Risk of Cervical Intraepithelial Neoplasia Grade 3 or Worse in HPV-Positive Women with Normal Cytology and Five-Year Type Concordance: A Randomized Comparison

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ABSTRACT

Background: In human papillomavirus (HPV)-based cervical screening programs, management of HPV-positive women with normal cytology is debated. Longitudinal information on HPV type persistence may be employed for risk stratification.

Methods: We assessed the risk of cervical intraepithelial neoplasia grade 3 or worse (CIN3+) after repeatedly testing positive for the same HPV type(s) in the randomized population-based screening study Amsterdam (POBASCAM). We compared 18-month CIN3+ risks in HPV-positive women (intervention, $n = 1{,}066$) to those in HPV-positive/cytology-negative women who tested HPV-positive in the next screening round (control, $n = 111$) five years later, stratified for HPV type concordance.

Results: The 18-month CIN3+ risk was 15% in HPV-positive women in the intervention group, 40% in the control group after two-round type concordance (relative risk 2.6, 95% confidence interval 1.9–3.4), and 20% in the control group after a type switch (1.3, 0.5–3.2). The relative increase in CIN3+ risk after two-round type concordance was similar in <35-year-old (3.0, 2.0–4.4) and older women (2.2, 1.4–3.5), and was high in high-risk HPV-positive women who were HPV16/18/31/33/45-negative in both rounds (9.9, 4.4–21.9).

Conclusions: Five-year HPV type concordance signals high CIN3+ risk and warrants referral for colposcopy without additional cytology triage.

Impact: HPV screening programs become highly efficient when HPV-positive women with negative triage testing at baseline are offered repeat HPV genotyping after five years.

Introduction

Given the causal role of human papillomavirus (HPV) infection in the development of cervical cancer (1), more and more countries are shifting toward HPV-based cervical screening programs (2–4). Diagnostic studies and screening trials have shown increased detection of cervical intraepithelial neoplasia grade 3 (CIN3) and better protection against invasive cervical cancer by HPV screening as compared with cytology (5, 6). On the other hand, HPV testing has a lower specificity than cytology (7), yielding a larger number of unnecessary colposcopy referrals and considerable extra demand of health resources.

To improve the efficiency of HPV-based screening, triage testing is recommended for HPV-positive women. Cytology is most often used as a first triage method, but there is still no consensus on how to best manage HPV-positive/cytology-negative women. This is a challenging problem, because their five-year risk for CIN3 or worse (CIN3+) is substantially higher than the five-year risk among HPV-negative women, even after a single negative repeat HPV test at 6 to 12 months (8).

HPV persistence over consecutive screening tests has been previously identified as a disease marker for HPV-positive/cytology-negative women (9, 10). However, its specificity depends on the time between tests, with shorter duration generally yielding increased number of referrals without CIN3+ (11). Retesting after three to five years for assessing (type) persistence instead of one year (12–14) as currently employed in some settings (14), may increase the efficiency of HPV-based screening programs, but the impact of postponing retesting beyond one year in women with positive HPV and negative cytology test results remains to be assessed.

In this study, we utilize a randomized setting with genotyping to demonstrate the potential of five-year HPV type persistence, here defined as type concordance, as a disease marker for HPV-positive/cytology-negative women. The Population-based Screening Amsterdam (POBASCAM) trial offers a unique opportunity to study risk profiles based on longitudinal HPV type concordance, because participating women received HPV (DNA) testing in two consecutive screening rounds five years apart (15). Women in the intervention group were managed by HPV and cytology in both rounds, whereas women in the control group were managed by cytology in the baseline round (with blinded HPV testing) and by HPV and cytology in the next round. This enabled us to study the effect of five-year HPV type concordance on the CIN3+ risk in a randomized way. More specifically, we estimated the CIN3+ risk in women with HPV type concordance between the baseline and next round and compared this with the CIN3+ risk in HPV-positive women in the baseline round.

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We repeated the analysis for end-point CIN grade 2 or worse (CIN2+) as well as in subgroups defined by HPV genotype and by age.

Materials and Methods

Study population and design

The POBASCAM study is a prospective randomized controlled trial (trial registration ID: NTR218) conducted in the setting of the regular cervical cancer screening program in the Netherlands. It was designed to assess whether HPV testing in screening decreases detection of CIN3 and cervical cancer in the next screening round five years later, as compared with cytology alone (15–18). In brief, 44,102 eligible consenting women aged 29 to 61 years were randomized (1:1) to cytology and HPV cotesting (intervention group) or cytology only (control group).

For this study, we included women who were positive at baseline on the generic HPV test and tested positive for at least one of the 14 high-risk HPV types (**Fig. 1**). In the intervention group, HPV-positive women with moderate dyskaryosis or worse (comparable with >ASC-US/LSIL; ref. 19) were immediately referred for colposcopy. HPV-positive women with negative cytology or borderline/mild dyskaryosis (ASC-US/LSIL) were advised to repeat both HPV and cytology testing after 6 and 18 months. HPV-positive women with negative cytology at baseline were referred for colposcopy if 6-month cytology was >ASC-US/LSIL. HPV-positive women with ASC-US/LSIL at baseline were referred for colposcopy if 6-month cytology was >ASC-US/LSIL or if 6-month test results were HPV-positive and ASC-US/LSIL. HPV-positive women with negative cytology or ASCUS/LSIL at baseline were also referred for colposcopy if 18-month HPV test result was positive and/or 18-month cytology was >ASC-US/LSIL. In the control group, cytology-negative women (blinded HPV test result) were invited to the next routine screening round after five years. At the second screen five years after baseline, women in both study groups were managed according to the intervention group protocol.

