HPV-based cervical screening

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Chapter 6

Management of HPV-positive women in cervical screening using results from two consecutive screening rounds

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Abstract

We studied whether triage of human papillomavirus (HPV)-positive women participating in an HPV-based screening programme can be improved by including the HPV result at the previous screen in the triage algorithm. We analyzed data of a subgroup of 366 women from the POBASCAM trial, screened by cytology and HPV co-testing. Women were included if they tested HPV-positive in the second HPV-based screening round. We evaluated the clinical performance of 16 strategies, consisting of cytology, HPV genotyping, and/or previous screen HPV result. The clinical endpoint was cervical precancer or cancer (CIN3+). The current Dutch triage testing policy for HPV-positive women is to refer women for colposcopy if they have abnormal cytology at baseline or after 6–18 months. In the second HPV-based screening round, this strategy yielded a negative predictive value (NPV) of 95.8% (95% confidence interval: 91.9–98.2) and colposcopy referral rate of 37.6% (32.3–43.2%). Replacing repeat cytology by the previous screen HPV result yielded a similar NPV (96.9%, 93.3–98.9) and colposcopy referral rate (38.8%, 33.4–44.4). A higher NPV (99.2%, 96.3–100%) at the cost of a higher colposcopy referral rate (49.2%, 43.6–54.8) was achieved when cytology was combined with HPV16/18 genotyping. The other 13 triage strategies yielded a lower NPV, a higher colposcopy referral rate or performed similarly but required additional testing. HPV-positive women in the second HPV-based screening round can be suitably managed by cytology, HPV16/18 genotyping and the HPV result at the previous screen, obviating the need for repeat testing of HPV-positive, cytology negative women.

What's new?

The management of HPV-positive women in the second HPV-based screening round may be different from the management in the first round. We showed that HPV-positive women in the second HPV-based screening round can be suitably managed by cytology, HPV16/18 genotyping and the HPV result at the previous screen, obviating the need for repeat testing of HPV-positive, cytology negative women.

Introduction

In several countries, HPV testing is being implemented as a primary test in cervical screening, alone or in combination with cytology.[1] As many HPV infections have a transient nature, only a small proportion of HPV-positive women have high-grade cervical intraepithelial neoplasia (CIN).[2,3] Therefore, triage testing of HPV-positive women is necessary in order to identify women with a high risk of cervical cancer and precancer (CIN3+) and to return the remainder of the group back to routine screening.

Multiple studies have evaluated triage strategies for HPV-positive women in large screening cohorts.[4–10] Strategies with immediate cytology, HPV16/18-genotyping, repeat HPV testing, and/or repeat cytology have been identified and adopted in current HPV-screening guidelines. In the Netherlands and Sweden, triage of HPV-positive women is performed with cytology testing only, whereas in Italy and the United States HPV-positive, cytology negative women are followed with repeat HPV testing and are returned to routine screening after viral clearance. The latter strategy yields a better safety against CIN3+, but comes at a cost of higher number of colposcopy referrals while the CIN3+ risk may still be elevated as compared to the CIN3+ risk in baseline HPV-negative women.[11,12] Therefore, there is not yet a uniformly best triage strategy for HPV-positive women and selection of a strategy depends on how safety against CIN3+ after a negative triage and the costs and harms of unnecessary colposcopy referrals are weighed.[13]

In the second HPV-based screening round, the CIN3+ risk in HPV-positive women may differ from that in the first round because the second screen consists of a larger proportion of short-term HPV infections.[14,15] This warrants a re-evaluation of triage strategies. Furthermore, in countries without follow-up HPV testing in the first round, the detection of CIN3+ in HPV-positive women in the second HPV-based screening round may be enhanced by stratifying according to the HPV test result in the first HPV-based screening round.

In our study, we conducted a post-hoc analysis within the POBASCAM (Population Based Screening Study Amsterdam) cohort[16,17] to identify feasible triage strategies for HPV-positive women in their second round of HPV-based screening. We evaluated the clinical performance of 16 different triage strategies for detection of CIN3+, using cytology, HPV genotyping, and the HPV result at the previous screen.

Material and Methods

Study population

The POBASCAM trial (Trial registration ID: NTR218) was designed to assess whether combined HPV and cytology testing in the first screening round decreases detection of CIN3+ in the second screening round 5 years later, as compared to cytology alone.[16–18] In brief, 44,938 women aged 29 to 61 years old were randomized (1:1) between cytology and HPV co-testing (intervention group) and conventional cytology (control group). Of these, 33,493 were included in the final analysis since they had a valid test result at the first screen and attended the second screen 5 years later.[17] For this post-hoc analysis, only women from the intervention group (n = 16,750) were included, since baseline HPV-positive women in the control group were not managed based on their HPV result. 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.

