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2019

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citation for published version (APA)

Polman, N. J. (2019). *HPV-based cervical screening: Challenges and future perspectives*. [PhD-Thesis - Research and graduation internal, Vrije Universiteit Amsterdam].

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Chapter 5

HPV-positive women with normal cytology remain at increased risk of CIN3 after a negative repeat HPV test

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British Journal of Cancer 2017; 117(10):1557-1561.

Abstract

Background: In human papillomavirus (HPV)-based screening, a repeat HPV test is often recommended for HPV-positive women with normal cytology (HPV-pos/cyt-neg), but its absolute risk of cervical precancer (CIN3+) over two screening rounds needs to be assessed.

Methods: We compared the 5-year risk of HPV infection and CIN3+ in HPV-pos/cyt-neg women with a negative repeat HPV test to the risk in HPV-negative women with normal cytology (double negatives) in the POBASCAM cohort. We obtained histology data from the Dutch pathology registry (PALGA).

Results: Human papillomavirus infection risk was 20.4% (19 of 93) in HPV-pos/cyt-neg, repeat HPV-negative women and 3.2% (294 of 9186; $P < 0.001$) in double negatives. Corresponding CIN3+ risks were 2.0% (4 of 199) and 0.2% (41 of 18 562; $P < 0.001$). Infection risks were also increased in type-specific analyses of HPV16, 31, 33, 39, 52, 56 and 58.

Conclusions: HPV-pos/cyt-neg women continue to have an increased CIN3+ risk, also when the repeat HPV test is negative. Therefore, intervals in primary HPV screening should be determined separately for HPV-positive and -negative women.

Introduction

Human papillomavirus (HPV) testing provides better protection against cervical cancer and high-grade cervical intraepithelial neoplasia (CIN) than cytology.^{1,2} Consequently, in several countries cytology is replaced by HPV (DNA) as primary screening test. Only a small proportion of HPV-positive women have cervical disease. To reduce the number of referrals, adjunct testing is required to detect the subset of HPV-positive women with CIN grade 3 or worse (CIN3+). However, there is still no general consensus about the most suitable triage strategy.

Options for stratification of HPV-positive women include reflex cytology, HPV16/18-genotyping and repeat HPV testing.^{3,5} Post hoc evaluations of data collected within one screening round indicate that repeat HPV testing is associated with a high number of colposcopy referrals.^{4,6} The absolute CIN3+ risk after a negative repeat HPV test is assumed to be low, but can only be assessed with data from two screening rounds as many women with a negative repeat HPV test are referred to routine screening.

In this study, we compare the 5-year risk of HPV infection and CIN3+ in HPV-positive women with normal cytology (HPV-pos/cyt-neg) and a negative repeat HPV test to the risk in HPV-negative women with normal cytology (double negatives). We used data from the intervention group of the POBASCAM (Population Based Screening Study Amsterdam) cohort^{7,8} in which women were screened with both HPV and cytology in two rounds 5 years apart.

Materials and methods

Study population and procedures

The POBASCAM trial (Trial registration ID: NTR218) was designed to assess whether HPV testing in the first screening round decreases detection of CIN3 and cervical cancer in the second screening round.^(7, 8) Women aged 29–61 years old were randomised (1 : 1) to cytology and HPV co-testing (intervention group) or cytology only (control group). The intervention group of the POBASCAM trial consists of 19 999 eligible women (≤56 years of age, no hysterectomy, no abnormal cytology in the preceding two years, valid HPV test). Thirty-three women without valid cytology and 680 women with abnormal cytology were excluded, leaving 19 286 women with normal cytology. In the second round after five years, women were managed according to the intervention group protocol. A detailed description of the

management is available.^{7,8} The POBASCAM trial was approved by the Medical Ethics Committee of the VU University Medical Centre (Amsterdam, the Netherlands; no 96/103) and the Ministry of Public Health (The Hague, the Netherlands; VWS no 328650). All participants provided written informed consent.

The HPV test (GP5+/6+-PCR EIA) detects 14 HPV types (16/18/31/33/35/39/45/51/52/56/58/59/66/68) and was done blinded to cytology.^{9,10} Human papillomavirus -positive samples were typed by a reverse line blot assay.¹¹

At colposcopy visit, biopsies were taken from suspected areas.^{12,13} Histological examination was done locally and samples were classified as CIN0, CIN1, CIN2, CIN3 or invasive cancer.¹⁴ Adenocarcinoma in situ was added to CIN3. Cytology and histology were identified through the nationwide network and registry of histology and cytopathology in the Netherlands (PALGA).¹⁵ Follow-up results were encrypted and linkage was conducted based on last name, year of birth, enrolment cytology registry number, date of sample collection and laboratory.

