*Original Research*

# High-Risk and Low-Risk Human Papillomavirus and the Absolute Risk of Cervical Intraepithelial Neoplasia or Cancer

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**OBJECTIVE:** To determine the absolute risk of cervical intraepithelial neoplasia (CIN) grade 3 or cervical cancer (CIN 3 or worse) after detection of low-risk human papillomavirus (HPV) and after a negative high-risk HPV test.

**METHODS:** In this prospective cohort study, consecutive liquid-based cervical cytology samples were collected from women screened for cervical cancer in Copenhagen, Denmark, during 2002–2005. Samples were tested with a clinical test for 13 high-risk and five low-risk HPV types. The cohort (N=35,539; aged 14–90 years) was monitored in a nationwide pathology register for up to 10.5 years for development of CIN 3 or worse.

**RESULTS:** The 8-year absolute risk of CIN 3 or worse was 1.1% (95% confidence interval [CI] 1.0–1.3%) for HPV-negative women; 1.7% (0.8–2.6%) for low-risk HPV-positive women without concurrent high-risk HPV; 17.4% (16.4–18.5%) for high-risk HPV-positive women without concurrent low-risk HPV; and 15.9% (13.5–18.3%) for women with concurrent high-risk and low-risk HPV. The 8-year absolute risk of CIN 3 or worse after a negative high-risk HPV test (irrespective of low-risk HPV status) was lower than after a normal cytology result among women aged younger than 30 years (3.5% [95% CI, 2.9–4.0%] compared with 6.9% [6.2–7.5%], $P<.001$) and women aged 30 years or older (0.7% [95% CI, 0.6–0.9%] compared with 1.8% [95% CI, 1.6–2.0%], $P<.001$).

**CONCLUSION:** A negative high-risk HPV test provides greater long-term reassurance against CIN 3 or worse than normal cytology. Detection of low-risk HPV does not predict CIN 3 or worse. Cervical cancer screening should not include testing for low-risk HPV types.

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Cervical cancer screening programs based on cytologic examinations have reduced the incidence of this cancer in most developed countries.[1] Primary screening for high-risk human papillomavirus (HPV) DNA is more sensitive, but less specific, than cytology in detecting high-grade cervical intraepithelial neoplasia (CIN) and cervical cancer.[2] Women testing negative for high-risk HPV DNA remain at low risk of high-grade CIN and cervical cancer for up to 18 years.[3–8] Therefore, several countries now incorporate high-risk HPV testing into cervical cancer screening.[9]





In contrast, cervical cancer screening guidelines do not recommend testing for low-risk HPV types.[10] These are rarely identified as single infections in prevalent cervical cancer cases[11–14] and case–control studies have found that they confer only a marginal[14] or no[15–17] increase in the risk of cervical cancer. However, only few prospective studies have estimated the absolute risk of CIN or cervical cancer after low-risk HPV infection.[5,18–20] Furthermore, low-risk HPV testing during cervical cancer screening appears to be relatively widespread in the United States; a survey among American health care providers who performed HPV testing reported that 25–31% tested for low-risk HPV.[21] The prognostic value of low-risk HPV testing in cervical cancer screening thus requires clarification.

In this prospective study of more than 35,000 women, we assessed the long-term absolute risk of CIN grade 3 or cervical cancer (CIN 3 or worse) by cervical cytology and high-risk and low-risk HPV status at baseline. Our aims were to compare the absolute risk of CIN 3 or worse among high-risk HPV-negative women with that of women with normal cytology and to determine the absolute risk of CIN 3 or worse after detection of low-risk HPV (with or without concurrent high-risk HPV) in cervical cytology samples.

## MATERIALS AND METHODS

The study was based on a cohort established in Copenhagen, Denmark, during 2002–2005. On random days, we collected 42,854 consecutive liquid-based cytology samples from the Department of Pathology at Copenhagen University Hospital, Hvidovre, which handles all cervical cytology samples from the greater Copenhagen area. The samples were obtained from both opportunistic screening and from the organized Danish screening program in which women aged 23–49 years are invited for cervical cytology every third year and women aged 50–64 years are invited every fifth year.[22,23] The samples were taken by general practitioners or gynecologists using the SurePath liquid-based cytology system and placed in vials containing CytoRich Preservative Fluid. Within 2 days of collection, samples were sent to the pathology department for routine cytologic diagnosis. After preparation of the cytologic slide, the residual cell material was suspended in 5 mL of alcohol (80%).

These residual samples were collected weekly by the Danish Cancer Society Research Center and sent to the Medical Virology Department, University Hospital of Tübingen, Tübingen, Germany, for HPV DNA testing. Samples were tested with the Hybrid Capture 2 test using the low-risk probe (probe set A) for HPV types 6, 11, 42, 43, and 44 and the high-risk probe (probe set B) for HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68.[24] Samples were centrifuged for 15 minutes at 3,220 g (4,000 rpm) in the original tubes, and the supernatant was discarded. The pellet was taken up in 500 microliters Specimen Transport Medium and then processed as described by the manufacturer. Samples containing 1.0 pg/mL or greater of HPV DNA were considered positive. Laboratory personnel were blinded to the clinical and demographic data related to each sample.

