

# Baseline Cytology, Human Papillomavirus Testing, and Risk for Cervical Neoplasia: A 10-Year Cohort Analysis

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**Background:** Annual Pap smear screening has been favored over less frequent screening in the United States to minimize the risk of cervical cancer. We evaluated whether simultaneous screening with a Pap test and human papillomavirus (HPV) testing is useful for assessing the risk for cervical intraepithelial neoplasia (CIN) 3 or cervical cancer. **Methods:** We enrolled 23 702 subjects in a study of HPV infection at Kaiser Permanente, Northwest Division, Portland, OR. Data were analyzed for 20 810 volunteers who were at least 16 years old (mean = 35.9 years) with satisfactory baseline Pap tests and suitable samples for HPV testing. Women were followed for up to 122 months (from April 1, 1989, to June 30, 1999) to determine the risk for histopathologically confirmed CIN3 or cancer. **Results:** Among 171 women with CIN3 or cancer diagnosed over 122 months, 123 (71.9%, 95% confidence interval [CI] = 65.2% to 78.7%) had baseline Pap results of atypical squamous cells or worse and/or a positive HPV test, including 102 (86.4%, 95% CI = 80.3% to 92.6%) of the 118 cases diagnosed within the first 45 months of follow-up. During this 45-month period, the cumulative incidence of CIN3 or cancer was 4.54% (95% CI = 3.61% to 5.46%) among women with a Pap test result of atypical squamous cells or worse, positive HPV tests, or both compared with 0.16% (95% CI = 0.08% to 0.24%) among women with negative Pap and HPV tests. Age, screening behavior, a history of cervical cancer precursors, and a history of treatment for CIN minimally affected results. **Conclusions:** Negative baseline Pap and HPV tests were associated with a low risk for CIN3 or cancer in the subsequent 45 months, largely because a negative HPV test was associated with a decreased risk of cervical neoplasia. Negative combined test results should provide added reassurance for lengthening the screening interval among low-risk women, whereas positive results identify a relatively small subgroup that requires more frequent surveillance. [J Natl Cancer Inst 2003;95:46-52]

Although cytologic screening programs using Pap smears have dramatically reduced cervical cancer incidence and mortality in developed nations, single Pap tests suffer from suboptimal sensitivity, limited reproducibility, and many equivocal results (1,2). Cytologic screening is effective because cervical cancer typically develops slowly, which permits a program of repeated testing combined with aggressive follow-up to compensate for the deficiencies of a single Pap test. Although less frequent screening of women with repeatedly negative Pap tests has been adopted as a practice by some clinicians (3), many patients and clinicians continue to favor annual screening because of concerns that even multiple negative tests do not ensure the safety of women for more than a year.

Research in the last decade has conclusively demonstrated that infection with carcinogenic types of human papillomaviruses (HPVs) represents a nearly universal event in cervical cancer development (4-6). Although natural history studies have demonstrated that most HPV infections produce only transient minor lesions (7,8), untreated infections may persist and progress to cervical intraepithelial neoplasia (CIN) 3, a cancer precursor. Without intervention, a sizable fraction of women with CIN3 will develop invasive cancer, in most instances, 10 years or more after the initial infection (9).

Testing methods capable of identifying low copy numbers of carcinogenic HPV DNA have demonstrated extremely high sensitivity for identifying prevalent CIN3 and cancer (1,10,11). Consequently, performing HPV testing and cytologic screening simultaneously should dramatically improve the detection of prevalent disease. Moreover, if negative cytologic and HPV testing results accurately predict a low future risk for cervical neoplasia, the screening interval for women with negative tests could be safely lengthened, and the subgroup of patients with positive results could be targeted for more frequent surveillance. If sufficiently specific, this approach would permit a large group of women with negative tests to avoid unnecessary frequent testing and permit screening programs to focus on women at highest risk.

Although there are abundant data related to the cross-sectional sensitivity of cytology and HPV testing, large studies that assess whether combined testing can predict the future development of disease are lacking. Accordingly, we assessed the risk for CIN3 and cancer associated with 1) a single baseline Pap smear reported as atypical squamous cells (ASC) or worse, 2) a positive HPV test, and 3) positive results for either or both tests during 10 years of follow-up. Our analysis used data from a large National Cancer Institute-sponsored cohort established at Kaiser Permanente Northwest Division, a health maintenance organization.