Statistical analysis

HPV-pos/cyt-neg women with a negative repeat HPV test were compared with double (HPV and cytology)-negative women (Figure 1). A negative repeat HPV result was defined as an HPV-negative test result at first repeat test scheduled at 6 months. In additional analyses, the subgroup of HPV-pos/cyt-neg women with an HPV-negative test result was extended with women with a positive repeat HPV test at 6 months followed by a negative repeat HPV test at 18 months.

Five-year risk of HPV infection and CIN3+/2+ were compared using Fisher's exact and the Mantel-Haenszel test, the latter one adjusting for age differences. Risk ratios (RR) were calculated. Analyses were performed with SPSS version 22. Five-year risk of HPV infection was based on HPV results at the second screening round. A screening test was assigned to the second screen when taken between 4 and 9 years after enrolment. Screening results and histology occurring more than 9 years after enrolment were excluded. For the HPV infection risk analysis, women with CIN2+ or uterus extirpation before 4 years were excluded. HPV infection risk was calculated separately for all 14 HPV types.

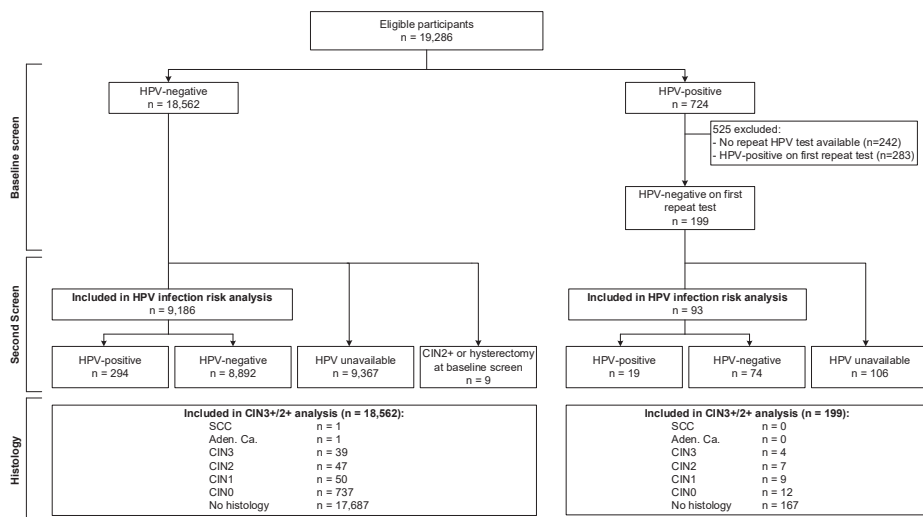


Figure 1. Flowchart of women in the POBASCAM intervention group with normal cytology, including information on HPV repeat testing, HPV result at second screen and histology.

Aden. Ca.=adenocarcinoma; CIN(2+)=cervical intraepithelial neoplasia (grade 2 or worse); HPV=human papillomavirus; SCC=squamous cell carcinoma.

Results

Study cohort characteristics

Seven hundred twenty-four out of 19 286 (3.8%) women with normal cytology had a positive HPV result at the baseline screen, 199 of whom had a negative HPV result at the first repeat test (Figure 1). Mean age was 37.9 (range 29–55). Mean time to first repeat HPV test was 9.8 months (range 3.0–29.7). Fifty-seven HPV-pos/cyt-neg women with an HPV positive result at the first repeat test had an HPV-negative result at the second repeat test (time from baseline to second repeat test: 19.9 months, range 12.0–27.6). Mean age of 18 562 women with a negative HPV result at baseline screen was 41.4 (range 29–56).

HPV infection risk

Women without an HPV result at the second screening round (n=9 473) and women with a CIN2+ or hysterectomy at baseline (n=9) were excluded, leaving 93 HPV-pos/cyt-neg women with a negative first repeat HPV result and 9186 HPV-neg/cyt-neg women (Figure 1). HPV-neg/cyt-neg women without an HPV result at the second screening round were slightly older than those with an HPV result (mean age: 41.8 vs 40.9; P<0.01). Among HPV-pos/cyt-neg, repeat HPV-negative women, age did not differ between those with and without an HPV result at the second screening round.

