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# Effect of Screening With Primary Cervical HPV Testing vs Cytology Testing on High-grade Cervical Intraepithelial Neoplasia at 48 Months

## The HPV FOCAL Randomized Clinical Trial

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**IMPORTANCE** There is limited information about the relative effectiveness of cervical cancer screening with primary human papillomavirus (HPV) testing alone compared with cytology in North American populations.

**OBJECTIVE** To evaluate histologically confirmed cumulative incident cervical intraepithelial neoplasia (CIN) grade 3 or worse (CIN3+) detected up to and including 48 months by primary HPV testing alone (intervention) or liquid-based cytology (control).

**DESIGN, SETTING, AND PARTICIPANTS** Randomized clinical trial conducted in an organized Cervical Cancer Screening Program in Canada. Participants were recruited through 224 collaborating clinicians from January 2008 to May 2012, with follow-up through December 2016. Women aged 25 to 65 years with no history of CIN2+ in the past 5 years, no history of invasive cervical cancer, or no history of hysterectomy; who have not received a Papanicolaou test within the past 12 months; and who were not receiving immunosuppressive therapy were eligible.

**INTERVENTIONS** A total of 19 009 women were randomized to the intervention (n = 9552) and control (n = 9457) groups. Women in the intervention group received HPV testing; those whose results were negative returned at 48 months. Women in the control group received liquid-based cytology (LBC) testing; those whose results were negative returned at 24 months for LBC. Women in the control group who were negative at 24 months returned at 48 months. At 48-month exit, both groups received HPV and LBC co-testing.

**MAIN OUTCOMES AND MEASURES** The primary outcome was the cumulative incidence of CIN3+ 48 months following randomization. The cumulative incidence of CIN2+ was a secondary outcome.

**RESULTS** Among 19 009 women who were randomized (mean age, 45 years [10th-90th percentile, 30-59]), 16 374 (8296 [86.9%] in the intervention group and 8078 [85.4%] in the control group) completed the study. At 48 months, significantly fewer CIN3+ and CIN2+ were detected in the intervention vs control group.

| | All Participants | | | Baseline Negative Screen | |
|---|---|---|---|---|---|
| | Incidence Rate/1000 (95% CI) at 48 mo | | Risk Ratio (95% CI) | Incidence Rate/1000 (95% CI) at 48 mo | Risk Ratio (95% CI) |
| | Intervention Group | Control Group | | | |
| CIN3+ | 2.3 (1.5-3.5) | 5.5 (4.2-7.2) | 0.42 (0.25-0.69) | 1.4 (0.8-2.4) | 0.25 (0.13-0.48) |
| CIN2+ | 5.0 (3.8-6.7) | 10.6 (8.7-12.9) | 0.47 (0.34-0.67) | | |

**CONCLUSIONS AND RELEVANCE** Among women undergoing cervical cancer screening, the use of primary HPV testing compared with cytology testing resulted in a significantly lower likelihood of CIN3+ at 48 months. Further research is needed to understand long-term clinical outcomes as well as cost-effectiveness.

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Cervical cancer screening with cytology is one of the most widely used cancer control interventions in high-income settings, and programs have decreased cervical cancer morbidity and mortality where appropriately deployed.[1] Despite this widespread use, it was estimated that 12 820 women in the United States would develop and approximately 4210 would die of cervical cancer in 2017, confirming a continued need to improve cervical cancer prevention.[2]

Approximately 99.7% of all cervical cancers are associated with a persistent cervical infection with an oncogenic human papillomavirus (HPV) genotype preceding the invasive tumor.[3] Although HPV vaccination holds potential as an effective cancer control strategy, given current vaccine uptake rates and costs, secondary prevention through screening will need to continue in the coming decades[4,5] and advances in improving screening remain a key priority for women's health.

For 20 years, cervical cancer screening using HPV testing has been evaluated in a variety of settings.[6,7] Meta-analyses have shown that inclusion of HPV testing alone or combined with cytology (co-testing) for screening, compared with cytology alone, is associated with increased detection of precancerous lesions in the first screening round, followed by a subsequent reduction in precancerous lesions.[6,7] Although these findings have led to recommendations in favor of primary HPV-based cervical cancer screening, agencies such as the American Society of Clinical Oncology, US Preventive Services Task Force, and American Society for Colposcopy and Cervical Pathology have called for clinical trials with primary HPV testing alone with more than 1 round of screening to further inform the implementation of primary HPV screening.[5,8-10]

This article reports the 48-month exit round results of the Human Papillomavirus For Cervical Cancer screening trial (HPV FOCAL), a publicly funded Canadian trial designed to compare the effect of primary HPV testing alone with liquid-based cytology (LBC) screening for the prevention of cervical intraepithelial neoplasia (CIN) grade 3 or worse (CIN3+) in the context of an organized screening program.

### Key Points

**Question** Does cervical cancer screening using primary cervical human papillomavirus (HPV) testing compared with cytology result in a lower likelihood of cervical intraepithelial neoplasia grade 3 or worse (CIN3+) at 48 months?

