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# Performance of Cervical Screening a Decade Following HPV Vaccination: The Costa Rica Vaccine Trial

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## Abstract

**Background:** We investigated the impact of human papillomavirus (HPV) vaccination on the performance of cytology-based and HPV-based screening for detection of cervical precancer among women vaccinated as young adults and reaching screening age. **Methods:** A total of 4632 women aged 25-36 years from the Costa Rica HPV Vaccine Trial were included (2418 HPV-vaccinated as young adults and 2214 unvaccinated). We assessed the performance of cytology- and HPV-based cervical screening modalities in vaccinated and unvaccinated women to detect high-grade cervical precancers diagnosed over 4 years and the absolute risk of cumulative cervical precancers by screening results at entry. **Results:** We detected 95 cervical intraepithelial neoplasia grade 3 or worse (52 in unvaccinated and 43 in vaccinated women). HPV16/18/31/33/45 was predominant (69%) among unvaccinated participants, and HPV35/52/58/39/51/56/59/66/68 predominated (65%) among vaccinated participants. Sensitivity and specificity of cervical screening approaches were comparable between women vaccinated as young adults and unvaccinated women. Colposcopy referral rates were lower in the vaccinated group for HPV-based screening modalities, but the positive predictive value was comparable between the 2 groups. **Conclusions:** Among women approaching screening ages, vaccinated as young adults, and with a history of intensive screening, the expected reduction in the positive predictive value of HPV testing, associated with dropping prevalence of HPV-associated lesions, was not observed. This is likely due to the presence of high-grade lesions associated with nonvaccine HPV types, which may be less likely to progress to cancer.

Cervical cancer (CC) is the fourth most common cancer among women worldwide (1), is causally associated with persistent human papillomavirus (HPV) infection (2), and is preventable by vaccination against HPV and screening/treatment of precancers (3). Vaccines have been adopted into immunization programs in more than 100 countries worldwide (4).

CC screening approaches in countries with high vaccination coverage must be carefully considered as vaccinated cohorts attain screening age. Reductions in vaccine-preventable HPV types and associated lesions could diminish the positive predictive value (PPV) and other characteristics of screening strategies (5-8). Lengthening of screening intervals may be possible according to guidelines benchmarking against precancer risk (9). Data on performance of screening and triage methods are required to define programmatic approaches, which may differ in high- and low-resource settings.

There are limited data from HPV-vaccinated cohorts reaching screening ages, and current guidelines do not differentiate programmatic recommendations based on the existence of vaccination programs (10-12). Thus far, most analyses from clinical and population-based studies focused on changes in cytology reporting profiles, colposcopy workload, and clinical activities in vaccinated women (5,13-17) or cost-effectiveness using health decision models (18-20). A randomized trial in Australia in a population with substantial uptake of HPV vaccination demonstrated a statistically significantly higher detection rate of high-grade precancers in HPV-screened women compared with cytology-screened women (21).

To inform cervical screening guidelines for HPV-vaccinated populations, we analyzed data from the Costa Rica (CR) HPV Vaccine Trial (CVT) and report performance of cytology- and HPV-based screening methods for predicting high-grade precancer and absolute risk of cumulative precancers by screening results, comparing unvaccinated women with women vaccinated as young adults whose attained age ranged from 25 to 36 years.

## Methods

### Study Population and Procedure

CVT was a phase III trial evaluating the efficacy of the HPV16/18 vaccine (Cervarix, GSK, Belgium) against cervical HPV16/18 infection and related precancers (NCT00128661), as previously described (22,23). Between 2004 and 2005, 7466 women in CR aged 18 to 25 years were randomly assigned (1:1) to receive 3 doses of HPV16/18 vaccine (HPV-vaccinated arm) or hepatitis A vaccine (control arm) and were followed-up annually for 4 years. At enrollment and follow-up, sexually active women had cervical cells collected for liquid-based cytology and HPV testing. Cytology guided colposcopy referral. Women with minor cytological abnormalities (including low-grade squamous intraepithelial lesion, atypical squamous cells of undetermined significance with HPV positive [ASC-US/HPV+], or inadequate cytology) were followed-up every 6 months, and women with high-grade squamous intraepithelial lesion (HSIL) or repeated minor abnormalities had colposcopy, biopsy, and treatment as needed. After colposcopy and/or treatment, women were followed-up every 6 months.

After the initial 4-year follow-up, women had cross-over vaccination and the HPV-vaccinated arm was invited to a long-term follow-up observational study for 7 additional years. To replace controls, a new screening-only unvaccinated control group (UCG, n = 2836) was recruited from identical birth cohorts and geographical regions. At enrollment, UCG women were screened using cytology and HPV testing and followed strict colposcopic algorithms to increase comparability with CVT women, who were previously screened for 4 years. UCG women with negative screening tests or HPV-negative ASC-US were rescreened in 2 years. Women with cytologic HSIL had colposcopy or treatment as necessary, and those with minor cytological abnormalities or positive or insufficient HPV test had cotesting 6 months later. If both tests were negative, they reverted to biennial screening; otherwise, they attended colposcopy. Comparability between control groups regarding women's characteristics and future HPV acquisition risk has been reported (23).