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 women gave informed written consent. The study was performed in accordance with the 1964 Declaration of Helsinki and its later amendments.

Laboratory testing

HPV DNA testing was done by a clinically validated generic HPV test (GP5+/6+ PCR-EIA), that detects 14 high-risk HPV types (types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68), blinded for cytology results (20). EIA-positive specimens were genotyped by reverse line blotting (21). A sample was considered to have a positive genotyping result when a positive probe signal for one or more of the 14 high-risk HPV types was seen on reverse line blot. Conventional cytologic smears were classified according to the CISOE-A framework used in the Netherlands (19). During colposcopy, biopsies were taken from suspected areas according to standard procedures in the Netherlands (22, 23). Histologic specimens were examined locally and classified as no dysplasia, CIN grade 1, 2, 3, or invasive cancer, according to international criteria (24). Adenocarcinoma *in situ* was

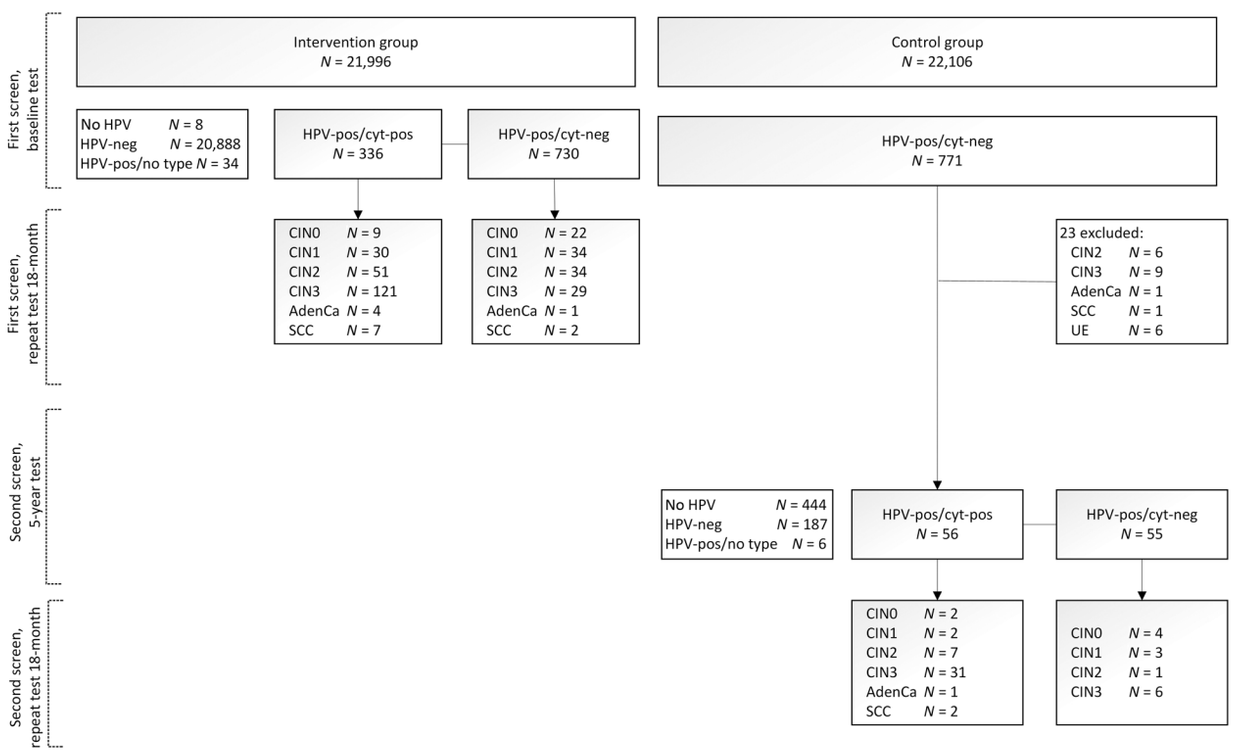

Figure 1.

Flowchart of the POBASCAM women included in this analysis, with information on test results at baseline and at second round and on histology. AdenCa, adenocarcinoma; CIN, cervical intraepithelial neoplasia; cyt, cytology; HPV, human papillomavirus; neg, negative; pos, positive; SCC, squamous cell carcinoma; UE, uterus extirpation.

added to CIN3. In this analysis, we included histology results up to nine years after baseline, which were obtained from the nationwide network and registry of histo- and cytopathology in the Netherlands (PALGA Foundation, Houten, the Netherlands).