Women in the cohort and clinicians were unaware of the HPV test results. Clinical management of the women was based on cervical cytology only, not on the HPV results. The study was approved by the Danish Scientific Ethics Committee and the Danish Data Protection Agency.

Follow-up was done passively using two nationwide Danish registers. In Denmark, all residents are registered in the computerized Civil Registration System with a unique personal identification number,[25] which contains information on sex and date of birth and is used throughout society, including in all health registries. The Civil Registration System database is updated daily and includes information on death, immigration, and emigration. By linking our cohort with this database, we collected information on vital and migration status of the women throughout follow-up.

Using the personal identification numbers as key identifiers, we also linked the cohort with the nationwide Pathology Data Bank. This database contains information on all cervical cytology, biopsies, and cones done in Denmark reported by pathology departments through an online, real-time system.[26] From the Pathology Data Bank, we collected information on the baseline smear diagnosis, all previous cervical examinations, and subsequent examinations until December 31, 2012, the maximum follow-up time being 10.5 years.

In the Pathology Data Bank, abnormal cervical diagnoses are mainly reported as atypia, mild dysplasia, moderate dysplasia, severe dysplasia, carcinoma in situ, or cancer. In 2012, the CIN nomenclature was introduced for histologic diagnoses. For our analysis, women diagnosed with atypia or worse on the baseline smear were considered to have had abnormal baseline cytology, whereas women with no abnormalities in the baseline smear were considered to have had normal baseline cytology. Histologic diagnoses of severe dysplasia, carcinoma in situ, CIN 3, or cervical cancer during follow-up were categorized as CIN 3 or worse.

Of the 42,854 liquid-based cytology samples collected, we excluded 184 samples because of technical errors or incomplete identification and 2,271 samples

that were duplicates from women already included in the study, resulting in a cohort of 40,399 individual women. We excluded 18 (less than 0.1%) women with missing baseline HPV or cytology result and 1,439 (3.6%) women being followed up for an abnormal cervical diagnosis in the year before baseline. Of the remaining 38,942 women, 3,403 had no cervical examinations during follow-up, leaving 35,539 women for analysis.

In the statistical analyses, we compared the following exposure groups: high-risk HPV-negative women compared with women with normal baseline cytology; women with concurrent high-risk and low-risk HPV compared with women with high-risk HPV alone; and women with low-risk HPV alone compared with HPV-negative women. Our primary end point was histologic diagnoses of CIN 3 or worse. However, in some analyses, invasive cervical cancer was used as the end point. Analyses were stratified by age at baseline (younger than 30 and 30 years or older).

We applied Turnbull's nonparametric estimator for interval-censored data to estimate the absolute risk with pointwise 95% confidence intervals (CIs).[27] The estimator takes into account that the exact failure times are unknown, because the outcome is only known to have occurred between the last negative examination in the Pathology Data Bank and the diagnosis date. In the primary analysis, a woman's follow-up time ended at the date of her last cervical record in the Pathology Data Bank, even if she was still alive and living in Denmark. In a sensitivity analysis, we did not require women to have a cervical examination in the Pathology Data Bank, but instead allowed follow-up time to continue until death, emigration, or end of follow-up (December 31, 2012).

Because Turnbull's estimator does not take into account competing risks, we repeated the analysis using the Aalen-Johansen estimator of the cumulative incidence function,[28] considering death and conization (without simultaneous CIN 3 or worse) as competing risks. In this analysis, the midpoint of each time interval was used as the failure time. The risk estimates according to the Aalen-Johansen model were virtually identical to the Turnbull estimates.

Lastly, to examine whether the intensity of follow-up differed by exposure group, the median number of smears during follow-up among women with no abnormalities was calculated for each group. All analyses were done in SAS 9.3.

## RESULTS

Of the 35,539 women included in the present analysis, 26,981 (75.9%) were negative for both high-risk and low-risk HPV types at baseline; 1,218 (3.4%) had low-risk HPV alone; 6,105 (17.2%) had high-risk HPV alone; and 1,235 (3.5%) had concurrent high-risk and low-risk HPV detected in their baseline cervical cytology sample. Atypia or worse was diagnosed in 1,993 women (5.6%) at baseline, whereas 33,546 women (94.4%) were cytologically normal (Table 1).

The age of the women ranged from 14 to 90 years at baseline (median 36 years). The median age was similar for high-risk HPV-negative women (38 years) and women with normal cytology (36 years) and that of women with concurrent high-risk and low-risk HPV (26 years) was similar to that of women with high-risk HPV alone (29 years). Women with low-risk HPV alone were slightly younger (median age 32 years) than those who didn't have either high-risk or low-risk HPV (median age 38 years) (Table 1).

We observed 1,187 cases of CIN 3 or worse during follow-up. There were 199 cases of CIN 3 or worse among women who were high-risk HPV-negative at baseline and 666 among women with normal baseline cytology. Cervical intraepithelial neoplasia grade 3 or worse was diagnosed in a lower proportion of high-risk HPV-negative women (0.7%) than in women with normal baseline cytology (2.0%), both for women aged younger than 30 years (1.4% compared with 3.8%) and those aged 30 years or older (0.5% compared with 1.3%). Of the 1,187 cases of CIN 3 or worse, 67 were cases of invasive cervical cancer. During follow-up, there were 20 cancer cases among high-risk HPV-negative women and 44 cancer cases among women with normal baseline cytology (Table 1).

