Petignat P, Faltin D, Goffin F, Billieux MH, Stucki D, Sporri S, Vassilakos PAge-related performance of human papillomavirus testing used as an adjunct to cytology for cervical car…

ARTICLE *in* CANCER · JUNE 2005

Impact Factor: 4.89 · DOI: 10.1002/cncr.21060

CANCER
CYTOPATHOLOGY

Age-Related Performance of Human Papillomavirus Testing Used as an Adjunct to Cytology for Cervical Carcinoma Screening in a Population with a Low Incidence of Cervical Carcinoma

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Supported by a grant from the "Foundation Against Cancer and for Biological and Medical Research."

The authors thank all of the women and gynecologists who participated in this study. They also thank Mrs. Rosemary Sudan for editorial assistance.

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Received November 1, 2004; revision received January 19, 2005; accepted February 2, 2005.

BACKGROUND. High-risk human papillomavirus (HR-HPV) testing has been proposed as a replacement for cytology or as an adjunct to cytology for primary cervical carcinoma screening. The objective of this study was to assess the age-specific prevalence of HR-HPV infection and the correlation between HR-HPV status and cytologic diagnosis.

METHODS. The authors enrolled 7254 women receiving routine cytologic screening in a cross-sectional study that was conducted during 12 months. Cervical samples were collected using liquid-based cytology to perform both Papanicolaou smears and HR-HPV testing. Analyses were performed using age stratification, and the cytologic results were considered as the reference diagnosis for parameter analysis tests.

RESULTS. The overall rate of HR-HPV infection was 11.4% (95% confidence interval, 9–12%) and was higher in younger women compared with older women (age $<$ 30 years vs. $\geq$ 30 years; 16% vs. 8.5%, respectively; $P < 0.0001$). The overall rate of abnormal cytology was 3.2% and, similarly, was more prevalent in younger women (6.1% vs. 2.4%; $P < 0.0001$). The best balance between sensitivity and specificity for high-grade lesions or worse occurred predominantly in older age groups (age $\geq$ 50 years).

CONCLUSIONS. The prevalence of HR-HPV was age-dependent, with the strongest correlation between HR-HPV positivity and disease observed among older women, who potentially may derive the most benefit. ***Cancer (Cancer Cytopathol)* 2005;105:126–32.** © *2005 American Cancer Society.*

KEYWORDS: cervical carcinoma, liquid-based cytology, Papanicolaou smear, human papillomavirus screening.

Cervical carcinoma screening based only on conventional cytology has important limitations, with a significant number of missed diagnoses, even in countries in which screening programs are organized well.[1–4] Hence, the emergence of new technologies, such as liquid-based cytology and high-risk human papillomavirus (HR-HPV) testing, to obtain improved levels of prognostic information and diagnostic accuracy.[5,6]

In Switzerland, there still are no epidemiologic data available with which to evaluate the HPV infection rate and its association with cervical neoplasia. Currently, to our knowledge only regional studies of the cervical carcinoma incidence have been performed by national cancer registries and have shown a low incidence ranging between 5.7 per 100,000 women and 9.3 per 100,000 women.[7] There is no orga-

DOI 10.1002/cncr.21060
Published online 8 April 2005 in Wiley InterScience (www.interscience.wiley.com).

nized screening program, and only an opportunistic screening is practiced; national recommendations are similar to those proposed by the U.S. and include an annual cervical cytology screening and, after 3 consecutive negative results, extending the interval to every 2–3 years.

Because HR-HPV prevalence varies markedly, depending on the population and age groups studied, test parameters, such as sensitivity, specificity, and predictive values, for the presence of cervical dysplasia may vary accordingly.[8–11] Therefore, epidemiologic surveys using HPV testing should help to improve our understanding of this infection, determine the current uncertainty regarding the rate of decline in age-specific HPV prevalence in women ages 30–40 years, provide baseline data for future studies, and generate debate on the use of this test as a replacement for cytology or as an adjunct to cytology for primary cervical cancer screening. The objective of this study was to assess the age-related prevalence of HPV infection and its association with cervical dysplasia in a European population with a low incidence of cervical carcinoma.