**Findings** In this randomized clinical trial that included 19 009 women, screening with primary HPV testing resulted in significantly lower likelihood of CIN3+ at 48 months compared with cytology (2.3/1000 vs 5.5/1000).

**Meaning** HPV-based screening resulted in lower likelihood of CIN3+ than cytology after 48 months, but further research is needed to understand long-term clinical outcomes as well as cost-effectiveness.

## Methods

The primary objective of this study was to evaluate primary HPV testing for cervical cancer screening in an organized program setting. Ethics approval was obtained from the University of British Columbia Clinical Research Ethics Board (H06-04032) and written consent was obtained from all participants. The full trial protocol and statistical analysis plan are available in Supplement 1.

### Participants

Inclusion criteria were women in British Columbia, Canada, with a personal health number, aged 25 to 65 years who had not had a Papanicolaou test in the previous 12 months, were not pregnant, were not HIV positive or receiving immunosuppressive therapy, and had no history of CIN2+ in the past 5 years; did not have invasive cervical cancer; or did not have total hysterectomy. Women who met inclusion criteria and were patients of 224 collaborating clinicians in Metro Vancouver and Greater Victoria were invited to participate.

### Randomization

Women were randomly assigned 1:1:1 to 1 of 3 (intervention, control, or safety) groups between January 2008 and December 31, 2010. Starting January 1, 2011, women were assigned 1:1 to the intervention or control when the safety group was closed.[11-14] Women and clinicians were blinded to group assignment until 24 months or if the baseline screen results were positive and required follow-up. The primary analysis for this study focuses on the intervention and control groups.

### Interventions

Participants randomized to HPV testing alone (intervention group) with negative test results were recalled at 48 months for exit with HPV and LBC testing. Participants randomized to LBC testing (control group) with negative test results were asked to return at 24 months for repeat testing with LBC in accordance with the cervical cancer screening guidelines in British Columbia. If LBC results were negative at this 24-month screen, participants were asked to return at 48 months for exit with HPV and LBC testing.

#### Intervention Group

Primary HPV testing was followed by reflex LBC in women with positive HPV test results. At baseline, if HPV positive and LBC negative, women were recalled in 12 months for HPV and LBC testing. At 12 months, if women were either HPV or LBC positive (≥atypical squamous cells of undetermined significance [ASCUS]), they were referred for colposcopy. If both HPV and LBC negative at 12 months, they were recommended for exit screen at 48 months. If the baseline reflex LBC result was greater than or equal to ASCUS, they were referred for immediate colposcopy and management.

#### Control Group

Primary LBC testing was followed by reflex HPV testing for women with ASCUS. If ASCUS and HPV positive at baseline, women were referred for immediate colposcopy. Women

with ASCUS and HPV-negative baseline results were recalled for LBC again at 12 months and were referred for colposcopy if their LBC result was greater than or equal to ASCUS. Women with baseline LBC low-grade squamous intraepithelial lesions or greater results were referred for colposcopy and management.

### Safety Group

Primary HPV testing was followed by reflex LBC in women with positive HPV test results, and they received the same management as the intervention group. However, in the safety group, HPV-negative women were recalled for exit screening with LBC at 24 months. The safety group was closed December 31, 2010, when the planned sample size for this group was achieved.[11]

### Intervention and Control Group Exit Screening

Exit screening for both the intervention and control groups occurred 48 months after baseline screening and consisted of HPV testing and LBC (exit co-testing).

### Procedures

All participants were invited to complete a demographic and behavioral questionnaire. From trial start through January 2010, the survey included sociodemographic, HPV vaccination status, reproductive, gynecological, and sexual health questions. After 2010, women completed an abbreviated survey that included questions regarding marital status, race/ethnicity, smoking, and lifetime sexual history. Race/ethnicity was captured based on fixed categories, self-reported, and collected as part of the sociodemographics to ensure randomization was true.

Participants underwent a pelvic examination, and cervical specimens were placed in a ThinPrep vial (Hologic Inc). Trial randomization was conducted at the laboratory on receipt of the enrollment specimen. HPV testing was performed with the Hybrid Capture 2 High Risk HPV DNA test (Qiagen), which detects high-risk HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68. To confirm specimen adequacy, 461 sequential ThinPrep specimens with valid HC2 results (34 HC2 positive and 427 negative) were tested with an in-house beta-globin polymerase chain reaction test and all were positive. As part of the trial protocol, samples with no visible cell pellet after conversion were rejected as inadequate. LBC slides were prepared using the ThinPrep 2000 (Hologic) processor and smears were screened manually by program cytotechnologists. Abnormal cytology test results were referred to a cytopathologist for final interpretation and reporting.