Since year 7 after vaccination (HPV-vaccinated group) and after 1 round of intensive screening (UCG), namely since the baseline visit of this analysis, both groups had 2 liquid-based cytology tests at 2-year intervals. Women with minor abnormalities had 6-month follow-up with cotesting. If tests were negative, women returned to cytologic biennial screening. If cytology was abnormal, they had colposcopy; if only HPV was positive, they had a second 6-month follow-up; and, if positive, they had colposcopy, as did women with HSIL cytology. At the last screening visit 4 years after the baseline visit of this analysis (11 years after vaccination for vaccinated group), women had cotesting with cytology and HPV testing. Those with minor abnormalities or only HPV positive had a 6-month cotesting follow-up, and if positive in either test they had colposcopy; otherwise, they were exited. Figure 1 shows the screening and follow-up procedures involved in this analysis.

Histologically confirmed cervical intraepithelial neoplasia grade 2 or worse (CIN2+; including CIN2, CIN3, adenocarcinoma in situ, and cancer) were treated mainly with large loop excision of transformation zone. For women with cytologic abnormalities or worrisome virologic patterns, a review panel including epidemiologists, clinicians, and pathologists assessed virologic, cytologic, colposcopic, and histologic findings for clinical safety and return to usual care. In some cases, diagnostic large loop excision of transformation zone was performed (eg, persistent HPV16 infection with cytologic abnormalities not histologically confirmed). CIN2+ diagnoses during colposcopic follow-up until December 2019 were included. The study was approved by Instituto Costarricense de Investigación y Enseñanza en Nutrición y Salud (INCIENSA) institutional review board in CR and National Cancer Institute institutional review board in the United States; all participants signed informed consent.

### Clinical Diagnosis and HPV Testing

Cytology was reported using the Bethesda system (24) at a central laboratory in CR. During the blinded CVT portion, abnormal slides plus 10% random negatives were reread in the United States. Upgrades triggered colposcopic referral. Given good agreement (kappa = 0.68, 95% CI = 0.66 to 0.69), this process stopped during long-term follow-up observational study. Histology was initially interpreted by a CR pathologist (Haemotoxylin and Eosin, CIN classification), and a US pathologist reviewed slides blinded to the CR diagnoses. Discrepant results were reviewed by another US pathologist, and majority rule defined the final diagnosis; p16 immunostaining was not used.

Hybrid Capture 2 (HC2; Qiagen, MD, USA) was used for DNA detection of any of 13 oncogenic types (HPV16/18/31/33/35/39/45/51/52/56/58/59/68) for clinical management and ASC-US triage. HC2 also detects high viral loads of genetically related types (25). At year 11, this test was replaced by Aptima HPV (Hologic, CA, USA), which detects HPV E6/E7 mRNA from 13 HPV types (HPV16/18/31/33/35/39/45/51/52/56/58/59/68) and HPV66 (26).