## SUBJECTS AND METHODS

### Study Subjects

We enrolled 23 702 women in a natural history study of HPV infection at the Kaiser Permanente prepaid health plan in Port-

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land, OR, between April 1, 1989, and November 2, 1990, as previously described (5). The cohort included a demographically representative sample of approximately 50% of women undergoing cervical cytologic screening at Kaiser, which served about one-quarter of the women residing in Portland during this time. Subjects were 16 years of age or older with a mean age of 35.9 years (range = 16 to 94 years).

The current analysis is restricted to 20810 women with both satisfactory baseline cervical smears and suitable samples for HPV testing. We excluded 1107 women who refused to participate and 1406 women who had undergone hysterectomy before enrollment. In addition, we excluded a total of 379 women for other causes: 67 women under 16 years of age, 128 who lacked an adequate sample for HPV testing, 85 with unsatisfactory or missing baseline cervical smears, and 99 who underwent colposcopy rather than screening at enrollment.

### Enrollment Examination

Briefly, subjects provided informed consent as required by institutional review boards at Kaiser and the National Institutes of Health and then underwent a routine pelvic examination. Experienced clinicians prepared a single ethanol-fixed Pap smear for each subject with exfoliative cervical cells collected by the use of an Ayre spatula and a cytobrush. Next, the cervix was rinsed with 10 mL of sterile saline via a 3.25-inch flexible intracatheter extender (1 inch = 2.54 cm). The pooled fluid was collected from the posterior vaginal fornix and processed for HPV testing as described below. Computerized records were reviewed to identify women who had a history of cervical abnormalities or treatment for cervical disease.

### Follow-up

During the study period, annual screening of women at Kaiser was standard practice. Smears were generally obtained at clinic visits if screening had not been performed within the prior 9 months or if there was clinical suspicion of a cervical abnormality. Participation in the health plan was relatively stable during the period of this study; women with negative baseline smears had a mean follow-up time of more than 6 years.

Patients with abnormal cytology were managed according to standard practice guidelines. As would be expected, women with baseline smears reported as ASC or worse had slightly more follow-up smears than those with negative cytology (for women with Pap smear results of ASC or worse,  $4.4 \pm 3.6$  smears (mean  $\pm$  standard deviation); for women with negative smear results,  $3.8 \pm 3.0$  smears). The results of HPV testing were not available to direct patient management. The average number of follow-up smears and length of follow-up were slightly less among HPV-positive women, who tended to be younger than HPV-negative women and, therefore, more likely to leave the health plan (data not shown). Overall, 83.6% of women had at least one follow-up Pap smear. Women who did not have repeat smears were included in the analysis to account for cases that were detected at a colposcopic examination prompted by an abnormal baseline smear without an intervening repeat.

### Pathology

Results of Pap smears were originally reported by a classification system that predated the development of the Bethesda System; we converted these interpretations into Bethesda 2001 terminology for this study (12). We reclassified women with

smears reported as “normal” or “benign reactive atypia” as “negative for intraepithelial lesion or malignancy (negative)” according to the Bethesda 2001 classification. Smears reported as “severe reactive atypia, possibly dysplasia” or “possible koilocytotic or condylomatous atypia” were classified as “atypical squamous cells (ASC).” Cytologic interpretations of dysplasia were reclassified as low- (LSIL) or high-grade squamous intraepithelial lesion (HSIL), and histologic diagnoses were converted into CIN nomenclature. Specifically, severe dysplasia and carcinoma *in situ* were categorized as CIN3.

### HPV Testing

Cervical lavage specimens were refrigerated within 1 hour of collection and transported to a laboratory for processing. A 1-mL aliquot was removed, frozen at  $-70^{\circ}$  C, and used subsequently for HPV testing with a polymerase chain reaction (PCR)-based method using MY09/11 primers (13). The remaining fluid was crudely divided in half and centrifuged, and then both pellets were frozen.

