EVALUATION OF 14 TRIAGE STRATEGIES FOR HPV DNA POSITIVE WOMEN IN POPULATION BASED CERVICAL SCREENING

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ABSTRACT

Introduction

High-risk human papillomavirus (hrHPV) testing has a higher sensitivity but lower specificity than cytology for detection of high-grade intraepithelial neoplasia (CIN). To avoid over-referral to colposcopy and overtreatment, hrHPV-positive women require triage testing and/or follow-up.

Methods

A total of 25,658 women (30–60 years) enrolled in a population-based cohort study had an adequate baseline Pap smear and hrHPV test. The end-point was cumulative two-year risk of CIN grade 3 or worse (CIN3+). In a post-hoc analysis, fourteen triage/follow-up strategies for hrHPV-positive women (n = 1,303) were evaluated for colposcopy referral rate, positive (PPV) and negative predictive value (NPV). Five strategies involved triage testing without a repeat test and nine strategies involved triage testing followed by one repeat testing. The tests were cytology, hrHPV, HPV16/18 genotyping and HPV16/18/31/33/45 genotyping.

Results

Results were adjusted for women in the cohort study who did not attend repeat testing. Of the strategies without repeat testing, combined cytology and HPV16/18/31/33/45 genotyping gave the highest NPV of 98.9% (95%CI 97.6–99.5%). The corresponding colposcopy referral rate was 58.1% (95%CI 55.4–60.8%). Eight of the nine strategies with retesting had an estimated NPV of at least 98%. Of those, cytology triage followed by cytology at 12 months had a markedly lower colposcopy referral rate of 33.4% (95%CI 30.2–36.7%) than the other strategies. The NPV of the latter strategy was 99.3% (95%CI 98.1–99.8%).

Conclusion

Triage hrHPV-positive women with cytology, followed by repeat cytology testing yielded a high NPV and modest colposcopy referral rate and appear to be the most feasible management strategy.

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Introduction

Strong evidence is now available that testing for high-risk human papillomavirus (hrHPV) infection is more sensitive than cytology in detecting high-grade cervical intraepithelial neoplasia (CIN).[1–7] However, hrHPV testing also detects more transient hrHPV infections than cytology,[1,8] which may lead to over-referral for colposcopy and thus overtreatment.[5] Management of hrHPV-positive women is, therefore, of major concern. In particular, in countries with an efficient cytology-based screening program and a moderate colposcopy referral rate such as the Netherlands and the United Kingdom, the increased burden on healthcare resources upon introduction of a less-specific screening test may be substantial. To control the number of colposcopy referrals, hrHPV-positive women should not be offered colposcopy immediately but should be further stratified by means of triage testing and repeat testing. Several triage suggestions have been made in the literature including cytology, hrHPV geno-typing and hrHPV-type specific persistence analysis and p16 staining,[1, 9, 10] but no uniform algorithm has emerged until now.

To evaluate the effectiveness of hrHPV testing in primary cervical screening, we set up the Vrije Universiteit Medical Centre- Saltro laboratory population-based cervical screening (VUSA-Screen study) within the routine cervical screening program in the Netherlands. Women participating in this cohort received combined hrHPV testing and cytology instead of cytology. We used the data from the cohort study for post-hoc analyses to determine feasible triage and followup testing schemes for hrHPV-positive women. We compared 14 triage/followup testing strategies using cytology, hrHPV testing and/or HPV genotyping. The end points were negative (NPV) and positive predictive values (PPV) for CIN grade 3 or worse (CIN3+) and colposcopy referral rates. The estimates of the end points were adjusted for women in the cohort study who did not attend repeat testing.

Material and Methods

Study design VUSA-screen

VUSA-screen is a population-based cohort study designed to evaluate the effectiveness of combined cervical cytology screening with hrHPV testing by HC2 hybridization assay (Qiagen, Gaithersburg, MD). The study was carried out in the Utrecht province of the Netherlands in the setting of the regular Dutch screening program that invites women aged 30–60 years to be screened every 5 years. The design of the study has been described elsewhere.[11] In brief, women invited for regular cervical screening were recruited between October 2003 and August 2005. Women with a history of cervical intraepithelial neoplasia grade 2 or worse (CIN2+) or abnormal cytology in the preceding 2 years were excluded from analysis. Participating women gave written informed consent. The VUSA-screen study was approved by the Ministry of Public Health (2002/02-WBO; ISBN-10: 90-5549-452-6) and registered in the trial register (NTR215, ISRCTN64621295).