During the blinded phase of CVT, HPV genotyping was performed using SPF10/DEIA/LiPA25 assay (version 1, Viroclinics-DDL, the Netherlands), detecting 14 oncogenic (HPV16/18/31/33/35/39/45/51/52/56/58/59/66, and HPV68/73) and 11 nononcogenic (HPV6/11/34/40/42/43/44/53/54/70/74) HPV types. HPV68/73 was treated as HPV68 for statistics because the 2 types cannot be discriminated. The test was replaced by the National Cancer Institute–developed, TypeSeq HPV method after demonstrating comparability with SPF10/DEIA/LiPA25 (27). TypeSeq is a next-generation sequencing-based assay using TypeSeq 3-PCR stage workflow and a custom Torrent Suite plugin. It provides a binary result (positive or negative) for 51 HPV types and a human DNA control.
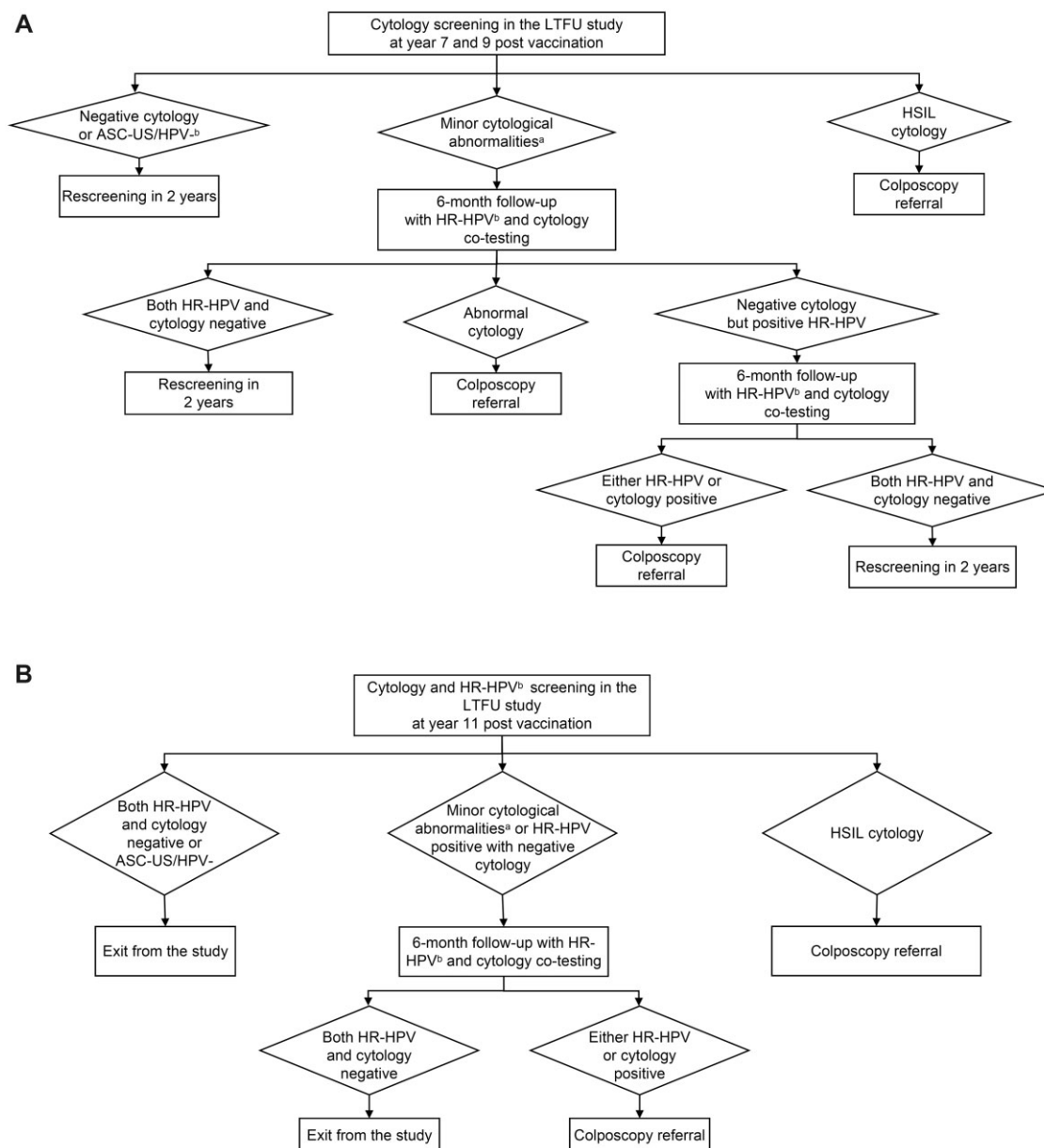
**Figure 1.** Flowchart of screening procedure in the long-term follow-up (LTFU) study. **A)** Flowchart of the screening procedure at year 7 and 9 post vaccination is shown. **B)** Flowchart of the screening procedure at year 11 post vaccination is shown. [a]Minor cytological abnormalities included low-grade squamous intraepithelial lesion, atypical squamous cells of undetermined significance with a positive high-risk human papillomavirus (HR-HPV) test (ASC-US/HPV+), or inadequate cytology. [b]Hybrid Capture 2 (Qiagen, MD, USA) was used for DNA detection of any of 13 oncogenic types (HPV16/18/31/33/35/39/45/51/52/56/58/59/68) for clinical management and ASC-US triage at year 7 and 9. At year 11, this test was replaced by Aptima HPV (Hologic, CA, USA), detecting HPV E6/E7 mRNA from 13 HPV types (HPV16/18/31/33/35/39/45/51/52/56/58/59/68) and HPV66. HSIL = high-grade squamous intraepithelial lesion.

## Analytical Cohorts

Our analytical population included 4632 women who attended the baseline screening visit (year 7 after vaccination or first visit after initial screening for UCG), were 25 years or older, and had at least 1 follow-up visit. Women with CIN2+ before baseline were excluded (Figure 2). A total of 2418 were vaccinated and 2214 were UCG. Analyses start at year 7 of the vaccinated cohort because 1) participants were aged 25 years to 36 years, within the targeted screening ages (172 women younger than 25 years were excluded); 2) the impact of vaccination is more pronounced as time passes after vaccination (14,28); and 3) intensive screening of the UCG had been completed and validation of comparability

of the groups performed. Follow-up time was stopped at diagnosis of CIN2+ or censored at the last follow-up.