We selected both frozen liquid aliquots and cell pellets for HPV testing, depending on their availability (5,13). The sensitivity of HPV testing for identifying women with CIN3 or cancer was similar, irrespective of the method of sample handling (data not shown). HPV testing (masked to cytology and clinical outcome) was performed with the Hybrid Capture 2 microplate assay (Digene, Gaithersburg, MD) at a detection threshold of 1.0 pg/mL (approximately 5000 copies of HPV DNA). The assay detected 13 carcinogenic types of HPV: HPV16, HPV18, HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV56, HPV58, HPV59, and HPV68, as described elsewhere (1,10,14).

### Statistical Analysis

We determined whether women with histopathologically confirmed CIN3 or cancer during a follow-up of up to 122 months from April 1, 1989, through June 30, 1999, had a baseline smear of ASC or worse and/or a positive baseline HPV test. The results for smears were assessed at two thresholds: 1) ASC or worse and 2) LSIL or worse.

Women with rigorously defined histopathologic CIN3 or cancer (including endocervical adenocarcinoma *in situ*) were designated as case patients. To avoid misclassification of less severe lesions as CIN3 or cancer, we restricted our case group to women who had received original histopathologic diagnoses of CIN3 or cancer on two different clinical specimens obtained on different dates (usually a diagnostic punch biopsy and a cone biopsy to remove the lesion, performed for treatment) or who met the following specific review criteria: 1) an original histopathologic diagnosis of CIN2 reviewed as CIN3 or worse or 2) an original histopathologic diagnosis of CIN3 or worse confirmed as at least CIN2. A single pathologist applying stringent criteria performed the reviews. In total, 171 (0.8%) of 20810 women, including 26 (0.1%) with invasive carcinoma, fulfilled this case definition.

Follow-up time was divided into an initial period of 9 months followed by yearly intervals for a total time of 122 months. These intervals roughly paralleled the intervals at which women returned for annual smears. We considered Pap tests that were performed within 9 months of enrollment to have been rapidly repeated, presumably prompted by a previous cytologic abnormality or suspicious symptoms. The classification of disease as

prevalent or incident in this study was purposely avoided, because data strongly indicate that even highly systematic multimodality evaluations by experts miss prevalent disease which, when detected at a later time, may be misclassified as incident (Schiffman M: unpublished observation). Accordingly, our goal was to determine the performance of screening tests during intervals that are meaningful for developing screening strategies (i.e., 45 and 122 months of follow-up), not to precisely define the interval between baseline testing and the development of CIN3 or cancer. For women who received histopathologic diagnoses of CIN3 or cancer at multiple times, the time of diagnosis was defined as the date of the first diagnosis.

The risk of cervical neoplasia was computed for each interval by dividing the number of cases diagnosed in that interval by the number of women screened during that interval (for summary, see Tables 2–4). We compiled all of the data for the first four intervals and then summarized data for the first 45 months and the entire 122-month study period. We calculated cumulative incidence and cumulative incidence ratios by using Kaplan–Meier methods. Conceptually, cumulative incidence among women with positive screening tests (i.e., Pap tests of ASC or worse and/or positive HPV tests) is comparable to positive predictive value for detection of disease (i.e., number of positive tests in women with disease divided by total number of positive tests × 100%, adjusted for loss to follow-up). Similarly, negative predictive value is equal to 100% minus the cumulative incidence among women with negative screening tests. The main analysis was repeated after stratifying by age (≤29 years, 30–39 years, and ≥40 years) and by a past medical history of important cervical abnormalities (defined as a record of histopathologic CIN2 or worse, cytologic HSIL, or treatment for CIN). Finally, we microscopically reviewed the diagnostic histopathology of case patients identified during the first 57 months of follow-up with negative or ASC baseline cytology and negative HPV tests to further characterize the lesions.

## RESULTS

### Baseline Results: Pap Smears and HPV Testing

In total, 654 (3.1%, 95% CI = 2.9% to 3.4%) women had enrollment or baseline Pap smears reported as ASC or worse, and 2979 (14.3%, 95% CI = 13.8% to 14.8%) tested positive for HPV (Table 1). HPV was detected among 417 (63.8%, 95% CI = 60.1% to 67.4%) of 654 women with smears interpreted as ASC or worse compared with 143 (80.3%, 95% CI = 74.5% to 86.2%) of 178 women with LSIL or more severe readings. Overall, 86.0% (95% CI = 84.8% to 87.2%) of HPV infections were detected among women with concurrently negative baseline Pap

smears, with the highest frequency among women 29 years of age or younger (data not shown).