Cervical scrapings were taken by a cytobrush (Rovers, Oss, the Netherlands), and after making a conventional cytological smear, the brush was placed in a container with 1 ml universal collection medium (Qiagen, Gaithersburg) for hrHPV HC2 testing (cocollection for HPV testing and conventional cytology). Cytology

results were reported, blinded to the hrHPV testing results, according to the CISOE-A classification, which is used in the Dutch screening program and can be easily converted into the 2001 Bethesda system.[12] Cytological results were grouped as normal, borderline or mild dyskaryosis (BMD) and >BMD (moderate dyskaryosis or worse). In the 2001 Bethesda system, BMD corresponds to atypical squamous cells of undetermined significance; atypical squamous cells, cannot rule out high-grade squamous intraepithelial lesions or low-grade squamous intraepithelial lesions and >BMD corresponds to high-grade squamous intraepithelial lesions.

Women with BMD or worse and positive in hrHPV test were directly referred for colposcopy. In women with normal cytology at baseline, a substudy was carried out. In this substudy, all hrHPV-positive women (n = 1,021) were included as well as a sub-set of n = 3,063 hrHPV-negative, cytologically normal women. Women with normal cytology were not informed about the hrHPV test result. HrHPV-positive women with normal cytology were offered cytology and a blinded hrHPV test at 12 months and combined hrHPV testing and cytology at 24 months. Women were referred at 12 months if cytology was abnormal and at 24 months in case of a positive hrHPV test and/or abnormal cytology (Fig. 1).

Of the women who were referred to a gynecologist for colposcopy, colposcopy-directed biopsies were taken for histological examination of suspicious areas on the cervix, according to standard procedures in the Netherlands.[13] Biopsy results were reported as normal, cervical intraepithelial neoplasia (CIN) grades 1, 2 and 3 or as invasive cancer, according to international criteria.[14,15] Glandular intraepithelial neoplasia grades 1, 2 and 3 were included in CIN. Cytology and histology results were retrieved from the nationwide network and registry of histopathology and cytopathology (PALGA; Bunnik, the Netherlands). We included all lesions diagnosed after the referral smear and within 3 years after baseline.

hrHPV DNA testing

hrHPV testing was performed by the HC2 hrHPV DNA test (13 hrHPV types) in an automated format on a rapid capture system (RCS; Qiagen, Gaithersburg, MD) as described before. Samples were considered positive if they attained or exceeded threshold of 1.0 RLU/CO (corresponding with 1 pg/mL HPV16 DNA).

HC2 positive samples were tested with GP5+/6+-PCR-EIA,[16] and all specimens tested positive by GP5+/6+-PCR-EIA were typed by reverse line blotting according to established protocols.[17]

Statistical Analysis

The primary endpoint of the study was cumulatively detected CIN grade 3 or worse (CIN3+). A secondary outcome was cumulatively detected CIN2+.

In our post-hoc analysis, 14 triage per followup strategies for hrHPV-positive women with no or a single repeat test were evaluated with respect to NPV, PPV, sensitivity, specificity and the colposcopy referral rate. For the repeat test, the 12-month screening test in the cohort study was used. The 24-month screening test was used if the 12-month test was missing. To gain insight into the impact of replacing cytological screening by hrHPV screening on the colposcopy referral rate in the entire screening

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population, we also calculated an overall colposcopy referral rate by multiplying the colposcopy referral rate in hrHPV-positive women by the hrHPV prevalence.

