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Polman, N.J.

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Chapter 7

Five-year cervical (pre)cancer risk of women screened by HPV and cytology testing

N.J. Polman*

M.H. Uijterwaal*

F.J. van Kemenade

S. van den Haselkamp

B.I. Witte

D. Rijkaart

J. Berkhof

P.J.F. Snijders

C.J.L.M. Meijer

* Both authors contributed equally to this work

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Abstract

Primary human papillomavirus (HPV)-based cervical screening will be introduced in the Netherlands in 2016. We assessed the 5-year cervical (pre)cancer risk of women with different combinations of HPV and cytology test results. Special attention was paid to risks for cervical intraepithelial neoplasia grade 3 and 2 or more (CIN3+/2+) of HPV-positive women with a negative triage test, because this determines the safety of a 5-year screening interval for HPV-positive, triage test-negative women. In addition, age-related effects were studied. A total of 25,553 women were screened by HPV testing and cytology in a screening setting. Women were managed on the presence of HPV and/or abnormal cytology. Five-year cumulative incidences for CIN3+/2+ were calculated. Five-year CIN3+(2+) risk was 10.0% (17.7%) among HPV-positive women. When stratified by cytology, the CIN3+ (CIN2+) risk was 7.9% (12.9%) for women with normal cytology and 22.2% (45.3%) for women with equivocal or mildly abnormal (i.e., BMD) cytology. For HPV-negative women, the 5-year CIN3+(2+) risk was 0.09% (0.21%). Additional triage of HPV-positive women with normal cytology by repeat cytology at 12 months showed a 5-year CIN3+(2+) risk of 4.1% (7.0%). HPV-non-16/18-positive women with normal cytology at baseline had comparable risks of 3.5% (7.9%). HPV-non 16/18-positive women with normal baseline cytology and normal repeat cytology had a 5-year CIN3+ risk of 0.42%. No age-related effects were detected. In conclusion, HPV-positive women with normal cytology and a negative triage test, either repeat cytology after 12 months or baseline HPV 16/18 genotyping, develop a non-negligible CIN3+ risk over 5 years. Therefore, extension of the screening interval over 5 years only seems possible for HPV screen-negative women.

Introduction

Data from clinical trials have shown that screening by HPV testing provides better protection against cervical cancer and high-grade precursors thereof, i.e., cervical intraepithelial neoplasia grade 2 and 3 (CIN2/3), than cytology screening.^{1,2} In the Netherlands, the Minister of Health has recently decided to convert the cytology-based screening program into an HPV-based screening program.

Because the HPV test has 2% to 4% lower specificity than cytology for cervical intraepithelial neoplasia grade 2 or worse (CIN2+), management on the basis of HPV test results alone would lead to over-referral and overtreatment of patients. Thus, proper triage algorithms using additional tests for HPV-positive women are necessary. Previously, we have determined the risk within 36 months for CIN2+ and CIN3 or worse (CIN3+) in the VUSA-Screen cohort.^{3,4} In this context, we have also evaluated several triage algorithms.⁵ Cytology testing at baseline and after 12 months was among the most attractive triage algorithms for HPV positive women, along with HPV 16/18 genotyping and a combination of genotyping and repeat cytology. Still, long-term data are needed to evaluate the safety of a negative triage.

We evaluated 5-year CIN2+/3+ risks of women screened by HPV testing and cytology. Special attention was paid to the risk of HPV-positive women with negative triage tests, because this risk determines the safety of a 5-year screening interval for these women. In addition, we analyzed age-related effects.

Materials and Methods

Patients and procedures

The VUSA-Screen study is a population-based study designed to evaluate the effectiveness of combined cervical cytology screening with hrHPV testing by the HC2 hybridization assay (Qiagen). The study design is outlined in Figure 1. Inclusion and exclusion criteria have been described previously.³ Briefly, 25,871 women participating in the regular cervical screening program were offered an HPV test in addition to cytology. Women with borderline or mild dyskaryosis (BMD, corresponding to ASC-US/LSIL) cytology and a HPV-positive test result were directly referred for colposcopy, as were women with worse than BMD cytology (irrespective of their HPV status). Women with BMD and a negative HPV test result were offered repeat cytology at 6 and 18 months, and were referred for colposcopy in case of abnormal cytology. In earlier studies on VUSA-Screen, we have analyzed the cumulative CIN2/3