## Statistical Analysis

We evaluated the performance of the cytology algorithm (with referral of ASC-US/HPV+ or more severe) and alternative approaches simulated using available data, separately for unvaccinated and vaccinated women, as follows: primary HPV screening methods, including 14 high-risk HPV (HR-HPV; HPV16/18/31/33/35/39/45/51/52/56/58/59/66/68) alone and 8-HPV typing (HPV16/18/31/33/35/45/52/58) alone; cotesting with HR-HPV and cytology; and triage methods
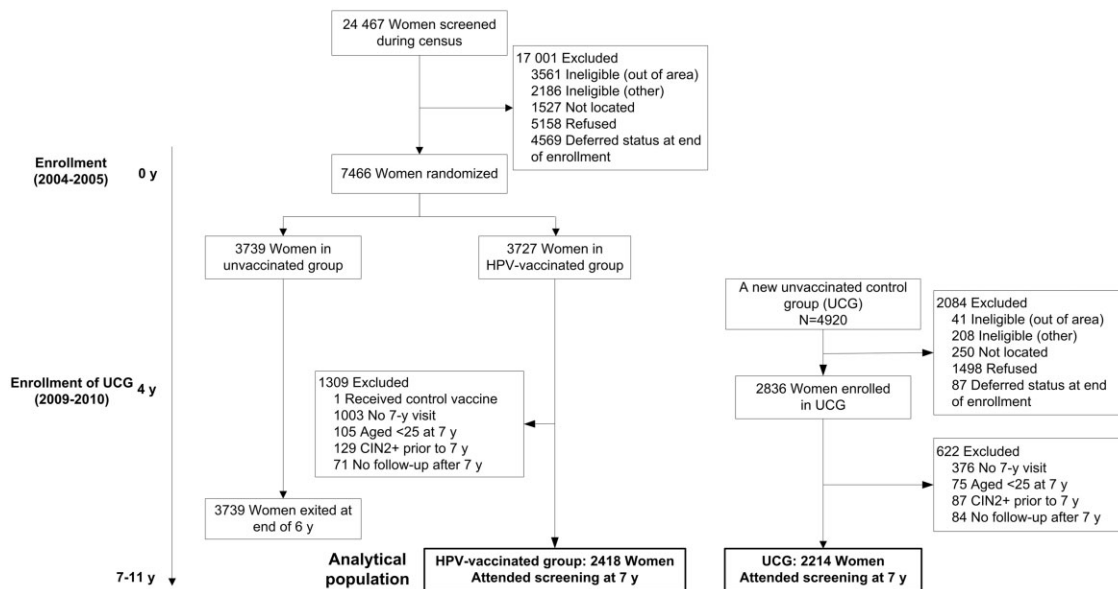
**Figure 2.** Selection of analytic cohorts. CIN2+ = cervical intraepithelial neoplasia grade 2 or worse; HPV = human papillomavirus; UCG = unvaccinated control group.

for HR-HPV–positive women: HR-HPV triaged with cytology (ASC-US+ threshold without knowledge of HPV status), with HPV16/18 and reflex cytology, and with 8-HPV typing and reflex cytology. We evaluated the HPV16/18/31/33/35/45/52/58 group as primary screening or triage for HR-HPV–positive women because these HPV types have high 3-year CIN3+ (including CIN3, adenocarcinoma in situ, and cancer) risk (29), whereas other HR-HPV types have lower risk despite high prevalence in some populations.

We assessed the performance of screening modalities at entry into the analytical cohort, including referral rate, sensitivity, specificity, PPV, and negative predictive value (NPV). The cumulative cervical precancer cases from year 7 to 11 were defined as the gold standard, with CIN3+ as primary and CIN2+ as secondary outcome. Women who always tested negative were considered negative. Mid-p corrected exact binomial 95% confidence intervals (CIs) of the above metrics were calculated. Ratios of the metrics and 95% confidence intervals between groups were reported by an asymptotic Wald method. Moreover, we performed sensitivity analysis using CIN3+ within 1 year after first colposcopy to assess the impact of repeated colposcopies on our assessments.

We also assessed cumulative risks of CIN2+/CIN3+ during follow-up by test results at entry and compared risks in the vaccinated group with those in the UCG. This statistic is calculated by dividing the number of outcomes in the strata by the total number of women in the strata. Ratios and 95% confidence intervals between groups were calculated by an asymptotic Wald method. Meanwhile, $\chi^2$ tests were used to compare HPV distributions among cumulative cervical precancers between groups. Statistical analyses were performed with SAS 9.4. All statistical tests were 2-sided, and P values less than .05 were considered statistically significant.

## Results

### Participant Characteristics

The median follow-up time was 4.3 years after entry in the analytical cohort (interquartile range = 3.7-4.9), and it was similar between the HPV-vaccinated group and the UCG

(Supplementary Table 1, available online). UCG women were similar with respect to age and lifetime number of sexual partners at entry compared with the vaccinated group but had more pregnancies, slightly lower reported oral contraceptive use, and a slightly lower rate of smoking. As expected, compared with the vaccinated group, the UCG had higher HR-HPV (23.0% vs 20.2%) and HPV16/18 positivity (6.2% vs 1.0%) at entry.

### Clinical Performance of Different Screening Modalities by Vaccination Status

When comparing each specific screening modality between the HPV-vaccinated and the UCG (Tables 1 and 2), somewhat higher sensitivities for detection of cumulative CIN3+ were observed in the vaccinated group for cytology alone (50.0% vs 40.8%; ratio = 1.22, 95% CI = 0.78 to 1.93) and consequently HR-HPV triaged with cytology (47.6% vs 40.8%, ratio = 1.17, 95% CI = 0.73 to 1.85), along with similar specificity. The PPVs and NPVs for all test combinations were comparable between the 2 groups. Referral rates were lower in vaccinated women when considering HPV-based modalities but not cytology-based ones.

The results for CIN3+ were generally comparable with those for CIN2+ (Supplementary Tables 2 and 3, available online) or restricting to the first year of colposcopy (Supplementary Tables 4 and 5, available online) or restricting to women aged 30 years and older (data not shown). The performance of the screening modalities in the vaccinated group when restricted to women who were HPV negative at vaccination (Supplementary Tables 6 and 7, available online) was similar to that of the vaccinated group including both HPV-positive and HPV-negative women (Tables 1 and 2).