HrHPV-positive women with normal cytology and without a valid repeat combination test result (repeat cytology and hrHPV test result) were considered as lost to followup. We accounted for this loss-to-followup by replacing raw counts by expected counts. The expectations were calculated under the assumption that women with normal cytology at baseline without a valid repeat test result had the same disease risk as women with a valid repeat test result. Furthermore, we classified a woman as having CIN0/1 if she was referred for colposcopy at baseline and had no detected CIN2+ or if she had a valid repeat test result and no detected CIN2+.
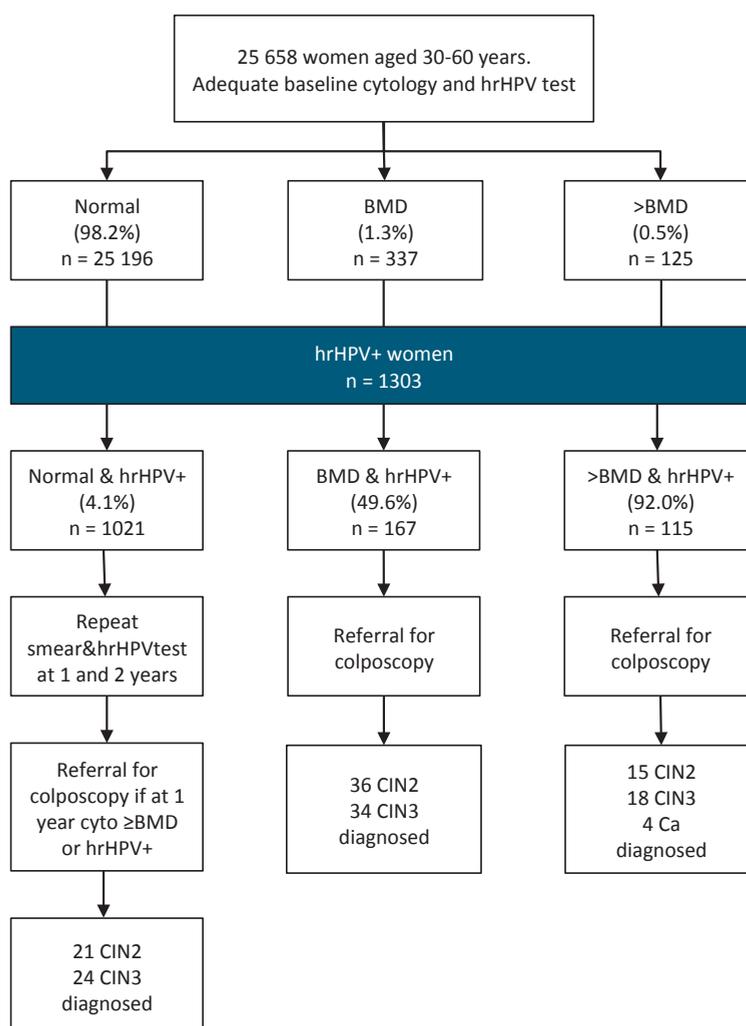

Figure 1 Flowchart of the screening profiles of hrHPV-positive women in the VUSA-Screen study.

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The latter implies that women with a double negative test at 24 months, which are returned to routine screening, are classified as CIN0/1.

For each new strategy, we estimated the expected number of colposcopy referrals and the expected number of CIN3+ and CIN2+ detected by the strategy. Furthermore, on the basis of the screening strategy used in the cohort study, we estimated the expected number of CIN3+ and CIN2+ in the whole cohort. We calculated the PPV and NPV for CIN3+ of a new strategy as:

$$PPV = \frac{\text{Expected number of CIN3 + in women referred for colposcopy}}{\text{Expected number of women referred for colposcopy}}$$

$$NPV1 = 1 - \frac{\text{Expected number of CIN3 + in women not referred for colposcopy}}{\text{Expected number of women not referred for colposcopy}}$$

The sensitivity and specificity for CIN3+ were calculated as

$$Sensitivity = \frac{\text{Expected number of CIN3 + in women referred for colposcopy}}{\text{Expected number of CIN3 + in cohort}}$$

$$Specificity = \frac{\text{Expected number of CIN0=1=2 in women not referred for colposcopy}}{\text{Expected number of CIN0=1=2 in cohort}}$$

Ninety-five percent confidence intervals were calculated for the outcome measures using the Wilson Score method,[18] where the sample size was set equal to the number of cases observed in the cohort study. To evaluate the strategies, we considered a NPV for CIN3+ of at least 98% (corresponding with a 2-year CIN3+ risk of at maximum 2%) to be a minimal requirement.[19]

