1	0.8	(0.0, 2.4)
	52	22	2	9.2	(0.0, 21.3)	2	9.2	(0.0, 21.3)
	39	65	4	6.0	(0.3, 11.8)	0	0.0	n/a
	59	23	1	4.4	(0.0, 12.9)	0	0.0	n/a
	56	72	2	2.9	(0.0, 6.8)	1	1.4	(0.0, 4.2)
	68	14	1	6.9	(0.0, 20.0)	0	0.0	n/a
High-Grade[1]	16	202	129	63.6	(57.0, 70.3)	101	49.6	(42.7, 56.5)
	33	19	12	63.2	(41.6, 84.9)	7	36.3	(14.7, 57.9)
	31	54	23	42.5	(29.3, 55.7)	13	24.1	(12.7, 35.6)
	18	37	22	59.1	(43.2, 75.0)	15	40.2	(24.4, 56.0)
	58	28	15	53.1	(34.6, 71.7)	8	28.3	(11.6, 44.9)
	35	17	8	47.2	(23.4, 71.0)	4	23.4	(3.3, 43.5)
	45	14	5	36.0	(10.8, 61.3)	3	22.1	(0.1, 44.1)
	51	30	8	26.7	(10.9, 42.6)	2	6.3	(0.0, 14.9)
	52	27	6	22.2	(6.5, 37.8)	6	22.2	(6.5, 37.8)
	39	11	7	63.9	(35.6, 92.3)	4	36.1	(7.7, 64.4)
	59	7	3	42.3	(5.8, 78.9)	1	14.1	(0.0, 39.8)
	56	4	0	0.0	n/a	0	0.0	n/a
	68	3	1	33.3	(0.0, 86.7)	1	33.3	(0.0, 86.7)

[1]High-grade cytology result includes HSIL, ASC-H, AGC, and Cancer.
[2]Confidence intervals are approximate and are not computed when there are no events (n/a).
[3]Because of sample weights, percentages for all ages combined cannot be calculated from the number of women and number of events.

Table 4. Risk of CIN2+ within 3 years of screening cytology that is positive for single HPV infection of type HPV16, HPV18, or other high-risk HPV type; all ages combined

Cytologic result	HPV16				HPV18				Other High-risk			
	Women	CIN2+	%	(95% CI)	Women	CIN2+	%	(95% CI)	Women	CIN2+	%	(95% CI)
Negative	505	28	3.6	(2.0, 5.2)	121	3	1.5	(0.0, 3.1)	1950	32	1.1	(0.6, 1.5)
ASC-US	229	53	23.3	(17.8, 28.8)	57	1	1.7[1]	(0.0, 5.0)	798	70	8.8	(6.8, 10.7)
LSIL	119	39	32.5	(24.1, 41.0)	31	6	19.6	(5.6, 33.7)	468	52	11.1	(8.2, 13.9)
AGC	22	9	41.3	(20.7, 62.0)	12	7	57.7[2]	(29.7, 85.8)	43	10	23.2	(10.6, 35.8)
ASC-H	67	39	57.5	(45.6, 69.3)	8	5	62.2	(28.6, 95.9)	96	31	32.2	(22.9, 41.6)
HSIL	113	81	71.6	(63.2, 79.9)	17	10	58.7	(35.2, 82.1)	75	47	62.3	(51.3, 73.3)

[1]Significantly different from risk for HPV16, $p = 0.0001$.
[2]Significantly different from risk for other high-risk HPV, $p = 0.02$.

to the other high-risk HPV types. However, this remains to be demonstrated and highlights the need for continued follow up to determine the value of separate typing for HPV 18 when HPV testing is used.

We did note a strong association of HPV18 positive AGC cytology with glandular disease, although the numbers were small. Previous studies have shown that HPV positive AGC is strongly associated with CIN2+ and especially AIS/

adenocarcinoma.[19–22] To our knowledge, this is the first time a link between HPV18-positive AGC cytology and AIS/adenocarcinoma has been reported.

In conclusion, we have reported the HPV type-specific 3-year risks of detecting CIN2+ and CIN3+ from a large, statewide sample of women undergoing opportunistic cervical cancer screening in the US. To our knowledge, this and the cohort at Kaiser Permanente Northern California[22] are the only cohorts of this magnitude in the US in which longitudinal HPV type-specific assessments of outcome can be determined. Importantly we have established the baseline risks of detecting CIN2+ and CIN3+ prior to widespread HPV vaccination against HPV16 and HPV18. In the future, as HPV vaccination coverage increases and HPV-vaccinated women reach the age of screening, we will be able to measure its impact on population risks to examine whether screening recommendations might need to be adjusted to rebalance benefits and harms of screening.[2] Specifically, we expect that the absolute risks following a positive screening test to be decreased significantly, as indirectly demonstrated in this analysis and other analyses,[12,13,23,24] for HPV16/18-vaccinated populations versus unvaccinated populations. We will also be able to evaluate any changes in the ecology and natural history of HPV in the absence of the two types that cause the majority of cancers, such as HPV-type replacement.

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