risk within 36 months after baseline, for HPV-positive women with normal cytology (n=1,021) and age-matched HPV-negative women with normal cytology (n=3,063). Women were evaluated by HPV-testing and cytology at 24 months after baseline.⁴ In the current study assessing the 5-year risk, the complete cohort of women was analyzed, consisting of 24,345 HPV-negative and 1,188 HPV-positive women. In case of abnormal results in follow-up (either HPV-positive or \geq BMD), women were referred for colposcopy. If colposcopic assessment showed suspect areas, biopsies were taken according to national and international guidelines.^{6,7} Histology was classified as normal, CIN1, 2, or 3 or as invasive cancer according to international criteria.^{6,8} Adenocarcinoma in situ of the cervix (ACIS) was counted as CIN3.

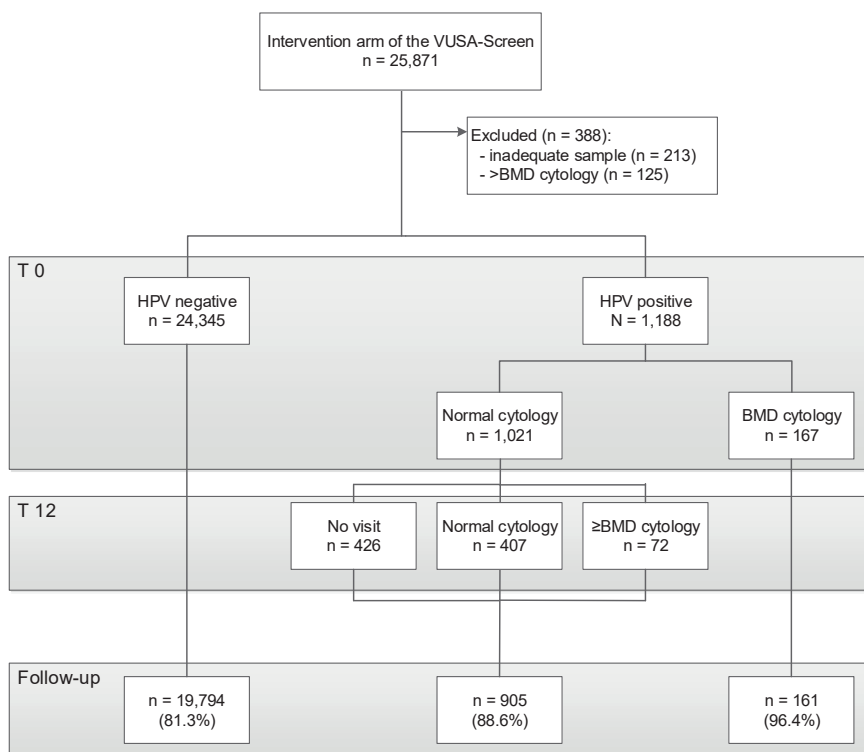


Figure 1. Flowchart of the intervention arm of the VUSA-Screen for women evaluated in this 5-year follow-up study. Women in the control group were excluded because they were only screened by cytology. Women with an inadequate sample or $>$ BMD cytology were not included in this follow-up study.

n = number of women; T0 = baseline; T12 = 12 months after baseline.

Participants had a cytologic smear taken with a cytobrush (Rovers Medical Devices B.V.). After preparation of the smear on a glass slide for cytology, the brush was placed in a vial containing 1 mL UCM (Universal Collection Medium; Qiagen) for HPV testing. Cytology results were read according to the CISOE-A classification, which can be easily converted to the 2001 Bethesda system.^{9,10} Cytologic results were grouped as normal, BMD, or >BMD.

High-risk HPV testing was performed by the HC2 high-risk HPV DNA test in an automated format on a rapid capture system according to the manufacturer's instructions (Qiagen). This test used a cocktail of probes to detect 13 high-risk HPV genotypes: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68. Samples were considered positive if they had an HC2 outcome of ≥ 1 RLU/CO. HC2-positive samples were typed by reverse line blot hybridization.¹¹ In the context of this study, HPV-positive women were classified into two groups of HPV 16/18-positive versus HPV 16/18-negative women (HPV-non 16/18-positive).

