

# Human papillomavirus DNA testing for the detection of cervical intraepithelial neoplasia grade 3 and cancer: 5-year follow-up of a randomised controlled implementation trial

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## Summary

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**Background** Tests for the DNA of high-risk types of human papillomavirus (HPV) have a higher sensitivity for cervical intraepithelial neoplasia grade 3 or worse (CIN3+) than does cytological testing, but the necessity of such testing in cervical screening has been debated. Our aim was to determine whether the effectiveness of cervical screening improves when HPV DNA testing is implemented.

**Methods** Women aged 29–56 years who were participating in the regular cervical screening programme in the Netherlands were randomly assigned to combined cytological and HPV DNA testing or to conventional cytological testing only. After 5 years, combined cytological and HPV DNA testing were done in both groups. The primary outcome measure was the number of CIN3+ lesions detected. Analyses were done by intention to treat. This trial is registered as an International Standard Randomised Controlled Trial, number ISRCTN20781131.

**Findings** 8575 women in the intervention group and 8580 in the control group were recruited, followed up for sufficient time ( $\geq 6 \cdot 5$  years), and met eligibility criteria for our analyses. More CIN3+ lesions were detected at baseline in the intervention group than in the control group (68/8575 vs 40/8580, 70% increase, 95% CI 15–151;  $p=0 \cdot 007$ ). The number of CIN3+ lesions detected in the subsequent round was lower in the intervention group than in the control group (24/8413 vs 54/8456, 55% decrease, 95% CI 28–72;  $p=0 \cdot 001$ ). The number of CIN3+ lesions over the two rounds did not differ between groups.

**Interpretation** The implementation of HPV DNA testing in cervical screening leads to earlier detection of CIN3+ lesions. Earlier detection of such lesions could permit an extension of the screening interval.

## Introduction

The implementation of organised cervical screening by cytological testing with a call and recall system has lowered the incidence of cervical cancer considerably.<sup>1–5</sup> However, the sensitivity of cytological testing for cervical intraepithelial neoplasia grade 3 and cervical cancer (CIN3+) is only moderate and this is compensated for by frequent screening.<sup>6</sup> High-risk types of human papillomavirus (HPV) are the causative agents for cervical cancer and improvements in the effectiveness of the cervical screening programme could be achieved by testing for the DNA of high-risk types of HPV as a primary screening tool. Several longitudinal studies have shown that being positive for the DNA of high-risk types of HPV is a predictor of cervical dysplasia in women without cytological abnormality,<sup>7–9</sup> and screening cohort studies have shown that HPV DNA testing has a higher sensitivity than does cytological testing for detecting cervical lesions, albeit at the cost of a slightly lower specificity.<sup>10–15</sup> Moreover, variability of HPV DNA testing, both between and within laboratories, is lower than that of cytological testing.<sup>11</sup> Because of the increased sensitivity of HPV DNA testing, the combined use of cytological and HPV DNA testing with Hybrid Capture 2 (Digene Corporation, Gaithersburg, MD, USA) in screening has been approved by the US Food and Drug Administration

for women aged 30 years and over.<sup>16</sup> However, whether the long-term effectiveness of cervical screening is improved when HPV DNA testing is implemented is unknown. At present, several randomised controlled trials are under way to assess the use of HPV DNA testing as a primary screening tool.<sup>17–22</sup> The aim of the Population Based Screening Study Amsterdam (POBASCAM) trial was to assess prospectively whether primary HPV DNA testing is more effective than cytological testing in the setting of a regular screening programme. Here, we present results from the first 17155 of the 44938 women enrolled in the POBASCAM trial.