Cervical intraepithelial neoplasia grade 3 or worse was diagnosed during follow-up in 15 of 1,218 (1.2%) women with low-risk HPV alone and 184 of 26,981 (0.7%) high-risk and low-risk HPV-negative women. None of the women with low-risk HPV alone had invasive cervical cancer. A slightly higher proportion of women with high-risk HPV alone (13.9%, n=848) were diagnosed with CIN 3 or worse than women with concurrent high-risk and low-risk HPV (11.3%, n=140). The patterns were similar in women aged younger than 30 and 30 years or older (Table 1).

Figure 1 presents the estimated absolute risks of CIN 3 or worse among high-risk HPV-negative women and women with normal baseline cytology for ages younger than 30 and 30 years or older, respectively. Among women aged younger than 30 years, the absolute risk was lower for those who were high-risk HPV-negative than for those with normal baseline cytology at 3 years (0.3%, 95% CI 0.2–0.4% compared with 0.6%, 95% CI 0.5–0.8%, $P<.01$), at

**Table 1. Number and Proportion of Women With Cervical Intraepithelial Neoplasia Grade 3 or Worse and Cervical Cancer During 10.5 Years Follow-Up According to Baseline Human Papillomavirus and Cytologic Status**

| | All Women | | | | Women Aged Younger Than 30 Y | | | Women Aged 30 Y or Older | | |
|---|---|---|---|---|---|---|---|---|---|---|
| HPV and Cytologic Status | n | Age (y) | CIN 3 or Worse | Cancer | n | CIN 3 or Worse | Cancer | n | CIN 3 or Worse | Cancer |
| Total | 35,539 | 36 (14–90) | 1,187 (3.3) | 67 (0.2) | 10,249 | 618 (6.0) | 12 (0.1) | 25,290 | 569 (2.2) | 55 (0.2) |
| Baseline HPV | | | | | | | | | | |
| Total high-risk HPV-negative | 28,199 | 38 (14–90) | 199 (0.7) | 20 (0.1) | 6,304 | 89 (1.4) | 1 (0.0) | 21,895 | 110 (0.5) | 19 (0.1) |
| High-risk and low-risk HPV-negative | 26,981 | 38 (14–90) | 184 (0.7) | 20 (0.1) | 5,841 | 82 (1.4) | 1 (0.0) | 21,140 | 102 (0.5) | 19 (0.1) |
| Low-risk HPV alone | 1,218 | 32 (17–76) | 15 (1.2) | 0 (0.0) | 463 | 7 (1.5) | 0 (0.0) | 755 | 8 (1.1) | 0 (0.0) |
| Total high-risk HPV-positive | 7,340 | 29 (14–81) | 988 (13.5) | 47 (0.6) | 3,945 | 529 (13.4) | 11 (0.3) | 3,395 | 459 (13.5) | 36 (1.1) |
| High-risk HPV alone | 6,105 | 29 (14–81) | 848 (13.9) | 43 (0.7) | 3,121 | 433 (13.9) | 8 (0.3) | 2,984 | 415 (13.9) | 35 (1.2) |
| Concurrent high-risk and low-risk HPV | 1,235 | 26 (15–79) | 140 (11.3) | 4 (0.3) | 824 | 96 (11.7) | 3 (0.4) | 411 | 44 (10.7) | 1 (0.2) |
| Baseline cytology | | | | | | | | | | |
| Normal | 33,546 | 36 (14–90) | 666 (2.0) | 44 (0.1) | 9,275 | 355 (3.8) | 7 (0.1) | 24,271 | 311 (1.3) | 37 (0.2) |
| Abnormal (atypia or worse) | 1,993 | 30 (15–83) | 521 (26.1) | 23 (1.2) | 974 | 263 (27.0) | 5 (0.5) | 1,019 | 258 (25.3) | 18 (1.8) |

HPV, human papillomavirus; CIN, cervical intraepithelial neoplasia.
Data are median (range) or n (%) unless otherwise specified.

5 years (0.7%, 95% CI 0.5–0.9% compared with 2.4%, 95% CI 2.1–2.8%, $P<.001$), and at 8 years of follow-up (3.5%, 95% CI 2.9–4.0% compared with 6.9%, 95% CI 6.2–7.5%, $P<.001$) (Fig. 1A). Likewise, among women aged 30 years or older, a negative high-risk HPV test gave greater reassurance against CIN 3 or worse than a normal cytology result at 3 years (0.1%, 95% CI 0.1–0.2% compared with 0.3%, 95% CI 0.2–0.3%, $P<.001$), at 5 years (0.3%, 95% CI 0.2–0.4% compared with 0.9%, 95% CI 0.8–1.0%, $P<.001$), and at 8 years of follow-up (0.7%, 95% CI 0.6–0.9% compared with 1.8%, 95% CI, 1.6–2.0%, $P<.001$) (Fig. 1B).

