Research Article
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Primary hrHPV DNA Testing in Cervical Cancer Screening: How to Manage Screen-Positive Women? A POBASCAM Trial Substudy

Maaike G. Dijkstra[1,3], Dirk van Niekerk[5], Dorien C. Rijkaart[1], Folkert J. van Kemenade[4], Daniëlle A.M. Heideman[1], Peter J.F. Snijders[1], Chris J.L.M. Meijer[1], and Johannes Berkhof[2]

Abstract

Background: High-risk human papillomavirus (hrHPV) testing has higher sensitivity but lower specificity than cytology for cervical (pre)-cancerous lesions. Therefore, triage of hrHPV-positive women is needed in cervical cancer screening.

Methods: A cohort of 1,100 hrHPV-positive women, from a population-based screening trial (POBASCAM: $n = 44,938$; 29–61 years), was used to evaluate 10 triage strategies, involving testing at baseline and six months with combinations of cytology, HPV16/18 genotyping, and/or repeat hrHPV testing. Clinical endpoint was cervical intraepithelial neoplasia grade 3 or worse (CIN3$^+$) detected within four years; results were adjusted for women not attending repeat testing. A triage strategy was considered acceptable, when the probability of no CIN3$^+$ after negative triage (negative predictive value, NPV) was at least 98%, and the CIN3$^+$ risk after positive triage (positive predictive value, PPV) was at least 20%.

Results: Triage at baseline with cytology only yielded an NPV of 94.3% [95% confidence interval (CI), 92.0–96.0] and a PPV of 39.7% (95% CI, 34.0–45.6). An increase in NPV, against a modest decrease in PPV, was obtained by triaging women with negative baseline cytology by repeat cytology (NPV 98.5% and PPV 34.0%) or by baseline HPV16/18 genotyping (NPV 98.8% and PPV 28.5%). The inclusion of both HPV16/18 genotyping at baseline and repeat cytology testing provided a high NPV (99.6%) and a moderately high PPV (25.6%).

Conclusions: Triaging hrHPV-positive women by cytology at baseline and after 6 to 12 months, possibly in combination with baseline HPV16/18 genotyping, seems acceptable for cervical cancer screening.

Impact: Implementable triage strategies are provided for primary hrHPV screening in an organized setting. *Cancer Epidemiol Biomarkers Prev; 23(1); 1–9. ©2013 AACR.*

Introduction

Cervical cancer develops through several intermediate steps, and cervical cancer prevention strategies exploit this knowledge by the timely identification and treatment of cervical intraepithelial neoplasia (CIN). The usual threshold for therapeutic intervention is CIN grade 2 (CIN2) or worse (CIN2$^+$); however, the histologic diagnosis of CIN2 is imprecise (1), and CIN2 often regresses (2, 3). Therefore, many argue that the risk of having or developing CIN grade 3 or worse (CIN3$^+$) should form the basis of cervical cancer prevention strategies (2, 4, 5).

Previously, randomized controlled trials and population-based cohort studies have shown that a negative high-risk human papillomavirus (hrHPV) DNA test carries a low risk (<2%) for CIN3$^+$, for even up to 5 years (5–11).

In addition, several studies have shown that primary hrHPV screening is more sensitive than cytology for detecting CIN2/3 lesions (6, 7, 12–17), and cervical cancer (9, 11, 18), but is less specific (2.5%–4%), and the resulting decreased positive predictive value (PPV) for CIN3$^+$ may lead to overreferral and overtreatment of patients (15). Thus, management of hrHPV screen–positive women remains a clinical dilemma (5).

Epidemiologic studies have indicated that detection of HPV16, HPV18, or both might be used to identify women with an increased risk for CIN3$^+$ (6, 10, 19). The results from the ATHENA trial also support the use of HPV16/18 genotyping, as positivity for these hrHPV types was associated with an increased CIN3$^+$ risk in women with normal cytology (20, 21). This is in line with the final results of the POBASCAM study (9), showing that the protective effect of HPV testing against CIN3$^+$ in the subsequent screening round, was largely attributable to

Authors' Affiliations: Departments of [1]Pathology and [2]Epidemiology and Biostatistics, VU University Medical Center; [3]Department of Obstetrics and Gynaecology, St. Lukas Andreas Ziekenhuis, Amsterdam; [4]Department of Pathology, Erasmus MC University Medical Center, Rotterdam, the Netherlands; and [5]Department of Pathology, British Columbia Cancer Agency, Vancouver, British Columbia, Canada