In British Columbia, all women are covered under the publicly funded health insurance program and cervical cancer screening is managed provincially by the BC Cervical Cancer Screening Program. All cytology screening specimens for the province, including those for this trial, were processed and tested at 1 centralized cytology laboratory in Vancouver, Canada. The Cervical Cancer Screening Program has 1 centralized registry that includes the cytology, histopathology, and treatment history for every woman ever screened in British Columbia. There is also 1 provincial cancer registry that captures all cancer diagnoses in British Columbia, permitting linkage between screening history and cervical cancer incidence for all women who participated.

The main trial objective was to compare the rates of cervical intraepithelial neoplasia (CIN) grade 3 or greater (CIN3+) 48 months after baseline screening with primary HPV vs LBC. Detailed trial methods and results have been previously described.[11-14] As outlined in **Figure 1**, round 1 refers to the baseline screen and any 12-month follow-up results in both the intervention and control groups. The 24-month screen round refers only to women in the control group because the intervention group did not receive 24-month screening, and this 24-month screen round included 24-month screen results and 36-month follow-up results. The 48-month exit round refers to 48-month exit screening results (plus 24-month results for the control group) and associated outcomes for both the intervention and control groups (eFigure in Supplement 2).

## Trial Outcomes

Rates of CIN3+ at 48 months in the intervention and control groups were the primary end points. Secondary trial end points included in this analysis are rates of CIN2+ at 48 months, the threshold for colposcopy referral and further evaluation, and evaluation of the impact of primary HPV testing on colposcopy services through evaluation of colposcopy referral rates in each group. Other secondary end points not included in this analysis are histologically confirmed CIN2+detected at 2 years in both the control and safety groups; clearance of HPV infection in women who were baseline HPV positive measured at 24 and 48 months; detection of histologically confirmed CIN3+ in HPV-positive women who received 12-month retesting measured at 24 months in the safety group; and total estimated cost per woman screened and total estimated cost per quality-adjusted life-year gained for each technology measured at 48 months.

All intervention and control group women who did not have a CIN2+ lesion detected during the trial or otherwise became ineligible (eg, hysterectomy, moved out of province) were invited for the 48-month exit screening. Women who were negative on both LBC and HPV co-testing at 48 months were deemed negative for CIN2+. Women who were either LBC of greater than or equal to ASCUS or HPV positive at 48 months were referred for colposcopy and biopsied to determine their status as CIN3+, CIN2+, less than or equal to CIN1, or normal.

## Statistical Methods

The sample size was based on a comparison of the rate of CIN3+ detected in the combined HPV and cytology screen in the intervention and control groups at 48 months. The HART trial[15] found that the rate of CIN3+ detection in women screened by combined HPV and cytology who had previously been screened by cytology was 8.1 per 1000. It was assumed that this rate would be applicable to the control group and the alternate hypothesis assumed that the rate in the intervention group would be 0.5 times this rate

Figure 1. Flow Diagram of HPV FOCAL Trial of HPV vs Cytology Screening to Detect Cervical Intraepithelial Neoplasia




ASCUS indicates atypical squamous cells of undetermined significance; CIN, cervical intraepithelial neoplasia; HPV, human papillomavirus; LBC, liquid-based cytology; LSIL, low-grade squamous intraepithelial lesion.

(ie, 4.0 per 1000). It was assumed that at least 80% of those randomized would be eligible and return for screening at 48 months. Specifying a 2-sided α = .05 and power = 0.90, then 9400 participants were required per arm in the control and intervention groups.

This article focuses on the primary study analysis, which is a comparison of the cumulative incidence rate in the intervention and control groups at 48 months. This analysis includes all participants from the intervention and control groups randomized and who had valid baseline and 48-month screening results. Disease detection and colposcopy referral rates at 48 months included all referrals and disease detected after round 1 screening in both groups and are reported throughout as rate per 1000. The denominator for the rate per 1000 is all women randomized into the intervention or control groups who also had valid baseline results. Confidence intervals were calculated using the Wilson method.[15] Comparisons were made using uncorrected $\chi^2$ test. Risk ratios were calculated using unconditional maximum likelihood with confidence intervals using normal approximation. Confidence intervals around absolute differences were constructed using the score intervals.

Cumulative disease incidence was plotted using 1 minus Kaplan-Meier estimates of disease-free probability. If a participant had an event (histopathology-confirmed CIN2+), the time to incidence was calculated as the difference between the date of disease detection and the randomization date. Those who did not have an event but became trial ineligible were censored. Time to censoring was the difference between the date the participant became ineligible and the randomization date. Participants who did not have an event or did not become ineligible were censored at the date of data extraction. Plots were truncated at 24 months after their 48-month screening and based on all women randomized regardless of attendance at all trial recommended screening.