For this study, we collected long-term follow-up data from all women with normal cytology or BMD at baseline. Women with an inadequate sample (n=213) were excluded. Women with >BMD at baseline (n=125) were also excluded because they had been directly referred for colposcopy, irrespective of HPV status.¹²

The VUSA-Screen study was approved by the Ministry of Public Health (2002/02-WBO; ISBN-10: 90-5549-452-6) and registered in the trial register (NTR215, ISRCTN64621295).

Data collection

The VUSA-Screen study was carried out between October 2003 and August 2005. Histo- and cytopathologic data from all 25,533 women with normal cytology and BMD cytology results at baseline were collected until December 2012, resulting in a follow-up period of at least 5 years. Within this time frame, all women should have had at least one follow-up visit, and therefore the risk within one round of the screening program can be determined. According to the screening program in the Netherlands, a subsequent screening round consisted of only cytology testing, and found place 5 years after the previous screening, irrespective of possible treatment and/or follow-up period. Follow-up results were retrieved from PALGA, the nationwide registry and network of all histopathologic and cytopathologic data.¹³ A total of 59,310 cytologic and histologic follow-up excerpts of gynecological origin were obtained. Non cervical data were discarded. In case of multiple histologic diagnoses in a single excerpt, the

worst diagnosis was taken. Every woman contributed from recruitment to CIN2+ detection, hysterectomy, or last follow-up visit before the end of the study.

Statistical analysis

The primary outcome measure was the proportion of histologically confirmed CIN3+ lesions found during the time span from intake up to the last recorded result. The secondary outcome measure was the proportion of histologically confirmed CIN2+ lesions. Interval censoring was used in case of a CIN2+/CIN3+ event and right censoring otherwise. This is a variant of the Kaplan–Meier approach that accounts for the fact that women had a different number of follow-up moments, at various time points during the study. The lower bound of the interval was the time of last normal cytology test, upper bound was the time of histologic diagnosis CIN2+. In case the first cytology test was BMD, and immediately after that a CIN2+ was diagnosed, the lower bound was set to baseline.

Separate analyses were performed for women between the ages of 29 and 33 and women of 34 years or older. These two age groups correspond to the first screen in the program and later screens. The chosen strategies for triaging HPV-positive women included (i) cytology followed by repeat cytology testing at 12 months (range, 6–18 months); (ii) cytology with HPV 16/18 genotyping; and (iii) cytology with HPV 16/18 genotyping followed by repeat cytology testing at 12 months. The cumulative incidences of CIN2+/3+ and 95% confidence intervals (CI) were calculated with the “interval” package in the statistical software program R (version 3.0.1¹⁴). Comparisons between curves were done using the R function IC test with default setting.

Results

A total of 25,533 women met the inclusion criteria (Figure 1). Of these, 20,860 (81.7%) women had a follow-up visit. Among HPV-positive women, follow-up results were available for 905 of 1,021 (88.6%) women with normal cytology and 161 of 167 (96.4%) women with BMD cytology. Median follow-up time of these women was 62.0 (range, 2–78) and 63.0 (range, 2–105) months, respectively. Mean age at inclusion was 38.2 (range, 29–60) and 37.8 (range, 29–61) years, respectively. Furthermore, 19,794 of 24,345 (81.3%) HPV-negative women had follow-up results. Median follow-up time was 62.0 (range, 0–107) months. The mean age at inclusion was 43.3 (range, 29–61) years.

In total, 138 women were diagnosed with CIN3+, including 7 adenocarcinomas and 4 squamous cell carcinomas, and 256 with CIN2+.

Five-year cumulative incidences of CIN3+ and CIN2+ are shown in Table 1 and Figure 2. Five-year CIN3+ risk for HPV-negative women was 0.09% (95% CI, 0.04%–0.14%). For HPV-positive women, 5-year CIN3+ risk was 10.0% (95% CI, 7.1%–11.5%). This was significantly higher than the risk of HPV-negative women ($P < 0.001$). Stratified by cytology, risks were 7.9% (95% CI, 4.4%–10.1%) and 22.2% (95% CI, 16.0%–28.0%; $P < 0.001$) for HPV-positive women with normal or BMD cytology, respectively. When only evaluating cytology results (i.e., normal or BMD cytology), the 5-year CIN3+ risk was 0.42% (95% CI, 0.28%–0.51%) for women with normal cytology and 11.6% (95% CI, 7.6%–15.5%) for women with BMD cytology ($P < 0.001$). For CIN2+, comparable differences in risks were found (Table 1).