### Risk of CIN3 and Cancer Associated With an Equivocal or Abnormal Baseline Smear

Among 171 case patients diagnosed with CIN3 or cervical cancer during follow-up, 59 (34.5%, 95% CI = 27.4% to 41.6%) had baseline Pap smears reported as ASC or worse, including 58 (49.2%, 95% CI = 40.1% to 58.2%) of 118 diagnosed within 45 months (Table 2). Among women with a baseline Pap test result of ASC or worse, the incidence of CIN3 or cancer was 7.82% (95% CI = 5.76% to 9.88%) in the first time interval and the cumulative incidence was 10.22% (95% CI = 7.56% to 12.88%) for the entire follow-up period. A positive baseline smear was reported for only one patient diagnosed with CIN3 or cancer between 21 and 45 months of follow-up and for only one more patient diagnosed after 45 months. The cumulative incidence ratio fell sharply from 103.53 (95% CI = 58.53 to 183.15) for the first time period to 39.25 (95% CI = 25.42 to 60.59) for the second period and then dropped consistently throughout follow-up to 7.38 (95% CI = 5.30 to 10.28) at the final time point. Baseline smear results of LSIL or worse were associated with a high cumulative incidence ratio at 122 months (16.90, 95% CI = 11.80 to 24.20), but at this threshold, only 39 (22.8%, 95% CI = 16.5% to 29.1%) of the 171 cases of cervical cancer would have been identified (data not shown).

The percentage of cases of CIN3 or cancer detected with a baseline Pap test result of ASC or worse was similar across age groups, although cumulative incidence ratios were higher among women 30 years of age or older. For women 29 years of age or younger, the cumulative incidence ratio was 3.34 (95% CI = 1.99 to 5.59), compared with 12.21 (95% CI = 6.97 to 21.41) for women 30–39 years of age and 13.47 (95% CI = 6.38 to 28.45) for women aged 40 years or older (data not shown). Stratifying on past medical history of cervical disease or treatment did not alter the results (data not shown).

### Risk of CIN3 and Cancer Associated With a Positive Baseline HPV Test

HPV was detected at enrollment among 110 (64.3%, 95% CI = 57.2% to 71.5%) of 171 women who later became case patients, including 89 (75.4%, 95% CI = 67.7% to 83.2%) of 118 women diagnosed within 45 months (Table 3). Among women with a positive HPV test, the incidence of CIN3 or cancer was 1.73% (95% CI = 1.26% to 2.20%) for the first time interval and 6.92% (95% CI = 5.49% to 8.35%) for the entire follow-up period. Unlike the Pap test results, the results for HPV testing indicated prolonged risk prediction; risk for each time

**Table 1.** Frequency of Pap smears reported as atypical squamous cells (ASC) or worse and positive tests for carcinogenic human papillomavirus (HPV) types at enrollment\*

Baseline Pap smear result	No. (column %; 95% CI)	Detection of oncogenic HPV DNA	
		No. negative (row %; 95% CI)	No. positive (row %; 95% CI)
Negative	20 156 (96.9; 96.6 to 97.1)	17 594 (87.3; 86.8 to 87.7)	2562 (12.7; 12.3 to 13.2)
ASC or worse	654 (3.1; 2.9 to 3.4)	237 (36.2; 32.6 to 39.9)	417 (63.8; 60.1 to 67.4)
ASC only	476 (2.3; 2.1 to 2.5)	202 (42.4; 38.0 to 46.9)	274 (57.6; 53.1 to 62.0)
LSIL or worse	178 (0.9; 0.7 to 1.0)	35 (19.7; 13.8 to 25.5)	143 (80.3; 74.5 to 86.2)
Total	20 810	17 831	2979

\*CI = confidence interval; LSIL = low-grade squamous intraepithelial lesion. Category of LSIL or worse is a subset of ASC or worse.