Randomization occurred at the central laboratory. Two primary laboratories were responsible for screening of histopathology samples, 1 each in Vancouver and Victoria, British Columbia. We compared the pathology outcomes between both laboratories and found no significant difference ($\chi^2$ $P$ = .36). Multiple imputation was used to account for missing outcomes at the exit screen. For imputation, enrollment screen results were dichotomized to be either negative (HPV or cytology negative) or positive (HPV or cytology positive [≥ASCUS]). Multiple imputation was based on logistic regression with the total number of imputations set to 25. For loss to follow-up, demographics of women who were lost to follow-up were compared between the study groups and no significant differences were found. All statistical tests were 2-sided with $P$ < .05 considered statistically significant. All analyses were conducted using SAS version 9.3 (SAS Institute) or R 3.3.2 (R Foundation).[16]

## Results

Recruitment occurred from January 2008 through May 2012. Through the BC Cervical Cancer Screening Program, 76 422 women were identified as trial eligible; 51 199 were excluded (43 645 did not respond to the invite, 5644 formally declined, and 1910 did not meet inclusion criteria). Between January 1, 2008, and December 31, 2010, 6104, 6214, and 6204 women were recruited to the control, safety, and intervention groups, respectively.[14] After January 1, 2011, a further 3353 and 3348 women were recruited to the control and intervention groups, respectively. In total, 25 223 women were enrolled (9457 to the control, 6214 to the safety, and 9552 to the intervention groups). At 48 months, 8296 women (86.9%) completed the intervention and 8078 women (85.4%) completed the control exit screenings (Figure 1). Trial exit samples were received and processed through December 2016.

Overall, 89% of women in the intervention and control groups completed the baseline survey (**Table 1**). There were no significant differences between the 2 groups with respect to the distributions of sociodemographic and lifestyle characteristics. Only 0.6% of women self-reported receipt of any doses of an HPV vaccine. Using the reverse Kaplan-Meier method, median follow-up time in the intervention group was 77.1 months (95% CI, 76.4-77.5) and in the control group, 76.8 months (95% CI, 76.1-77.5).

**Table 1. Sociodemographic and Lifestyle Characteristics of the Intervention and Control Participants**

| Characteristic | No. (%) | |
|---|---|---|
| | Intervention (HPV Testing) | Control (LBC) |
| Age at baseline, y | n = 9552 | n = 9457 |
| 25-29 | 829 (8.7) | 834 (8.8) |
| 30-34 | 1037 (10.9) | 1046 (11.1) |
| 35-39 | 1388 (14.5) | 1303 (13.8) |
| 40-44 | 1522 (15.9) | 1496 (15.8) |
| 45-49 | 1553 (16.3) | 1530 (16.2) |
| 50-54 | 1365 (14.3) | 1385 (14.7) |
| 55-59 | 1083 (11.3) | 1079 (11.4) |
| 60-65 | 775 (8.1) | 784 (8.3) |
| Education level | n = 8443 | n = 8336 |
| High school or less | 1455 (17.2) | 1406 (16.9) |
| Trade school or college | 2423 (28.7) | 2419 (29.0) |
| Some university | 4565 (54.1) | 4511 (54.1) |
| Ethnicity[a] | n = 8510 | n = 8378 |
| Aboriginal | 257 (3.0) | 260 (3.1) |
| Chinese | 1190 (14.0) | 1173 (14.0) |
| European origin | 6510 (76.5) | 6361 (75.9) |
| Other Asian | 710 (8.3) | 732 (8.7) |
| Other | 365 (4.3) | 384 (4.6) |
| Lifetime sexual partners | n = 8343 | n = 8255 |
| 0-5 | 4679 (56.1) | 4711 (57.1) |
| 6-10 | 1928 (23.1) | 1817 (22.0) |
| >10 | 1736 (20.8) | 1727 (20.9) |
| Smoke ever | n = 8391 | n = 8292 |
| No | 5399 (64.3) | 5282 (63.7) |

Abbreviations: HPV, human papillomavirus; LBC, liquid-based cytology.

[a] Percentage adds up to more than 100% because participants were allowed to choose multiple ethnicities.

### Primary End Points

As previously reported[14] in the first round of screening, significantly more CIN3+ cases were detected in the intervention (HPV tested) compared with the control group.[13] The round 1 risk ratio for CIN3+ was 1.61 (95% CI, 1.09 to 2.37) in the intervention vs control group and the absolute difference in the incidence rate was 2.67/1000 (95% CI, 0.53-4.88) (eTable 1 in Supplement 2).

By 48 months, significantly fewer CIN3+ cases were detected overall and across all age groups in the intervention compared with the control group. The CIN3+ rate was 2.3/1000 (95% CI, 1.5-3.5) in the intervention group (**Table 2** and eTable 1 in Supplement 2). The risk ratio for CIN3+ at the exit round in the intervention compared with control group was 0.42 (95% CI, 0.25-0.69) and the absolute difference in the incidence rate for CIN3+ was −3.22/1000 (95% CI, −5.12 to −1.48).

Cumulative CIN3+ incidence curves show no significantly different disease detection across trial groups (**Figure 2**A). In the intervention group, the cumulative incidence was higher earlier in the trial at 18 months and 42 months compared with the control group. In this trial, all women in the intervention and control groups had the same intervention at the 48-month exit (HPV and cytology co-testing). By the end of trial follow-up (72 months), incidence was similar across both groups.