Table 1. Five-year cumulative incidences for CIN3+ and CIN2+.

	CIN3+				CIN2+		
	n	n	Cumulative Incidence	95% CI	n	Cumulative Incidence	95% CI
HPV-negative	19,794	26	0.09%	(0.04-0.14%)	64	0.21%	(0.13-0.28%)
HPV-positive	1,066	112	10.0%	(7.1-11.5%)	192	17.7%	(14.8-20.1%)
HPV-positive, normal cytology	905	72	7.9%	(4.4-10.1%)	114	12.9%	(9.6-16.0%)
HPV-positive, BMD cytology	161	40	22.2%	(16.0-28.0%)	78	45.3%	(36.5-51.6%)
Normal cytology	20,537	95	0.42%	(0.28-0.51%)	172	0.72%	(0.58-0.83%)
BMD cytology	323	43	11.6%	(7.6-15.5%)	84	24.1%	(18.8-28.8%)

n = number of women

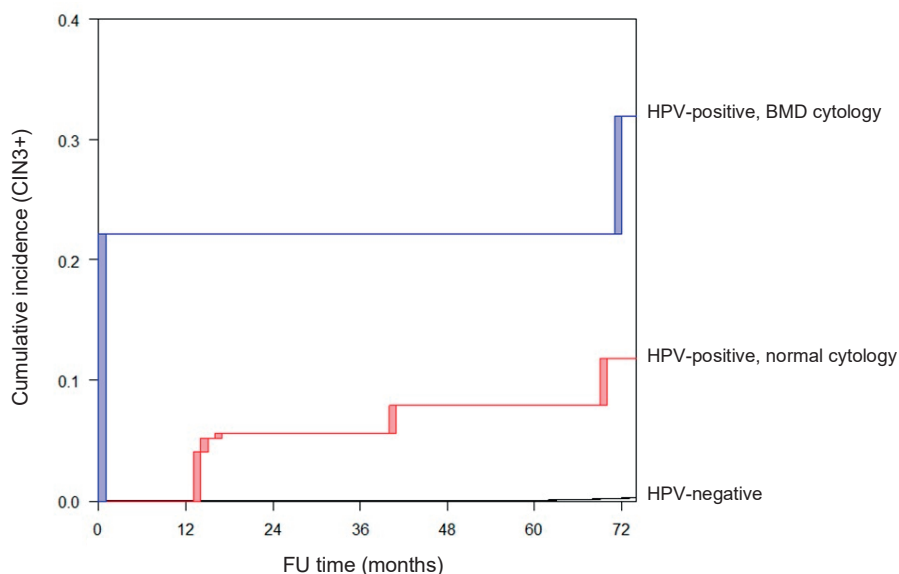


Figure 2. Cumulative incidences for CIN3+.
CIN = cervical intraepithelial neoplasia.

We next evaluated the risk of HPV-positive women triaged by different strategies, with special attention to the risk of those with a negative triage result. The associated 5-year CIN3+ and CIN2+ risks are shown in Table 2 and Figure 3. First, 5-year risks of HPV-positive women with normal cytology triaged by repeat cytology testing were calculated. In case of a normal repeat cytology test, women had a 5-year CIN3+ risk of 4.1% (95% CI, 0.44%–5.9%), which was significantly lower than the risk of HPV-positive women with normal cytology without repeat cytology testing (7.9%; 95% CI, 4.4%–10.1%; $P=0.007$). Five-year risks of women with \geq BMD repeat cytology are shown in Table 2.