### Cumulative Risk of Cervical Precancers by Vaccination Status and Screening Results

Cumulative risk of cervical precancers over the 4 years including repeated colposcopic evaluations was higher in the UCG

**Table 1.** Comparison of screening performance across groups (HPV-vaccinated group vs UCG) using primary screening tests for CIN3+ detection over 4 years

| Primary screening method | Group | Total screened, No. | CIN3+ detection, No. (%) | Sensitivity, % (95% CI) | Specificity, % (95% CI) | PPV, % (95% CI) | NPV, % (95% CI) | Referral rate, % (95% CI) |
|---|---|---|---|---|---|---|---|---|
| Cytology[a] | UCG | 2117 | 20 (0.9) | 40.8 (27.8 to 54.9) | 91.9 (90.7 to 93.0) | 10.7 (6.8 to 15.8) | 98.5 (97.9 to 99.0) | 8.8 (7.7 to 10.1) |
| | HPV vaccinated | 2320 | 21 (0.9) | 50.0 (35.1 to 64.9) | 92.4 (91.2 to 93.4) | 10.8 (7.0 to 15.7) | 99.0 (98.5 to 99.4) | 8.4 (7.3 to 9.6) |
| Ratio compared with UCG | | | | 1.22 (0.78 to 1.93) | 1.00 (0.99 to 1.02) | 1.01 (0.56 to 1.80) | 1.01 (1.00 to 1.01) | 0.95 (0.79 to 1.15) |
| HR-HPV[b] | UCG | 2213 | 38 (1.7) | 73.1 (59.9 to 83.8) | 78.2 (76.4 to 79.8) | 7.5 (5.4 to 10.0) | 99.2 (98.7 to 99.5) | 23.0 (21.3 to 24.8) |
| | HPV vaccinated | 2413 | 32 (1.3) | 74.4 (59.9 to 85.7) | 80.8 (79.2 to 82.3) | 6.6 (4.6 to 9.0) | 99.4 (99.0 to 99.7) | 20.2 (18.6 to 21.8) |
| Ratio compared with UCG | | | | 1.02 (0.80 to 1.30) | 1.03 (1.00 to 1.06) | 0.88 (0.56 to 1.39) | 1.00 (1.00 to 1.01) | 0.88 (0.78 to 0.98) |
| 8-HPV genotyping[c] | UCG | 2213 | 31 (1.4) | 59.6 (45.9 to 72.3) | 85.7 (84.1 to 87.1) | 9.1 (6.4 to 12.5) | 98.9 (98.3 to 99.3) | 15.4 (14.0 to 17.0) |
| | HPV vaccinated | 2413 | 26 (1.1) | 60.5 (45.4 to 74.2) | 91.2 (90.0 to 92.3) | 11.1 (7.5 to 15.6) | 99.2 (98.8 to 99.5) | 9.7 (8.6 to 10.9) |
| Ratio compared with UCG | | | | 1.01 (0.73 to 1.41) | 1.07 (1.04 to 1.09) | 1.22 (0.75 to 2.00) | 1.00 (1.00 to 1.01) | 0.63 (0.54 to 0.74) |
| Cotesting with HR-HPV[b] and cytology (ASC-US+) | UCG | 2116 | 39 (1.8) | 79.6 (66.6 to 89.1) | 73.1 (71.1 to 75.0) | 6.6 (4.8 to 8.8) | 99.3 (98.8 to 99.7) | 28.1 (26.2 to 30.1) |
| | HPV vaccinated | 2319 | 33 (1.4) | 78.6 (64.3 to 89.0) | 75.5 (73.6 to 77.2) | 5.6 (3.9 to 7.7) | 99.5 (99.0 to 99.7) | 25.5 (23.8 to 27.3) |
| Ratio compared with UCG | | | | 0.99 (0.80 to 1.22) | 1.03 (1.00 to 1.07) | 0.85 (0.54 to 1.33) | 1.00 (1.00 to 1.01) | 0.91 (0.82 to 1.00) |

[a]ASC-US+ = atypical squamous cells of undetermined significance or worse; CI = confidence interval; CIN3+ = cervical intraepithelial neoplasia grade 3 or worse; HC2 = Hybrid Capture 2; HR-HPV = high-risk human papillomavirus; NPV = negative predictive value; PPV = positive predictive value; UCG = unvaccinated control group. Cytology screening algorithm refers to cytology plus HC2 triage of ASC-US.
[b]HR-HPV includes all 14 oncogenic HPV types: HPV16/18/31/33/35/39/45/51/52/56/58/59/66/68.
[c]8-HPV genotyping refers to HPV16/18/31/33/35/45/52/58.