Corresponding author: Chris J.L.M. Meijer, Department of Pathology, VU University Medical Center, P.O. Box 7057, 1007 MB Amsterdam, the Netherlands. Phone: 31-20-444-4070; Fax: 31-20-444-2964; E-mail: cjlm.meijer@vumc.nl

the timely identification and treatment of HPV16-positive lesions in the baseline round. Yet, in a recent hrHPV screening trial (22), direct triage with cytology and repeat cytology testing at 12 months emerged as a suitable implementation strategy. Thus, there is no consensus on the best way to manage hrHPV DNA–positive women.

Here, we performed an analysis of data from a recently completed population-based randomized controlled trial (POBASCAM), in which, 44,938 women between 29 and 61 years of age were enrolled. Our study's aim was to evaluate triage strategies based on hrHPV genotyping and cytology, with regard to $CIN3^+$ detected within 4 years, to provide directives on how to manage hrHPV DNA–positive women in the setting of nationwide cervical cancer screening.

Materials and Methods

Study population

We evaluated the data from the population-based screening study Amsterdam (POBASCAM; trial registration ID NTR218). The study design and results have been published elsewhere (6, 9, 23). Briefly, women between 29 and 61 years of age were invited to participate in cervical screening and were randomized, either to a conventional cytology–based control arm, or to the intervention arm, in which women were managed on the basis of cytology plus hrHPV DNA test results (both scored blinded for each other). In total, 44,938 women were enrolled, of whom 22,420 were randomized to the intervention arm, and evaluation of this cohort forms the basis of this study (Fig. 1).

A group of 242 family practitioners participated in POBASCAM. They collected samples for cytology using a Rovers Cervex-Brush or a cytobrush. After preparing a conventional cytology smear, the brush was placed in a vial containing collection medium for hrHPV testing.

Management

The management of combined cytology and hrHPV DNA results at baseline has been described previously (6, 9). In short, all hrHPV-positive women with moderate dyskaryosis or worse (>BMD, borderline or mild dyskaryosis) cytology, corresponding to high-grade squamous intraepithelial lesions in the Bethesda system (TBS; ref. 24), were directly referred for colposcopy. Of note, hrHPV-positive women with borderline or mild dyskaryosis (BMD) cytology at baseline were advised to repeat cytology and hrHPV testing at 6 and 18 months. These women were referred for colposcopy at 6 months, if they had >BDM cytology, or BMD cytology in combination with an hrHPV-positive test result, whereas they were referred at 18 months if they had >BMD cytology and/or an hrHPV-positive test result. Women with normal cytology at baseline were also advised to repeat cytology and hrHPV testing at 6 and 18 months. They were referred at 6 months if they had >BMD cytology, and were referred at 18 months if they had >BMD cytology and/or a positive hrHPV test result.

Colposcopy and histology

Colposcopy-guided biopsies of the cervix were taken by gynecologists according to the guideline of the Dutch Society of Obstetrics and Gynaecology (Utrecht, the Netherlands; ref. 25). Histologic biopsies were only taken when cervical abnormalities were seen. Histologic follow-up was obtained from 4 participating laboratories, and data were also tracked through the nationwide pathology database [Pathologisch Anatomisch Landelijk Geautomatiseerd Archief (PALGA); ref. 26], with a follow-up time of 78 months. Histology was examined locally and classified as normal, CIN grade 1, 2, and 3, or invasive cancer according to international criteria (27). Adenocarcinoma *in situ* was included in the CIN grade 3 group. To account for variations around the targeted screening interval length of 5 years, CIN or cancer cases detected during the first 48 months were labeled as cases detected at the baseline round, whereas $CIN3^+$ cases detected at a later time were labeled as detected at the subsequent screening round (9). Treatment of abnormalities was according to protocols (25).

hrHPV testing

All hrHPV tests ($GP5^+/6^+$-PCR EIA) were carried out in duplicate in the Department of Pathology at VU University Medical Center (Amsterdam, the Netherlands), without knowledge of cytology results, as described previously (23, 28). hrHPV-positive samples were subsequently typed, using a previously published reverse line blot assay (29).