We considered the following five strategies for hrHPV-positive women with triage at baseline but without a repeat test: (*i*) cytology (threshold BMD), (*ii*) HPV16/18 genotyping, (*iii*) HPV16/18/31/33/45 genotyping, (*iv*) combined cytology and HPV16/18 genotyping and (*v*) combined cytology and HPV16/18/31/33/45 genotyping (Table 1). We considered nine strategies with baseline triage followed by one round of repeat testing. In the first five of these strategies, baseline triage testing consisted of cytology only: (*vi*) cytology triage at baseline and cytological testing at 12 months; (*vii*) cytology triage at baseline and hrHPV testing at 12 months; (*viii*) cytology triage at baseline and HPV type persistence at 12 months; (ix) cytology triage at baseline and combined cytology and hrHPV testing at 12 months and (*x*) cytology triage at baseline and combined cytology and HPV16/18 genotyping at 12 months. In the last four strategies, baseline triage testing consisted of cytology combined with hrHPV16/18 genotyping. For the repeat tests, strategies (*xi–xiv*) were similar to strategies (*vi–ix*) (Table 2).

Analyses were done with SPSS version 15.0 (LEAD Technologies Inc., Haddonfield, NJ), Excel (Microsoft Corporation, Redmond, WA) and MATLAB version 7.9 (The MathWorks Inc., Natick, MA).

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Table 1 Sensitivity, specificity, NPV, PPV and colposcopy referral rate of five baseline triage strategies for hrHPV-positive women, adjusted for non-attendance at repeat testing.

Triage strategy	hrHPV-positive women									Total screening population
	Endpoint CIN3+				Endpoint CIN2+				Colpo referral rate % (95% CI)	Colpo referral rate % (95% CI)
	Sensitivity % (95%CI)	Specificity % (95%CI)	NPV % (95%CI)	PPV % (95%CI)	Sensitivity % (95% CI)	Specificity % (95% CI)	NPV % (95% CI)	PPV % (95% CI)		
i Cytology	70.6 (62.7-77.4)	85.6 (82.8-88.1)	95.1 (93.0-96.7)	42.2 (36.6-48.0)	62.7 (56.2-68.8)	89.1 (86.4-91.4)	90.1 (87.3-92.3)	60.3 (54.5-65.8)	21.6 (19.0-24.6)	1.09 (0.96-1.22)
ii HPV 16/18	65.4 (57.4-72.7)	72.5 (69.0-75.8)	93.4 (91.0-95.2)	26.1 (21.4-31.4)	58.6 (52.1-64.9)	74.5 (70.8-77.8)	87.3 (84.2-89.9)	37.6 (32.2-43.3)	32.4 (29.7-35.2)	1.65 (1.49-1.80)
iii HPV 16/18/31/33/45	81.2 (74.1-86.8)	53.8 (50.0-57.5)	95.1 (92.4-96.8)	20.7 (17.2-24.7)	77.8 (71.9-82.7)	56.4 (52.3-60.3)	90.6 (87.3-93.2)	31.8 (27.6-36.3)	50.7 (47.7-53.8)	2.58 (2.38-2.77)
iv Cytology & HPV16/18	87.4 (81.1-91.9)	63.2 (59.5-66.7)	97.1 (94.9-98.4)	26.0 (22.1-30.4)	81.5 (75.9-86.1)	66.6 (62.8-70.3)	93.2 (90.3-95.3)	39.0 (34.6-43.7)	43.4 (40.2-46.5)	2.21 (2.03-2.39)
v Cytology & HPV 16/18/31/33/45	96.3 (91.9-98.4)	47.4 (44.2-50.6)	98.9 (97.6-99.5)	20.7 (17.5-24.3)	95.0 (91.3-97.2)	51.0 (47.7-54.4)	97.6 (96.0-98.6)	32.4 (28.6-36.5)	58.1 (55.4-60.8)	2.95 (2.75-3.17)

HPV= human papillomavirus; CI= confidence interval; CIN= cervical intraepithelial neoplasia (grade 2 and 3 or higher); NPV= negative predictive value; PPV= positive predictive value.

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Results

VUSA-screen characteristics

Of the 25,871 women participating in the VUSA-screen study, 25,658 (99.2%) had an adequate baseline Pap smear and hrHPV test. The median age of participating women was 44.0 years (range 29–61 years). Among the women with an adequate Pap smear, 98.2% had normal cytology, 1.3% had BMD and 0.5% had >BMD (Fig. 1). The proportion of women with a positive hrHPV HC2test was 4.1% among those with normal cytology, 49.6% in those with BMD and 92.0% in women with >BMD. Overall, 5.1% (1,303/25,658) of the women tested hrHPV positive.