**Table 2.** Risk for cervical intraepithelial neoplasia 3 (CIN3) and cancer associated with a Pap smear of atypical squamous cells (ASC) or worse\*

Follow-up	Pap smear result	No. of women	No. of case patients	Risk during follow-up interval, % (95% CI)	Cumulative incidence, % (95% CI)	Cumulative incidence ratio (95% CI)
0–9 mo	≥ASC	652	51	7.82 (5.76 to 9.88)	7.82 (5.76 to 9.88)	103.53 (58.53 to 183.15)
	Neg	19 854	15	0.08 (0.04 to 0.11)	0.08 (0.04 to 0.11)	
9–21 mo	≥ASC	368	6	1.63 (0.34 to 2.92)	9.32 (6.97 to 11.68)	39.25 (25.42 to 60.59)
	Neg	11 099	18	0.16 (0.09 to 0.24)	0.24 (0.15 to 0.32)	
21–33 mo	≥ASC	295	1	0.34 (0.00 to 1.00)	9.63 (7.21 to 12.05)	24.68 (16.79 to 36.28)
	Neg	9800	15	0.15 (0.08 to 0.23)	0.39 (0.28 to 0.50)	
33–45 mo	≥ASC	231	0	0.00 (0.00 to 0.00)	9.63 (7.21 to 12.05)	18.29 (12.73 to 26.27)
	Neg	8764	12	0.14 (0.06 to 0.21)	0.53 (0.39 to 0.66)	
Total (0–45 mo)	≥ASC	654	58	N/A	9.63 (7.21 to 12.05)	18.29 (12.73 to 26.27)
	Neg	20 132	60	N/A	0.53 (0.39 to 0.66)	
Overall (0–122 mo)	≥ASC	654	59	N/A	10.22 (7.56 to 12.88)	7.38 (5.30 to 10.28)
	Neg	20 156	112	N/A	1.38 (1.10 to 1.67)	

\*CI = confidence interval; Neg = negative; N/A = not applicable (instead, see cumulative incidence ratio adjusted for person-time); ≥ASC = ASC or a more severe cytologic interpretation.

**Table 3.** Risk for cervical intraepithelial neoplasia 3 (CIN3) and cancer associated with a positive test for carcinogenic human papillomavirus (HPV) types\*

Follow-up	HPV test result	No. of women	No. of case patients	Risk during follow-up interval, % (95% CI)	Cumulative incidence, % (95% CI)	Cumulative incidence ratio (95% CI)
0–9 mo	Positive	2946	51	1.73 (1.26 to 2.20)	1.73 (1.26 to 2.20)	20.27 (11.41 to 35.99)
	Negative	17 560	15	0.09 (0.04 to 0.13)	0.09 (0.04 to 0.13)	
9–21 mo	Positive	1600	20	1.25 (0.71 to 1.79)	2.96 (2.25 to 3.67)	23.50 (13.92 to 39.68)
	Negative	9867	4	0.04 (0.00 to 0.08)	0.13 (0.07 to 0.18)	
21–33 mo	Positive	1336	9	0.67 (0.24 to 1.11)	3.61 (2.79 to 4.44)	17.56 (11.04 to 27.94)
	Negative	8759	7	0.08 (0.02 to 0.14)	0.21 (0.12 to 0.29)	
33–45 mo	Positive	1102	9	0.82 (0.29 to 1.35)	4.40 (3.44 to 5.36)	18.06 (11.60 to 28.10)
	Negative	7893	3	0.04 (0.00 to 0.08)	0.24 (0.15 to 0.34)	
Total (0–45 mo)	Positive	2976	89	N/A	4.40 (3.44 to 5.36)	18.06 (11.60 to 28.10)
	Negative	17 810	29	N/A	0.24 (0.15 to 0.34)	
Overall (0–122 mo)	Positive	2979	110	N/A	6.92 (5.49 to 8.35)	8.00 (5.61 to 11.41)
	Negative	17 831	61	N/A	0.87 (0.62 to 1.12)	

\*CI = confidence interval; N/A = not applicable (instead, see cumulative incidence ratio adjusted for person-time).

interval remained elevated, though diminished, 105 months after enrollment (data not shown). The cumulative incidence ratio was 18.06 (95% CI = 11.60 to 28.10) at 45 months and 8.00 (95% CI = 5.61 to 11.41) at 122 months. Although cumulative incidence ratios associated with a positive HPV test were higher among women aged 30 years or older than among younger women, the increase was not monotonic with increasing age. For women 29 years of age or younger, the cumulative incidence ratio was 4.07 (95% CI = 2.37 to 6.99) compared with 10.93 (95% CI = 5.84 to 20.43) for women 30–39 years of age and 8.77 (95% CI = 4.20 to 18.34) for women aged 40 years or older. Results for HPV testing were not substantially affected by stratifying on prior history of cervical abnormalities or treatment.