Management

Management of POBASCAM study participants has been published before.[17,18] Women in the intervention group were managed based on HPV and cytology testing at the first screening round and at the second screening round 5 years later. Women with a negative HPV result and normal cytology were referred back to routine screening. Women with worse than borderline or mild dyskaryosis (>BMD) cytology were referred for colposcopy irrespective of the HPV result. HPV-positive women with normal cytology or borderline or mild dyskaryosis (BMD) cytology, (comparable to atypical squamous cells of undetermined significance [ASC-US] in the Bethesda classification)[19] were advised to repeat both HPV and cytology testing after 6 and 18 months. In case of a positive HPV test or in case of ≥BMD cytology at repeat testing women were referred for colposcopy.

Procedures

HPV tests (HR HPV GP5+/6+ PCR EIA) were done in duplicate, blinded for cytology results.[16,20] Typing of EIA-positive samples was done by a reverse line blot assay, which detects 14 high-risk HPV types (i.e. HPV type 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68).[21]

During colposcopy, biopsies were taken from suspected areas according to standard procedures in the Netherlands.[22,23] Histological specimens were examined at local hospitals and classified as no dysplasia, CIN grade 1, 2, or 3, or as invasive cancer,

according to international criteria.[24] Adenocarcinoma in situ was added to CIN3. Cytology and histology results up to 9 years after baseline were collected through the nationwide network and registry of histo- and cytopathology in the Netherlands (PALGA).[25]

In- and exclusion criteria

Women were eligible for this post-hoc analysis if they tested HPV-positive at the second HPV-based screening round. Women were excluded if they had a histological diagnosis prior to the second screen or if they violated the study protocol (i.e. had normal cytology but were referred for colposcopy-directed biopsy). In total, 366 HPV-positive women from the intervention group were included in this post-hoc analysis. A study flowchart is shown in Figure 1.

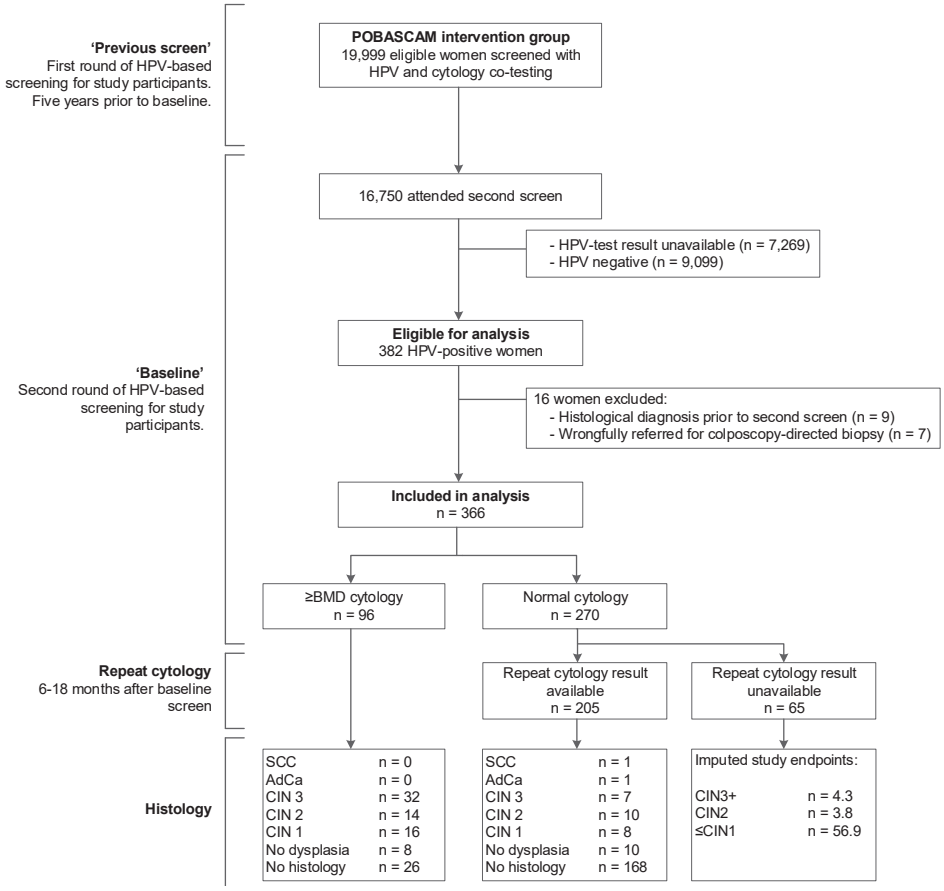

Figure 1. Study flowchart of the POBASCAM intervention group, including information on women included in this post-hoc analysis.