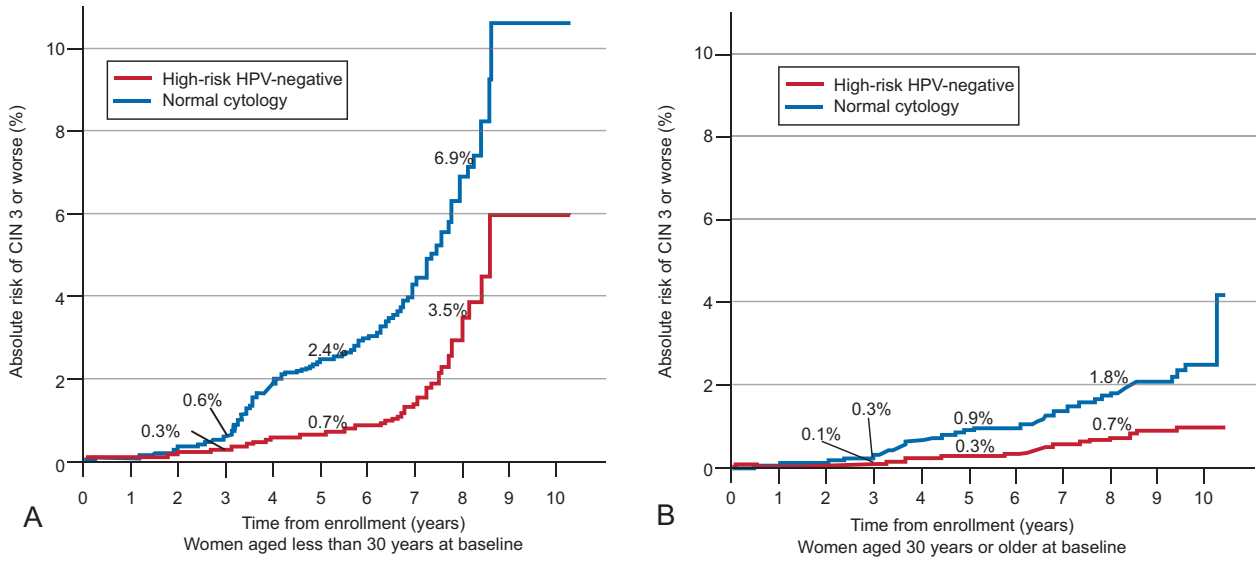
The pattern was the same when invasive cervical cancer was used as the end point, but numbers were small and most differences statistically insignificant. The absolute risks of invasive cervical cancer for high-risk HPV-negative women compared with women with normal baseline cytology were 0.02% (95% CI 0.00–0.04%) compared with 0.03% (95% CI 0.01–0.05%) at 3 years ($P=.48$), 0.05% (95% CI 0.02–0.07%) compared with 0.08% (95% CI 0.05–0.11%) at 5 years ($P=.13$), and 0.11% (95% CI 0.06–0.15%) compared with 0.19% (95% CI 0.14–0.25%) at 8 years of follow-up ($P=.03$) (data not shown).

Figure 2 presents the estimated absolute risks of CIN 3 or worse by high-risk and low-risk HPV status at baseline. High-risk HPV status was the main predictor of the future risk of CIN 3 or worse (Fig. 2A and B). Having low-risk HPV at baseline did not substantially alter the subsequent risk of CIN 3 or worse neither among high-risk HPV-positive (Fig. 2A) nor high-risk HPV-negative women (Fig. 2B).

Among high-risk HPV-positive women (Fig. 2A), the estimated absolute risk of CIN 3 or worse was slightly lower for women with concurrent high-risk and low-risk HPV than for those with only high-risk HPV, but 95% CIs of the two absolute risk curves were overlapping. At 5 years of follow-up, the absolute risk was 9.6% (95% CI 7.9–11.3%) among women with concurrent high-risk and low-risk HPV and 12.0% (95% CI 11.2–12.9%) among those with only high-risk HPV ($P=.01$). At 8 years of follow-up, the corresponding risk estimates were 15.9% (95% CI 13.5–18.3%) and 17.4% (95% CI 16.4–18.5%) ($P=.25$) (Fig. 2A).

Among high-risk HPV negative women (Fig. 2B), the absolute risk of CIN 3 or worse remained low throughout follow-up irrespective of low-risk HPV status at baseline. Although low-risk HPV-positive women appeared to have a slightly increased risk toward the end of follow-up, this tendency was based on few cases, and the 95% CI for the absolute risk curve for low-risk HPV-positive women overlapped




| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| High-risk HPV-negative | | | | | | | | | | | |
| At risk (n) | 6,304 | 6,251 | 6,188 | 6,085 | 5,682 | 5,303 | 4,389 | 2,064 | 456 | 5 | 2 |
| Cases (n) | 6 | 3 | 9 | 15 | 6 | 10 | 16 | 18 | 6 | 0 | 0 |
| Normal cytology | | | | | | | | | | | |
| At risk (n) | 9,275 | 9,215 | 9,100 | 8,948 | 8,281 | 7,662 | 6,306 | 2,982 | 643 | 5 | 2 |
| Cases (n) | 6 | 20 | 25 | 110 | 46 | 40 | 57 | 41 | 10 | 0 | 0 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| High-risk HPV-negative | | | | | | | | | | | |
| At risk (n) | 21,895 | 21,574 | 21,279 | 20,812 | 18,072 | 16,740 | 13,908 | 8,918 | 4,934 | 1,789 | 301 |
| Cases (n) | 13 | 8 | 3 | 24 | 12 | 6 | 27 | 9 | 5 | 3 | 0 |
| Normal cytology | | | | | | | | | | | |
| At risk (n) | 24,271 | 23,962 | 23,418 | 20,408 | 18,917 | 15,807 | 10,204 | 5,787 | 2,341 | 690 | 334 |
| Cases (n) | 15 | 22 | 27 | 88 | 39 | 17 | 52 | 29 | 13 | 8 | 1 |

**Fig. 1.** Absolute risk of cervical intraepithelial neoplasia (CIN) grade 3 or worse in high-risk human papillomavirus (HPV)-negative women and women with normal baseline cytology, by age. **A**. Women younger than 30 years at baseline. **B**. Women aged 30 years or older at baseline. CIN 3 or worse, CIN grade 3 or cervical cancer.

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with that for HPV-negative women (Fig. 2B). At 5 years of follow-up, the absolute risk was 0.7% (95% CI 0.2–1.2%) among women with low-risk HPV alone and 0.4% (95% CI 0.3–0.5%) among high-risk and low-risk HPV-negative women (*P*=.19). At 8 years of follow-up, the corresponding risk estimates were 1.7% (95% CI 0.8–2.6%) and 1.1% (95% CI 1.0–1.3%) (*P*=.19) (Fig. 2B).

In an age-stratified analysis (younger than 30 and 30 years or older), high-risk HPV positivity was the main predictor of CIN 3 or worse in both age groups, whereas being low-risk HPV-positive did not substantially alter the risk estimates. For example, among women aged 30 years or older, the 5-year absolute risk of CIN 3 or worse was 0.6% (95% CI 0.0–1.2%) for women with low-risk HPV alone and 0.3% (95% CI 0.2–0.4%) for HPV-negative women (*P*=.33). The corresponding 8-year risks were 1.6% (95% CI 0.6–2.6%) and 0.7% (95% CI 0.6–0.8%), respectively (*P*=.08) (data not shown).

Sensitivity analyses in which women were monitored until death, emigration, or end of follow-up, regardless of whether they had a cervical examination, resulted in slightly lower risk estimates than the primary analyses. However, the relative pattern for high-risk HPV-negative women compared with women with normal cytology was unchanged. Likewise, the relative patterns were unchanged for women with concurrent high-risk and low-risk HPV compared with women with high-risk HPV alone and for women with low-risk HPV alone compared with women without either high-risk or low-risk HPV (data not shown). In our analysis of follow-up intensity, we found that the median number of smears during follow-up among women with no abnormalities was the same in all exposure groups (median two examinations) (data not shown).

## DISCUSSION

In this prospective study of more than 35,000 women monitored for up to 10.5 years, having low-risk HPV in the baseline cervical cytology sample did not increase the risk of CIN 3 or worse either among high-risk HPV-positive or high-risk HPV-negative women.

This result is in line with case–control[14–17] and prevalence[11–13,29] studies supporting that low-risk HPV types rarely, if ever, cause cervical cancer. The few previous prospective studies that have estimated the absolute risk of CIN 3 or worse[18–20] or carcinoma in situ and invasive cervical cancer[5] after low-risk HPV infection also found that risks were low[5,19,20] or