The 1,021 hrHPV-positive women with normal baseline cytology received a recommendation to return at 12 and 24 months for a smear and a hrHPV test. A total of 535 of these women had at least one round of followup with a hrHPV test and a smear for evaluation of cytology. Among women who attended at repeat testing, the average time to the first followup test was 15.0 months with a standard deviation of 4.7 months. The followup time ranged from 1.3 to 28.6 months.

Evaluation of triage strategies for hrHPV-positive women

Detailed results of the five strategies without a repeat test are presented in Table 1. The highest NPV of 98.9% (95%CI: 97.6–99.5%) was obtained with the strategy of combined cytology and HPV16/18/31/33/45 genotyping. None of other four strategies met the NPV threshold for CIN3+ of 98% and, therefore, these strategies were not acceptable. However, the strategy with combined cytology and HPV16/18/31/33/45 genotyping resulted in a high colposcopy referral rate of 58.1% (95%CI: 55.4–60.8%) among hrHPV-positive women. This would translate into a referral rate of 2.95% (95%CI: 2.75–3.17%) in the total screening population, which is almost threefold higher than obtained with baseline cytology triage only (colposcopy referral rate of 1.09%; 95%CI: 0.96–1.22%).

Table 2 shows detailed results of the nine strategies with baseline triage testing followed by one round of repeat testing. Eight strategies had a NPV for CIN3+ above the threshold of 98%. The PPVs of these strategies were acceptable, ranging from 17.7 to 37.5%. Strategies with hrHPV testing at followup showed high colposcopy referral rates (ranging from 65.7 to 73.0%).

The lowest colposcopy referral rate in hrHPV-positive women was obtained with cytology triage at baseline followed by repeat cytology testing at 12 months (33.4%). In the total screening population, this would result in a colposcopy referral rate of 1.70% (95%CI:1.54–1.85%), a 1.55-fold increase compared with baseline cytology triage only.

The NPV and PPV of the 14 strategies are graphically displayed in Figure 2. Nine strategies had an estimated NPV greater than 98%, eight strategies with and one without repeat testing. Strategies with hrHPV testing in followup (strategies vii, ix, xii and xiv) showed not only the highest NPV but also the lowest PPV. The strategy with cytology triage followed by cytology testing showed both a high PPV and high NPV (Fig. 2, upper right corner).

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Table 2 Sensitivity, specificity, NPV, PPV and colposcopy referral rate of nine triage strategies for hrHPV-positive women based on baseline and one round of repeat testing, adjusted for non-attendance at repeat testing.