HPV = human papillomavirus; BMD = borderline or mild dyskaryosis; SCC = squamous cell carcinoma; AdCa = adenocarcinoma; CIN = cervical intraepithelial neoplasia; CIN3+ = CIN3 or worse; ≤CIN1 = CIN1 or less.

Statistical analysis

In this post-hoc analysis, the second HPV-based screen was considered as baseline. The HPV result in the first HPV-based screening round was considered as previous screen HPV result (see Figure 1).

Sixteen different strategies for triage of HPV-positive women in the second HPV-based screening round were evaluated. For triage strategies that consisted of two or more additional tests, the result was considered positive if at least one of the tests was positive. Table 1 presents an overview of results of triage testing algorithms among HPV-positive women in the second HPV-based screen that result in referral for colposcopy.

The following tests were used in HPV-positive women: Strategy 1) no further testing, but immediate referral for colposcopy: 2) cytology (threshold ≥ BMD), 3) HPV16/18-genotyping; 4) cytology and HPV16/18-genotyping, 5) cytology and repeat cytology, and 6) cytology, HPV16/18-genotyping and repeat cytology. In the subsequent five triage strategies, the HPV result at the previous screening round was one of the triage tests: 7) HPV previous screen, 8) HPV previous screen and cytology, 9) HPV previous screen and HPV16/18-genotyping, 10) HPV previous screen, cytology and HPV16/18-genotyping, and 11) HPV previous screen, cytology and repeat cytology. In the last five triage strategies, HPV type persistence over two screening rounds was included. HPV type persistence was defined as having an HPV infection with at least one HPV type that was also detected at the previous screen. The five strategies with HPV type persistence were: 12) HPV type persistence, 13) HPV type persistence and cytology, 14) HPV type persistence and HPV16/18-genotyping, 15) HPV type persistence, cytology and HPV16/18-genotyping, and 16) HPV type persistence, cytology and repeat cytology.
Out of the 270 women with normal cytology, 65 (24.1%) women did not have cytology and histological follow-up. These missing data were imputed under the assumption that the probability of a repeat cytology and histology result depends on earlier screening test results in the first and second HPV-based screening round, but does not depend on other, unobserved factors (missing at random).[26] The imputed histology was added to Figure 1.

Table 1. Overview of results of triage testing among HPV-positive women in the second HPV-based screen that result in referral for colposcopy.

	Triage strategy		HPV result previous screen (i.e. first HPV-based screen)	Baseline triage test result (i.e second HPV based screen)	Repeat cytology result (6-18 months after baseline)
1.	No further testing	I.	-	-	-
2.	Cytology	I.	-	Abnormal cytology*	-
3.	HPV16/18-genotyping	I.	-	HPV16/18-positive	-
4.	Cytology and HPV16/18-genotyping	I.	-	Abnormal cytology* AND/OR HPV16/18-positive	-
5.	Cytology and repeat cytology	I.	-	Abnormal cytology*	-
		II.	-	Normal cytology	Abnormal cytology*
6.	Cytology, HPV16/18-genotyping and repeat cytology	I.	-	Abnormal cytology* AND/OR HPV16/18-positive	-
		II.	-	Normal cytology and HPV16/18-negative	Abnormal cytology*
7.	HPV previous screen	I.	HPV-positive	-	-
8.	HPV previous screen and cytology	I.	HPV-positive	-	-
		II.	HPV-negative	Abnormal cytology*	-
9.	HPV previous screen and HPV16/18-genotyping	I.	HPV-positive	-	-
		II.	HPV-negative	HPV16/18-positive	-
10.	HPV previous screen, cytology and HPV16/18-genotyping	I.	HPV-positive	-	-
		II.	HPV-negative	Abnormal cytology* AND/OR HPV16/18-positive	-
11.	HPV previous screen, cytology and repeat cytology	I.	HPV-positive	-	-
		II.	HPV-negative	Abnormal cytology*	-
		III.	HPV-negative	Normal cytology	Abnormal cytology*
12.	HPV type persistence	I.	HPV-positive - same type as baseline	-	-
13.	HPV type persistence and cytology	I.	HPV-positive - same type as baseline	-	-
		II.	HPV-positive - different type than baseline	Abnormal cytology*	-

Table 1. Overview of results of triage testing among HPV-positive women in the second HPV-based screen that result in referral for colposcopy. (*continued*)

Triage strategy		HPV result previous screen (i.e. first HPV-based screen)	Baseline triage test result (i.e second HPV based screen)	Repeat cytology result (6-18 months after baseline)
14. HPV type persistence and HPV16/18-genotyping	I.	HPV-positive - same type as baseline	-	-
	II.	HPV-positive - different type than baseline	HPV16/18-positive	-
15. HPV type persistence, cytology and HPV16/18-genotyping	I.	HPV-positive - same type as baseline	-	-
	II.	HPV-positive - different type than baseline	Abnormal cytology* AND/OR HPV16/18-positive	-
16. HPV type persistence, cytology and repeat cytology	I.	HPV-positive - same type as baseline	-	-
	II.	HPV-positive - different type than baseline	Abnormal cytology*	-
	III.	HPV-positive - different type than baseline	Normal cytology	Abnormal cytology*

*Abnormal cytology: ≥ASC-US (atypical squamous cells of undetermined significance).