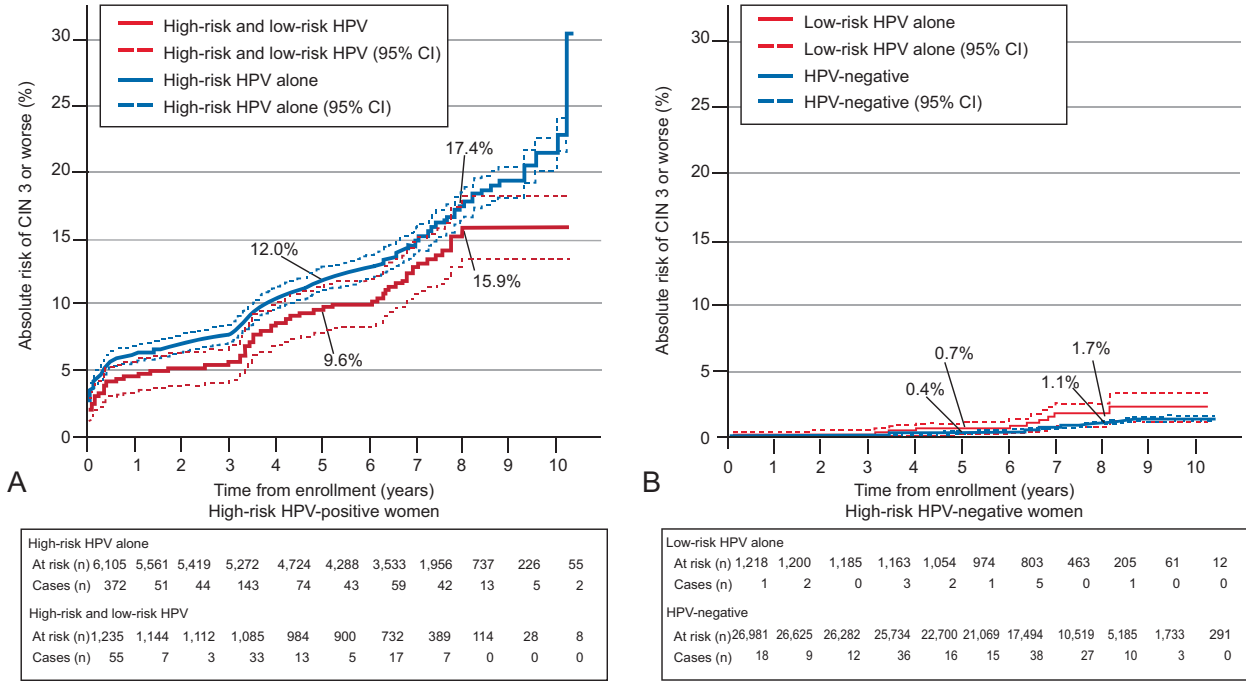


**Fig. 2.** Absolute risk of cervical intraepithelial neoplasia (CIN) grade 3 or worse according to high-risk and low-risk human papillomavirus (HPV) status at baseline. **A**. High-risk HPV-positive women. **B**. High-risk HPV-negative women. CI, confidence interval. CIN 3 or worse, CIN grade 3 or cervical cancer.

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even zero.[18] These prospective studies used polymerase chain reaction-based HPV detection methods, which are mainly used for research, whereas we used a clinical HPV test.[30] The previous prospective studies either excluded women with prevalent disease[5,18] or were restricted to women with cytologic abnormalities at baseline.[19,20] In contrast, our analysis included all women regardless of baseline cytologic status, thus providing clinically relevant estimates of the prognostic value of low-risk HPV testing during cervical cancer screening.

Our results support current recommendations[10] that screening for cervical cancer should not include testing for low-risk HPV types. Screening for HPV types unrelated to cervical cancer may cause improper follow-up procedures, waste of resources, and unnecessary psychological distress for patients. Therefore, reimbursement systems should discourage the use of low-risk HPV testing and initiatives to eliminate availability of the clinical low-risk HPV test should be considered.[21,31]

Some studies have suggested an antagonistic effect between certain high-risk and low-risk HPV types, resulting in a decreased risk of CIN grade 2 (CIN 2) or worse[19] or of cervical cancer[32,33] among women concurrently infected with HPV 16 and HPV 6,[32] HPV 16 and HPV 6/11,[33] or HPV 16 and a pool of 24 low-risk HPV types.[19] Our finding of a slightly (although not statistically significant) lower risk of CIN 3 or worse among women with concurrent high-risk and low-risk HPV than in those with only high-risk HPV may indicate an antagonistic effect. This could potentially be caused by cross-protective cell-mediated immunity between HPV types.[33] Exploration of such type–type interactions will require HPV genotype-specific analyses, which are currently underway for this cohort.

In accordance with previous European[7,8] and American[3,4] studies, this study also showed that a negative high-risk HPV test at baseline (irrespective of low-risk HPV status) provides greater long-term reassurance against CIN 3 or worse than a normal cytology result. This was found for both older (30 years or older) and younger (younger than 30 years) women. Our results thus support that in screening programs currently based on cytology,[34] screening intervals for screen-negatives may be extended if primary high-risk HPV testing is introduced.

The strengths of our study include the large sample (greater than 35,000 women) and the long

follow-up (greater than 10 years). Because the women were monitored in a national pathology register with virtually 100% coverage,[26] our study had little loss to follow-up; more than 90% of our cohort had at least one follow-up record in the Pathology Data Bank, and 94% remained under observation (alive and living in Denmark) throughout follow-up. As a result of our large sample size, the 95% CIs are narrow, indicating that our estimates of the absolute risk of CIN 3 or worse have a high degree of precision. For example, among women with low-risk HPV alone at baseline, the upper bound of the 95% CI at 5 years of follow-up was only 1.2%. On this basis, we are confident in concluding that low-risk HPV testing has no clinical predictive value in cervical cancer screening.

The study also had some limitations. First, because of the passive follow-up, our estimates of the absolute risk of CIN 3 or worse reflect the screening pattern in Denmark, where cytology screening at 3- or 5-year intervals (depending on the woman's age) is recommended.[22,23] Based on these observational data, we cannot entirely rule out that extending the screening interval would result in additional cases of CIN 3 or worse developing between screens. Furthermore, aggressive clinical management of lower grade lesions might have prevented some cases of CIN 3 or worse, resulting in an underestimate of the absolute risk. The effect of this would, however, be minimal, because Danish guidelines for CIN treatment are conservative, with recommendations for observation without excisional treatment of CIN 1 and even CIN 2 in women with a visible transformation zone.[35] Furthermore, because the screening intensity was similar in all exposure groups, we believe that the relative patterns were not biased by differential follow-up.