## Methods

### Patients and procedures

POBASCAM is a population-based randomised controlled implementation trial to assess the effectiveness of cervical screening with HPV DNA testing combined with cytological testing (intervention group) compared with conventional cytological testing only (control group, HPV DNA test results blinded). The trial was done within the regular Dutch nationwide screening programme that invites women aged 30–60 years to be screened every 5 years. The design, methods, and baseline results of the trial have been described previously.<sup>23</sup> Briefly, between January, 1999, and September, 2002, women invited for

the regular cervical screening programme were asked to participate in the POBASCAM trial. Women were eligible if they lived in a defined semi-urbanised region to the southwest of Amsterdam and if they were willing and able to give written informed consent for the study. Women were excluded if they had a history of cervical intraepithelial neoplasia grade 2 or worse (CIN2+) or abnormal cytological test results in the preceding 2 years, if they had undergone hysterectomy, or if they were aged 57 years or over at baseline

Eligible women were randomly assigned in a 1:1 manner by use of computer generated random numbers to the intervention or control group after the cervical specimen had been taken and administrative data entered into the central study database. Neither the molecular technicians nor the cytotechnicians had access to the central study database, and consequently were unaware of assignment to either the intervention or control group. HPV DNA test results were automatically updated in the central study database. For women in the control group, the user interface did not show HPV DNA test results, but instead stated that they were blinded, to ensure that even the operator of the central study database had no access to the HPV DNA status of women assigned to the control group.

Women assigned to the intervention group were advised at baseline and subsequent rounds according to both cytological testing and HPV DNA results. Women with moderate dyskaryosis or worse (high-grade squamous intraepithelial lesions according to the 2001 Bethesda system)<sup>24</sup> were immediately referred to colposcopy, irrespective of the HPV DNA result. Women with normal cytological results and a negative HPV DNA test were recalled at the subsequent screening round (after 5 years). Repeat testing after 6 and 18 months was advised to women with normal cytological results and a positive HPV DNA test, and to women with borderline or mild dyskaryosis (corresponding to atypical squamous cells of undetermined significance; atypical squamous cells, cannot rule out high-grade squamous intraepithelial lesions; or low-grade squamous intraepithelial lesions on the 2001 Bethesda system).<sup>24</sup> Women with borderline or mild dyskaryosis at baseline were referred to colposcopy at 6 months if the repeat test result was borderline dyskaryosis or worse and HPV DNA positive, or moderate dyskaryosis or worse, whereas women with normal cytological results and a positive HPV DNA test at baseline were only referred to colposcopy at 6 months if the repeat test result was moderate dyskaryosis or worse. Women were also referred to colposcopy if the second repeat smear at 18 months was HPV DNA positive or cytological results were moderate dyskaryosis or worse. Women with cytological results that were borderline or mild dyskaryosis or better and were HPV DNA negative at 18 months were recalled at the subsequent screening round.

Women assigned to the control group were advised at the baseline round according to the current guidelines