Statistical analysis

Ten triage strategies were evaluated, and analyses of our data were performed for disease endpoints of $CIN3^+$ and $CIN2^+$, cumulatively detected at 48 months. The primary endpoint was $CIN3^+$. Sensitivity, specificity, negative predictive value (NPV), PPV, and colposcopy referral rates were computed together with two-tailed 95% confidence intervals (CI) using the Wilson score method (30). SPSS software version 15.0 (LEAD Technologies Inc.) and Excel (Microsoft Corporation) were used. A time window of 48 months was chosen, because it allows sufficient time for follow-up investigations within one round and does not include CIN detected after the next screening invitation.

Cytology was dichotomized, and a positive cytology result was considered as BMD or worse (BMD^+), which corresponds to atypical squamous cells of undetermined significance or worse in the Bethesda 2001 nomenclature. Time to first follow-up, expressed in months, was calculated along with the mean, maximum, minimum, and SD. Women who were colposcopically verified at baseline, or who had at least one repeat visit with complete test results, were included. To correct for loss to follow-up, observed proportions in cases with follow-up were applied to cases with missing data. Of note, 95% CIs were calculated using corrected proportions, but

Figure 1. Flow diagram of POBASCAM intervention arm, depicting the baseline and follow-up results after 4 years.
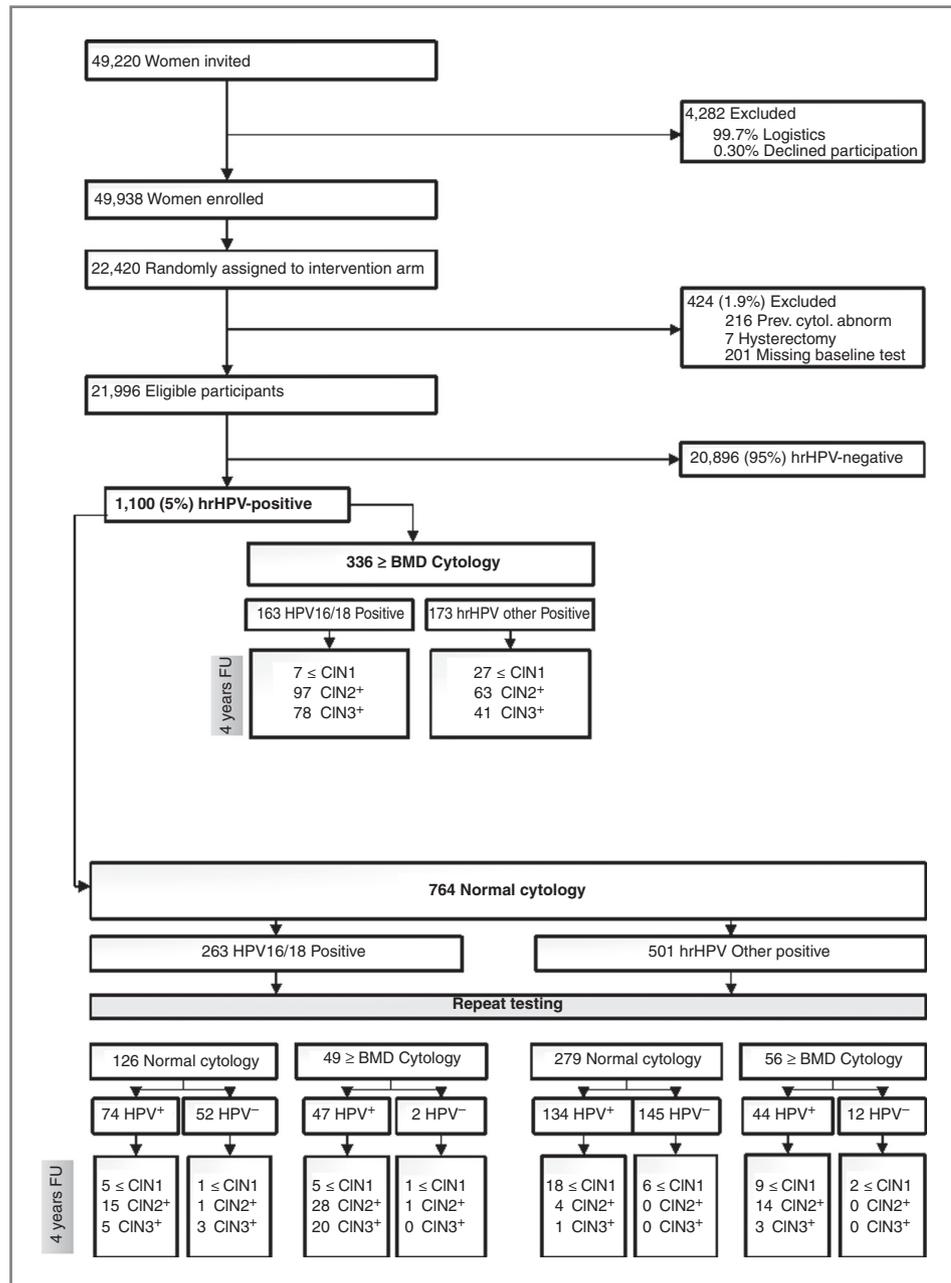
using only directly observed cases for sample size values. To judge the performance of triage strategies, we defined acceptability thresholds for NPV and PPV for CIN3$^+$. We considered a triage strategy as acceptable when the probability of no CIN3$^+$ (cumulatively detected within 48 months) after negative triage (NPV) was at least 98%, and the CIN3$^+$ risk after positive triage (PPV) was at least 20%. The threshold for NPV was based on the current CIN3$^+$ risk of women with BMD at baseline, and normal cytology at 6 and 18 months follow-up (1.2%), which is presently accepted in the Netherlands (31). Furthermore, we considered a PPV of 20% as acceptable, as this would translate into a chance of