Statistical analysis

We calculated 18-month CIN3+ risks in the baseline round and next round of the POBASCAM cohort. Regarding the calculations for the baseline round, only high-risk HPV-positive women in the intervention group were included. Regarding the calculations for the next round, women from the control group with a high-risk HPV-positive/cytology-negative result in the baseline round and no CIN2+ or uterus extirpation (UE) before the next round were included (**Fig. 1**). The term 18-month risk reflects that repeat testing in the POBASCAM study was scheduled at 6 and 18 months, but we included histology up to four years after the corresponding screening visit. This means that in the intervention group the results of the next round after five years did not contribute to the analysis, whereas in the control group histology up to nine years after the baseline screen was included for risk calculation (15). The 18-month risks in the next round were calculated separately for women with HPV type concordance between the baseline and next round and for women with a type switch. Here, type concordance is defined as positivity at the next screen for at least one of the high-risk HPV types present at the baseline screen and type switch is defined as positivity for all different type(s) than detected at baseline. Relative risks (RR) for detection of CIN3+ were calculated, together with 95% confidence intervals (CI), where the comparison was between intervention and control group. We adopted an intention-to-treat analysis, which means that we did not adjust the absolute risk estimates for loss-to-follow-up and verification bias.

The CIN3+ risks were also compared in women with normal cytology in the baseline round (intervention group) and in the next round (control group), and in women aged <35 and ≥35 years at baseline. The cut-off of 35 years was used to separate the first screening round from later rounds of the Dutch screening program. The CIN3+ risks were also compared in the subgroups defined by HPV16, by HPV16/18, by the subgroup of the five HPV types most prevalent in cervical cancer (25), that is, HPV16/18/31/33/45, and other high-risk HPV types. All analyses were repeated for end-point CIN2+.

The RR were evaluated by means of χ^2 testing. Fisher's exact test was used when at least one cell frequency was <5. Heterogeneity of RR across age was assessed by Mantel–Haenszel testing. Analyses were performed using STATA version 14.1 (StataCorp).

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Results

Study subjects

One thousand sixty-six of 21,996 women from the intervention group had a positive HPV test result and a positive genotyping result at the baseline screen, 730 of whom (3.3% of total) had a negative cytology triage. Histology outcomes are shown in **Fig. 1**. Mean age of the 1,066 HPV-positive women in the intervention group was 37.8 (range 29–60) years and 38.4 (range 29–60) years in the subset of 730 HPV-positive/cytology-negative at baseline. Seven hundred seventy-one of 22,106 women from the control group (3.5%) had a positive HPV test result with a positive genotyping result and negative cytology at the baseline screen. The mean age was 38.6 (range 29 − 61). Twenty-three women developed CIN2+ or had UE during the baseline round and were excluded. Of the remaining 748 HPV-positive/cytology-negative women from the control group, 59% (444/748) had no HPV test result at the next screen, 25% (187/748) were HPV-negative, 15% (111/748) were HPV-positive with a positive genotyping result, and the remaining <1% (6/748) were HPV-positive by the generic high-risk HPV test but negative for any of the 14 high-risk HPV types at genotyping. Of the HPV-positive group with positive genotyping results, mean age was 37.0 (range 29–55) years, 82% (91/111) had a type concordant infection as compared with the baseline screen, and 50% (55/111) had negative cytology.

Progression to CIN3+ and CIN2+

HPV-positive women from the intervention group had a 15% 18-month CIN3+ risk in the baseline round (**Table 1**). HPV-positive/cytology-negative women from the control group had an increased 18-month CIN3+ risk in the next round after two-round type concordance (40%, RR 2.6, 95% CI, 1.9–3.4; $P < 0.001$), but not after a type switch (20%, $P > 0.1$). The RR for endpoint CIN3+ after type concordance was not related to age (RR 3.0, 95% CI, 2.0–4.4 if age <35 vs. 2.2, 95% CI, 1.4–3.5 if age ≥35; $P = 0.33$). For endpoint CIN2+, increased risks were also observed after two-round type concordance ($P < 0.001$), but not after type switch ($P > 0.1$).

Results stratified for HPV genotype(s) are presented in **Table 2**. The 18-month CIN3+ risk was 30% in HPV16-positive women from the intervention group, and a substantially higher risk was observed in HPV16-positive/cytology-negative women from the control group after two-round HPV16 concordance (61%, RR 2.0, 95% CI, 1.5–2.7; $P < 0.001$). Stratified for age, the RR for endpoint CIN3+ was 2.3 (95% CI, 1.6–3.2) if age <35 and 1.7 (95% CI, 1.0–2.9) if age ≥35. In women with a high-risk HPV-positive/HPV16-negative result, the 18-month CIN3+ risk in the intervention group was 8% and the 18-month CIN3+ risk was substantially elevated in cytology-negative women from the control group who were HPV16-negative at the next screen and had a two-round type concordant test result (22%, RR 2.7, 95% CI, 1.5–4.7; $P = 0.001$). Increased CIN3+ risks after

Table 1. CIN3+ and CIN2+ in HPV-positive women in the baseline round (intervention group) versus next round (control group, in bold).