### Risk of CIN3 and Cancer Associated With a Baseline Smear of ASC or Worse and/or a Positive HPV Test

The cohort included 3216 (15.5%, 95% CI = 15.0% to 16.0%) women who had baseline Pap test results of ASC or worse, positive HPV tests, or both. A total of 123 (71.9%, 95% CI = 65.2% to 78.7%) case patients had baseline smears of ASC or worse, positive HPV test results, or both, including 102 (86.4%, 95% CI = 80.3% to 92.6%) of 118 case patients diagnosed within 45 months. Among women considered positive by a combined testing strategy, the cumulative incidence of CIN3 or cancer was 4.54% (95% CI = 3.61% to 5.46%) at 45 months, compared with 0.16% (95% CI = 0.08% to 0.24%) among

women with negative Pap tests and negative HPV tests. The cumulative incidence at 122 months for women considered positive by combined testing was 6.83% (95% CI = 5.50% to 8.16%) compared with 0.79% (95% CI = 0.54% to 1.04%) for those considered negative, roughly paralleling results for HPV testing alone. The negative predictive value for combined testing was 99.21%. Considering the entire follow-up period, women who tested positive at baseline with Pap and HPV testing had a cumulative incidence ratio of 8.67 (95% CI = 5.98 to 12.56) compared with women for whom both tests were negative; for the first 45 months of follow-up, the cumulative incidence ratio was 28.85 (95% CI = 16.67 to 49.95; Table 4). The two tests identified 20 (76.9%) of 26 women diagnosed with carcinoma during follow-up. Consideration of age or past medical history did not substantially alter these results. The cumulative incidence ratio was 4.36 (95% CI = 2.48 to 7.67) among women 29 years of age or younger, 12.47 (95% CI = 6.45 to 24.11) among women 30–39 years of age, and 8.73 (95% CI = 4.17 to 18.28) for women 40 years of age or older (data not shown).

Unmasked histopathologic review of the available diagnostic material for 22 cases of CIN3 or cancer detected within 57 months after negative or ASC baseline smears and negative HPV tests demonstrated that most of these lesions were small. Two cases of endocervical adenocarcinoma *in situ* and two cases of carcinomas of uncertain origin, possibly not cervical, were also found among these case patients.

**Table 4.** Risk for cervical intraepithelial neoplasia 3 (CIN3) and cancer associated with an enrollment Pap smear of atypical squamous cells (ASC) or worse and/or a positive human papillomavirus (HPV) test\*

Follow-up	Pap result of $\geq$ ASC or HPV positive	No. of women	No. of case patients	Risk during follow-up interval, % (95% CI)	Cumulative incidence, % (95% CI)	Cumulative incidence ratio (95% CI)
0–9 mo	Yes	3182	61	1.92 (1.44 to 2.39)	1.92 (1.44 to 2.39)	66.42 (26.71 to 165.17)
	No	17 324	5	0.03 (0.00 to 0.05)	0.03 (0.00 to 0.05)	
9–21 mo	Yes	1748	22	1.26 (0.74 to 1.78)	3.15 (2.46 to 3.85)	63.75 (28.59 to 142.15)
	No	9719	2	0.02 (0.00 to 0.05)	0.05 (0.01 to 0.09)	
21–33 mo	Yes	1459	10	0.69 (0.26 to 1.11)	3.82 (3.01 to 4.62)	32.09 (17.54 to 58.74)
	No	8636	6	0.07 (0.01 to 0.13)	0.12 (0.05 to 0.19)	
33–45 mo	Yes	1197	9	0.75 (0.26 to 1.24)	4.54 (3.61 to 5.46)	28.85 (16.67 to 49.95)
	No	7798	3	0.04 (0.00 to 0.08)	0.16 (0.08 to 0.24)	
Total (0–45 mo)	Yes	3213	102	N/A	4.54 (3.61 to 5.46)	28.85 (16.67 to 49.95)
	No	17 573	16	N/A	0.16 (0.08 to 0.24)	
Overall (0–122 mo)	Yes	3216	123	N/A	6.83 (5.50 to 8.16)	8.67 (5.98 to 12.56)
	No	17 594	48	N/A	0.79 (0.54 to 1.04)	