one in five to detect high-grade CIN among referred women.

Results

Characteristics of the POBASCAM intervention arm

In the POBASCAM intervention arm, 1,100 women were hrHPV-positive, and in Fig. 1 baseline and follow-up results are presented. Histology was available for 194 (58%) of the 336 women with abnormal cytology (BMD$^+$) at baseline, cumulative after one screening round (4 years). Furthermore, 510 of 764 women with normal baseline cytology attended at least one repeat visit of hrHPV and cytology cotesting, with an average time after

Table 1. Age distribution of hrHPV-positive women ($n = 1,100$) and CIN2$^+$ and CIN3$^+$ detected within 4 years

Age, y	hrHPV-positive baseline				4 Years		
	N	%	CIN2$^+$	%	CIN3$^+$	%
29–33	372	34	83	37	58	39
34–38	312	28	71	32	39	26
39–43	136	12	29	13	23	16
44–48	96	9	20	9	14	9
49–53	77	7	9	4	7	5
54–58	64	6	4	2	3	2
59–61	43	4	7	3	4	3
Total	1,100	100	223	100	148	100

baseline of 8.75 months and a SD of 3.96. The minimum follow-up time in the screening round was 0 months (a single case), and the maximum time was 30 months. Histology was available for 110 (22%) of these women after 4 years.

The age distribution of the cohort and the numbers of detected CIN3$^+$ and CIN2$^+$ per age group are shown in Table 1.

Strategies to triage hrHPV-positive women

The performances of three baseline triage strategies, without a follow-up test, are shown in Table 2. Triage of hrHPV-positive women at baseline, with cytology only, yielded an NPV for CIN3$^+$ of 94.3%, clearly below our threshold of 98%, and therefore this strategy was not deemed acceptable. Baseline cytology testing combined with HPV16 genotyping met the NPV and PPV thresholds, with an NPV of 98.1% and a PPV of 30.5%. The sensitivity and specificity for detection of CIN3$^+$ were 94.1% and 58.8%, respectively. The colposcopy referral rate was 49.7% of the hrHPV positives, translating into a referral rate of 2.49% of women in the POBASCAM intervention arm. Results for baseline cytology testing combined with HPV16 and HPV18 genotyping were similar, although the NPV was slightly higher (98.8%), the PPV was slightly lower (28.5%), sensitivity and referral rate were moderately higher, and the specificity was lower.