			CIN3+			CIN2+		
Baseline round	**Next round**	***N***	***n***	**%**	**RR (95% CI)**	***n***	**%**	**RR (95% CI)**
HPV-pos		1,066	164	15%	1.0 (ref)	249	23%	1.0 (ref)
HPV-pos/cyt-neg		730	32	4%		66	9%	
HPV-pos/cyt-neg	**HPV-pos**	**111**	**40**	**36%**	**2.3 (1.8–3.1)**	**48**	**43%**	**1.9 (1.5–2.4)**
	type concordance	**91**	**36**	**40%**	**2.6 (1.9–3.4)**	**43**	**47%**	**2.0 (1.6–2.6)**
	type switch	**20**	**4**	**20%**	**1.3 (0.5–3.2)**	**5**	**25%**	**1.1 (0.5–2.3)**

Abbreviations: cyt, cytology; HPV, human papillomavirus; neg, negative; pos, positive.

Table 2. CIN3+ and CIN2+ in women with HPV type(s) 16, 16/18, 16/18/31/33/45 or other high-risk type(s) infection in the baseline round (intervention group) versus type concordant infection in the next round (control group, in bold).

			CIN3+			CIN2+		
Baseline round	**Next round**	***N***	***n***	**%**	**RR (95% CI)**	***n***	**%**	**RR (95% CI)**
HPV16-pos		343	103	30%	1.0 (ref)	140	41%	1.0 (ref)
HPV16-pos/cyt-neg		211	24	11%		40	19%	
HPV16-pos/cyt-neg	**HPV16-pos**	**38**	**23**	**61%**	**2.0 (1.5–2.7)**	**24**	**63%**	**1.5 (1.2–2.0)**
hrHPV-pos and HPV16-neg		723	61	8%	1.0 (ref)	109	15%	1.0 (ref)
hrHPV-pos/cyt-neg and HPV16-neg		519	8	2%		26	5%	
hrHPV-pos/cyt-neg and HPV16-neg	**At least one concordant type and HPV16-neg**	**49**	**11**	**22%**	**2.7 (1.5–4.7)**	**16**	**33%**	**2.2 (1.4–3.4)**
HPV16/18-pos		427	113	26%	1.0 (ref)	161	38%	1.0 (ref)
HPV16/18-pos/cyt-neg		263	27	10%		48	18%	
HPV16/18-pos/cyt-neg	**At least one concordant type**	**49**	**25**	**51%**	**1.9 (1.4–2.6)**	**27**	**55%**	**1.5 (1.1–1.9)**
hrHPV-pos and HPV16/18-neg		639	51	8%	1.0 (ref)	88	14%	1.0 (ref)
hrHPV-pos/cyt-neg and HPV16/18-neg		467	5	1%		18	4%	
hrHPV-pos/cyt-neg and HPV16/18-neg	**At least one concordant type and HPV16/18-neg**	**39**	**10**	**26%**	**3.2 (1.8–5.8)**	**14**	**36%**	**2.6 (1.6–4.1)**
HPV16/18/31/33/45-pos		697	147	21%	1.0 (ref)	214	31%	1.0 (ref)
HPV16/18/31/33/45-pos/cyt-neg		456	31	7%		60	13%	
HPV16/18/31/33/45-pos/cyt-neg	**At least one concordant type**	**76**	**29**	**38%**	**1.8 (1.3–2.5)**	**34**	**45%**	**1.5 (1.1–1.9)**
hrHPV-pos and HPV16/18/31/33/45-neg		369	17	5%	1.0 (ref)	35	9%	1.0 (ref)
hrHPV-pos/cyt-neg and HPV16/18/31/33/45-neg		274	1	0.4%		6	2%	
hrHPV-pos/cyt-neg and HPV16/18/31/33/45-neg	**At least one concordant type and HPV16/18/31/33/45-neg**	**11**	**5**	**45%**	**9.9 (4.4–21.9)**	**6**	**55%**	**5.8 (3.1–10.7)**

Abbreviations: cyt, cytology; HPV, human papillomavirus; hr, high-risk; neg, negative; pos, positive.

two-round type concordance were also observed in subgroups of women who were HPV16/18-positive, HPV16/18/31/33/45-positive, high-risk HPV-positive/HPV16/18-negative, or high-risk HPV-positive/HPV16/18/31/33/45-negative at the baseline screen. In the subgroup of high-risk HPV-positive/HPV16/18/31/33/45-negative women, women from the intervention group had an 18-month CIN3+ risk of only 5% and cytology-negative women from the control group with a two-round type concordant test result had an increased 18-month CIN3+ risk of 45% (RR 9.9, 95% CI, 4.4–21.9; $P < 0.001$). Similar results were also obtained when stratifying according to nonavalent HPV vaccine types HPV16/18/31/33/45/52/58, where vaccine type–negative women in the intervention group and control group had CIN3+ risks in the baseline round and after two-round type concordance of 3% and 33% ($P = 0.018$), respectively. The results for endpoint CIN2+ were similar and are shown in **Table 2**.