Another potential limitation is that HPV testing was performed on SurePath liquid-based cytology residual samples. In a case report, concern has been raised that this procedure may provide false-negative results.[36] However, a recent study found identical test performances for Hybrid Capture 2 testing of SurePath specimens compared with Hybrid Capture 2 testing of liquid-based cytology samples placed in a U.S. Food and Drug Administration–approved collection medium.[37] In our study, false-negative test results would have led us to overestimate the absolute risk of CIN 3 or worse after a negative HPV test. Therefore, in theory, a negative high-risk HPV test using a U.S. Food and Drug Administration–approved collection medium may provide even greater reassurance against CIN 3 or worse than we observed. Furthermore, our estimates of the absolute risk among low-risk HPV-positive women should not be affected by false-negative results.

A third potential limitation is that outcomes were not verified by an expert pathologist. Use of CIN 3 or worse as the end point (instead of CIN 2 or worse) should, however, have minimized misclassification, because clinical diagnoses of CIN 3 are much more reproducible than CIN 2.[38] Lastly, because we measured HPV at baseline only, we had no information on duration of infection or on the predictive values of HPV testing compared with cytology after multiple screening rounds.[4]

In conclusion, this study confirms that a negative test for high-risk HPV DNA provides greater long-term reassurance against CIN 3 or worse than normal cytology. Furthermore, we found that detection of low-risk HPV does not increase the risk of CIN 3 or worse. Therefore, screening for low-risk HPV types should be abandoned outside of research settings.

## REFERENCES


1. Vizcaino AP, Moreno V, Bosch FX, Munoz N, Barros-Dios XM, Borras J, et al. International trends in incidence of cervical cancer: II. Squamous-cell carcinoma. Int J Cancer 2000;86:429–35.
2. Arbyn M, Ronco G, Anttila A, Meijer CJ, Poljak M, Ogilvie G, et al. Evidence regarding human papillomavirus testing in secondary prevention of cervical cancer. Vaccine 2012;30(suppl 5):F88–99.
3. Castle PE, Glass AG, Rush BB, Scott DR, Wentzensen N, Gage JC, et al. Clinical human papillomavirus detection forecasts cervical cancer risk in women over 18 years of follow-up. J Clin Oncol 2012;30:3044–50.
4. Katki HA, Kinney WK, Fetterman B, Lorey T, Poitras NE, Cheung L, et al. Cervical cancer risk for women undergoing concurrent testing for human papillomavirus and cervical cytology: a population-based study in routine clinical practice. Lancet Oncol 2011;12:663–72.
5. Chen HC, Schiffman M, Lin CY, Pan MH, You SL, Chuang LC, et al. Persistence of type-specific human papillomavirus infection and increased long-term risk of cervical cancer. J Natl Cancer Inst 2011;103:1387–96.
6. Kjær SK, Frederiksen K, Munk C, Iftner T. Long-term absolute risk of cervical intraepithelial neoplasia grade 3 or worse following human papillomavirus infection: role of persistence. J Natl Cancer Inst 2010;102:1478–88.
7. Mesher D, Szarewski A, Cadman L, Cubie H, Kitchener H, Luesley D, et al. Long-term follow-up of cervical disease in women screened by cytology and HPV testing: results from the HART study. Br J Cancer 2010;102:1405–10.
8. Dillner J, Rebolj M, Birembaut P, Petry KU, Szarewski A, Munk C, et al. Long term predictive values of cytology and human papillomavirus testing in cervical cancer screening: joint European cohort study. BMJ 2008;337:a1754.
9. Castle PE, de Sanjosé S, Qiao YL, Belinson JL, Lazcano-Ponce E, Kinney W. Introduction of human papillomavirus DNA screening in the world: 15 years of experience. Vaccine 2012;30(suppl 5):F117–22.
10. Saslow D, Solomon D, Lawson HW, Killackey M, Kulasingam SL, Cain J, et al. American Cancer Society, American Society for Colposcopy and Cervical Pathology, and

American Society for Clinical Pathology screening guidelines for the prevention and early detection of cervical cancer. Am J Clin Pathol 2012;137:516–42.