Among baseline HPV or LBC-negative women, rates of CIN3+ at 48 months were significantly higher across all age groups in the control compared with the intervention group (Table 2). The CIN3+ risk ratio for the intervention group compared with the control group was 0.25 (95% CI, 0.13-0.48). The absolute difference in the incidence rate was −4.03/1000 (95% CI, −5.88 to −2.41) for CIN3+. Cumulative incidence curves show that women who were HPV negative at baseline had a significantly lower risk of CIN3+ at 48 months compared with cytology-negative women (**Figure 3**A).

### Secondary End Points

As previously reported,[14] in the first round of screening, significantly more CIN2+ cases were detected in the intervention group (HPV tested) compared with the control group. The round 1 risk ratio for CIN2+ in the intervention vs control group was 1.61 (95% CI, 1.24-2.09) and the absolute difference in the incidence rates was 5.84/1000 (95% CI, 2.70-9.07) for CIN2+ (eTable 1 in Supplement 2). By 48 months, significantly fewer CIN2+ cases were detected overall and across all age groups in the intervention group compared with the control group. The CIN2+ rate was 5.0/1000 (95% CI, 3.8-6.7) (Table 2 and eTable 1 in Supplement 2). The risk ratio for CIN2+ at the exit round in the intervention group compared with control group was 0.47 (95% CI, 0.34-0.67). The absolute difference in the incidence rate for CIN2+ was −5.60/1000 (95% CI, −8.21 to −3.13).

Cumulative CIN2+ incidence curves show no significantly different disease detection across trial groups (Figure 2B). In the intervention group, cumulative incidence was higher earlier in the trial at 18 and 42 months compared with the control group. In this trial, all women in the intervention and control groups had the same intervention at the 48-month exit (HPV and cytology co-testing). By the end of trial follow-up (72 months), incidence was similar across both groups.

Among baseline HPV or LBC-negative women, rates of CIN2+ at 48 months were significantly higher across all age groups in the control group compared with the intervention group (Table 2). The CIN2+ risk ratio for the intervention compared with the control group was 0.36 (95% CI, 0.24-0.54). The absolute difference in the incidence rate was −6.38/1000 (95% CI, −8.91 to −4.02) for CIN2+. Cumulative incidence curves show that women who were HPV negative at baseline had a significantly lower risk of CIN2+ at 48 months compared with cytology-negative women (Figure 3B).

Colposcopy referral rates in the intervention group were significantly higher in round 1 (intervention: 57.0 [95% CI, 52.5-61.9] vs control: 30.8 [95% CI, 27.5-34.5]; absolute difference between intervention and control: 26.2 [95% CI, 20.4-32.1]). However, by 48 months, rates were lower in the intervention group compared with the control group for all ages (intervention: 49.2 [95% CI, 45.0-53.7]; control: 70.5 [95% CI, 65.5-75.8]; absolute difference between intervention and control: −21.3 [95% CI, −28.3 to −14.8]). Cumulative colposcopy referral rates were similar between both groups (intervention: 106.2 [95% CI, 100.2-112.5]; control: 101.5 [95% CI, 95.6-107.8]; absolute difference between intervention and control: 4.7 [95% CI, −4.0 to 13.4]).

In our investigation of the effect of missing outcome data for participants not attending the exit screen through multiple imputation, we did not find any significant differences in comparison of control and intervention groups for trial primary and secondary end points (eTable 2 in Supplement 2).

## Discussion

In this trial, by 48 months, among women screened for cervical cancer with HPV testing without cytology, there were significantly fewer CIN3+ and CIN2+ cases compared with women who were screened with cytology alone at baseline. Women who were HPV negative at baseline were significantly less likely to have CIN3+ and CIN2+ at 48 months compared with women who were cytology negative at baseline. These results have demonstrated that primary HPV testing detects cervical neoplasia earlier and more accurately than cytology.

Although cervical screening guidelines from a number of organizations[8,17] have recommended primary HPV testing based on the natural history of cervical cancer,[3] cross-sectional studies,[18] studies where HPV-based screening was part of a screening group,[7,19] or where studies ultimately evolved into primary HPV evaluations,[19,20] none of these studies were designed specifically to examine HPV testing as the primary screening modality. This trial, which compares primary HPV testing vs LBC with standardized triage and colposcopy follow-up, found primary HPV testing detected significantly more CIN3+ and CIN2+ cases in the first round and significantly reduced CIN3+ and CIN2+ rates 48 months later. This trial also confirmed that women who were HPV negative at baseline have lower rates of CIN2+ at 48 months than

Table 2. High-grade CIN Rates per 1000 Patients Detected at 48-Month Exit and Cumulatively[a]