Table 2. Five-year cumulative incidences for CIN3+ and CIN2+ in HPV-positive women with normal cytology triaged with different triage strategies

Baseline	Repeat-cytology (after 12 months)	n	CIN3+			CIN2+		
			n	Incidence	95% CI	n	Incidence	95% CI
HPV-positive, normal cytology	-	905	72	7.9%	(4.4-10.1%)	114	12.9%	(9.6-16.0%)
HPV-positive, normal cytology	Normal cytology	407	15	4.1%	(0.44-5.9%)	25	7.0%	(3.3-9.2%)
HPV-positive, normal cytology	\geq BMD	72	23	31.1%	(19.5-40.9%)	38	53.2%	(39.3-66.8%)
HPV 16/18-positive, normal cytology	-	259	48	18.1%	(9.4-33.9%)	62	24.6%	(16.7-30.2%)
HPV 16/18-positive, normal cytology	Normal cytology	104	10	11.3%	(0.00-19.2%)	12	13.4%	(2.8-21.0%)
HPV 16/18-positive, normal cytology	\geq BMD	32	14	40.9%	(22.3-59.1%)	22	69.6%	(50.3-87.5%)
HPV-non16/18- positive, normal cytology	-	646	24	3.5%	(1.7-5.3%)	52	7.9%	(5.7-10.4%)
HPV-non16/18- positive, normal cytology	Normal cytology	303	5	0.42%	(0.00-1.4%)	13	3.5%	(0.00-6.2%)
HPV-non16/18- positive, normal cytology	\geq BMD	40	9	23.3%	(10.3-38.7%)	16	41.3%	(23.1-58.6)

n = number of women

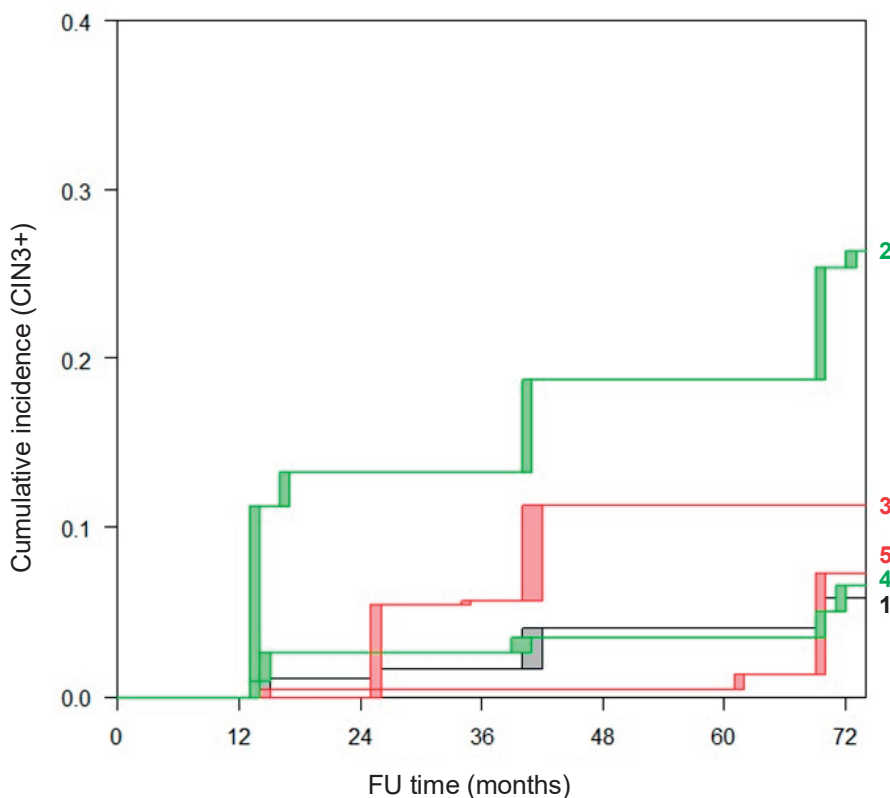


Figure 3. Cumulative incidence curves for CIN3+ in HPV-positive women with normal cytology, triaged by HPV 16/18 genotyping and/or repeat cytology.

1. HPV-positive, normal cytology with normal repeat cytology; 2. HPV 16/18-positive, normal cytology; 3. HPV 16/18-positive, normal cytology with normal repeat cytology; 4. HPV-non 16/18-positive, normal cytology; 5. HPV-non 16/18-positive, normal cytology with normal repeat cytology.

CIN = cervical intraepithelial neoplasia.