Negative predictive value (NPV), positive predictive value (PPV), sensitivity and specificity for detection of CIN3+ were calculated from cross-tabulations of test results and histological endpoints, together with Wilson 95% confidence intervals (CI's). A criterion for acceptability of a triage strategy is that the NPV for end-point CIN3+ is at least 98% and the PPV is at least 20%.[4,5] However, these NPV and PPV thresholds were used for evaluating triage strategies in the first round of HPV-based screening. In the second round of HPV-based screening, the proportion of short-term HPV infections is expected to be higher than in the first round, and for this reason thresholds were not interpreted as stringent as before.[14,15] The analyses were repeated for secondary outcome CIN grade 2 or worse (CIN2+). Analyses were performed in IBM SPSS Statistics version 22.0.

Results

Study cohort characteristics

Two-hundred seventy (73.8%) out of 366 HPV-positive women, included in the final analysis, had normal cytology at baseline and 96 (26.2%) had BMD cytology or worse (≥BMD) at baseline. Histology outcomes are shown in Figure 1. Mean age at baseline was 43.5 years. The previous screen was on average 5.0 years (range 4.0–6.9) prior to baseline. Mean time between baseline and histological diagnosis was 12.2 months (range 0.2–46.6). Among 205 women with normal cytology who did have a repeat cytology result, the mean time between baseline and repeat cytology was 13.2 months (range 1.4–29.3).

Test performance characteristics

The results of the 16 triage strategies for detection of CIN3+ are shown in Table 2. The reference strategy without further testing in HPV positive women (strategy 1) had a PPV of 12.4% which lies far below the threshold of 20% and indicates that further testing in HPV-positive women is required. Among the first five strategies that did not include HPV information of the previous screen (strategy 2–6), strategies that combined cytology with HPV16/18 genotyping had NPV estimates above the 98% threshold: cytology and HPV16/18-genotyping (strategy 4; 99.2%) and cytology, HPV16/18-genotyping and repeat cytology (strategy 6; 99.2%). The strategy consisting of baseline cytology and repeat cytology had an NPV estimate below 98% (strategy 5; 95.8%), but the 95% confidence interval still included 98%. The NPV estimates of two strategies were below 98%: cytology (strategy 2; 95.1%) and HPV16/18-genotyping (strategy 3; 92.4%). Strategies 2–6 all had PPV estimates above 20%. Among five strategies that used the previous screen HPV result (strategy 7–11), the NPV estimate

Table 2. Clinical performance of different triage strategies for detection of CIN3+ in HPV-positive women (n=366) in the second HPV-based screening round.