Lowering the endpoint threshold from CIN3$^+$ to CIN2$^+$ yielded 5% to 10% lower sensitivities for all strategies without repeat HPV DNA testing at 6 months. The clinical relevance of those extra-detected CIN2 cases is not clear (Table 2). Within the subgroup of women with negative cytology and a positive HPV16/18 genotyping test result at baseline, the PPV was 14.3% (95% CI, 9.5%–20.4%), which is about twice as low as the PPV in women with abnormal cytology and a positive HPV16/18 result. Seven triage strategies, combining baseline testing with one round of follow-up at 6 months, were evaluated (Table 3). All of these strategies had estimated NPVs for CIN3$^+$ risk above 98%. The PPV estimates ranged from 20.0% to 34.0%, and colposcopy referral rates varied between

44.8% and 80.3% of hrHPV positives, corresponding with referral rates between 2.24% and 4.02% in the total intervention arm.

Baseline cytology testing, followed by repeat cytology screening for women with negative baseline cytology, had an estimated NPV for CIN3$^+$ of 98.5%, combined with the highest estimated PPV (34.0%) and the lowest referral rate (44.8%). The sensitivity and specificity for detection of CIN3$^+$ were 94.4% and 64.7%, respectively, and for CIN2$^+$, these were 89.2% and 70.1%, respectively. The inclusion of HPV16/18 genotyping at baseline, whereas retaining repeat cytology testing at follow-up resulted in high NPV for CIN3$^+$ (99.6%) at the cost of a slightly lower PPV (25.6%). In addition, the sensitivity increased to 99.2%, whereas the specificity was lower, i.e., 45.0%. For detection of CIN2$^+$, these were 97.8% and 50.0%, respectively. The referral rate increased to 62.1% for hrHPV-positive women.

All four strategies with repeat hrHPV testing at follow-up yielded very high sensitivities (and NPVs) for both CIN3$^+$ and CIN2$^+$, yet specificities were much lower, i.e., all were below 35.0%. More importantly, these strategies resulted in substantially higher colposcopy referral rates (i.e., 71.3%–80.3% of hrHPV-positive women).

The NPV and PPV of the 10 triage strategies for hrHPV-positive women are graphically shown in Fig. 2.

Discussion

The intervention arm of the POBASCAM trial provided us with an opportunity to assess the clinical performance of triage strategies for hrHPV-positive women, in a large screening population followed for 4 years within one screening round. Ten triage strategies were evaluated, and most of these met the thresholds for NPV and PPV of 98% and 20%, respectively. The three strategies that showed the best balance between the safety of a strategy (NPV), and the burden of screening on patients and clinicians (PPV and referral rate), were (i) cytology and HPV16/18 genotyping at baseline without repeat testing, (ii) cytology at baseline with repeat cytology testing after 6

Table 2. Sensitivity, specificity, NPV, PPV, and colposcopy referral rate of three baseline triage strategies; CIN2$^+$ and CIN3$^+$ cases detected within 4 years were included

Baseline triage strategy	Endpoint CIN3$^+$				Endpoint CIN2$^+$					Total screening population
	Sensitivity (95% CI)	Specificity (95% CI)	NPV (95% CI)	PPV (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)	NPV (95% CI)	PPV (95% CI)	Colposcopy referral rate (95% CI)	Colposcopy referral rate (95% CI)
I. Cytology	75.4% (67.9–81.7)	78.0% (74.6–81.1)	94.3 (92.0–96.0)	39.7% (34.0–45.6)	66.0% (59.6–71.9)	81.4% (78.0–84.4)	87.96% (84.5–90.2)	54.5% (48.5–60.4)	30.5% (27.5–33.7)	1.53% (1.38–1.69)
II. Cytology/HPV16	94.1% (89.1–96.9)	58.8% (54.9–62.6)	98.1% (96.2–99.1)	30.5% (26.2–35.1)	86.1% (80.9–90.0)	62.6% (58.5–66.5)	93.0% (90.0–95.2)	43.8% (39.0–48.6)	49.7% (46.5–53.0)	2.49% (2.33–2.65)
III. Cytology/HPV16/18	96.6% (92.3–98.5)	53.6% (49.7–57.5)	98.8% (97.0–99.5)	28.5% (24.4–32.8)	90.3% (85.7–93.5)	57.6% (53.3–61.7)	94.6% (91.7–96.6)	41.8% (37.3–46.6)	54.5% (51.2–57.6)	2.73% (2.56–2.88)