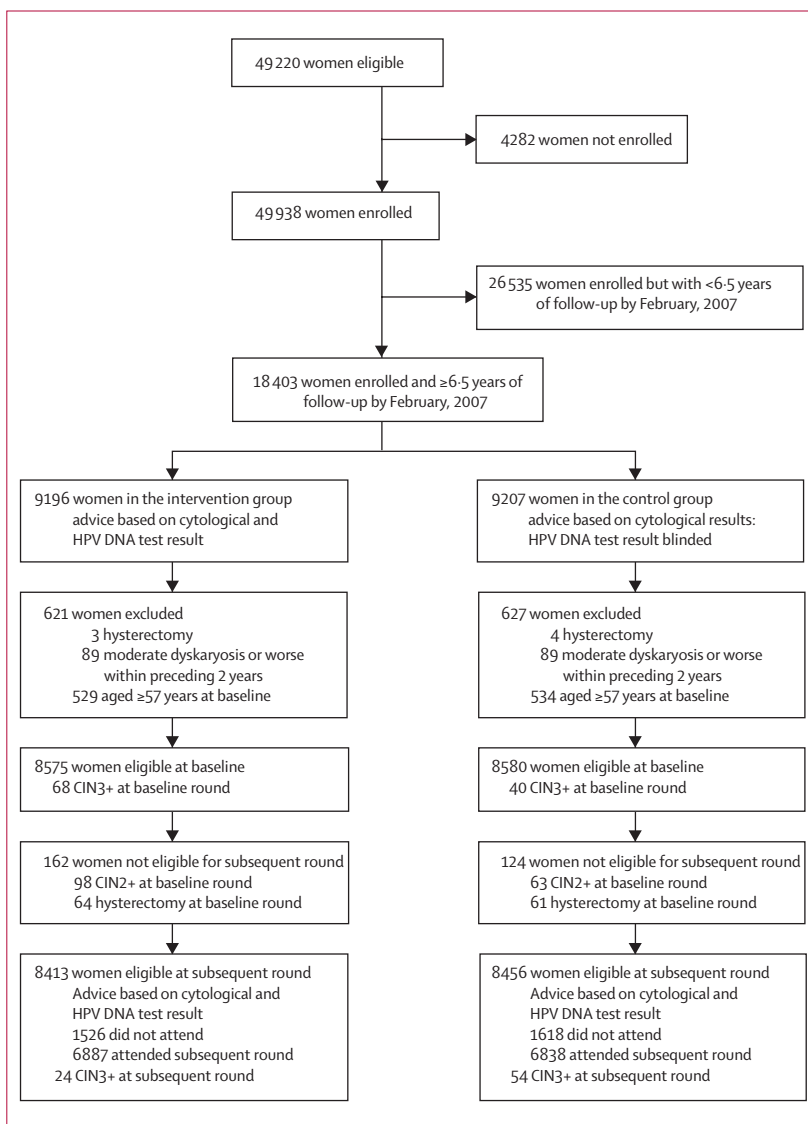


Figure 1: Trial profile

for cervical screening in the Netherlands.<sup>24,25</sup> Advice was given on the basis of cytological results alone (HPV DNA test result blinded). As in the intervention group, women with cytological results of moderate dyskaryosis or worse were immediately referred to colposcopy. Women with normal cytological results were recalled at the subsequent screening round after 5 years, and women with borderline or mild dyskaryosis were advised to repeat the tests after 6 and 18 months. If one of those repeat tests was abnormal, women were referred to colposcopy. Women with normal cytological results after 6 and 18 months were recalled at the subsequent screening round. At the subsequent screening round, women were managed according to the screening protocol for the intervention group—ie, by combined HPV DNA and cytological testing.

Conventional cytological smears were taken with a Cervex-Brush (Rovers, Oss, Netherlands) or a cytobrush. The brush was placed in a vial containing phosphate-buffered saline for HPV DNA testing. Cervical smears were classified, blinded to the HPV DNA testing results, according to the CISOE-A (National Proforma reporting on Composition, Inflammation, Squamous, Other and endometrium, and Endocervical cylindrical epithelium, and Adequacy) classification used in the Netherlands. The results can easily be converted into the Bethesda system.<sup>24</sup> Cytological results were grouped as normal, borderline or mild dyskaryosis, and moderate dyskaryosis or worse.

Detection of HPV DNA was done, blinded to cytological results, by GP5+/6+ PCR followed by enzyme immunoassay detection of 14 high-risk types (ie, HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) with a cocktail of oligonucleotide probes.<sup>26</sup> Validation of this assay and inter-method and intra-method comparisons have been described previously.<sup>27,28</sup> Participants and all medical personnel involved—ie, general practitioners, cytotechnicians, gynaecologists, and pathologists—were blinded to the HPV DNA results of women in the control group.

Colposcopically directed biopsies were taken for histological examination from suspected areas on the cervix according to standard procedures in the Netherlands.<sup>29,30</sup> Histological examination was done locally and classified as cervical intraepithelial neoplasia grade 0, 1, 2, or 3, or as invasive cancer, according to international criteria.<sup>31,32</sup> Confirmation of CIN3+ diagnosis by two independent pathologists gave high concordance with the original CIN3+ diagnoses (97%). The original diagnoses were used in the analyses. Cytological and histological results were identified through the nationwide network and registry of histopathology and cytopathology in the Netherlands (PALGA; Belga, Netherlands).

The primary outcome measure was the number of histologically confirmed CIN3+ lesions detected.

The trial was approved by both the Medical Ethics Committee of the VU University Medical Centre (no 96/103) and the Ministry of Public Health (VWS no 328650). All women gave written informed consent.