**Table 2.** Comparison of screening performance across groups (HPV-vaccinated group vs UCG) using screening algorithms with triage for HR-HPV[a] positive women for CIN3+ detection over 4 years

| Screening method (Primary test/ triage test) | Group | Total screened, No. | CIN3+ detection, No. (%) | Sensitivity, % (95% CI) | Specificity, % (95% CI) | PPV, % (95% CI) | NPV, % (95% CI) | Referral rate, % (95% CI) |
|---|---|---|---|---|---|---|---|---|
| HR-HPV[a]/ cytology (ASC-US+) | UCG | 2116 | 20 (0.9) | 40.8 (27.8 to 54.9) | 93.5 (92.4 to 94.5) | 13.0 (8.3 to 19.0) | 98.5 (97.9 to 99.0) | 7.3 (6.2 to 8.4) |
| | HPV vaccinated | 2319 | 20 (0.9) | 47.6 (32.9 to 62.6) | 94.0 (93.0 to 94.9) | 12.8 (8.2 to 18.8) | 99.0 (98.5 to 99.3) | 6.7 (5.8 to 7.8) |
| Ratio compared with UCG | | | | 1.17 (0.73 to 1.85) | 1.01 (0.99 to 1.02) | 0.99 (0.55 to 1.76) | 1.00 (1.00 to 1.01) | 0.92 (0.75 to 1.15) |
| HR-HPV[a]/ HPV16/18 and reflex cytology (ASC-US+) | UCG | 2116 | 24 (1.1) | 49.0 (35.2 to 62.8) | 89.3 (87.9 to 90.6) | 9.8 (6.5 to 14.0) | 98.7 (98.1 to 99.1) | 11.6 (10.3 to 13.0) |
| | HPV vaccinated | 2319 | 23 (1.0) | 54.8 (39.6 to 69.2) | 93.5 (92.4 to 94.4) | 13.4 (8.9 to 19.1) | 99.1 (98.6 to 99.5) | 7.4 (6.4 to 8.5) |
| Ratio compared with UCG | | | | 1.12 (0.75 to 1.66) | 1.05 (1.03 to 1.07) | 1.37 (0.80 to 2.34) | 1.00 (1.00 to 1.01) | 0.64 (0.53 to 0.77) |
| HR-HPV[a]/ 8-HPV genotyping[b] and reflex cytology (ASC-US+) | UCG | 2116 | 34 (1.6) | 69.4 (55.5 to 81.0) | 83.4 (81.7 to 84.9) | 9.0 (6.4 to 12.2) | 99.1 (98.6 to 99.5) | 17.8 (16.2 to 19.5) |
| | HPV vaccinated | 2319 | 31 (1.3) | 73.8 (59.0 to 85.4) | 87.9 (86.5 to 89.2) | 10.1 (7.1 to 13.9) | 99.5 (99.1 to 99.7) | 13.2 (11.9 to 14.7) |
| Ratio compared with UCG | | | | 1.06 (0.82 to 1.38) | 1.05 (1.03 to 1.08) | 1.12 (0.70 to 1.78) | 1.00 (1.00 to 1.01) | 0.74 (0.65 to 0.85) |

[a]8-HPV genotyping refers to HPV16/18/31/33/35/45/52/58. ASC-US+ = atypical squamous cells of undetermined significance or worse; CI = confidence interval; CIN3+ = cervical intraepithelial neoplasia grade 3 or worse; HPV = human papillomavirus; HR-HPV = high-risk human papillomavirus; NPV = negative predictive value; PPV = positive predictive value; UCG = unvaccinated control group. HR-HPV includes all 14 oncogenic HPV types: HPV16/18/31/33/35/39/45/51/52/56/58/59/66/68.
[b]8-HPV genotyping refers to HPV16/18/31/33/35/45/52/58.

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**Table 3.** HPV type distribution in CIN2 and CIN3+ diagnosed over 4 years by vaccination status[a]

| HPV genotypes | CIN2 (n = 66) | | CIN3+ (n = 95) | |
|---|---|---|---|---|
| | UCG No. (%) | HPV-vaccinated group No. (%) | UCG No. (%) | HPV-vaccinated group No. (%) |
| HPV16/18 | 18 (38.3) | 1 (5.3) | 22 (42.3) | 4 (9.3) |
| HPV31/33/45 | 12 (25.5) | 1 (5.3) | 14 (26.9) | 9 (20.9) |
| HPV35/52/58 | 9 (19.1) | 7 (36.8) | 11 (21.2) | 18 (41.9) |
| HPV39/51/56/59/66/68 | 7 (14.9) | 5 (26.3) | 3 (5.8) | 10 (23.3) |
| Nononcogenic HPV | 0 (0.0) | 2 (10.5) | 1 (1.9) | 1 (2.3) |
| HPV negative/unknown | 1 (2.1) | 3 (15.8) | 1 (1.9) | 1 (2.3) |

[a]CIN2 = cervical intraepithelial neoplasia grade 2; CIN3+ = cervical intraepithelial neoplasia grade 3 or worse; HPV = human papillomavirus; UCG = unvaccinated control group.

than in the vaccinated group (CIN3+: 2.3% vs 1.8%, $P = .17$; CIN2+: 4.5% vs 2.6%, $P < .001$). When restricting to the first year of colposcopy, these estimates decreased by approximately one-third in the UCG and by one-fourth in the vaccinated group.

For the 95 CIN3+, HPV16/18/31/33/45 were predominant (69.2%) among the UCG cases, whereas HPV35/52/58/39/51/56/59/66/68 predominated (65.2%) among the vaccinated participants (Table 3). A similar pattern was observed in the 66 CIN2. All lesions associated with HPV16/18 in the vaccinated group occurred in women HPV16/18 positive before vaccination.