Discussion

Our results convincingly show that women who test HPV-positive at two consecutive screens five years apart have an increased risk of CIN3+ in case of type concordance. The absolute CIN3+ risk in our study population was 40% after two-round type concordance, well above an informal CIN3+ risk threshold for colposcopy referral of 20% (18, 26), and similar to the CIN3+ risk in HPV-positive women with concurrent abnormal cytology in the same trial (18). In the new Dutch HPV-based screening program, direct colposcopy referral is recommended for the latter group (27), and hence a similar recommendation seems warranted after having observed type concordance after five years. Apparently, this holds for all high-risk HPV types, as the short-term (18-month) CIN3+ risk in cytologically normal women with a high-risk HPV infection other than HPV16/18/31/33/45 increased from 0.4% to 45% after a five-year type concordant result. This provides further support for directly referring women with long-term type-specific persistence for colposcopy, irrespective of HPV type (11). Such a strategy retains a high positive predictive value for CIN3+ after referral for colposcopy, while it circumvents limitations of repeat cytology testing, as currently recommended in some countries (including the Netherlands). In particular, identification of cytologically poorly detectable lesions may be enhanced through repeat HPV testing (7, 28).

An important finding of our study is that after five-year type persistence, the CIN3+ risks were high in all subgroups of HPV types. As expected, the CIN3+ risk after five-year type persistence was highest in women with HPV16 (61%), but still a CIN3+ risk of 45% was observed in HPV-positive women without HPV16/18/31/33/45, and a CIN3+ risk of 33% was observed in women with a non-nonavalent HPV vaccine type. HPV16-positive infections are associated with lower clearance rates and higher CIN3+ risks than other high-risk HPV infections (11, 29, 30), but apparently also less aggressive HPV types eventually lead to CIN3+ when they persist for a longer time. This is consistent with the literature indicating that women with a high-risk HPV infection who do not clear the infection eventually develop CIN2+ (10, 31). A further implication of this finding is that our results support the use of five-year type persistence as a marker for immediate colposcopy in a setting where a substantial proportion of women is immunized and consequently the majority of HPV-positive women is infected by a nonvaccine type.

The assessment of type-specific concordance requires full genotyping of HPV-positive women. If full genotyping comes with the primary high-risk HPV test, then type concordance can be performed without additional costs, but at the moment only two HPV DNA tests offer full genotyping and have been validated according to international criteria (32). In the near future, more full genotyping tests are expected to enter the market, because there is wide interest in full genotyping in vaccinated cohorts for monitoring of vaccine effectiveness.

In real-world screening programs, partial genotyping may be used as an alternative to full genotyping because 82% of the women with five-year HPV persistence have a concordant type (91/111, **Table 1**). Therefore, measuring high-risk HPV persistence instead of type-specific persistence only has a limited effect on the number of colposcopy referrals. Partial genotyping is still needed because the CIN3+ risk in HPV-positive/cytology-negative women is too high (4%, **Table 1**) to dismiss them from further follow-up. In our analysis, CIN3+ risk in HPV-positive/cytology-negative women decreased to only 1% in the baseline round when the result was HPV16/18-negative and to only 0.4% when the result was HPV16/18/31/33/45-negative, supporting the use of partial genotyping for returning women to routine screening. Notably, referring all women with one-year high-risk HPV persistence to colposcopy leads to a high number of colposcopy referrals and a low positive predictive value for CIN2+ as was observed in regional Italian primary HPV screening programs (14, 33–35).

A particularly interesting feature of molecular techniques such as genotyping is that they can be used on self-collected samples so that provider-sampling for triage testing of HPV-positive women by cytology can be avoided. Besides partial genotyping, several other molecular techniques are currently in development including host and viral gene methylation techniques. It is interesting to compare genotyping and other molecular techniques, but we think that genotyping and methylation can be used in conjunction to triage HPV-positive women. For example, host cell DNA methylation can be used to select women for immediate referral for colposcopy, but methylation-negative women require surveillance (36). The results of this study suggest that partial genotyping may be used to determine the time at which a methylation-negative woman should be offered repeat HPV testing.

Many researchers have studied the association between HPV type persistence and CIN2+ or CIN3+ (37–40), but only few have evaluated the role of HPV type persistence in the management of HPV-positive women with normal cytology. In an early prospective population study within the Scottish cytology-based program (9), it was shown that HPV-positive/cytology-negative women at baseline who tested HPV-positive for the same type (41%) two to three years later were significantly more likely to develop abnormal cytology than those who were sequentially positive for different types (8%). In another follow-up analysis of HPV-positive/cytology-negative women of the Swedescreen trial, conducted in five cities in Sweden (10), women who had not been treated for CIN2+ were selected and invited for yearly repeat HPV tests followed by colposcopy in case of type persistence. This study showed that all women who continuously showed persistence at each retest eventually developed CIN2+ and almost all cases occurred before six years. It was recognized that the intensive follow-up with yearly HPV tests had limited benefits and a less intensive management was suggested. The role of type persistence was also studied in an analysis of the second round of the ARTISTIC trial, conducted in the Manchester area (41). A main difference with our study was that the analysis of the ARTISTIC study did not exclude women with abnormal cytology and did not consider a randomized comparison of different screening strategies at the baseline screening round. Nonetheless, similar to our study, the two-and-a-half-year CIN3+ risk was 17.6% in women with a type concordant HPV infection over two rounds with an interval of three years, whereas among 155 women with a type switch between the two screening rounds, no CIN3+ cases were found in the second screening round. The CIN3+ risk was particularly high (30.3%) in women with concordant HPV16.