### Statistical analysis

The main analyses were done by intention to treat. Although CIN3+ was used as primary endpoint, we repeated the calculations with CIN2+ as the endpoint because treatment of CIN2 is normal clinical practice. In the calculation of the number of CIN3+ and CIN2+ lesions, cases of adenocarcinoma in situ and adenocarcinoma were included. Overall numbers of CIN3+ identified in the intervention and control group were calculated for the baseline screening round, the subsequent screening round, and the two rounds combined. For the subsequent round, we included only those women who had not reached CIN2+ and were eligible for subsequent screening. Each woman was followed for at least 6.5 years to cover the full period of two screening rounds plus possible repeat calls at 6 and 18 months. To account for variation around the targeted screening interval length of 5 years, CIN3+ cases detected during the period 0–48 months were labelled as CIN3+ cases detected at the baseline round whereas CIN3+ cases detected at a later time were labelled as CIN3+ cases detected at the subsequent round. The 5-year risk of CIN3+ after a negative test result was calculated as the cumulative risk of CIN3+ over two screening rounds. Both crude and adjusted risks were calculated. Adjusted CIN3+ risks were obtained by adjusting for women who did not attend the subsequent round and women who did not attend repeat testing during the subsequent round. The adjusted risks were calculated from a decision tree. Separate nodes were defined for the cytological outcomes at the subsequent round. In this way, the risk of CIN3+ was adjusted for loss to follow-up, even when the degree of loss to follow-up depended on cytological results.<sup>33</sup>

The number of referrals to colposcopy and the number of CIN3+ lesions per referral were also calculated. Differences between the two screening strategies in the number of CIN3+ lesions and number of referrals were examined by  $\chi^2$  tests. Analyses were done with SPSS version 12.0.

The study size of 44000 women enrolled in the entire trial was powered to detect a difference in the number of CIN3+ lesions in the subgroups of women with a smear

	Intervention group			Control group		
	Total	Tested for HPV DNA	HPV DNA positive*	Total	Tested for HPV DNA	HPV DNA positive*
Inadequate	9 (0.1%)	9	0 (0%)	12 (0.1%)	11	0 (0%)
Normal	8331 (97.2%)	8260	280 (3.4%)	8330 (97.1%)	8261	279 (3.4%)
Borderline or mild dyskaryosis	179 (2.1%)	174	58 (33.3%)	184 (2.1%)	184	61 (33.2%)
Moderate dyskaryosis or worse	56 (0.7%)	56	48 (85.7%)	54 (0.6%)	53	45 (84.9%)
Total	8575 (100%)	8499	386 (4.5%)	8580 (100%)	8509	385 (4.5%)

Data are n (%) or n. Analyses included women eligible for the subsequent round. \*For each cytology category, proportion of women with an HPV DNA positive test result among those with an available HPV DNA test result.

**Table 1: Cytological and HPV DNA test results at baseline round**

	Intervention group			Control group		
	Total	Tested for HPV DNA	HPV DNA positive*	Total	Tested for HPV DNA	HPV DNA positive*
Inadequate	24 (0.3%)	14	0 (0%)	24 (0.4%)	18	0 (0%)
Normal	6697 (97.2%)	4421	109 (2.5%)	6633 (97.0%)	4356	112 (2.6%)
Borderline or mild dyskaryosis	128 (1.9%)	94	27 (28.7%)	131 (1.9%)	83	25 (30.1%)
Moderate dyskaryosis or worse	38 (0.6%)	18	14 (77.8%)	50 (0.7%)	27	21 (77.8%)
Total	6887 (100%)	4547	150 (3.3%)	6838 (100%)	4484	158 (3.5%)

Data are n (%) or n. Analyses included women eligible for the subsequent round. \*For each cytology category, proportion of women with an HPV DNA positive test result among those with an available HPV DNA test result.