Baseline triage test	Repeat test at 12months	hrHPV-positive women										Total screening population
		Endpoint CIN3+				Endpoint CIN2+						
		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 tests, % (95% CI)	Colpo referral rate % (95% CI)	Colpo referral rate % (95% CI)
vi Cytology	Cytology	96.6 (92.3-98.6)	76.0 (72.7-79.1)	99.3 (98.1-99.8)	37.5 (32.6-42.6)	90.9 (86.3-94.0)	81.7 (78.4-84.6)	97.1 (95.2-98.3)	56.7 (51.5-61.7)	78.4 (76.0-80.5)	33.4 (30.2-36.7)	1.70 (1.54-1.85)
vii Cytology	hrHPV (HC2)	98.9 (95.5-99.7)	39.2 (35.6-42.9)	99.6 (97.6-99.9)	19.5 (16.5-22.9)	98.6 (96.0-99.5)	42.9 (39.0-46.9)	99.1 (96.9-99.8)	31.2 (27.6-35.1)	78.4 (76.0-80.5)	65.7 (62.4-68.9)	3.34 (3.12-3.56)
viii Cytology	HPV type persistence	89.8 (83.8-93.8)	58.0 (54.3-61.7)	97.5 (95.2-98.7)	24.1 (20.5-28.2)	88.0 (83.1-91.7)	62.3 (58.4-66.1)	95.2 (92.4-97.0)	38.0 (33.7-42.5)	78.4 (76.0-80.5)	48.2 (44.7-51.6)	2.45 (2.26-2.64)
ix Cytology	Cytology/ hrHPV (HC2)	100 (97.4-100)	38.4 (34.8-42.1)	100 (98.3-100)	19.4 (16.4-22.8)	100 (98.3-100)	42.2 (38.3-46.2)	100 (98.3-100)	31.2 (27.6-35.1)	78.4 (76.0-80.5)	66.6 (63.3-69.9)	3.38 (3.16-3.60)
x Cytology	Cytology& HPV16/18	97.7 (93.8-99.2)	62.4 (58.7-66.0)	99.5 (98.1-99.9)	27.9 (23.9-32.2)	94.4 (90.5-96.7)	67.5 (63.6-71.2)	97.9 (95.8-98.9)	43.3 (38.8-47.9)	78.4 (76.0-80.5)	45.4 (42.0-48.8)	2.31 (2.12-2.49)
xi Cytology &HPV16/18	Cytology	98.9 (95.5-99.7)	57.4 (53.6-61.1)	99.7 (98.4-99.9)	25.6 (21.9-29.7)	94.3 (90.5-96.7)	61.8 (57.8-65.6)	97.6 (95.4-98.8)	39.3 (35.0-43.7)	56.6 (53.9-59.3)	49.9 (46.7-53.0)	2.53 (2.34-2.73)
xii Cytology &HPV16/18	hrHPV (HC2)	100 (97.4-100)	31.5 (28.1-35.1)	100 (98.0-100)	17.8 (15.0-21.0)	100 (98.3-100)	34.6 (30.9-38.5)	100 (98.0-100)	28.6 (25.2-32.3)	56.6 (53.9-59.3)	72.6 (69.6-75.3)	3.69 (3.46-3.92)
xiii Cytology &HPV16/18	HPV type persistence	94.3 (89.2-97.0)	47.9 (44.2-51.7)	98.3 (96.0-99.3)	21.2 (17.9-24.8)	92.2 (87.9-95.0)	51.6 (47.6-55.6)	96.2 (93.3-97.9)	33.3 (29.4-37.4)	56.6 (53.9-59.3)	57.5 (54.4-60.6)	2.92 (2.71-3.13)
xiv Cytology &HPV16/18	Cytology &hrHPV (HC2)	100 (97.4-100)	31.0 (27.6-34.6)	100 (97.9-100)	17.7 (14.9-20.8)	100 (98.3-100)	34.1 (30.4-38.0)	100 (97.9-100)	28.4 (25.1-32.1)	56.6 (53.9-59.3)	73.0 (70.1-75.7)	3.71 (3.48-3.94)

HrHPV = high-risk human papillomavirus; CI = confidence interval; CIN = cervical intraepithelial neoplasia (grade 2 or 3 or higher); Gray: the strategies which met the criteria for NPV at a CIN3+ threshold of 98%.
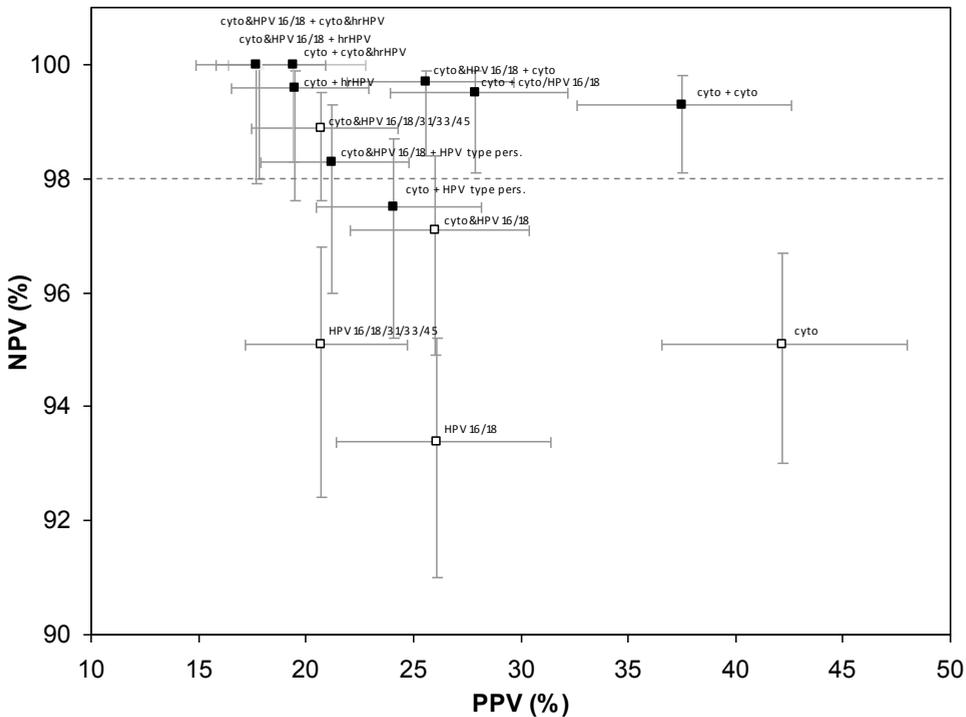
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Figure 2 NPV and PPV for CIN3+ of the 14 triage/follow-up strategies for hrHPV-positive women, adjusted for non-attendance at repeat testing. PPV = positive predictive value; NPV = negative predictive value ; o triage strategy without a repeat test ; n triage strategy followed by on round of repeat testing; Bars represent 95% confidence intervals; cyto = strategy (i); HPV16/18 = strategy (ii); HPV16/18/31/33/45 = strategy (iii); cyto&HPV16/18 = strategy (iv); cyto&HPV16/18/31/33/45 = strategy (v); cyto + cyto = strategy (vi); cyto + hrHPV = strategy (vii); cyto + HPV type pers = strategy (viii); cyto + cyto&hrHPV = strategy (ix); cyto + cyto&HPV16/18 = strategy (x); cyto&hrHPV16/18 + cyto = strategy (xi); cyto&hrHPV16/18 + hrHPV = strategy (xii); cyto&hrHPV16/18 + HPV type pers = strategy (xiii) ; cyto&hrHPV16/18 + cyto&hrHPV = strategy (xiv).