Second, 5-year risks of women triaged by HPV 16/18 genotyping at baseline were calculated. HPV 16/18-positive women with normal cytology had a 5-year CIN3+ risk of 18.1% (95% CI, 9.4%–33.9%). When further triaging these women with cytology after 12 months, the 5-year CIN3+ risk after negative repeat cytology tended to be lower (11.3%; 95% CI, 0.00%–19.2%; $P=0.057$). For CIN2+, the 5-year risk in HPV 16/18-positive women with normal cytology was significantly lower after a negative repeat cytology test (24.6%; 95% CI, 16.7%–30.2% vs. 13.4%; 95% CI, 2.8%–21.0%; $P=0.011$).

Interestingly, HPV-non 16/18-positive women with normal cytology at baseline had a 5-year CIN3+ risk of 3.5% (95% CI, 1.7%–5.3%). This is comparable with the risk of women with a negative repeat cytology triage test result (4.1%; 95% CI, 0.44%–5.9%;

P=0.89). When these HPV-non 16/18-positive women with normal cytology had a negative repeat cytology test at 12 months, 5-year CIN3+ risk became very low (0.42%; 95% CI, 0.00–1.4%), although this was not significantly lower (P=0.11). For CIN2+, HPV-non 16/18-positive women had a borderline significantly lower 5-year risk after a negative repeat cytology test (7.9%; 95% CI, 5.7%–10.4% vs. 3.5%; 95% CI, 0.00%–6.2%; P=0.049).

Finally, we stratified women into two age groups: women invited for screening for the first time (i.e., 29–33 years) and women who were invited previously (i.e., ≥34 years). Five-year CIN3+ and CIN2+ risks for these groups are shown in Table 3 and Supplementary Figure S1. For younger women with a negative HPV test, the 5-year CIN3+ risk was 0.10% (95% CI, 0.0%–0.22%). This risk was comparable with the risk of their older counterparts 0.08% (95% CI, 0.02–0.14%). For CIN2+, a statistically significant difference between these age groups was found: 0.42% (95% CI, 0.06%–0.68%) versus 0.16% (95% CI, 0.08%–0.22%), respectively (P=0.006). For younger and older women with a positive HPV test, no differences in 5-year CIN3+ risks were found. For those with normal cytology, 5-year CIN3+ risks were 9.5% (95% CI, 3.6%–12.8%) and 6.9% (95% CI, 3.5%–9.4%), respectively (P=0.206). For those with BMD cytology, CIN3+ risks were 27.5% (95% CI, 13.7%–43.1%) and 20.0% (95% CI, 11.8%–26.4%), respectively (P=0.243).

Table 3. Five-year cumulative incidences for CIN3+ and CIN2+ in first-screened and previously screened women.

		n	CIN3+			CIN2+		
			n	Incidence	95% CI	n	Incidence	95% CI
First-screened women (29-33 years)	HPV-negative	3.227	4	0.10%	(0.0-0.22%)	20	0.42%	(0.06-0.68%)
	HPV-positive, normal cytology	308	30	9.5%	(3.6-12.8%)	44	14.6%	(10.0-19.4%)
	HPV-positive, BMD cytology	51	16	27.5%	(13.7-43.1%)	28	49.0%	(35.3-62.8%)
Previously screened women (≥34 years)	HPV-negative	16.567	22	0.08%	(0.02-0.14%)	44	0.16%	(0.08-0.22%)
	HPV-positive, normal cytology	597	42	6.9%	(3.5-9.4%)	70	11.7%	(7.8-14.7%)
	HPV-positive, BMD cytology	110	24	20.0%	(11.8-26.4%)	50	43.7%	(34.8-51.9%)

n = number of women

Discussion

Immediate referral of HPV-positive women with \geq BMD (ASCUS/LSIL) for colposcopy is based on the high short-term CIN2+ risk (3-year risk, $>20\%$). Here, we evaluated the 5-year CIN2+/3+ risk of HPV-positive women triaged by three different triage strategies, which has been shown to be acceptable in terms of safety and costs.^{5,15} Highest 5-year risks were found for HPV-positive women. HPV-positive women with BMD (ASC-US/LSIL) cytology had a 5-year CIN3+risk of 22.2%, for those with normal cytology this risk was 7.9%. HPV-negative women displayed substantially lower CIN3+ risks, i.e., 0.09%. The 5-year risks found in our study imply that the presence of HPV, regardless of the associated cytology test result, indicates a high risk for the development of high-grade CIN lesions. This is in agreement with other studies.^{15,16}