\*CI = confidence interval; N/A = not applicable (instead, see cumulative incidence ratio adjusted for person-time);  $\geq$ ASC = ASC or a more severe cytologic interpretation.

## DISCUSSION

This article examined the risk of CIN3 or cancer among a group of 20810 members in a health care maintenance organization who were followed for more than a decade. In this screening cohort, a single baseline smear of ASC or worse and/or a positive HPV test defined a subgroup consisting of 15.5% of the total cohort, which included 71.9% of all women diagnosed with CIN3 or cancer and 86.4% of those diagnosed with CIN3 or cancer within 45 months.

Enrollment or baseline Pap smear results of ASC or worse were identified in 3.1% of women, including 34.5% of those who became case patients. However, all but one case patient identified by a Pap test was diagnosed early in follow-up, suggesting that the Pap test was useful in detecting lesions that were present at enrollment but was insensitive in assessing future risk. The cumulative incidence (positive predictive value adjusted for women lost to follow-up) of CIN3 or cancer among women with a baseline Pap test reported as ASC or worse was 7.82% for the first 9 months and 10.22% for the entire follow-up period compared with 1.73% and 6.92% for HPV testing, respectively, at these time points. The higher positive predictive value of Pap testing compared with HPV testing reflected the comparatively low frequency at which ASC or worse was detected among women who were not case patients, which included women with pathology results ranging from normal to CIN2. However, enrollment HPV testing was much more sensitive than Pap testing, especially for later occurring cases, identifying the majority of women who became case patients over the entire 122-month period.

Combined baseline Pap and HPV testing divided the cohort into two groups with strikingly different risks for disease. Among women with ASC or worse, a positive HPV test, or both, the cumulative incidence at 122 months of CIN3 or cancer was 6.83% compared with 0.79% among women with both a negative Pap smear and a negative HPV test (negative predictive value = 99.21%). Over the first 45-month period, the cumulative incidence among women with negative results for both tests was only 0.16% (95% CI = 0.08% to 0.24%), compared with a substantially higher figure of 0.53% (95% CI = 0.39% to 0.66%) among women with negative cytology only. The low risk of CIN3 and cancer among women with both a negative Pap test and a negative HPV test during follow-up reflects mainly the

high sensitivity of HPV testing over the entire 122-month period. Therefore, negative results with simultaneous, combined screening by Pap and HPV testing provided strong reassurance that prevalent disease was absent; the negative HPV test alone predicted that the future risk for disease was low.

The data from this large cohort with lengthy follow-up provide powerful confirmation that HPV infection precedes and is causally associated with the development of cervical neoplasia. A nested case-control study (13) previously performed in this cohort demonstrated that enrollment HPV testing by PCR was associated with a relative risk of 12.7 for the development of high-grade disease within 5 years. Other studies have yielded similar findings. In a cohort of 2011 teenage subjects followed for a median of 29 months, Woodman et al. (15) reported that HPV-positive women with a normal Pap test were at 13-fold increased risk for moderate or severe dyskaryosis. Rozendaal et al. (16) reported that, among women with a mean age of 42 years followed for a mean of 40 months, HPV detection was associated with 116-fold increased risk for the development of CIN3. Retrospective HPV testing performed on archival smears has also demonstrated that HPV detection strongly predicts risk for future development of cervical neoplasia (17,18). Results from our study and others (15–18) highlight the central etiologic role of HPV infection in cervical neoplasia by establishing that viral infection precedes the development of disease and, therefore, support the perspective that HPV testing effectively stratifies patients according to cancer risk.