Mean time from baseline to second screen was 59.9 months (range 48.1–81.6) in HPV-pos/cyt-neg women with an HPV-negative repeat test, and 60.8 months (range 48.0–102.8) in double negatives. HPV-pos/cyt-neg women who tested HPV-negative at the first repeat test had a 20.4% (19 of 93) HPV infection risk at the second screen 5 years later (Table 1, left columns). In comparison, HPV infection risk in double-negative women was 3.2% (294 of 9186) and significantly lower (RR 6.4; $P < 0.001$). After correction for age, the relative risk remained statistically significant (RR 10.8; $P < 0.001$). In 10 of 16 women with valid HPV genotypes in the baseline and second screening round, the HPV types detected in the second round were also found at baseline. In HPV-pos/cyt-neg women who tested HPV-negative at the first or second repeat test, similar relative risks were obtained (Table 1, right columns).

HPV16-pos/cyt-neg women with an HPV16-negative repeat test had a 5-year HPV16 infection risk of 17.6%, compared with 0.8% in HPV16-neg/cyt-neg women (RR 22.1; $P < 0.001$). Type-specific infection risks were also increased for HPV type 31, 33, 39, 52, 56 and 58 (Table 1, left columns).

CIN3+ and CIN2+ risk

Thirty-two (16.1%) of 199 HPV-pos/cyt-neg women with a negative repeat HPV result and 875 out of 18 562 (4.7%) double negatives had a histology diagnosis within 9 years (Figure 1). Mean time from baseline to histology diagnosis was 52.4 months (range 0.03–107.4). Among 57 HPV-pos/cyt-neg women with an HPV-negative result at second repeat test, 11 (19.3%) had a histology diagnosis within 9 years (mean time from baseline to histology diagnosis was 51.7 months, range 5.6–86.2). Cervical intraepithelial neoplasia grade 3+ and CIN2+ risks are shown in Table 2. Cumulative CIN3+ risk in HPV-pos/cyt-neg women with a negative first repeat HPV result was 2.0%, and significantly higher than the 0.2% risk in double-negative women (RR 9.1, $P < 0.001$). For CIN2+, risks were 5.5% and 0.5% (RR 11.7, $P < 0.001$), respectively.

In six HPV-pos/cyt-neg women with a negative repeat HPV result and CIN2+, HPV typing results were available in the sample prior to CIN2+ (16, 31, 33, 58, 59, and multiple infection 18 of 52). The same HPV types were found in the corresponding baseline sample.

Table 1, Risk of (type-specific) HPV infection at second screen in HPV-pos/cyt-neg women with negative repeat HPV result and in HPV-neg/cyt-neg women,

Baseline test result	Repeat test result	HPV-negative test result at first repeat test			HPV-negative test result at first or second repeat test		
		Total	n	%	Total	n	%
HPV-positive (all types)	HPV-negative	93	19	20.4%	131	24	18.3%
HPV-negative (all types)	-	9,186	294	3.2%	9,186	294	3.2%
				sign (p<0.001)			sign (p<0.001)
HPV16-positive	HPV16-negative	17	3	17.6%	26	4	15.4%
HPV16-negative	-	9,262	74	0.8%	9,291	74	0.8%
				sign (p<0.001)			sign (p<0.001)
HPV18-positive	HPV18-negative	6	0	0.0%	9	1	11.1%
HPV18-negative	-	9,273	25	0.3%	9,308	25	0.3%
				n.s.			sign (p=0.030)
HPV31-positive	HPV31-negative	11	1	9.1%	15	1	6.7%
HPV31-negative	-	9,268	30	0.3%	9,302	30	0.3%
				sign (p=0.036)			sign (p=0.049)
HPV33-positive	HPV33-negative	3	1	33.3%	4	1	25.0%
HPV33-negative	-	9,276	13	0.1%	9,313	13	0.1%
				sign (p=0.005)			sign (p=0.006)
HPV35-positive	HPV35-negative	4	0	0.0%	6	0	0.0%
HPV35-negative	-	9,275	10	0.1%	9,311	10	0.1%
				n.s.			n.s.
HPV39-positive	HPV39-negative	5	1	20.0%	9	2	22.2%
HPV39-negative	-	9,274	16	0.2%	9,308	16	0.2%
				sign (p=0.009)			sign (p<0.001)
HPV45-positive	HPV45-negative	5	0	0.0%	10	1	10.0%
HPV45-negative	-	9,274	26	0.3%	9,307	26	0.3%
				n.s.			sign (p=0.029)
HPV51-positive	HPV51-negative	7	0	0.0%	10	0	0.0%
HPV51-negative	-	9,272	28	0.3%	9,307	28	0.3%
				n.s.			n.s.
HPV52-positive	HPV52-negative	7	2	28.6%	10	2	20.0%
HPV52-negative	-	9,272	20	0.2%	9,307	20	0.2%
				sign (p<0.001)			sign (p<0.001)