Colposcopy referral policies are determined based on short-term CIN 2/3+ risks. In the Netherlands, cytology at baseline and repeat cytology at 6–12 months have been shown to be a good and feasible triage strategy, with a short-term CIN3+risk of $<2\%$ and a positive predictive value (PPV) of $>20\%$.^{4,5} Therefore, this algorithm will be used in the Netherlands after the implementation of primary HPV-based screening in 2016.¹⁷ However, because the Netherlands maintains a 5-year screening interval for HPV-positive, triage test–negative women, it is important to know the 5-year CIN3+ risk of women who were HPV screen–positive, but were negative for the chosen triage strategy. Our study showed that HPV-positive women with normal cytology and normal repeat cytology after 12 months had a 5-year CIN3+ risk of 4.1%. HPV-positive women with normal cytology and a negative baseline HPV 16/18 genotyping test had a comparable 5-year CIN3+ risk of 3.5%. This shows that HPV-positive women with normal cytology and a negative triage test, either repeat cytology or baseline HPV 16/18 genotyping still have a non-negligible 5-year CIN3 risk of about 4%. Therefore, these women must be followed-up in the next screening round. Given these data, extension of the screening interval over 5 years only seems possible for HPV screen–negative women who had a very low 5-year CIN3+ risk of 0.09%.

Triaging HPV-positive women with cytology and baseline HPV 16/18 genotyping has the advantage, compared with repeat cytology, that it identifies women with a high risk of underlying high-grade disease at baseline.^{5,15,18,19} Therefore, no additional doctor's visit is required, and no associated risk of losing women to follow-up during triage is present.^{20,21} A disadvantage of this triage algorithm is that previous studies, comparing triage strategies, show that baseline HPV 16/18 genotyping leads to a higher referral rate than repeat cytology does (43.3%–54.5% for HPV 16/18 genotyping

and 33.4%– 44.8% for repeat cytology^{5,15}), resulting in higher costs and a greater risk of overtreatment and cervical morbidity. Also, in this study, the referral rate of HPV-positive women with normal cytology triaged by baseline HPV 16/18 genotyping was 28.6%, whereas this was 15.0% in case of repeat cytology triage ($P < 0.001$).

The acceptance of risks may vary between countries, and the preferred triage strategy will depend on the resources available.

Another point of discussion is whether HPV testing leads to overdiagnosis of high-grade CIN lesions in younger women.²² The CIN3+ and CIN2+ risks of HPV-positive women with both normal cytology or BMD cytology between younger and older women were comparable. This indicates that HPV screening does not lead to overdiagnosis in younger women, which is supported by results from previous studies.^{23,24} However, younger HPV-negative women had a significantly higher CIN2+ risk than their older counterparts. We hypothesize that this difference can be explained by the fact that younger women are more likely to become infected with HPV and subsequently develop CIN2 lesions. However, for both younger and older HPV-negative women, risks were so low (0.42% vs. 0.16%) that they do not require different management. Furthermore, this study shows that HPV screen-negative women have a very low 5-year risk (i.e., 0.09% (0.21%) for CIN3+(2+)). This is in line with results from other studies with even longer follow-up and supports further extension of the screening interval above 5 years for HPV-negative women.^{23,25–30}

In this study, there are a number of differences in comparison with the HPV-based screening program starting in the Netherlands in 2016. First, conventional cytology was used. Studies show that conventional cytology performs similar to liquid-based cytology in terms of sensitivity and specificity. Therefore, it is unlikely that this will have influenced the study results.^{31–33} Second, cytotechnicians in this study were blinded to the HPV test results. In a randomized trial where cytotechnicians were informed about HPV test results, this information had only a small effect on cytology assessment.³⁴ In order to completely overcome this influence, we suggest the use of more objective tests, such as molecular markers.³⁵

The strengths of this study are the large size of the cohort of women, its setting within the regular screening program, making results easily convertible to the general population, and the relatively long follow-up. A disadvantage is the relatively low attendance rate at repeat testing of the HPV-positive women with normal cytology (i.e., 59.7% within 3 years). In the original design of the study, women with normal cytology were not informed about their HPV status, which was done to maximize

attendance at repeat testing among HPV-negative women.⁴ Nevertheless, program compliance was fairly good, with an attendance at the next screening round of 81.7%.