Table 3. Sensitivity, specificity, NPV, PPV, colposcopy referral rate, and mean number of repeat tests of seven strategies with baseline triage and repeat testing at 6 months; CIN2$^+$ and CIN3$^+$ cases detected within 4 years were included

| Baseline triage test	Repeat test (t = 6 months)	Endpoint CIN3$^+$				Endpoint CIN2$^+$						Total screening population
		Sensitivity (95% CI)	Specificity (95% CI)	NPV (95% CI)	PPV (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)	NPV (95% CI)	PPV (95% CI)	Repeat test (95% CI)	Colposcopy referral rate (95% CI)	Colposcopy referral rate (95% CI)
IV. Cytology	HPV	100% (97.5–100)	34.1% (30.6–38.0)	100.0% (98.2–100)	22.5% (19.3–26.2)	98.8% (96.5–97.7)	38.1% (34.1–42.2)	99.1% (96.6–99.7)	35.0% (31.2–39.0)	69.5% (66.7–72.1)	71.3% (68.1–74.2)	3.57% (3.41–3.71)
V. Cytology	Cytology	94.9% (90.1–97.5)	64.7% (60.9–68.4)	98.5% (96.8–99.3)	34.0% (29.4–39.0)	89.2% (84.5–92.6)	70.1% (62.2–78.8)	95.1% (95.5–96.8)	50.2% (45.1–55.2)	69.5% (66.7–72.1)	44.8% (41.5–48.2)	2.24% (2.08–2.41)
VI. Cytology	Cytology/HPV	100.0% (97.5–100)	32.0% (24.8–35.7)	100% (98.1–100)	22.0% (18.8–25.5)	99.5% (97.4–99.9)	35.7% (31.8–39.9)	99.5% (97.2–99.9)	34.3% (30.6–38.3)	69.5% (66.7–72.1)	73.2% (70.1–76.0)	3.66% (3.51–3.80)
VII. Cytology/HPV16	Cytology	98.3% (94.7–99.5)	49.5% (45.6–53.3%)	99.4% (97.7–98.8)	27.2% (23.4–31.4)	95.2% (91.5–97.3)	54.3% (50.1–58.4)	97.1% (94.6–98.5)	41.3% (37.0–45.8)	50.3% (47.3–53.2)	58.2% (55.0–61.4)	2.91% (2.75–3.07)
VIII. Cytology/HPV16	Cytology/HPV	100.0% (97.5–100)	25.0% (21.7–28.5)	100% (97.6–100)	20.4% (17.4–23.7)	99.5% (97.4–99.9)	27.9% (24.3–31.7)	99.4% (96.4–99.9)	31.8% (28.3–35.6)	50.3% (47.3–53.2)	79.1% (76.3–81.6)	3.96% (3.82–4.08)
IX. Cytology/HPV16/18	Cytology	99.2% (96.0–98.8)	45.0% (41.2–48.9)	99.6% (98.0–99.9)	25.6% (22.0–29.6)	97.85 (95.0–99.1)	50.0% (45.8–54.1)	98.6% (96.4–99.4)	39.7% (35.5–44.1)	45.5% (42.6–48.5)	62.1% (58.9–65.1)	3.11% (2.95–3.26)
X. Cytology/HPV16/18	Cytology/HPV	100.0% (97.5–100)	23.5% (20.3–26.9)	100% (97.4–100)	20.0% (17.0–23.3)	100.0% (98.3–100)	26.4% (22.9–30.2)	100% (97.4–100)	31.4% (27.9–35.1)	45.5% (42.6–48.5)	80.3% (77.6–82.7)	4.02% (3.88–4.14)
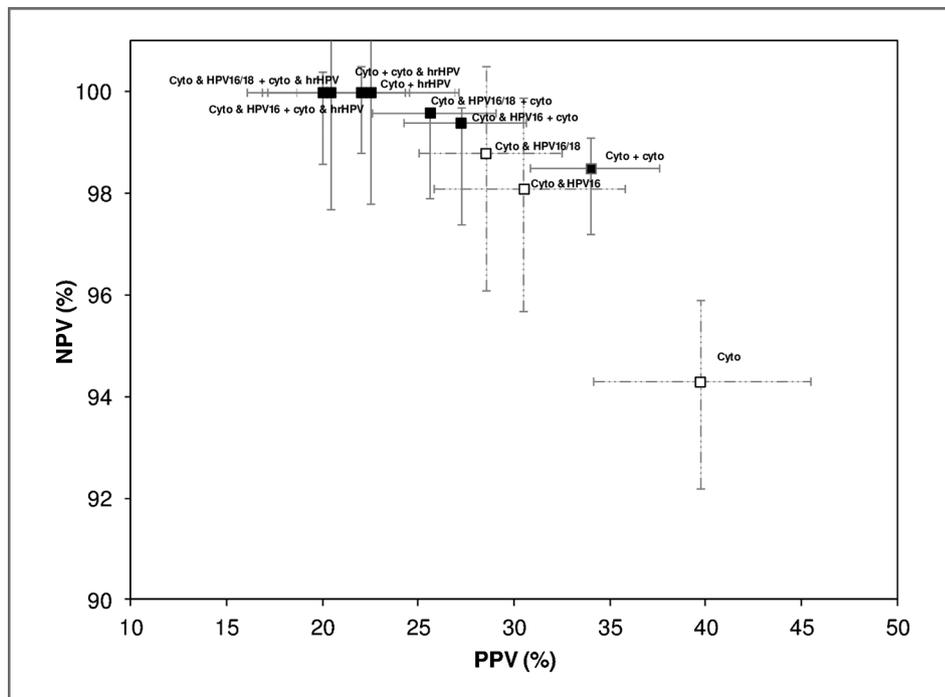
Figure 2. NPV and PPV for CIN3$^+$ of the ten triage strategies for hrHPV-positive women. □, triage strategy without a repeat test; ■, triage strategy with one round of repeat testing; bars, 95% CIs; cyto, strategy I; cyto and HPV16, strategy II; cyto and HPV16/18, strategy III; cyto + hrHPV, strategy IV; cyto + cyto, strategy V; cyto + cyto and hrHPV, strategy VI; cyto and HPV16 + cyto, strategy VII; cyto and HPV16 + cyto and hrHPV, strategy VIII; cyto and HPV16/18 + cyto, strategy IX; cyto and HPV16/18 + cyto and hrHPV, strategy X.