	Triage strategy	NPV	PPV	Sensitivity	Specificity	Colposcopy referral rate
1.	No further testing	-	12.4% (9.0 - 16.5%)	100%	0.0%	100%
2.	Cytology	95.1% (91.4 - 97.5%)	33.3% (24.7 - 43.2%)	70.6% (55.4 - 82.5%)	80.0% (74.8 - 84.5%)	26.2% (21.5 - 31.4%)
3.	HPV16/18-genotyping	92.4% (88.0 - 95.5%)	22.0% (14.8 - 30.7%)	59.6% (44.2 - 73.2%)	70.0% (64.3 - 75.4%)	33.6% (28.5 - 39.1%)
4.	Cytology and HPV16/18-genotyping	99.2% (96.3 - 100%)	24.4% (18.3 - 31.6%)	97.1% (86.8 - 99.8%)	57.6% (51.5 - 63.5%)	49.2% (43.6 - 54.8%)
5.	Cytology and repeat cytology	95.8% (91.9 - 98.2%)	26.0% (19.1 - 34.2%)	79.0% (64.4 - 89.0%)	68.2% (62.3 - 73.6%)	37.6% (32.3 - 43.2%)
6.	Cytology, HPV16/18-genotyping and repeat cytology	99.2% (95.7 - 100%)	21.2% (15.7 - 27.6%)	97.1% (86.8 - 99.8%)	48.9% (42.8 - 54.9%)	56.8% (51.2 - 62.3%)
7.	HPV previous screen	92.3% (87.6 - 95.9%)	29.3% (22.6 - 37.7%)	51.7% (36.7 - 66.2%)	82.3% (77.3 - 86.6%)	21.9% (17.5 - 26.8%)
8.	HPV previous screen and cytology	96.9% (93.3 - 98.9%)	27.0% (20.1 - 35.1%)	84.8% (70.9 - 93.0%)	67.7% (61.8 - 73.1%)	38.8% (33.4 - 44.4%)
9.	HPV previous screen and HPV16/18-genotyping	94.4% (89.8 - 97.2%)	20.2% (14.3 - 27.4%)	75.7% (60.9 - 86.6%)	57.7% (51.6 - 63.6%)	46.4% (40.9 - 52.1%)
10.	HPV previous screen, cytology and HPV16/18-genotyping	100% (97.2 - 100%)	21.5% (16.1 - 27.9%)	100% (91.8 - 100%)	48.3% (42.3 - 54.4%)	57.7% (52.0 - 63.1%)
11.	HPV previous screen, cytology and repeat cytology	97.9% (94.3 - 99.6%)	23.9% (17.8 - 31.2%)	90.9% (78.4 - 96.8%)	59.2% (53.1 - 65.0%)	47.0% (41.4 - 52.7%)
12.	HPV type persistence	91.7% (87.7 - 94.7%)	31.9% (21.1 - 44.6%)	45.0% (30.7 - 60.0%)	86.4% (81.8 - 90.1%)	17.5% (13.5 - 22.1%)
13.	HPV type persistence and cytology	95.8% (91.7 - 98.4%)	27.2% (20.8 - 34.9%)	78.1% (66.2 - 85.5%)	70.5% (64.4 - 76.1%)	35.5% (30.3 - 41.1%)
14.	HPV type persistence and HPV16/18-genotyping	94.7% (90.4 - 97.4%)	21.6% (15.3 - 29.2%)	75.7% (60.9 - 86.6%)	61.1% (55.1 - 66.9%)	43.4% (37.9 - 49.1%)
15.	HPV type persistence, cytology and HPV16/18-genotyping	100% (97.3 - 100%)	22.3% (16.7 - 29.0%)	100% (91.8 - 100%)	50.8% (44.8 - 56.9%)	55.5% (49.8 - 61.0%)
16.	HPV type persistence, cytology and repeat cytology	96.5% (92.5 - 98.8%)	23.7% (17.4 - 31.1%)	84.3% (70.3 - 92.7%)	61.6% (55.5 - 67.3%)	44.1% (38.6 - 49.8%)

CIN3+ = cervical intraepithelial neoplasia grade 3 or worse; HPV = human papillomavirus; NPV = negative predictive value; PPV = positive predictive value.

was above 98% when both cytology and HPV16/18 genotyping were included (strategy 10; 100%). When only baseline cytology was added to previous screen HPV result, NPV estimate was 96.9% (strategy 8) and when both baseline and repeat cytology were added, the NPV estimate was 97.9% (strategy 11). In both cases, the 95% confidence interval included the threshold value of 98%. When cytology was not included in triage testing, NPV estimates were clearly below 98% (strategies 7 and 9). Again, strategies 7–11 all had PPV estimates above 20%. Among strategies with acceptable NPV estimates, HPV previous screen combined with cytology had the highest PPV estimate (27.0%), with the lower bound of the 95% confidence interval above 20%.

Among strategies with HPV type persistence (strategies 12–16), an NPV estimate of 100% was observed when both cytology and HPV16/18-genotyping were included (strategy 15), and NPV estimates of 95.8% and 96.5% were observed for strategies with only cytology (strategy 13 and 16, respectively). The 95% confidence intervals of the latter two strategies included 98%. Strategies without cytology yielded NPV estimates that were clearly below 98% (91.7% and 94.7% for strategy 12 and 14, respectively). All three strategies that included cytology had PPV estimates above 20%.

When CIN2+ was used as endpoint, 2–8% lower NPV estimates and 8–16% higher PPV estimates were observed (Supplementary Table S1).

Colposcopy referral rates

Colposcopy referral rates are also presented in Table 2. A graphical representation of the colposcopy referral rates together with NPV and PPV thresholds can be found in the Supplementary material (Supplementary Figure S1). Among strategies with acceptable NPV estimates (strategies 4–6, 8, 10–11, 13, 15–16), the lowest colposcopy referral rates were estimated for cytology combined with repeat cytology (strategy 11; 37.6%), HPV previous screen and cytology (strategy 8; 38.8%), and HPV type persistence and cytology (strategy 13; 35.5%). Strategies that included HPV16/18-genotyping and strategies that consisted of a combination of three triage tests had substantially higher colposcopy referral rates (44.1–57.7%) than the other strategies. Notably, the differences in colposcopy referral rate were more pronounced than the corresponding differences in PPV. For instance, the PPV estimates of the strategy consisting of cytology with HPV16/18 genotyping (strategy 4) and the strategy consisting of previous screen HPV result and cytology (strategy 8) differed by 2.6% (resp. 24.4% vs. 27.0%), but colposcopy referral rates differed by about 10% (resp. 49.2% vs. 38.8%). When type persistence was used (strategy 12–16) instead of the

previous screen HPV result (strategy 7–11), a minimal reduction in colposcopy referral rates was observed.