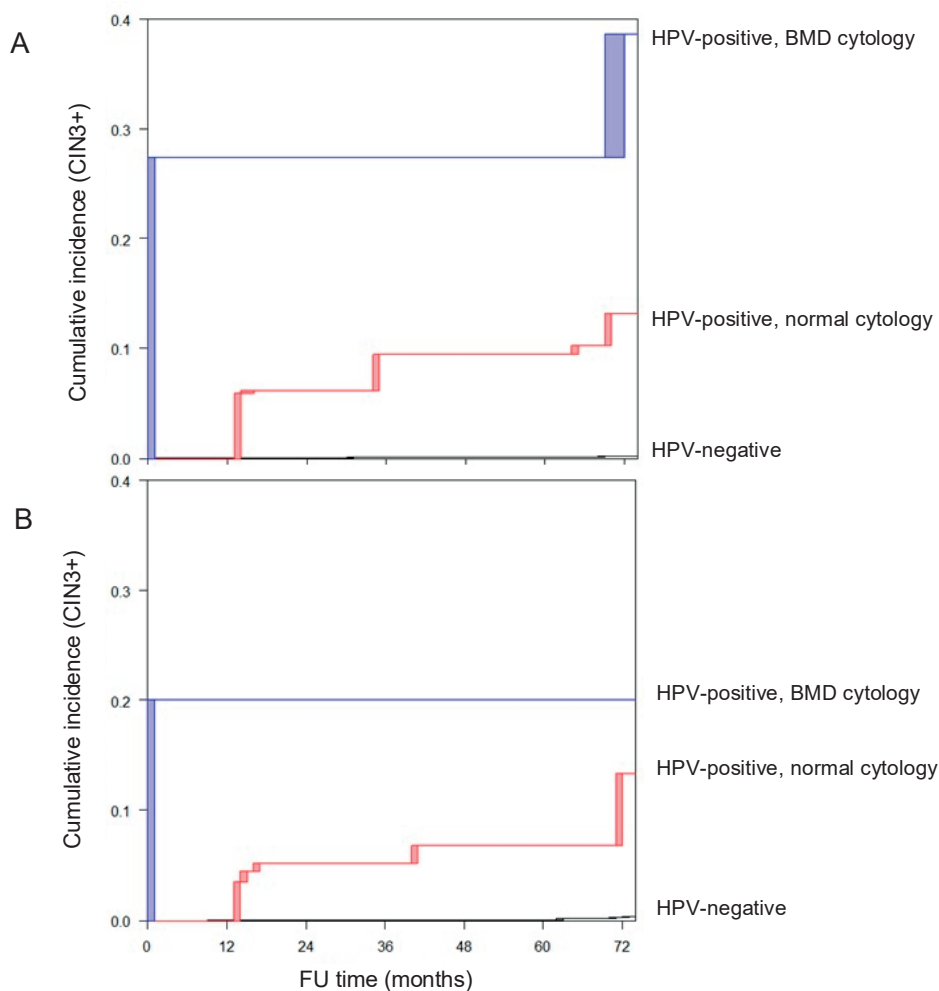
In conclusion, HPV-positive women have a high risk for developing high-grade CIN lesions. Because the Netherlands maintains a 5-year screening interval for HPV-positive, triage test-negative women, it is important to know the 5-year CIN3+ risk of women who were HPV screen-positive, but were negative for the chosen triage strategy. Longitudinal analysis shows that HPV-positive women with normal cytology and a negative triage test, either repeat cytology after 12 months or baseline HPV 16/18 genotyping, develop a non-negligible CIN3+ risk over 5 years. Therefore, these women must be followed-up in the next screening round. Extension of the screening interval over 5 years only seems possible for HPV screen-negative women.

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Supplementary Figure S1. Cumulative incidence curves for CIN3+ of (A) women invited for the first time (29-33 years) and (B) older women (>34 years).

CIN = cervical intraepithelial neoplasia (grade 3 or higher); HPV = human papillomavirus; BMD = borderline/mild dyskaryosis.