A unique and key feature of the presented analysis is the randomized comparison between the intervention group at baseline and the control group at the next screen after five years, where no action was taken after negative cytology in the baseline round. This enabled us to clearly show that by postponing the repeat testing moment for HPV-positive/cytology-negative women beyond 18 months, CIN3+ risks become high enough to warrant immediate referral. Other strengths of our study are the fact that the POBASCAM trial was conducted within the national Dutch screening program with a screening interval of five years, which has been proposed for primary HPV screening programs in various countries (e.g., Australia, the Netherlands, the United Kingdom, and Italy). Moreover, our study was large enough to verify that the type concordance effect is present both in HPV16-positive and HPV16-negative women and in women below and above age 35 years, which suggests that the effect is not mediated by genotype or age (42–44).

We identified the following limitations of our study. First, only about 40% of the HPV-positive/cytology-negative women had an HPV test result at the next round after five years. The main reason for this is that some general practitioners did not send in a sample for HPV testing but only a slide for (conventional) cytology testing. We do not expect that this biases our estimates, although it lowers the statistical power for assessing risk differences in subgroups. Second, we do not have information about colposcopy procedures as our data were tracked through the nationwide histology and cytology registry PALGA. Therefore, absolute CIN3+ risks may be underestimated as some women may not have adhered to the colposcopy recommendation. Besides, some women may not have completed the full scheme of triage by repeat testing, lowering the estimates of the absolute CIN3+ risk. However, follow-up protocols were equal in the intervention and control group so that no substantial bias is expected in the estimated relative disease risks. Third, as women in our control group were managed according to cytology, we were not able to evaluate the psychosocial impact of repeat HPV testing with a long interval. A potential concern is that repeat HPV testing leads to anxiety, distress, and worries about cancer. However, multiple studies have indicated that anxiety and distress are short-lived and repeated exposure to the same test (twice HPV testing) seemed to normalize anxiety (45, 46). Fourth, a potential bias in the comparison of the two randomization groups is that 23 CIN2+ cases detected on the basis of their baseline screening results were excluded. However, this bias is likely to be small because in the POBASCAM study, women were excluded when they had had a CIN2+ in the two years before enrollment (15). Besides, if the 23 CIN2+ cases had not been detected in the baseline round but in the next round, the number of CIN2+ cases detected in the next round would have been higher, which would have provided further support for immediate referral to colposcopy after five-year persistence.

To summarize, we capitalized on a randomized comparison between immediate and delayed referral on the basis of HPV test results to show the diagnostic value of repeatedly testing positive for the same HPV type(s) in cervical screening. Women with a concordant HPV type after five years have a CIN3+ risk that is high enough to warrant immediate referral for colposcopy without additional triage testing, also for less aggressive high-risk HPV types. In settings of HPV-based screening with intervals of five years, two-round HPV type concordance is a promising marker for risk stratification of HPV-positive/cytology-negative women, and immediate referral would translate into a reduced demand of adjunct cytology testing.

Authors' Disclosures

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Authors' Contributions

F. Inturrisi: Conceptualization, data curation, formal analysis, investigation, visualization, methodology, writing–original draft, project administration, writing–review and editing. **J.A. Bogaards:** Conceptualization, supervision, validation, investigation, visualization, writing–review and editing. **D.A.M. Heideman:** Resources, data curation, investigation, methodology, writing–review and editing. **C.J.L.M. Meijer:** Resources, data curation, investigation, writing–review and editing. **J. Berkhof:** Conceptualization, resources, supervision, funding acquisition, validation, investigation, visualization, methodology, writing–original draft, project administration, writing–review and editing.