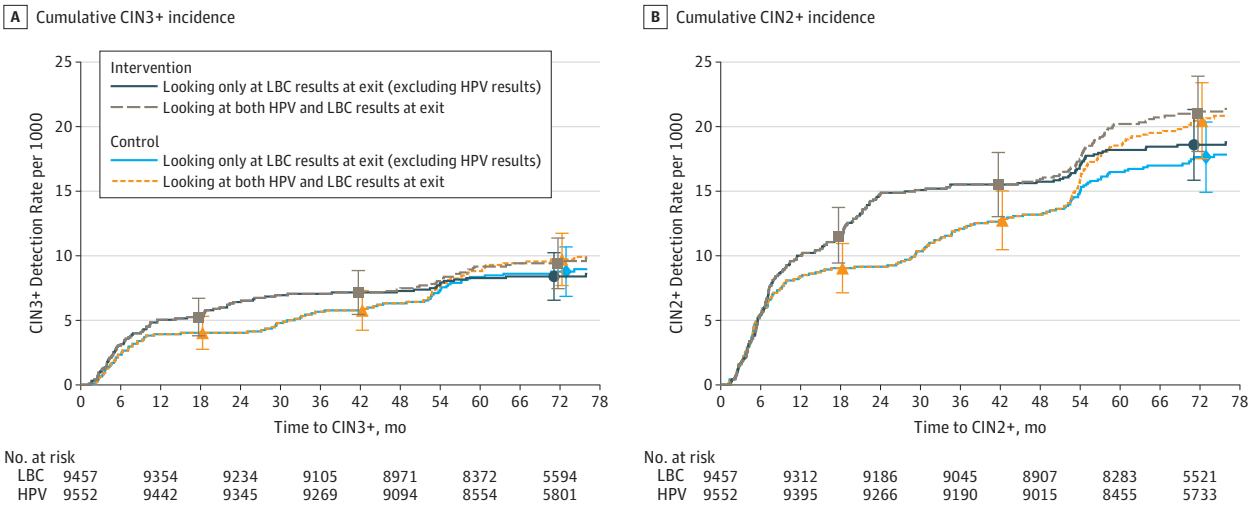
| Age at Baseline, y | 48-mo Exit Round | | | | | | | Round 1 and 48-mo Exit Round Combined | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Intervention (HPV Testing) | | Control (LBC)[b] | | | | | Intervention (HPV Testing) | | Control (LBC) | | | | |
| | No./ Total No. | Incidence / Rate/1000 (95% CI) | No./ Total No. | Incidence / Rate/1000 (95% CI) | Absolute Difference (95% CI) (HPV-LBC) | Risk Ratio (95% CI) (HPV vs LBC) | *P* Value | No./ Total No. | Incidence / Rate/1000 (95% CI) | No./ Total No. | Incidence / Rate/1000 (95% CI) | Absolute Difference (95% CI) (HPV-LBC) | Risk Ratio (95% CI) (HPV vs LBC) | *P* Value |
| **All Participants Attending 48-mo Screening** | | | | | | | | | | | | | | |
| CIN3+ primary outcome | | | | | | | | | | | | | | |
| 25-29 | 6/826 | 7.3 (3.3 to 15.8) | 15/828 | 18.1 (11.0 to 29.7) | −10.85 (−23.22 to −0.06) | 0.40 (0.16 to 1.02) | .05 | 26/826 | 31.5 (21.6 to 45.7) | 29/828 | 35.0 (24.5 to 49.8) | −3.55 (−21.43 to 14.16) | 0.90 (0.53 to 1.51) | .69 |
| ≥30 | 16/8714 | 1.8 (1.1 to 3.0) | 37/8580 | 4.3 (3.1 to 5.9) | −2.48 (−4.27 to −0.86) | 0.43 (0.24 to 0.76) | .003 | 63/8714 | 7.2 (5.7 to 9.2) | 64/8580 | 7.5 (5.8 to 9.5) | −0.22 (−2.82 to 2.35) | 0.97 (0.69 to 1.37) | .86 |
| All | 22/9540 | 2.3 (1.5 to 3.5) | 52/9408 | 5.5 (4.2 to 7.2) | −3.22 (−5.12 to −1.48) | 0.42 (0.25 to 0.69) | <.001 | 89/9540 | 9.3 (7.6 to 11.5) | 93/9408 | 9.8 (8.1 to 12.1) | −0.55 (−3.37 to 2.24) | 0.94 (0.71 to 1.26) | .69 |
| CIN2+ secondary outcome | | | | | | | | | | | | | | |
| 25-29 | 14/826 | 16.9 (10.1 to 28.2) | 27/828 | 32.6 (22.5 to 47.0) | −15.66 (−31.78 to −0.70) | 0.52 (0.27 to 0.98) | .04 | 59/826 | 71.4 (55.8 to 91.0) | 53/828 | 64.0 (49.3 to 82.8) | 7.42 (−17.02 to 32.03) | 1.11 (0.78 to 1.60) | .54 |
| ≥30 | 34/8714 | 3.9 (2.8 to 5.4) | 73/8580 | 8.5 (6.8 to 10.7) | −4.61 (−7.07 to −2.31) | 0.46 (0.31 to 0.69) | <.001 | 136/8714 | 15.6 (13.2 to 18.4) | 137/8580 | 16.0 (13.5 to 18.8) | −0.36 (−4.11 to 3.37) | 0.98 (0.77 to 1.24) | .85 |
| All | 48/9540 | 5.0 (3.8 to 6.7) | 100/9408 | 10.6 (8.7 to 12.9) | −5.60 (−8.21 to −3.13) | 0.47 (0.34 to 0.67) | <.001 | 195/9540 | 20.4 (17.8 to 23.5) | 190/9408 | 20.2 (17.5 to 23.2) | 0.24 (−3.79 to 4.28) | 1.01 (0.83 to 1.23) | .90 |
| **Baseline Screening Result Negative (Cytology or HPV Negative)** | | | | | | | | | | | | | | |
| CIN3+ primary outcome | | | | | | | | | | | | | | |
| 25-29 | 4/635 | 6.3 (2.5 to 16.1) | 15/758 | 19.8 (12.0 to 32.4) | −13.49 (−26.89 to −1.46) | 0.32 (0.11 to 0.95) | .03 | | | | | | | |
| ≥30 | 8/8134 | 1.0 (0.5 to 1.9) | 34/8316 | 4.1 (2.9 to 5.7) | −3.10 (−4.82 to −1.65) | 0.24 (0.11 to 0.52) | <.001 | | | | | | | |
| All | 12/8769 | 1.4 (0.8 to 2.4) | 49/9074 | 5.4 (4.1 to 7.1) | −4.03 (−5.88 to −2.41) | 0.25 (0.13 to 0.48) | <.001 | | | | | | | |
| CIN2+ Secondary Outcome | | | | | | | | | | | | | | |
| 25-29 | 10/635 | 15.7 (8.6 to 28.7) | 25/758 | 33.0 (22.4 to 48.2) | −17.23 (−34.33 to −0.79) | 0.48 (0.23 to 0.99) | .04 | | | | | | | |
| ≥30 | 22/8134 | 2.7 (1.8 to 4.1) | 66/8316 | 7.9 (6.2 to 10.1) | −5.23 (−7.59 to −3.07) | 0.34 (0.21 to 0.55) | <.001 | | | | | | | |
| All | 32/8769 | 3.6 (2.6 to 5.1) | 91/9074 | 10.0 (8.2 to 12.3) | −6.38 (−8.91 to −4.02) | 0.36 (0.24 to 0.54) | <.001 | | | | | | | |