MATERIALS AND METHODS

Study Group

Between September 2001 and August 2002, we recruited a cohort of 7366 women in the western region of Switzerland who had a routine cervical Papanicolaou examination. Study participants were between ages 13–96 years (mean age, 42.2 years). Liquid-based samples were obtained from a public hospital and from an office-based gynecologic practice. Participants attending for annual control had a cervical specimen taken for cytology and HR-HPV testing. No selection criteria were applied to the cohort. The study was approved by the local ethics committee (intercantonal ethics committee for health research of the cantons of Jura, Neuchâtel, and Fribourg) and women attending for routine annual gynecologic control were informed about the purpose of the study and gave their consent to use residual material from their samples for HPV testing.

Cytology Interpretation

Participants had a cervical specimen taken for cytology and HR-HPV DNA testing. Within 48 hours of collection, a thin-layer cervicovaginal slide was prepared using the robotic AutoCyte PREP system (AutoCyte Inc., Elon College, Elon, NC) according to the manufacturer's instructions. All slides initially were processed according to the routine screening procedure of the Institute of Pathology (Geneva). Suspicious slides were reviewed by a senior cytotechnician and were forwarded to the cytopathologist (P.V.) for diagnosis. We defined a negative Papanicolaou test as a test that was interpreted as normal or that indicated the presence of infection or reactive change. A positive test was defined as a test that indicated the presence of atypical squamous cells of undetermined significance (ASCUS) or worse ($\geq$ ASCUS). Cytologic diagnoses were classified according to the Bethesda nomenclature system and were interpreted without prior knowledge of the women's HPV status.

HPV Detection

After preparation of the AutoCytePrep slides, the remaining sample was used for HPV DNA detection on a routine basis using the Hybrid Capture II (HC-2) assay (Digene Diagnostics, Inc., Silver Spring, MD). After preparation of the thin-layer slides, the residual fluid from the sample was centrifuged at $\times$ 800 $\pm$ 15 *g* for 10 minutes $\pm$ 1 minute. The supernatant fluid was decanted, and the residual was removed by blotting the inverted tube on clean, absorbent paper. Specimen transport medium (STM; Digene) (200 μL) was added to each cervical cell pellet, and the tube was vortexed for 15 seconds to resuspend the pellet. Denaturation reagent of 100 μL was then added to produce single-stranded DNA that reacted with 13 full-length RNA probes recognizing HR-HPV types 16 (HR-HPV-16), HR-HPV-18, HR-HPV-31, HR-HPV-33, HR-HPV-35, HR-HPV-39, HR-HPV-45, HR-HPV-51, HR-HPV-52, HR-HPV-56, HR-HPV-58, HR-HPV-59, and HR-HPV-68; vortexed for 5 seconds; and incubated in a 65 °C $\pm$ 2 °C water bath for 90 minutes $\pm$ 5 minutes. Then, the denatured specimen was tested immediately using the HC-2 assay according to the manufacturer's instructions.

A test was considered positive for the presence of HR-HPV DNA if the relative light unit (RLU) obtained from the luminometer equaled or exceeded the mean of the 3 positive control values (cut-off value = 1.0 pg/mL). An RLU measurement $<$ 1.0 pg/mL indicated either the absence of the 13 HR-HPV types (HR-HPV-16, HR-HPV-18, HR-HPV-31, HR-HPV-33, HR-HPV-35, HR-HPV-39, HR-HPV-45, HR-HPV-51, HR-HPV-52, HR-HPV-56, HR-HPV-58, HR-HPV-59, and HR-HPV-68) or HPV-DNA levels below the threshold of detection.

Statistical Analysis

All patient characteristics, together with the results from both tests, were entered into a Microsoft Access data base for analysis. Analysis was performed first using the total population; then the analysis was stratified into 5-year and 10-year age groups; and, finally, the analysis was performed separately for women