Discussion

Implementation of hrHPV testing as a primary screening test in cervical screening may soon be reality. However, at the moment there is still debate about how to manage hrHPV test-positive women, because hrHPV testing is less specific than cytology.[7] Referring all hrHPV-positive women to colposcopy will result in over-diagnosis, over-treatment and high costs. Therefore, triage and/or followup strategies for hrHPV-positive women are needed. The VUSA-Screen cohort study enabled us to examine this issue in more detail.

Five triage strategies without repeat test were investigated, with cytology, hrHPV, HPV16/18 genotyping and HPV16/18/31/33/45 genotyping as triage tests. A triage strategy was considered feasible if the NPV was equal to or exceeded a predefined threshold of 98%.[19] Of the five triage strategies, one strategy (cytology combined with HPV16/18/31/33/45) met the NPV criterion of 98%. This strategy would be attractive for implementation, because no follow-up is needed. However, a

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major disadvantage is the high overall colposcopy referral rate in the total population (2.95%). In addition to the increase in the costs that would result from this strategy, it also poses a large burden on the colposcopy capacity of gynecologists. By adding one repeat test at 12 months, it is possible to reduce this referral rate markedly. The most attractive strategy was cytology triage at baseline followed by repeat cytology testing at 12 months, with an overall colposcopy referral rate in the total population of 1.70 and a NPV of 99.3 (95%CI: 98.1–99.8%).

Loss-to-followup may be a problem when implementing a screening strategy with a repeat test. In this study, the loss-to-followup of women with normal cytology at baseline was about 40%. Other studies have also shown that attendance at repeat testing is poor, particularly after a cytologically normal test.[7, 20] Therefore, adequate communication is necessary to improve attendance at repeat testing. In the POBASCAM trial in the Amsterdam area in the Netherlands, follow-up at repeat testing was 77%.[7] Depending on the magnitude of envisaged loss to follow-up, a direct triage strategy without a repeat visit could be an alternative option if the almost threefold increase in colposcopy rate compared with baseline cytology only triage testing could be accommodated. Such an increase in colposcopy rate is expected to lead to capacity problems in the Netherlands where the annual number of colposcopy referrals is low due to a well-functioning cytological screening program (about 12,000 per year). Therefore, for the Netherlands, it is preferable to implement the strategy with cytology triage at baseline and a repeat cytology test at 12 months. This can be implemented against much less extra colposcopies. Moreover, this increase in colposcopy rate is accompanied by an almost similar increase in the number of detected CIN3+. Furthermore, after a few rounds of hrHPV screening a decline in colposcopy referrals is expected because the extra detection of high-grade lesions (with subsequent treatment) will lead to a reduced rate of abnormalities in subsequent screening rounds.[4, 7, 20]