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References

1. zur Hausen H. Condylomata acuminata and human genital cancer. Cancer Res 1976;36:794.
2. Cuschieri K, Ronco G, Lorincz A, Smith L, Ogilvie G, Mirabello L, et al. Eurogin roadmap 2017: Triage strategies for the management of HPV-positive women in cervical screening programs. Int J Cancer 2018;143:735–45.
3. Cancer Council Australia Cervical Cancer Screening Guidelines Working Party. National cervical screening program: guidelines for the management of screen-detected abnormalities, screening in specific populations and investigation of abnormal vaginal bleeding. Available from: https://wiki.cancer.org.au/australia/Guidelines:Cervical_cancer/Screening.
4. National Institute for Public Health and the Environment (RIVM). Framework for the execution of cervical cancer population screening. Available from: https://www.rivm.nl/sites/default/files/2018-11/Framework%20for%20the%20Execution%20of%20Cervical%20Cancer%20Population%20Screening%20(EN).pdf.
5. 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:F88–99.
6. Ronco G, Dillner J, Elfström KM, Tunesi S, Snijders PJF, Arbyn M, et al. Efficacy of HPV-based screening for prevention of invasive cervical cancer: follow-up of four European randomised controlled trials. Lancet North Am Ed 2014;383:524–32.
7. Koliopoulos G, Nyaga VN, Santesso N, Bryant A, Martin-Hirsch PP, Mustafa RA, et al. Cytology versus HPV testing for cervical cancer screening in the general population. Cochrane Database Syst Rev 2017;8:CD008587.
8. Polman NJ, Veldhuijzen NJ, Heideman DAM, Snijders PJF, Meijer C, Berkhof J. HPV-positive women with normal cytology remain at increased risk of CIN3 after a negative repeat HPV test. Br J Cancer 2017;117:1557–61.
9. Cuschieri KS, Cubie HA, Whitley MW, Gilkison G, Arends MJ, Graham C, et al. Persistent high risk HPV infection associated with development of cervical neoplasia in a prospective population study. J Clin Pathol 2005;58:946–50.
10. Elfgren K, Elfstrom KM, Naucler P, Arnheim-Dahlstrom L, Dillner J. Management of women with human papillomavirus persistence: long-term follow-up of a randomized clinical trial. Am J Obstet Gynecol 2017;216:264.
11. Demarco M, Hyun N, Carter-Pokras O, Raine-Bennett TR, Cheung L, Chen X, et al. A study of type-specific HPV natural history and implications for contemporary cervical cancer screening programs. EClinicalMedicine 2020;22:100293.
12. Bulkmans NW, Berkhof J, Bulk S, Bleeker MC, van Kemenade FJ, Rozendaal L, et al. High-risk HPV type-specific clearance rates in cervical screening. Br J Cancer 2007;96:1419–24.
13. Naucler P, Ryd W, Törnberg S, Strand A, Wadell G, Elfgren K, et al. Efficacy of HPV DNA testing with cytology triage and/or repeat HPV DNA testing in primary cervical cancer screening. J Natl Cancer Inst 2009;101:88–99.
14. Zorzi M, Frayle H, Rizzi M, Fedato C, Rugge M, Penon MG, et al. A 3-year interval is too short for re-screening women testing negative for human papillomavirus: a population-based cohort study. BJOG 2017;124:1585–93.
15. Rijkaart DC, Berkhof J, Rozendaal L, van Kemenade FJ, Bulkmans NWJ, Heideman DAM, et al. Human papillomavirus testing for the detection of high-grade cervical intraepithelial neoplasia and cancer: final results of the POBASCAM randomised controlled trial. Lancet Oncol 2012;13:78–88.
16. Bulkmans NW, Rozendaal L, Snijders PJ, Voorhorst FJ, Boeke AJ, Zandwijken GR, et al. POBASCAM, a population-based randomized controlled trial for implementation of high-risk HPV testing in cervical screening: design, methods and baseline data of 44,102 women. Int J Cancer 2004;110:94–101.
17. Bulkmans NWJ, Berkhof J, Rozendaal L, van Kemenade FJ, Boeke AJP, Bulk S, et al. Human papillomavirus DNA testing for the detection of cervical intraepithelial neoplasia grade 3 and cancer: 5-year follow-up of a randomised controlled implementation trial. Lancet North Am Ed 2007;370:1764–72.
18. Dijkstra MG, van Niekerk D, Rijkaart DC, van Kemenade FJ, Heideman DA, Snijders PJ, et al. Primary hrHPV DNA testing in cervical cancer screening: how to manage screen-positive women? A POBASCAM trial substudy. Cancer Epidemiol Biomarkers Prev 2014;23:55–63.
19. Bulk S, van Kemenade FJ, Rozendaal L, Meijer C. The Dutch CISOE-A framework for cytology reporting increases efficacy of screening upon standardisation since 1996. J Clin Pathol 2004;57:388–93.
20. Jacobs MV, Snijders PJ, van den Brule AJ, Helmerhorst TJ, Meijer CJ, Walboomers JM. A general primer GP5+/GP6(+)-mediated PCR-enzyme immunoassay method for rapid detection of 14 high-risk and 6 low-risk human papillomavirus genotypes in cervical scrapings. J Clin Microbiol 1997;35:791–5.
21. van den Brule AJ, Pol R, Fransen-Daalmeijer N, Schouls LM, Meijer CJ, Snijders PJ. GP5+/6+ PCR followed by reverse line blot analysis enables rapid and high-throughput identification of human papillomavirus genotypes. J Clin Microbiol 2002;40:779–87.
22. Hopman EH, Rozendaal L, Voorhorst FJ, Walboomers JM, Kenemans P, Helmerhorst TJ. High risk human papillomavirus in women with normal cervical cytology prior to the development of abnormal cytology and colposcopy. BJOG 2000;107:600–4.
23. Hopman EH, Voorhorst FJ, Kenemans P, Meyer CJ, Helmerhorst TJ. Observer agreement on interpreting colposcopic images of CIN. Gynecol Oncol 1995;58:206–9.