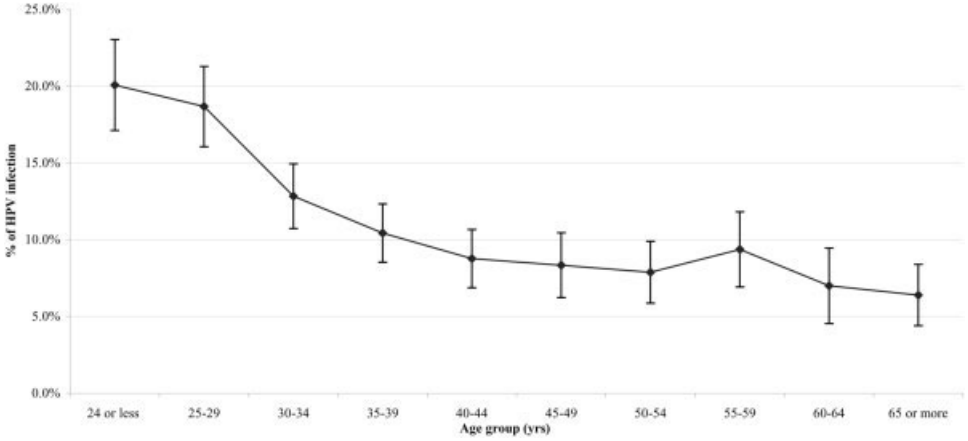

FIGURE 1. This chart illustrates the prevalence of human papillomavirus (HPV) infection by 5-year age groups. Note that data prints represent the percent and I bars represent the 95% confidence interval

TABLE 1
Cervical Cytology Diagnosis and High-Risk Human Papillomavirus Infection Status by Age

Age (yrs)	No. of patients	No. of patients (%)				
		Cytology+	ASCUS	LSIL	HSIL+	HPV+
≤ 29	1558	89 (5.7)	19 (1.2)	65 (4.2)	5 (0.3)	301 (19.3)
30–39	1962	72 (3.7)	29 (1.5)	36 (1.8)	7 (0.4)	228 (11.6)
40–49	1514	43 (2.8)	15 (1.0)	22 (1.5)	6 (0.4)	130 (8.6)
50–59	1228	18 (1.5)	10 (0.8)	6 (0.5)	2 (0.2)	105 (8.6)
≥ 60	992	11 (1.1)	7 (0.7)	2 (0.2)	2 (0.2)	66 (6.7)
Total	7254	233 (3.2)	80 (1.1)	131 (1.8)	22 (0.3)	830 (11.4)

Cytology+: positive cytology results; ASCUS: atypical squamous cells of undetermined significance or atypical glandular cells of undetermined significance; LSIL: low-grade squamous intraepithelial lesion; HSIL+: positive for high-grade squamous intraepithelial lesion or carcinoma; HPV+: positive for human papillomavirus.

age < 30 years and for women age ≥ 30 years. Sensitivities, specificities, positive predictive values, and negative predictive values were determined using cytology as the reference diagnostic. Data were analyzed as a contingency table with 2-sided *P* values using crude odds ratio with 95% confidence intervals (95% CIs) and odds ratios adjusted for age. The receiver operating curves (ROC) were performed and plotted as specificity (false-positive) on the x-axis versus sensitivity (true-positive) on the y-axis, with different ages used as cut-off points. Data analysis was performed with Stata software, version 8.0 (Stata Corporation, College Station, TX).

RESULTS

POPULATION AND AGE-RELATED HR-HPV PREVALENCE

Among the 7366 women who enrolled between September 2001 and August 2002, 112 women were excluded because of incomplete data. Therefore, the study included 7254 women who had a cytology and HPV-DNA test. Overall, 830 of 7254 women (11.4%; 95% CI, 10–12%) were positive for HR-HPV. The prevalence of HR-HPV by 5-year age groups is plotted in Figure 1. A peak prevalence of 20.1% (95% CI, 17–23%) was found among women age ≤ 24 years. This rate then declined to < 10% among women age > 40 years (Fig. 1). There was an inverse relation between age and HR-HPV status that was statistically significant (chi-square test = 116.6; $P < 0.0001$). A second, weaker peak of prevalence was observed among women ages 55–59 years, who had a rate of 9.4% (95% CI, 7–12%).

Population Prevalence of Abnormal Cytology

Abnormal cervical cytology was found in 233 women (3.2%; 95% CI, 2.8–3.6%); details are shown in Tables 1 and 2. The prevalence of abnormal findings decreased from 5.7% in the "younger" group to 1.1% in the group age > 60 years (chi-square test for trend of odds = 58.3; $P < 0.001$). The prevalence of ASCUS or atypical glandular cells of undetermined significance decreased from 1.2% in "younger" women to 0.7% in women age 60 years, with a peak of 1.5% in women ages 30–39 years (chi-square test for trend of odds =

TABLE 2
Crude and Age-Adjusted Odds Ratios and 95% Confidence Intervals for Cervical Sample Results by Cervical High-Risk Human Papillomavirus Status