Discussion

The aim of our study was to evaluate the performance of strategies for triage of HPV-positive women in the second HPV-based screening round with and without taking the HPV result of the previous screen into account. Four strategies had NPV estimates for detection of CIN3+ above the 98% threshold (strategy 4, 6, 10 and 15), and an additional five strategies had NPV estimates just below the 98% threshold with 95% confidence intervals including 98% (strategy 5, 8, 11, 13 and 16). PPV estimates of these nine strategies all met the 20% threshold, however, colposcopy rates were substantially lower for strategies without HPV16/18-genotyping.

Results of our study are important to health decision makers as many countries are introducing HPV-based screening. Current triage strategies for HPV-positive women have been selected based on studies in which results from only one HPV-based screening round were evaluated.[4–10] To our knowledge, this is the first study evaluating the performance of triage strategies in the second HPV-based screening round with a screening interval of 5 years.

The high risk of high-grade CIN among women who test HPV-positive in two consecutive screening rounds suggests that a substantial proportion of infections in these women are persistent.[27,28] HPV type persistence over two consecutive screens could provide a more precise distinction between women with persistent and incident HPV type infection[29] but in our analysis, colposcopy referral rates between the strategies with previous screen HPV result (strategy 7–11) and the strategies with HPV type persistence (strategy 12–16) were similar. Hence, a strategy in which all previous screen HPV-positive women are directly referred for colposcopy seems acceptable, less costly, and is easier to implement as it obviates the need for full genotyping of HPV-positive samples.

The use of the previous screen HPV result for triage of HPV-positive women in the second HPV-based screening round is particularly attractive because it lowers the need for repeat cytology testing. Replacing the strategy consisting of cytology combined with repeat cytology, currently used in the Dutch primary HPV screening programme, by previous screen HPV result combined with cytology hardly changed the NPV, PPV and colposcopy referral rate, however, led to a 55% decrease in the

number of cytological evaluations. This clearly indicates that the previous screen HPV result is useful for triage of HPV-positive women, and probably more attractive than a strategy with repeat cytology. Our results also show that a strategy combining the previous screen HPV result with cytology and repeat cytology is not likely to substantially improve the algorithm: compared to the strategy with previous screen HPV result and baseline cytology, an increase of only 1% in NPV was observed but at the cost of an 8 % increase in number of colposcopy referrals.

HPV16/18-genotyping was a strong risk stratifier in women with normal cytology. The strategy combining cytology with HPV16/18 genotyping had a 2% higher NPV than cytology combined with HPV result at the previous screen, but at the cost of a 10% increase in number of colposcopy referrals. HPV16/18 genotyping is easy to implement as a variety of clinically validated HPV assays have separate HPV16 and HPV18 channels, and choosing for HPV16/18 genotyping therefore depends on how many extra colposcopy referrals are considered acceptable for detection of an extra CIN3+. Finally, a strategy combining cytology, HPV16/18 genotyping, and previous screen HPV result could be considered when maximum safety is desired, although this did lead to an increase in number of colposcopy referrals to 58%.

In our study, triage strategies with an NPV of 98% and a PPV of 20% for endpoint CIN3+ were considered acceptable. The NPV threshold was based on the CIN3+ risk among women with BMD cytology at baseline and normal cytology at 6 and 18 months follow-up. In the previous Dutch cytology-based screening programme, these women had a CIN3+ risk of 1.2% and were referred back to routine screening.[30] An NPV risk of 2% was also used in previous studies that evaluated triage strategies for HPV-positive women,[4,5] and one of the evaluated triage strategies that met this threshold, i.e. two times cytology testing after an HPV-positive result, has been implemented in the new Dutch HPV-based screening program which started in 2017. The PPV threshold of 20% has also been used in previous evaluations.[4,5] In the cytology-based screening programme, the CIN3+ risk in triage-positive women was at least 30%, indicating that in three out of every 10 women referred for colposcopy a CIN3+ was diagnosed.[17,30] If the previous evaluations[4,5] had used a PPV threshold below 20%, strategies which allow for a substantial increase in the number of colposcopy referrals and costs as compared to the cytology-based program, would have been judged favorably. It is unlikely that such strategies are considered acceptable by the health authorities. Notably, in the new Dutch HPV-based screening programme, the probability of CIN3+ in triage-positive women is at least 30% so that the PPV in the cytology-based program is maintained in the HPV-based program.[4,5]

In the second round of HPV-based screening, the proportion of short-term HPV infections is expected to be higher than in the first round, and for this reason we did not interpret thresholds as stringent as before. However, point estimates of the NPV and PPV were still close to the respective thresholds of 98% and 20%. The performance of triage strategies and the judgment of acceptable risks and number of colposcopy referrals varies considerably between countries. Therefore, different thresholds may be used in other countries where different values are attributed to CIN3+ risks and screening-related harms and costs.