Abbreviations: CIN, cervical intraepithelial neoplasia; HPV, human papillomavirus; LBC, liquid-based cytology.

[a] Multiple imputations were conducted and confirmed the observed difference of CIN2+ rates between the HPV and LBC-tested groups persist with similar scale as calculated from initial unimputed data presented in this table (eTable 2 in Supplement 2).

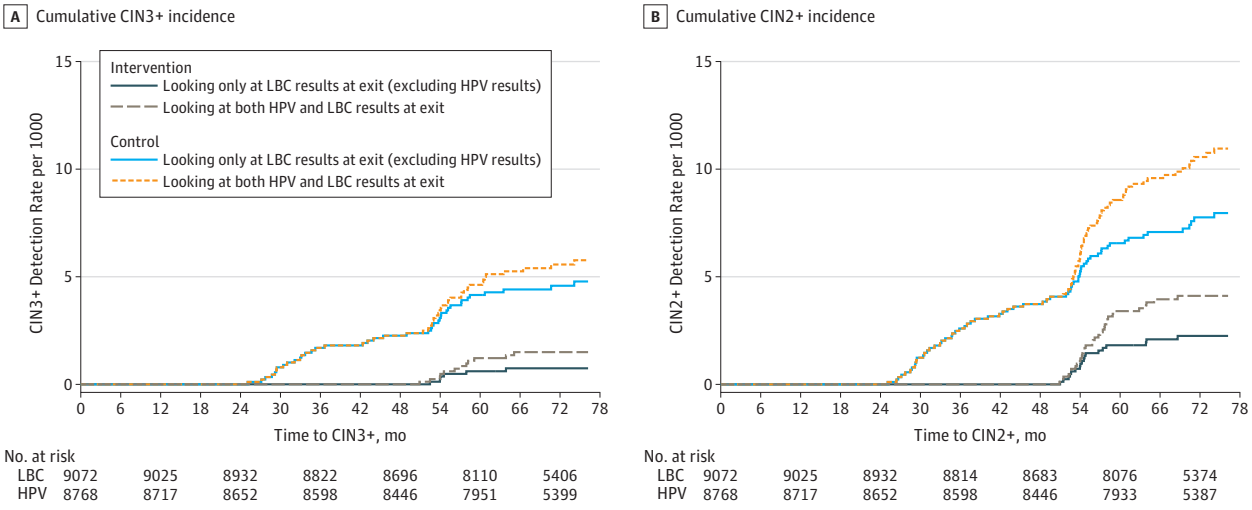
[b] Exit results at 48 months for the control group include disease detected at the 24-month screening.