months, and (iii) cytology and HPV16/18 genotyping at baseline followed by repeat cytology examination at 6 months.

A great benefit of direct triage at baseline with cytology and HPV16/18 genotyping is the avoidance of follow-up testing, because this results in 20% to 40% loss to follow-up, particularly after normal cytology (6, 9, 32). In our study, the attendance rate at repeat testing was 77% (6, 9).

An important disadvantage of baseline triage is that the PPV is limited, indicating a considerable risk of overtreatment. The burden of screening will be especially high for HPV16/18 positives with normal cytology, as the PPV for CIN3$^+$ in this subgroup was only 14.3%. Stated differently, 86% of these referred women will not have underlying CIN3$^+$. Second, physicians might find it difficult to communicate the low risk of non-16, non-18 hrHPV infections to women that are triage test negative (hrHPV16/18–negative and normal cytology). Therefore, adequate education of physicians is essential when implementing this strategy.

In contrast, triage by cytology testing at baseline followed by repeat cytology at 6 months for women with normal baseline cytology is easy to communicate: hrHPV testing is used to define the risk population, whereas cytology indicates the presence of a precursor lesion. Normal cytology at baseline and at rescreening should reassure hrHPV-positive women that a return to routine screening is acceptable. Another advantage of baseline and repeat cytology testing is the low referral rate, and, in addition, the highest PPV for CIN3$^+$ (34.0%). It should be kept in mind that, in countries with less efficient cytology screening, and thus higher cytologic abnormality rates than in the Netherlands, or in countries with more frequent screening (yearly), the PPV advantage of cytology

may not hold, and therefore direct (baseline) genotyping may be considered for triage instead (ATHENA trial; ref. 20). Adding HPV16/18 genotyping to cytology testing at baseline, while retaining follow-up cytology testing, might be considered in these countries, as is the safest strategy (highest NPV), whereas the increase in referral rate is unlikely to cause capacity problems. However, the eventual triage strategy should also take into account potential overtreatment induced by the increase in referral rate.

An even more rigorous screening approach, with baseline cytology examination followed by repeat hrHPV and cytology cotesting, may not be efficient, and the PPV will be relatively low. In our study, only six CIN3$^+$ lesions were detected within 48 months, among 208 participants with normal cytology at baseline and at follow-up, who tested persistently hrHPV-positive.