An important aspect that should be taken into account when selecting a triage strategy is whether the strategy is acceptable to women. In HPV-based programmes, having an HPV infection may cause anxiety, and it is important to reassure women that HPV infections are common and harmless and that viral persistence is required for the development of cervical precancer. Reassurance is particularly important in countries where HPV-positive, cytology negative women are not followed-up with repeat HPV testing. In those countries, information about HPV clearance or persistence can be obtained by linking the HPV results of two subsequent screening rounds. The strong association between CIN3+ and HPV persistence over two screening rounds and the possibility to reassure women who have cleared the HPV infection implies that countries which currently have different views on the suitability of short-term viral persistence for managing HPV-positive women likely agree on the role of long-term persistence. Besides, including persistence over two consecutive screening rounds as a triage marker is attractive because it does not require additional testing. However, individual linkage of results from different rounds of screening has not yet been implemented in every country with a cervical cancer screening programme. In the Netherlands, a central information technology system named ScreenIT has recently been implemented which facilitates individual linkage of screening results over time.[31]

Strengths of our study are the long follow-up and the fact that the POBASCAM trial is conducted within the Dutch screening programme, which makes results translatable to a population-based screening setting. A limitation of our study is that only a small subgroup of the large, original trial were eligible for this post-hoc analysis, but it is to our knowledge one of the first cohorts in which women have been screened with HPV-based screening in two consecutive screening rounds. Furthermore, results only apply to unvaccinated women, but in the coming three decades, there will still be unvaccinated birth cohorts that rely on screening as the only cervical cancer prevention method. Nevertheless, vaccinated cohorts are reaching screening ages and additional research will have to be performed to re-evaluate the screening

interval and performance of triage strategies in those cohorts and whether separate programmes are needed for unvaccinated and vaccinated women the same age.

In conclusion, the HPV result at the previous screen is a useful marker for risk stratification of HPV-positive women in the second HPV-based screening round. When combined with cytology triage, it obviates the need for repeat cytology testing. Cytology combined with either HPV16/18-genotyping or the previous screen HPV result showed high NPV and PPV values for detection of CIN3+ and are easy to implement. The combination of cytology and HPV16/18-genotyping had the highest NPV and therefore may be preferred for reasons of safety, but the strategy combining cytology with previous screen HPV result had a lower colposcopy referral rate and is expected to lead to a lower number of unnecessary treatments. The eventual choice depends on factors such as disease burden, preferences, and resources which vary across settings.

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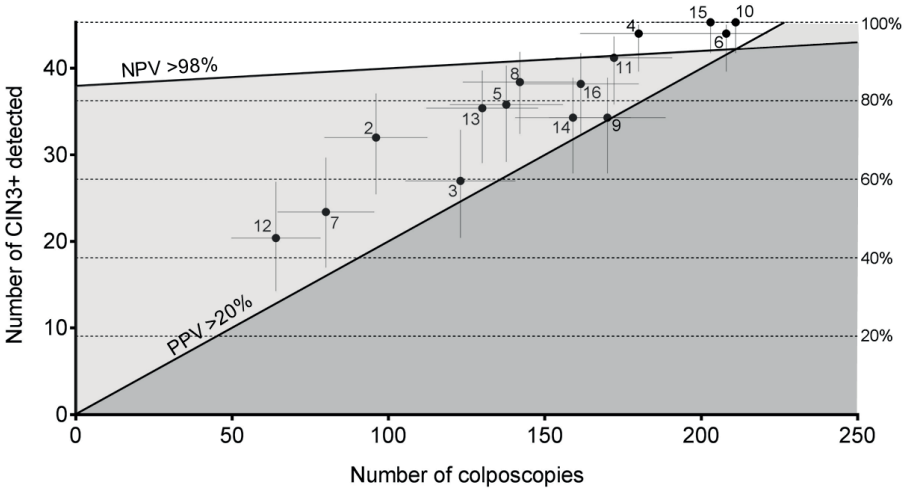
Supplementary Table S1. Clinical performance of different triage strategies for detection of CIN2+ in HPV–positive women (n = 366) the second HPV–based screening round.