Cytology	No. of patients	HPV+ (%)	OR (95% CI)	
			Crude	Age-adjusted
Normal	7021	677 (9.6)	1.0	1.0
ASCUS	80	28 (35.0)	5.0 (3.2–8.1)	5.1 (3.1–8.3)
LSIL	131	106 (80.9)	39.7 (24.9–63.3)	32.0 (19.6–52.3)
HSIL	20	17 (85.0)	53.1 (15.3–183.7)	66.8 (15.6–286.0)
Carcinoma	2	2 (100)	—	—

HPV+: positive for human papillomavirus; OR: odds ratio; 95% CI: 95% confidence interval; ASCUS: atypical squamous cells of undetermined significance or atypical glandular cells of undetermined significance; LSIL: low-grade squamous intraepithelial lesion; HSIL: high-grade squamous intraepithelial lesion.

2.17; $P = 0.14$). Similarly, the prevalence of low-grade squamous intraepithelial lesions (LSIL) decreased from 4.2% in "younger" women to 0.2% in women age $>$ 60 years (chi-square test for trend of odds = 75.6; $P < 0.0001$). The prevalence of high-grade squamous intraepithelial lesions and carcinoma ($\geq$ HSIL) was 0.3%, with no trend observed across all age categories; however, the sample size had only a 15% power to detect a trend from 0.05% to 0.02%.

Cytology and HPV Status

A strong association exists between HR-HPV and abnormal cytology, because women who had been HR-HPV-positive had a higher risk (risk ratio, 14.8; 95% CI, 11–19%) of having an abnormal smear compared with HR-HPV-negative women. Moreover, HR-HPV-positive women had a risk of 2.3% that they would have an HSIL or carcinoma diagnosis compared with a risk of 0.05% for HR-HPV-negative women (risk ratio, 49.0; 95% CI, 14–165). There also was a clear correlation between HPV positivity and the severity of the lesion; women classified with ASCUS, LSIL, HSIL, or carcinoma had infection rates of 35%, 81%, 85%, and 100%, respectively (Table 2).

Test Parameters of HC-2 by Defining Cytology as the Reference Diagnosis

By defining cytology as the "reference diagnosis" in calculating sensitivity, specificity, and predictive value of the HPV test, we found that the HC-2 predictive characteristics changed across age categories (Fig. 2). Table 3 shows the test parameters for the detection of positive cytology ($\geq$ ASCUS). The sensitivity for "younger" versus "older" women was 81% (95% CI, 71–88%) and 56% (95% CI, 48–64%), respectively, with a specificity of 84% (95% CI, 72–86%) and 92% (95% CI, 91–93%), respectively. Table 4 shows the test parameters for the detection of HSIL or carcinoma. The sensitivity for "younger" versus "older" women was 100% (95% CI, 48–100%) and 82% (95% CI, 57–96%), respectively, with a specificity of 81% (95% CI, 79–83%) and 91% (95% CI, 90–92%), respectively. By applying the ROC, the best balance between sensitivity and specificity for $\geq$ ASCUS lesions was found in women age $<$ 30 years. However, if only $\geq$ HSIL lesions were considered, older age groups (women age $\geq$ 50 years) seemed to derive more benefit from the test (Fig. 2).

DISCUSSION

HR-HPV prevalence analysis showed an overall infection rate relatively high, with 11.4% of women having an infection. Age was a strong demographic predictor of HR-HPV infection, in that it was highly prevalent in younger women, infecting about 1 in 5, with a subsequent rapid decline to a lower rate in women age $>$ 40 years. At around age 40 years, again, we observed a slight decline of prevalence until age 50 years, with a slight increase in the group ages 55–59 years. By restricting HR-HPV testing to women age $\geq$ 30 years, approximately 8% of women still were positive.

Previous studies performed in other high-incidence and low-incidence areas have shown marked geographic variation in HPV incidence.[1,5,12–15] For example, in Hong Kong and Costa Rica, the prevalence among women age $\geq$ 35 years ranged between 3% and 4%; in our population, the rate was approximately double.[9,10] The strength of our study is that we were able to determine the age-specific prevalence of HR-HPV at a community level by including patients of gynecologists in office-based gynecologic practice and in a public hospital under "real-life" screening conditions and in an unselected population, compared with many previous studies, which were performed in research settings. Second, to our knowledge this is one of the largest studies assessing the prevalence of HR-