24. Anderson MC. Premalignant and malignant squamous lesions of the cervix, in Obstetrical and gynaecological pathology. In: Fox H, Wells M, editors. Obstetrical and gynaecological pathology. Vol 36. 5th ed. Edinburgh, UK: Churchill Livingstone; 2004. p. 292–97.
25. 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.
26. Rijkaart DC, Berkhof J, van Kemenade FJ, Coupe VM, Hesselink AT, Rozendaal L, et al. Evaluation of 14 triage strategies for HPV DNA-positive women in population-based cervical screening. Int J Cancer 2012;130:602–10.
27. National Institute for Public Health and the Environment (RIVM). Cervical cancer screening programme. Available from: https://www.rivm.nl/en/cervical-cancer-screening-programme.
28. Gyllensten U, Sanner K, Gustavsson I, Lindell M, Wikström I, Wilander E. Short-time repeat high-risk HPV testing by self-sampling for screening of cervical cancer. Br J Cancer 2011;105:694–7.
29. Khan MJ, Castle PE, Lorincz AT, Wacholder S, Sherman M, Scott DR, et al. The elevated 10-year risk of cervical precancer and cancer in women with human papillomavirus (HPV) type 16 or 18 and the possible utility of type-specific HPV testing in clinical practice. J Natl Cancer Inst 2005;97:1072–9.
30. Gilham C, Sargent A, Peto J. Triaging women with human papillomavirus infection and normal cytology or low-grade dyskaryosis: evidence from 10-year follow up of the ARTISTIC trial cohort. BJOG 2020;127:58–68.
31. 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.
32. Arbyn M, Snijders PJ, Meijer CJ, Berkhof J, Cuschieri K, Kocjan BJ, et al. Which high-risk HPV assays fulfil criteria for use in primary cervical cancer screening? Clin Microbiol Infect 2015;21:817–26.
33. Maggino T, Sciarrone R, Murer B, Dei Rossi MR, Fedato C, Maran M, et al. Screening women for cervical cancer carcinoma with a HPV mRNA test: first results from the Venice pilot program. Br J Cancer 2016;115:525–32.
34. Pasquale L, Giorgi Rossi P, Carozzi F, Pedretti C, Ruggeri C, Scalvinoni V, et al. Cervical cancer screening with HPV testing in the valcamonica (Italy) screening programme. J Med Screen 2015;22:38–48.
35. Passamonti B, Gustinucci D, Giorgi Rossi P, Cesarini E, Bulletti S, Carlani A, et al. Cervical human papilloma virus (HPV) DNA primary screening test: Results of a population-based screening programme in central Italy. J Med Screen 2017;24:153–62.
36. De Strooper LM, Meijer CJ, Berkhof J, Hesselink AT, Snijders PJ, Steenbergen RD, et al. Methylation analysis of the FAM19A4 gene in cervical scrapes is highly efficient in detecting cervical carcinomas and advanced CIN2/3 lesions. Cancer Prev Res 2014;7:1251–7.
37. Koshiol J, Lindsay L, Pimenta JM, Poole C, Jenkins D, Smith JS. Persistent human papillomavirus infection and cervical neoplasia: a systematic review and meta-analysis. Am J Epidemiol 2008;168:123–37.
38. Munoz N, Hernandez-Suarez G, Mendez F, Molano M, Posso H, Moreno V, et al. Persistence of HPV infection and risk of high-grade cervical intraepithelial neoplasia in a cohort of Colombian women. Br J Cancer 2009;100:1184–90.
39. Kjaer 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.
40. Castle PE, Rodriguez AC, Burk RD, Herrero R, Wacholder S, Hildesheim A, et al. Long-term persistence of prevalently detected human papillomavirus infections in the absence of detectable cervical precancer and cancer. J Infect Dis 2011;203:814–22.
41. Gilham C, Sargent A, Kitchener HC, Peto J. HPV testing compared with routine cytology in cervical screening: long-term follow-up of ARTISTIC RCT. Health Technol Assess 2019;23:1–44.
42. Howell-Jones R, Bailey A, Beddows S, Sargent A, de Silva N, Wilson G, et al. Multi-site study of HPV type-specific prevalence in women with cervical cancer, intraepithelial neoplasia and normal cytology, in England. Br J Cancer 2010;103:209–16.
43. Porras C, Rodríguez AC, Hildesheim A, Herrero R, González P, Wacholder S, et al. Human papillomavirus types by age in cervical cancer precursors: predominance of human papillomavirus 16 in young women. Cancer Epidemiol Biomarkers Prev 2009;18:863–5.
44. McKenna M, McMenamin M, McDowell A. HPV16 and HPV18 genotyping triage in young women with borderline cytology or mild dyskaryosis: effect of age on genotype-specific risk of high-grade CIN. Cytopathology 2016;27:261–8.
45. McBride E, Marlow LAV, Forster AS, Ridout D, Kitchener H, Patnick J, et al. Anxiety and distress following receipt of results from routine HPV primary testing in cervical screening: The psychological impact of primary screening (PIPS) study. Int J Cancer 2020;146:2113–21.
46. McCaffery KJ, Irwig L, Turner R, Chan SF, Macaskill P, Lewicka M, et al. Psychosocial outcomes of three triage methods for the management of borderline abnormal cervical smears: an open randomised trial. BMJ 2010;340:b4491.