Previously, two other prospective trials have also compared strategies with triage screen-positive women following primary hrHPV DNA–based screening (22, 33). Data from our study match well with those of the Vrije Universiteit Medical Center Saltro Laboratory population-based cervical screening (VUSA)-screen study, performed in the Netherlands, in which triage by cytology testing at baseline followed by cytology at 12 months also showed the highest PPV in combination with a high NPV (22). As a result of the similar results between these studies, we believe that in the triage strategy with baseline cytology followed by cytology retesting, the time to follow-up testing can be either 6 or 12 months. A limitation of the VUSA-screen study, however, was the duration of follow-up, which was 3 years, whereas cervical screening is usually offered every 5 years in the Dutch screening program.

Furthermore, in a nested evaluation of the Swedish SWEDESCREEN study, the efficacy of 11 screening strategies was evaluated in 6,257 women cotested with hrHPV and cytology (33). That study suggested to follow-up hrHPV-positive women with normal cytology at baseline, by one repeat hrHPV DNA test. This strategy showed comparable results in terms of sensitivity for $CIN3^+$ (96.0%), however, with somewhat lower PPV (22.0%) than obtained with the three preferred strategies in our study. Furthermore, our study indicates that implementation of the suggested strategy leads to a substantial increase in colposcopy referral rate and thus possible overtreatment. In addition, a limitation of the Swedish study was the relatively narrow age range (32–38 years) of the study participants, which makes generalizations to whole screening populations less compelling. Other trials evaluating baseline cytology triage (17, 20) have revealed substantially lower PPVs than in both studies performed in the Netherlands [i.e., the VUSA-screen study (ref. 22) and the present study]. This might be explained by the efficiency of the Dutch screening program, with an abnormal cytology rate of only 1.8% (34), a long screening interval (5-year), a high age at which screening starts (at the age of 30), and a low percentage of excess smear use (35).

Advantages of this study include the large size, the long follow-up, and the age range of study participants (30–60 years), which is the age for which screening by hrHPV testing is most widely advocated (36). Furthermore, the study is nested within a population-based screening program, indicating that results should be scalable to whole populations.

There were also limitations to the study. First, not all of 764 hrHPV positives with normal cytology at baseline returned for follow-up. In total, 510 (66.8%) completed at least one follow-up test. We corrected for loss to follow-up by extrapolating observed rates among subjects with at least one repeat test, to subjects without repeat testing. Such a procedure corrects for participants who did not attend repeat testing, but does not distinguish between complete and incomplete repeat testing. We studied this extensively and observed that an additional adjustment for incomplete repeat testing had only a minor effect on the estimates; these were therefore not presented. Second, even with correction for loss to follow-up, there is still a possibility of negative verification bias, as only 22% of the attendants ultimately had biopsy verification after 48 months. This effect is mitigated by the fact that a further 197 (38.6%) of the 510 hrHPV-positive women with normal cytology had at least one repeat visit, which showed negative cytology and hrHPV test results. Previous studies have shown that a double-negative cotest result is associated with an extremely low risk for $CIN3^+$ (6–9, 12). Another limitation of our study was that some of the HPV16/18-positive $CIN3^+$ lesions that were used to calculate the PPV of baseline triage with cytology and hrHPV genotyping were actually detected at follow-up, which may positively bias the PPV. Furthermore, in our

study, we have solely evaluated triage strategies based on cytology, hrHPV genotyping, and combinations thereof, though, it is likely that the role of cytology becomes more limited in future screening and validated molecular biomarkers gain attention; among these, $p16^{INK4a}$/Ki-67 double staining and host genome, or viral DNA methylation markers seem to be promising (37–41). These markers could also be of value, if in a particular country cytologic reading does not meet quality criteria, and adjunct testing is required. Further validation in prospective studies is needed before these tests can be considered for screening.

In summary, triaging hrHPV-positive women by cytology at baseline and repeat cytology testing after 6 to 12 months, possibly in combination with baseline HPV16/18 genotyping, seems safe and yields an acceptable colposcopy referral rate. The weights placed on safety and screening-related burden, as well as the quality of cytology in a particular country, will likely determine the eventual management of hrHPV-positive women.

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Maaike G. Dijkstra, Dirk van Niekerk, Dorien C. Rijkaart, et al.

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