	Triage strategy	NPV		PPV		Sensitivity		Specificity		Colposcopy referral rate	
1.	No further testing	-		20.0%	(15.8 - 24.8%)	100.0%		0.0%		100.0%	
2.	Cytology	90.0%	(85.2 - 93.6%)	47.9%	(38.2 - 57.8%)	62.9%	(50.8 - 73.8%)	82.9%	(77.7 - 87.3%)	26.2%	(21.5 - 31.4%)
3.	HPV16/18-genotyping	85.5%	(80.0 - 89.9%)	30.8%	(22.5 - 40.2%)	51.8%	(39.8 - 63.6%)	70.9%	(64.9 - 76.4%)	33.6%	(28.5 - 39.1%)
4.	Cytology and HPV16/18-genotyping	95.5%	(91.1 - 98.2%)	36.1%	(28.9 - 43.7%)	88.8%	(78.9 - 94.6%)	60.7%	(54.3 - 66.8%)	49.2%	(43.6 - 54.8%)
5.	Cytology and repeat cytology	93.4%	(88.9 - 96.5%)	42.4%	(34.2 - 51.1%)	79.2%	(67.9 - 87.5%)	73.2%	(67.3 - 78.5%)	37.3%	(31.9 - 42.8%)
6.	Cytology, HPV16/18-genotyping and repeat cytology	97.4%	(93.1 - 99.5%)	33.3%	(26.8 - 40.4%)	94.4%	(86.2 - 98.2%)	52.8%	(46.4 - 59.1%)	56.6%	(51.0 - 62.1%)
7.	HPV previous screen	85.0%	(80.0 - 89.2%)	37.9%	(27.3 - 49.6%)	41.5%	(30.1 - 53.6%)	83.0%	(77.8 - 87.4%)	21.9%	(17.5 - 26.8%)
8.	HPV previous screen and cytology	92.5%	(87.7 - 95.8%)	39.6%	(31.7 - 48.2%)	77.0%	(65.5 - 85.8%)	70.7%	(64.7 - 76.3%)	38.8%	(33.4 - 44.4%)
9.	HPV previous screen and HPV16/18-genotyping	88.8%	(83.1 - 93.0%)	30.1%	(23.0 - 38.0%)	70.0%	(58.0 - 79.9%)	59.4%	(53.1 - 65.5%)	46.4%	(40.9 - 52.1%)
10.	HPV previous screen, cytology and HPV16/18-genotyping	97.4%	(93.1 - 99.5%)	32.8%	(26.3 - 39.8%)	94.7%	(86.4 - 98.3%)	51.6%	(45.2 - 57.9%)	57.7%	(52.0 - 63.1%)
11.	HPV previous screen, cytology and repeat cytology	95.0%	(90.4 -97.8%)	37.0%	(29.7 - 44.8%)	86.6%	(76.4 - 93.1%)	63.1%	(56.8 - 69.1%)	46.8%	(41.2 - 52.5%)
12.	HPV type persistence	84.2%	(79.1 - 88.3%)	39.5%	(27.8 - 52.4%)	34.6%	(24.0 - 46.7%)	86.8%	(82.0 - 90.6%)	17.5%	(13.5 - 22.1%)
13.	HPV type persistence and cytology	91.6%	(86.8 - 95.1%)	41.0%	(32.7 - 49.8%)	72.9%	(61.1 - 82.4%)	73.8%	(67.9 - 79.1%)	35.5%	(30.3 - 41.1%)
14.	HPV type persistence and HPV16/18-genotyping	88.4%	(82.8 - 92.6%)	30.9%	(23.6 - 39.2%)	67.3%	(55.2 - 77.6%)	62.5%	(56.2 - 68.5%)	43.4%	(37.9 - 49.1%)
15.	HPV type persistence, cytology and HPV16/18-genotyping	97.5%	(93.4 - 99.5%)	34.1%	(27.4 - 41.3%)	94.7%	(86.4 - 98.3%)	54.3%	(47.9 - 60.6%)	55.5%	(49.8 - 61.0%)
16.	HPV type persistence, cytology and repeat cytology	93.8%	(89.0 - 96.9%)	37.5%	(30.1 - 45.6%)	82.5%	(71.7 - 90.1%)	65.8%	(59.5 - 71.6%)	43.9%	(38.4 - 49.5%)

CIN3+ = cervical intraepithelial neoplasia grade 3 or worse; HPV = human papillomavirus; NPV = negative predictive value; PPV = positive predictive value.



Supplementary Figure S1. Representation of the colposcopy referral rates with NPV and PPV thresholds.

CIN3+ = Cervical Intraepithelial Neoplasia grade 3 or worse; NPV = Negative predictive value; PPV = Positive predictive value.