Table 1, Risk of (type-specific) HPV infection at second screen in HPV-pos/cyt-neg women with negative repeat HPV result and in HPV-neg/cyt-neg women, (continued)

Baseline test result	Repeat test result	HPV-negative test result at first repeat test			HPV-negative test result at first or second repeat test		
		Total n	n	%	Total n	n	%
HPV56-positive	HPV56-negative	8	1	12.5%	12	1	8.3%
HPV56-negative	-	9,271	24	0.3%	9,305	24	0.3%
				sign (p=0.021)			sign (p=0.032)
HPV58-positive	HPV58-negative	9	1	11.1%	12	2	16.7%
HPV58-negative	-	9,270	12	0.1%	9,305	12	0.1%
				sign (p=0.013)			sign (p<0.001)
HPV59-positive	HPV59-negative	4	0	0.0%	5	0	0.0%
HPV59-negative	-	9,275	11	0.1%	9,312	11	0.1%
				n.s.			n.s.
HPV66-positive	HPV66-negative	10	0	0.0%	13	0	0.0%
HPV66-negative	-	9,269	30	0.3%	9,304	30	0.3%
				n.s.			n.s.
HPV68-positive	HPV68-negative	4	0	0.0%	4	0	0.0%
HPV68-negative	-	9,275	2	0.0%	9,313	2	0.0%
				n.s.			n.s.

HPV = human papillomavirus; NS = not significantly different when compared with HPV-neg/cyt-neg women; sign = significantly different when compared with HPV-neg/cyt-neg women.

Table 2. Risk of CIN3+/2+ in HPV-pos/cyt-neg women and in HPV-neg/cyt-neg women

Baseline	Repeat test result	Total	CIN3+		CIN2+		
		n	n	%	n	%	
HPV-pos/ cyt-neg	HPV-negative test result at first repeat test	199	4	2.0%	sign (p=0.001)	11	5.5% (p<0.001)
HPV-pos/ cyt-neg	HPV-negative test result at first or second repeat tests	256	9	3.5%	sign (p<0.001)	18	7.0% (p<0.001)
HPV-neg/ cyt-neg	-	18,562	41	0.2%	-	88	0.5% -

CIN3/2+ = cervical intraepithelial neoplasia grade 3/2 or worse; HPV = human papillomavirus; NS = not significantly different when compared with HPV-neg/cyt-neg women; sign = significantly different when compared with HPV-neg/cyt-neg women.

Discussion

The results show that HPV-pos/cyt-neg, repeat HPV-negative women have a significantly higher risk of overall and type-specific HPV infection and CIN3+ compared with double negatives. There are at least two possible explanations for the alternating HPV-positive, -negative, -positive pattern. When the new infection is of a different type than found in the baseline round, the pattern reflects viral clearance followed by a newly acquired infection. In our study, this only holds for a minority of the infections and only those without CIN2+. When the same HPV type is observed in the baseline and second round, the pattern may still reflect reinfection as natural immunity after clearance offers only limited protection¹⁶, but it may also be caused by a temporary decrease in viral load below the detection threshold.¹⁷⁻²⁰ The latter explanation is important for defining screening algorithms. It suggests that once infected, women remain at increased HPV and CIN3+ risk, also after a negative repeat HPV test, and that screening intervals in primary HPV screening programmes should be determined separately for HPV-positive and -negative women.

A limitation of the POBASCAM study is that histology diagnosis was done by local pathologists. However, interobserver reliability of CIN3+ was very high.¹⁰ Another limitation is that follow-up screening was incomplete, mainly because only a cytological assessment was requested by the general practitioners in accordance with the standard screening guidelines in the Netherlands at that time. Attendance in the second round was 88% in HPV-pos/cyt-neg, repeat HPV test negative women and 85% in double negatives. Second round HPV was missing in 53.3% and 50.4% in the two strata, respectively. Together, this means that the incomplete follow-up may negatively bias CIN risk estimates, but similarly in the two study strata.

The results of this study show that HPV-pos/cyt-neg women continue to have an increased CIN3+ risk, also when the repeat HPV test is negative. Therefore, intervals in primary HPV screening should be determined separately for HPV-positive and -negative women, and a triage testing algorithm should optimally weigh CIN3+ risk and colposcopy referral rate. In our viewpoint, a triage strategy that uses repeat cytology seems appropriate as the colposcopy referral rate will be approximately 40% lower in comparison with repeat HPV testing.^{4,5}

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