**Figure 2. Cumulative Cervical Intraepithelial Neoplasia Grade 3 or Greater (CIN3+) and Grade 2 or Greater (CIN2+) Incidence for All Intervention and Control Group Participants Attending 48-Month Exit**




Incidence at 18, 42, and 72 months is marked by a point and the confidence intervals around it are shown as the point range. Points are jittered with respect to the x-axis to avoid overlays. Groups are artificially divided at 48 months to show the incidence in same participants if they were to be tested using liquid-based cytology alone. Hence, the number at risk is the same across partitions within primary groups. A, Cumulative CIN3+ incidence for intervention and control groups for all participants attending 48-month exit screen. B, Cumulative CIN2+ incidence for intervention and control groups for all participants attending 48-month exit screen. If cases were CIN3+ (in panel A) or CIN2+ (in panel B) at the initial screen but did not attend the exit screen, they contribute as an event at that time point.

**Figure 3. Cumulative Cervical Intraepithelial Neoplasia Grade 3 or Greater (CIN3+) and Grade 2 or Greater (CIN2+) Incidence for Baseline Human Papillomavirus (HPV) and Cytology-Negative Participants Attending 48-Month Exit Screen**




Incidence of CIN3+ (A) and CIN2+ (B) for baseline HPV and cytology-negative participants attending 48-month exit screen. Groups are artificially divided at 48 months to show the incidence in the same participants if they were to be tested using liquid-based cytology alone.

cytology-negative women at baseline. Previous studies found the benefit of HPV and cytology co-testing was based primarily on the contribution of HPV,[21] which this trial now prospectively validates. Further analyses modeling the cost-effectiveness of HPV primary screening using parameters from this study will be carried out to assess the potential economic effect of moving to HPV-based screening.

One of the concerns for adopting HPV-based screening is the lower CIN2+ specificity of HPV testing compared with cytology, leading to higher screen positive rates and the resulting need for more colposcopies and biopsies. Unnecessary colposcopies potentially cause unintended harm for women and increased costs to health care systems.[22-24] In this trial, round 1 colposcopy rates in the HPV-tested group

were significantly higher than the cytology-tested group. However, by 48 months, the colposcopy rate in the intervention group was reduced while the control group rate increased. This increase is partly a result of HPV and cytology co-testing at trial end.[11] Of the 513 control group women referred for colposcopy at exit, 304 (59%) were cytology negative and HPV positive. In the HPV-tested group, the colposcopy rate decreased in the second round of screening, which more accurately reflects the ongoing impact of HPV-based screening on a colposcopy program. The baseline colposcopy referral rate reflects what happens when HPV-based screening is first implemented, when both prevalent and incident infections will be detected.

To have an unbiased verification of the extent of disease left undiagnosed at trial exit, this trial included HPV and cytology co-testing for all participants at the 48-month screen.[25,26] As a result, with ongoing future monitoring of trial participants, it is anticipated some of the cervical cancer detected in the cytology group follow-up of other trials will be reduced in this trial, due to the fact that cytology-tested participants had added HPV testing at exit, permitting detection of lesions missed earlier in the trial.

This trial has several strengths. It was embedded in a well-established centralized cervical screening program, where all cytology in an entire Canadian province is analyzed at 1 certified laboratory by experienced staff, minimizing interobserver bias. Opportunistic screening not recommended through the trial was minimized by active notification and follow-up with clinicians by trial staff. Histopathological assessment was blinded to HPV and cytology results. Colposcopy procedures were standardized for all participants. These design factors reduced bias and limited variation in clinical procedures within trial groups.[14]

### Limitations

This study has several limitations. First, to provide a complete census of events at 48 months, women in both groups received HPV and cytology co-testing. Therefore, the exit intervention was not the same as the baseline intervention. However, by adding cytology to the intervention group, an additional 3 CIN2+ lesions were detected in HPV-negative women. In contrast, by adding HPV testing to the control group, HPV testing detected 25 CIN2+ lesions that would not have been detected by cytology alone. The addition of cytology to HPV testing detected very few additional events. Second, although the women from this clinical trial are participants in the population-based screening program, there is the potential for selection bias. The cohort was highly educated and primarily from 2 geographic regions in the province with limited representation from rural and remote populations. Therefore, results may underestimate the effect of the trial findings by underrepresentation of underscreened women who may face the highest risk of cervical cancer.

## Conclusions

Among women undergoing cervical cancer screening, the use of primary HPV testing compared with cytology testing resulted in a significantly lower likelihood of CIN3+ at 48 months. Further research is needed to understand long-term clinical outcomes as well as cost-effectiveness.


### ARTICLE INFORMATION

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**Author Contributions:** Drs Ogilvie and Coldman had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.
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