**Table 2: Cytological and HPV DNA test results at subsequent round**

that showed borderline or mild dyskaryosis.<sup>23</sup> Overall, a sample size of 18000 enrolled women was sufficient to achieve a power of 80% to show a three times decrease in the number of CIN3+ lesions at the subsequent round when comparing women in the intervention group with those in the control group. This calculation was based on the assumption that, in women with normal cytological results, the relative risk of developing CIN3+ associated with a positive HPV DNA test was at least 13 (lower bound 95% CI).<sup>34</sup>

This trial is registered as an International Standard Randomised Controlled Trial, with the number ISRCTN20781131.

### Role of the funding source

The funding source had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

### Results

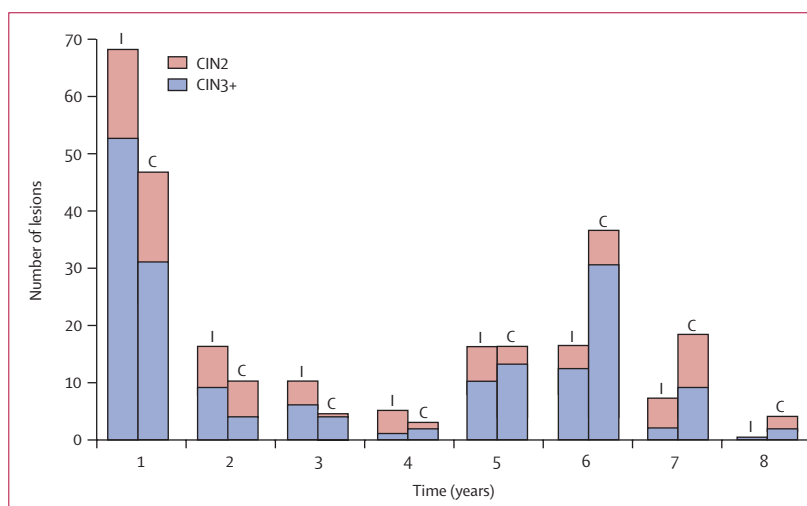
Between January, 1999, and September, 2002, 44938 women were randomly assigned to the intervention or control group. By February, 2007, 18403 women had completed the required follow-up of 6.5 years. 1248 women (621 in the intervention group and 627 in the control group) were not eligible for analyses and further analyses were based on an intervention group of 8575 women and a control group of 8580 women (figure 1).

Median follow-up time was 7.2 (range 6.5–8.5) years and median age was 41.0 (range 29–56) years; neither median follow-up time nor median age differed between the intervention and control group (data not shown). Baseline results for cytological and HPV DNA tests did not differ between groups (table 1). Of the women who were eligible for screening at the subsequent round and had not reached CIN2+, 82% (6887/8413) women in the intervention group and 81% (6838/8456) in the control group attended subsequent screening (figure 1). Attendance rates were not significantly different between groups. In the baseline round, 82% (376/459) of the women in the intervention group and 94% (173/184) of

	CIN2	CIN3	ACIS	AdCa	SCC	CIN3+	CIN2+
<b>Intervention group</b>							
Baseline round (n=8575)	30	60	3	1	4	68 (0.8, 0.6–1.0)	98 (1.1, 0.9–1.4)
Subsequent round (n=8413*)	15	22	0	0	2	24 (0.3, 0.2–0.4)	39 (0.5, 0.3–0.6)
Both rounds (n=8575)	45	82	3	1	6	92 (1.1, 0.9–1.3)	137 (1.6, 1.4–1.9)
<b>Control group</b>							
Baseline round (n=8580)	23	37	1	1	1	40 (0.5, 0.4–0.6)	63 (0.7, 0.6–0.9)
Subsequent round (n=8456*)	20	44	3	2	5	54 (0.6, 0.5–0.8)	74 (0.9, 0.7–1.1)
Both rounds (n=8580)	43	81	4	3	6	94 (1.1, 0.9–1.3)	137 (1.6, 1.4–1.9)

Data are n or n (%), 95% CI. ACIS=adenocarcinoma in situ. AdCa=adenocarcinoma of endocervical epithelium. CIN2=cervical intraepithelial neoplasia grade 2; CIN3=cervical intraepithelial neoplasia grade 3. SCC=squamous cell carcinoma. \*Women were excluded when they had reached the endpoint CIN2+ or had undergone hysterectomy during the baseline round.