Compared with the UCG, vaccinated women with negative cytology, negative HR-HPV, or both cytology and HR-HPV negative at entry had a statistically non-significant approximately 20% to 30% decreased risk of cumulative CIN3+ (Table 4) and at least 50% decreased risk of cumulative CIN2+ (Supplementary Table 8, available online). Comparable decreased risks of cumulative CIN3+ and CIN2+ in vaccinated women were observed for women with only HR-HPV positive or with only non-HPV16/18 positive. There were no reductions in cumulative risks among women with cytological HSIL+, or HPV16/18 or non-HPV16/18 plus ASC-US+. These patterns were similar for diagnoses within 1 year of colposcopy, with lower incidences (Supplementary Table 9, available online). Cumulative risk of CIN3+ among vaccinated women who were HPV negative at vaccination (Supplementary Table 10, available online) was comparable with that in the entire group including HPV-positive and -negative participants. The patterns were again comparable for women 30 years and older (data not shown).

## Discussion

We modeled performance of cytology- and HPV-based screening modalities over 4 years. Among previously screened young women, sensitivity and specificity of screening modalities were comparable between vaccinated and unvaccinated women. Interestingly, the PPV for detection of CIN3+ was similar in the 2 groups for all screening approaches, including those where triage methods were used. However, HPV types in lesions were very different, with predominance of vaccine and cross-protected types in the unvaccinated and of other types in the vaccinated. Nonvaccine HPV types are less frequent in CC, and approximately 50% of precancers are caused by non-16/18 HPV types compared with approximately 30% of cancers (30). Thus, in vaccinated women, a positive screening test result, despite predicting histologically confirmed CIN3+, may be associated with lower cancer risk than in unvaccinated women, given different progression risks associated with different HPV types. This has implications for screening vaccinated cohorts because

numbers of lesions currently considered cancer precursors may not be reduced as expected. Additional natural history studies of nonvaccine HPV types and associated lesions are required, despite design difficulties. NPV was similar, but vaccinated women had lower colposcopy referral using HPV-based screening approaches due to lower detection of HPV infections.

As previously observed in unvaccinated women (31-33), cytology was less sensitive than HPV for precancer detection and had limited value as an addition to HPV. We observed somewhat higher sensitivity of cytology in vaccinated compared with unvaccinated women, but sexual behavior, size of lesions, and virology did not appear to explain it (data not shown). Overall, using HC2, the HPV positivity of ASC-US was 52%, consistent with cytology with proper sensitivity and specificity balance.

A global meta-analysis reported the sensitivity of HPV testing and cytology for CIN3+ detection was greater than 90% and greater than 70% (31), respectively, but reported sensitivity of cytology from Latin American laboratories has been lower (34). Sensitivity of HPV testing in our study was lower than expected, possibly resulting from the extensive management of abnormalities and detection of cumulative precancers. However, performance was similar when restricting to cases detected during the first year of colposcopy. Also, both groups had been extensively screened, and many of the HPV infections are likely incident, possibly affecting test performance. Furthermore, genotyping was conducted with a more sensitive test than those in clinical use, possibly influencing modalities including genotyping. Nevertheless, the lower sensitivity observed should not have an impact on the comparison of clinical performance of each screening modality between the unvaccinated and the vaccinated groups, which was the main objective of our study, and no impact on the ranking of metrics among screening modalities.

Part of the explanation of the similar PPV in 2 groups is probably that women were vaccinated between ages 18 and 25 years, emulating catch-up, and most vaccinated women (82%) were sexually active at vaccination, with 23.0% of HR-HPV positivity, 6.2% of HPV16/18 infection and thus higher risk of precancer than cohorts of girls vaccinated younger. Some studies have demonstrated that PPV of cytology decreases among women vaccinated at younger ages (5,7,15).

Restricting to the 8 most carcinogenic HPV types (8-HPV genotyping) for primary screening and 3 triage methods using cytology alone or combined with HPV16/18 or 8-HPV genotyping for HPV-positive women yielded increased specificity and a decreased colposcopy referral compared with HR-HPV testing in vaccinated and unvaccinated women. However, as expected, 8-