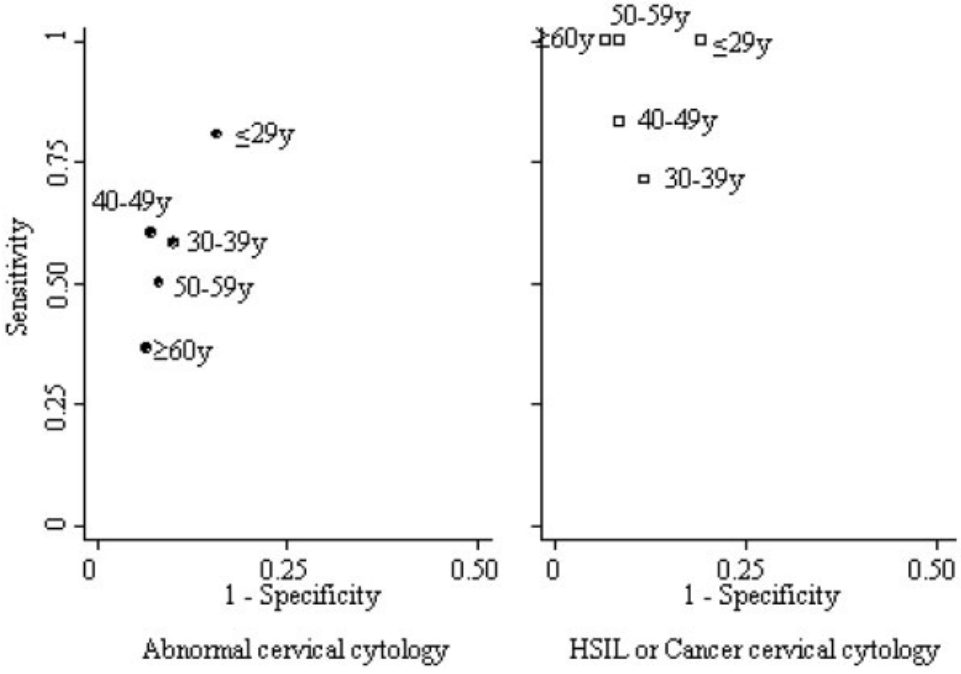

FIGURE 2. These plots indicate the sensitivity and specificity of the high-risk human papillomavirus test for predicting abnormal cervical cytology and high-grade squamous epithelial lesions (HSIL) or worse.

TABLE 3
High-Risk-Human Papillomavirus DNA Test Parameters for the Prediction of Abnormal Cervical Cytology

Age (yrs)	No. of patients	No. of patients (%)				Sensitivity	Specificity	PPV	NPV
		HPV−/cytology−	HPV−/cytology+	HPV+/cytology−	HPV+/cytology+				
≤ 29	1558	1240 (79.6)	229 (14.7)	17 (1.1)	72 (4.6)	80.9	84.4	23.9	98.7
30–39	1962	1704 (86.9)	186 (9.5)	30 (1.5)	42 (2.1)	58.3	90.2	18.4	98.3
40–49	1514	1367 (90.3)	104 (6.9)	17 (1.1)	26 (1.7)	60.5	92.9	20.0	98.8
50–59	1228	114 (90.7)	96 (7.8)	9 (0.7)	9 (0.7)	50.0	92.1	8.6	99.2
≥ 60	992	919 (92.6)	62 (6.3)	7 (0.7)	4 (0.4)	36.4	93.7	6.1	99.2
Total	7254	6344 (87.5)	677 (9.3)	80 (1.1)	153 (2.1)	65.7	90.4	18.4	98.8

HPV−: negative for human papillomavirus; cytology−: negative cytology results; cytology+: positive cytology results; HPV+: positive for human papillomavirus; PPV: positive predictive value; NPV: negative predictive value.

HPV infection across all age ranges, with a sample size $>$ 400 women in each 5-year age group. Third, it is a regional survey in an area with a low incidence of cervical carcinoma and brings forth essential information both for future studies and for discussion on screening based on HPV testing as a replacement for cytology or as an adjunct to cytology for primary cervical cancer screening.

By defining cytology as the "reference diagnosis" in calculating sensitivity, specificity, and predictive value of the HPV test, we observed that the performance of HR-HPV testing was influenced by age, with higher sensitivity and poor specificity for younger age groups and with lower sensitivity but improved specificity in older age groups. By applying ROC curves for the different age groups, we observed that the best balance between specificity and sensitivity for the detection of $\geq$ HSIL lesions can be obtained predominantly for women ages $>$ 50 years.