A point of consideration in our current study is the low followup at repeat testing. HrHPV-positive women with normal cytology and a representative age-matched subcohort of hrHPV-negative women with normal cytology were invited for repeat testing after 1 and/or 2 years and were referred for colposcopy if they presented with abnormal cytology and/or positive hrHPV test. In our study, women with normal cytology were not informed about the hrHPV status at baseline. The concealment was necessary to maximize attendance at repeat testing among hrHPV-negative women with normal cytology. It is likely that the attendance rate in our study would have been higher if women had been informed about their hrHPV test result. In addition to the women, cytotechnicians were not informed about the hrHPV test result. In a single hrHPV testing strategy with cytology triage for hrHPV-positive women, the hrHPV test result may be known to the cytotechnician. Awareness of the negative or positive hrHPV test result may affect the criteria for defining abnormalities because the interpretation of cytology is subjective. In a recent Finnish randomized trial,[21] cytotechnicians in the hrHPV screening arm were informed about the hrHPV screening test result. However, the hrHPV test information in that study only had a small effect on cytology assessment, and, therefore, on the CIN3+ detection rate and the number of colposcopies.

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Furthermore, implementation of HPV vaccination may have an effect on the cost effectiveness of screening programs. In particular, it is expected that vaccination reduces the PPV of cytological screening since fewer women will develop high-grade cervical lesions.[22, 23] In particular, the reading performance for cytological slides is likely to deteriorate when the occurrence of an abnormal smear becomes less common.[24] However, HPV vaccination will have a stronger effect on PPV of a primary cytological screening test than on the PPV of triage or repeat cytology in hrHPV-positive women. The reason is that the prevalence of hrHPV will also decrease after vaccination, and this partly compensates the decrease in PPV if cytological evaluation is limited to hrHPV-positive women. Therefore, we think that the proposed strategy with cytological triage at baseline and repeat cytology at 1 year will also be an interesting triage strategy for vaccinated women.

A strong point of our study is the longitudinal design and the older age range of study participants (30–60 year), which is the age for which hrHPV testing is most widely advocated.[4, 7, 25, 26] In our study, we took into account all the cytology and histological follow-up data derived from the national wide network of registry within 3-years after baseline. The study was population-based and part of a routine organised screening activity in a low-risk population, indicating that the results could be implemented in routine practice.

Other studies have suggested to follow-up hrHPV-positive women with normal cytology by one repeat hrHPV test10 or by a combined cytology and hrHPV test.[27] Our study indicates that implementation of these strategies lead to a substantial increase in colposcopy referral rate, eventually referring two third of all hrHPV-positive women. However, it should be kept in mind that in the Netherlands, the cytological screening program is very efficient, which is demonstrated by a cytology abnormality rate of 1.8% (Fig. 1)[28] with a similarly low colposcopy referral rate. However, in countries with already higher cytological abnormality, and related colposcopy rates, such triage strategies would, depending on the screening interval, have a much lower impact on the current number of colposcopies in those countries.[21, 29]

Finally, in addition to CIN3+ risk considerations, implementation of a triage and follow-up strategy for hrHPV-positive women asks for acceptability by physicians and women. To prevent anxiety among women who are directed back to routine screening, it is essential that the last screening test is negative. A repeat visit for cytology only at 1 year, meets this requirement, but, e.g., implementation of HPV16/18 genotyping after 1 year seems less straightforward as women who will be hrHPV-positive but HPV16/18 negative at a repeat visit, may feel uncomfortable about their mixed test result even if their CIN3+ risk is low enough to return to the next screening round. This may be alleviated with a triage test that directly detects HPV16/18 at baseline, rather than using a consensus test first. Finally, the logistics of a triage and follow-up strategy in national programme should preferably be simple.

In conclusion, our post-hoc analysis of triage strategies for hrHPV-positive women, based on the VUSA-screen cohort strongly points to the use of cytological testing both at baseline and at 12 months in countries with low rates of cytological abnormalities and long screening intervals (5 years). This is a feasible triage strategy because it has a high NPV for CIN3+, modest colposcopy referral rate and is easy to communicate to physicians and women. However, in countries with higher rates of

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cytological abnormalities, shorter screening intervals and higher colposcopy rates, other triage strategies involving for instance combined cytology and HPV16/18/31/33/45 genotyping at baseline without repeat testing might be considered for implementation.

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