Improved methods for preparing exfoliated cells for cytologic interpretation and for collecting samples to test for viruses have been developed in the decade since this cohort was established. Specifically, meta-analyses (19,20) suggest that thin-layer cytology is more sensitive than conventional Pap smears. In addition, cervical scrapes seem to provide a better sample than cervicovaginal lavage to test for viruses. In this study, 77.3% of case patients diagnosed within 9 months of enrollment were identified by HPV testing, whereas, in several other large studies that have used similar HPV assays to test cervical scrapes (as opposed to lavages), cross-sectional sensitivity for CIN3 exceeded 90% (1,10,11). Our analysis suggested that our lavage technique was less effective than direct cervical scraping in sampling the endocervical canal. Most CIN3 lesions and cancers develop at the squamocolumnar junction or “transformation

zone," which is known to recede with advancing age and, therefore, may be difficult to sample. The unexpected decline in the sensitivity of HPV testing from 69.7% among women aged 29 years or younger to 50.0% among those 40 years or older (one-third of our subjects) suggests that the transformation zone was inadequately sampled among our oldest subjects. Finally, incident HPV infection after enrollment, with rapid development of CIN3 as previously reported (15,21), probably accounted for some cases detected in follow-up that were associated with negative baseline HPV tests.

Cost and logistics precluded systematically timed follow-up of more than 20 000 healthy women for over a decade in the United States; consequently, subjects in this study were followed according to prevailing clinical practice standards. Our calculated cumulative incidence for CIN3 and cancer associated with HPV detection may represent a low estimate if the insensitivity of Pap testing or loss to follow-up disproportionately affected HPV-positive undiagnosed case patients compared with HPV-positive women who did not become case patients. Clearly, this possibility would represent a modest limitation because it would tend to underestimate the value of the combined testing approach. In addition, we speculate that biopsy and treatment of lower grade lesions may have interrupted the natural history of HPV infection in some potential cases, although proof of this assertion is lacking. It is also possible that some cases of CIN3 were missed among control subjects secondary to underdiagnosis; insensitive screening was extremely unlikely to have affected our conclusions, given the consistent bias of Kaiser pathologists to diagnose lesions as more severe than the independent reviewer (data not shown). Finally, some cases with positive baseline HPV tests (particularly those diagnosed late in follow-up) may have been caused by HPV infections other than those that were present at enrollment.

Studies often demonstrate that the frequency of HPV infection among women with normal Pap test results declines with increasing age (22–24), however, HPV infections among older women are more likely to be persistent (25). Persistent HPV infection is a risk factor for developing CIN3 and cancer (26–29). Theoretically, this observation suggests that the positive predictive value of a sensitive HPV test should increase with age, because the ratio of true positives (positive tests among women with CIN3 and cancer) to false positives (positive tests among women with CIN2 or less severe pathology) should increase among older women. Although we observed this expected result among women with equivocal cytology in a prospective trial examining the clinical utility of HPV testing (30), we did not find an age-related improvement in predictive value in this analysis. The decline in sensitivity (true positives) among older women led to a loss of positive predictive value that was not compensated for by the dramatic decline in the overall percentage of older women who tested positive (data not shown).

An ideal screening program for cervical cancer would target women at greatest risk for close surveillance while sparing others the inconvenience, cost, and potential morbidity associated with annual testing and excessive treatment. Reallocation of resources in this manner requires a screening approach that detects prevalent disease with high sensitivity and also provides strong reassurance that interval cancers will not develop between screens. We conclude that simultaneous combined screening with Pap and HPV testing sensitively detects women with prevalent disease and that a negative HPV test provides reassurance

that cancer is unlikely to develop for several years. Therefore, among women with a suitable gynecologic history and negative Pap tests, HPV testing has immediate promise as an adjunct test indicating that the screening interval can be lengthened. Women with a positive HPV test and a negative Pap test remain at risk and should continue to participate in routine screening. Further evaluation of the efficacy of primary screening approaches with HPV testing, both alone and in combination with Pap testing, is the focus of ongoing randomized clinical trials.

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## NOTES

*Editor's note:* Dr. Attila Lorincz is the Chief Scientific Officer of Digene and holds Digene stock and stock options. Dr. Iwona Mielzynska-Lohnas is an employee of Digene and holds stock options. Dr. David Scott holds stock in Digene and Cytyc corporations.

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