Younger women (age $<$ 30 years) also have an acceptable balance for specificity with a smaller decrease in sensitivity. In this age group, we found that women with $\geq$ HSIL were 100% HR-HPV positive. This means that the potential risk of missing abnormalities and the risk of a false-negative result with the HR-HPV test is very low in this population. However, the problem lies in the specificity in those age groups; because, even if the incidence of HSIL peaks among women in their late 20s and early 30s, cervical carcinoma is rare in women age $<$ 25 years.[12,16] There is now extensive supporting literature and widespread agreement that the vast majority of HPV infections are transient in

TABLE 4
High-Risk-Human Papillomavirus DNA Test Parameters for the Prediction of Cervical High-Grade Squamous Intraepithelial Lesion or Carcinoma

Age (yrs)	No. of patients	No. of patients (%)				Sensitivity	Specificity	PPV	NPV
		HPV−/cytology−	HPV−/cytology+	HPV+/cytology−	HPV+/cytology+				
≤ 29	1558	1257 (80.7)	0 (0.0)	296 (19.0)	5 (0.3)	100.0	80.9	1.7	100.0
30–39	1962	1732 (88.3)	2 (0.1)	223 (11.4)	5 (0.3)	71.4	88.6	2.2	99.88
40–49	1514	1383 (91.4)	1 (0.07)	125 (8.3)	5 (0.3)	83.3	91.7	3.9	99.93
50–59	1228	1123 (91.5)	0 (0.0)	103 (8.4)	2 (0.2)	100	91.6	1.9	100.0
≥ 60	992	926 (93.4)	0 (0.0)	64 (6.5)	2 (0.2)	100	93.5	3.0	99.6
Total	7254	6421 (88.5)	3 (0.04)	811 (11.2)	19 (0.3)	86.4	88.8	2.3	99.95

HPV−: negative for human papillomavirus; cytology−: negative cytology results; cytology+: positive cytology results; HPV+: positive for human papillomavirus; PPV: positive predictive value; NPV: negative predictive value.

young women, in that those infections are cleared by the immune system within a few months without any detectable lesion.[17–18] Therefore in the younger age groups, problems with specificity most likely have more clinical importance than sensitivity, because most lesions are acute or transient, and very few will progress to carcinoma. Therefore, the use of HR-HPV testing to improve or replace cytologic screening probably has limited usefulness in younger patients.[19–21]

A limitation that should be discussed is that the diagnostic endpoint used in the current study was the cytologic diagnosis and not the results of histology. Therefore, it is reasonable to assume that the obtained sensitivity may have been slightly lower and that HSIL lesions may have been identified by HPV testing but missed by cytology. Over the next 3 years, the follow-up will be completed, and the data collected will provide information on missed diagnoses. However, the current study was conducted under routine clinical conditions to evaluate the correlation between HR-HPV and cytologic diagnosis, and we considered that the histologic diagnosis was outside the scope of the current study.

Among women ages ≥ 30 years, a double-negative test (negative cytology and negative HR-HPV results) was observed in 80% of those between ages 30 years and 40 years and in > 90% of those age > 40 years. According to the recent publication of the interim guidance report for women who have double-negative results, this suggests that most of our population should not be rescreened before 3 years.[21] Again, according to the same report, 9.5% of younger women (ages 30–39 years) and 6–8% of older women in the current series will be concerned with rescreening (Table 3).

The issues that arise in this study that are important to determine in the debate are, first, that we found a very low prevalence of ≥ HSIL (0.3%) in our population. Second, large numbers of women (9.6%), whether young or old, were positive for HR-HPV without abnormal cytology but, due to their positive test results, will require diagnostic and follow-up measures, because that they are at the greatest risk of developing ≥ HSIL.[18,22] Finally, in Switzerland, cervical screening is not organized in a national program but is based only on opportunistic screening, mostly performed annually. Therefore, to recommend the use of either the HPV test as a replacement of cytology or as an adjunct to cytology outside an organized approach undoubtedly can be achieved only at the expense of an important amount of over screening.

In the current study, we found large differences in HPV prevalence among the different age groups, but mostly with no cytologic abnormality. The strongest correlation between HPV positivity and disease was found for older women (age ≥ 50 years), who potentially may derive the most benefit.

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