**Table 4.** Cumulative risks of CIN3+ over 4 years by screening results and vaccination status[a]

| Screening results | UCG | | HPV-vaccinated group | | Ratio compared with UCG (95% CI) |
|---|---|---|---|---|---|
| | No. of CIN3+/No. of women | % (95% CI) | No. of CIN3+/No. of women | % (95% CI) | |
| Negative cytology | 26/1857 | 1.4 (0.9 to 2.0) | 21/2043 | 1.0 (0.7 to 1.5) | 0.73 (0.41 to 1.30) |
| ASC-US/HC2- | 3/73 | 4.1 (1.1 to 10.8) | 0/82 | 0.0 (0.0 to 3.6) | 0.00 (NA) |
| ASC-US/HC2+ | 8/84 | 9.5 (4.5 to 17.3) | 7/85 | 8.2 (3.7 to 15.6) | 0.86 (0.33 to 2.28) |
| LSIL | 8/90 | 8.9 (4.2 to 16.2) | 9/98 | 9.2 (4.6 to 16.2) | 1.03 (0.42 to 2.56) |
| HSIL+ | 4/13 | 30.8 (10.6 to 58.7) | 5/12 | 41.7 (17.2 to 69.8) | 1.35 (0.47 to 3.89) |
| HR-HPV negative | 14/1703 | 0.8 (0.5 to 1.3) | 11/1926 | 0.6 (0.3 to 1.0) | 0.69 (0.32 to 1.53) |
| HR-HPV negative and ASC-US+ | 3/105 | 2.9 (0.7 to 7.6) | 1/121 | 0.8 (0.0 to 4.0) | 0.29 (0.03 to 2.74) |
| Both HR-HPV and cytology negative | 10/1521 | 0.7 (0.3 to 1.2) | 9/1727 | 0.5 (0.3 to 1.0) | 0.79 (0.32 to 1.95) |
| HR-HPV positive and ASC-US+ | 20/154 | 13.0 (8.3 to 19.0) | 20/156 | 12.8 (8.2 to 18.8) | 0.99 (0.55 to 1.76) |
| HR-HPV positive and cytology negative | 16/336 | 4.8 (2.8 to 7.5) | 12/315 | 3.8 (2.1 to 6.4) | 0.80 (0.38 to 1.66) |
| HPV16/18 positive | 16/138 | 11.6 (7.0 to 17.8) | 4/24 | 16.7 (5.5 to 35.5) | 1.44 (0.53 to 3.93) |
| Non-HPV16/18 HR-HPV positive and ASC-US+ | 9/110 | 8.2 (4.1 to 14.5) | 19/149 | 12.8 (8.1 to 18.9) | 1.56 (0.73 to 3.31) |
| Non-HPV16/18 HR-HPV positive and cytology negative | 12/245 | 4.9 (2.7 to 8.2) | 9/299 | 3.0 (1.5 to 5.5) | 0.61 (0.26 to 1.43) |

[a]ASC-US+ = atypical squamous cells of undetermined significance or worse; CI = confidence interval; CIN3+ = cervical intraepithelial neoplasia grade 3 or worse; HC2 = Hybrid Capture 2; HPV = human papillomavirus; HR-HPV = high-risk human papillomavirus; HSIL+ = high-grade squamous intraepithelial lesion or worse; LSIL = low-grade squamous intraepithelial lesion; NA = not applicable; UCG = unvaccinated control group.

HPV genotyping had lower sensitivity compared with HR-HPV testing because it misses precancers by other HPV types.

Triaging using 8-HPV genotyping and reflex cytology had better sensitivity than other approaches, with the expected loss in specificity and higher referrals. Yet, HPV genotyping needs reassessment among girls vaccinated before sexual debut attaining screening age, where reductions in infections and precancers are likely more pronounced (35-37). In fact, in our study, all HPV16/18-associated CIN3s in the vaccinated cohort were present at vaccination. More sensitive and specific biomarkers for triage, such as p16/Ki-67 dual staining and host and viral methylation, among others, are needed (21,38-43). Artificial intelligence algorithms using cervical images offer promise for triage and possibly for primary screening (44).

Cumulative risk of CIN3+ among vaccinated women was approximately 20% to 30% lower compared with unvaccinated women following negative cytology, negative HR-HPV, or both cytology and HR-HPV negative. However, absolute cumulative risk of CIN3+ appears relatively high, likely because our participants were at peak ages of CIN3 incidence (45) and some underwent colposcopic follow-up for years after initial referral. Due to this possible overestimate, we could not assess the impact of extending screening intervals for screen-negative women (9). Interestingly, cumulative risk estimates were comparable restricting to HPV-negative women at vaccination.

The main strengths of this study include comprehensive evaluation of screening modalities in a relatively large cohort of vaccinated and unvaccinated women reaching screening ages and a reliable gold standard with expert-reviewed diagnoses. However, groups were not randomly assigned and had a different screening history that we attempted to make equal by intensively screening the UCG (23). In addition, colposcopic referral was triggered during initial follow-up by cytology and not HPV test, possibly introducing verification bias. However, at the last visit, women were also referred based on HPV results, allowing capture of previously missed cases in women with only HR-HPV positive, to reduce the possibility of bias (46,47). Some verification bias may remain if probability of colposcopic referral is higher for women with abnormal cytology compared with HPV.

Our findings concur with recommendations favoring HPV testing for primary screening among women vaccinated as young adults. More research is needed to inform screening guidelines in HPV-vaccinated cohorts, especially among those vaccinated as young girls, because differences in baseline cytologic abnormalities, vaccination coverage, vaccination age, vaccine types, and sexual behaviors will affect performance of screening methods. The finding of limited impact of vaccination on the PPV of screening warrants further exploration in other datasets and can provide important clues to define research priorities and for design of future screening programs.

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## Data Availability

Outside collaborators can apply to access our protocols and data from the blinded phase of the Costa Rica Vaccine Trial (CVT, NCT00128661) for research purposes in accordance with institutional review boards on reasonable request to the Working Group of CVT. Data for the long term follow-up phase are not yet available. A trial summary, current publications, and contact information are available online at https://dceg.cancer.gov/research/who-we-study/cohorts/costa-rica-vaccine-trial.

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