11. Smith JS, Lindsay L, Hoots B, Keys J, Franceschi S, Winer R, et al. Human papillomavirus type distribution in invasive cervical cancer and high-grade cervical lesions: a meta-analysis update. Int J Cancer 2007;121:621–32.
12. de Sanjose S, Quint WG, Alemany L, Geraets DT, Klaustermeier JE, Lloveras B, et al. Human papillomavirus genotype attribution in invasive cervical cancer: a retrospective cross-sectional worldwide study. Lancet Oncol 2010;11:1048–56.
13. Muñoz N, Bosch FX, Castellsagué X, Díaz M, de Sanjosé S, Hammouda D, et al. Against which human papillomavirus types shall we vaccinate and screen? The international perspective. Int J Cancer 2004;111:278–85.
14. Wheeler CM, Hunt WC, Joste NE, Key CR, Quint WG, Castle PE. Human papillomavirus genotype distributions: implications for vaccination and cancer screening in the United States. J Natl Cancer Inst 2009;101:475–87.
15. Dahlström LA, Ylitalo N, Sundstrom K, Palmgren J, Ploner A, Eloranta S, et al. Prospective study of human papillomavirus and risk of cervical adenocarcinoma. Int J Cancer 2010;127:1923–30.
16. Sundström K, Eloranta S, Sparén P, Arnheim Dahlström L, Gunnell A, Lindgren A, et al. Prospective study of human papillomavirus (HPV) types, HPV persistence, and risk of squamous cell carcinoma of the cervix. Cancer Epidemiol Biomarkers Prev 2010;19:2469–78.
17. Muñoz N, Bosch FX, de Sanjosé S, Herrero R, Castellsagué X, Shah KV, et al. Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl J Med 2003;348:518–27.
18. Schiffman M, Herrero R, Desalle R, Hildesheim A, Wacholder S, Rodriguez AC, et al. The carcinogenicity of human papillomavirus types reflects viral evolution. Virology 2005;337:76–84.
19. Wheeler CM, Hunt WC, Schiffman M, Castle PE; Atypical Squamous Cells of Undetermined Significance/Low-Grade Squamous Intraepithelial Lesions Triage Study Group. Human papillomavirus genotypes and the cumulative 2-year risk of cervical precancer. J Infect Dis 2006;194:1291–9.
20. Castle PE, Solomon D, Schiffman M, Wheeler CM. Human papillomavirus type 16 infections and 2-year absolute risk of cervical precancer in women with equivocal or mild cytologic abnormalities. J Natl Cancer Inst 2005;97:1066–71.
21. Lee JW, Berkowitz Z, Saraiya M. Low-risk human papillomavirus testing and other nonrecommended human papillomavirus testing practices among U.S. health care providers. Obstet Gynecol 2011;118:4–13.
22. Danish National Board of Health. Screening against cervical cancer–recommendations [in Danish]. Copenhagen (Denmark): Danish National Board of Health; 2007.
23. Danish National Board of Health. Screening against cervical cancer–recommendations [in Danish]. Copenhagen (Denmark): Danish National Board of Health; 2012.
24. Iftner T, Villa LL. Chapter 12: human papillomavirus technologies. J Natl Cancer Inst Monogr 2003:80–8.
25. Pedersen CB. The Danish Civil Registration System. Scand J Public Health 2011;39(suppl):22–5.
26. Bjerregaard B, Larsen OB. The Danish Pathology Register. Scand J Public Health 2011;39(suppl):72–4.
27. Turnbull BW. The empirical distribution function with arbitrarily grouped, censored and truncated data. J R Stat Soc Ser B 1976;38:290–5.
28. Aalen OO, Johansen S. Empirical transition matrix for non-homogenous Markov-chains based on censored observations. Scand J Stat 1978;5:141–50.
29. Nielsen A, Iftner T, Nørgaard M, Munk C, Junge J, Kjaer SK. The importance of low-risk HPV infection for the risk of abnormal cervical cytology/histology in more than 40 000 Danish women. Sex Transm Infect 2012;88:627–32.
30. Schiffman M, Wentzensen N, Wacholder S, Kinney W, Gage JC, Castle PE. Human papillomavirus testing in the prevention of cervical cancer. J Natl Cancer Inst 2011;103:368–83.
31. Castle PE. Abuses in human papillomavirus DNA testing. Obstet Gynecol 2011;118:1–3.
32. Silins I, Wang Z, Avall-Lundqvist E, Frankendal B, Vikmanis U, Sapp M, et al. Serological evidence for protection by human papillomavirus (HPV) type 6 infection against HPV type 16 cervical carcinogenesis. J Gen Virol 1999;80:2931–6.
33. Luostarinen T, af Geijersstam V, Bjørge T, Eklund C, Hakama M, et al. No excess risk of cervical carcinoma among women seropositive for both HPV16 and HPV6/11. Int J Cancer 1999;80:818–22.
34. Anttila A, Ronco G; Working Group on the Registration and Monitoring of Cervical Cancer Screening Programmes in the European Union; within the European Network for Information on Cancer (EUNICE). Description of the national situation of cervical cancer screening in the member states of the European Union. Eur J Cancer 2009;45:2685–708.
35. Barken SS, Rebolj M, Andersen ES, Lynge E. Frequency of cervical intraepithelial neoplasia treatment in a well-screened population. Int J Cancer 2012;130:2438–44.
36. Naryshkin S, Austin RM. Limitations of widely used high-risk human papillomavirus laboratory-developed testing in cervical cancer screening. Drug Healthc Patient Saf 2012;4:167–72.
37. Zhao FH, Hu SY, Bian JJ, Liu B, Peck RB, Bao YP, et al. Comparison of ThinPrep and SurePath liquid-based cytology and subsequent human papillomavirus DNA testing in China. Cancer Cytopathol 2011;119:387–94.
38. Carreon JD, Sherman ME, Guillén D, Solomon D, Herrero R, Jerónimo J, et al. CIN2 is a much less reproducible and less valid diagnosis than CIN3: results from a histological review of population-based cervical samples. Int J Gynecol Pathol 2007;26:441–6.