**Table 3: Number of CIN3+ and CIN2+ detected**



**Figure 2: Year of diagnosis**  
I=intervention, C=control.

the women in the control group who were advised to attend repeat testing showed up at least once for repeat testing. In the subsequent round, attendance rates for at least one repeat test were 78% (186/237) in the intervention group and 85% (207/243) in the control group. 58% (268/459) of the women in the intervention group

	Intervention group Baseline cytological and HPV DNA testing							Control group Baseline cytological and HPV DNA testing						
	Normal		Borderline or mild dyskaryosis		Moderate dyskaryosis or worse		Total	Normal		Borderline or mild dyskaryosis		Moderate dyskaryosis or worse		Total
	HPV DNA positive	HPV DNA negative	HPV DNA positive	HPV DNA negative	HPV DNA positive	HPV DNA negative		HPV DNA positive	HPV DNA negative	HPV DNA positive	HPV DNA negative	HPV DNA positive	HPV DNA negative	
<b>Baseline round</b>														
CIN3+ detected	13	2*	14	0	36	3	68	1*	2*	8	1	28	0	40
CIN2+ detected	25	4*	21	2	41	5	98	2*	5*	15	1	37	3	63
<b>Subsequent round</b>														
CIN3+ detected	12	8	3	0	1	0	24	32	16	5	0	1	0	54
After normal cytology/ HPV DNA positive	0	0	0	0	0	0	0	5	0	1	0	0	0	6
Borderline dyskaryosis or worse/HPV DNA positive	5	3	2	0	0	0	10	17	3	0	0	1	0	21
Borderline dyskaryosis or worse/HPV DNA negative	1	1	0	0	0	0	2	2	3	0	0	0	0	5
Borderline dyskaryosis or worse/no HPV DNA result	4	3	1	0	1	0	9	7	8	4	0	0	0	19
Other†	2	1	0	0	0	0	3	1	2	0	0	0	0	3
CIN2+ detected	15	18	5	0	1	0	39	38	25	7	2	1	1	74
After normal cytology/ HPV DNA positive	0	2	1	0	0	0	3	5	3	1	0	0	0	9
Borderline dyskaryosis or worse/HPV DNA positive	6	7	2	0	0	0	15	19	5	1	0	1	0	26
Borderline dyskaryosis or worse/HPV DNA negative	1	1	0	0	0	0	2	2	3	0	0	0	0	5
Borderline dyskaryosis or worse/no HPV DNA result	6	6	2	0	1	0	15	10	11	4	1	0	1	27
Other†	2	2	0	0	0	0	4	2	3	1	1	0	0	7

\*CIN3+ and CIN2+ cases also include cases that were found by opportunistic screening, this explains the reported cases found after negative test results. †Includes women without an indication for follow-up or referral—ie, normal HPV DNA negative, normal without HPV DNA test result, inadequate cytology, no cytological result.

**Table 4: Number of CIN3+ and CIN2+ detected, stratified for cytological and HPV DNA test results**

and 66% (122/184) of the women in the control group completed repeat testing in the baseline round. In the subsequent round, the corresponding figures were 52% (123/237) and 53% (128/243). The proportion of women without abnormalities at baseline who repeated the smear before receiving a repeat invitation after 5 years (opportunistic screening) was 21% (1690/7980) in the intervention group and 21% (1776/8330) in the control group. As in the baseline round, cytological and HPV DNA results at the subsequent round did not differ between women in the intervention group and those in the control group (table 2). 66% (9031/13725) of the women who attended the subsequent round had an HPV DNA test result.

Table 3 shows the overall number of CIN3+ lesions detected during the baseline and subsequent screening round in the intervention and control groups. In the baseline round, the number of detected CIN3+ was 70% (95% CI 15–151, p=0.007) higher in the intervention group than in the control group; at the subsequent round, the number of CIN3+ lesions in the intervention group was 55% (28–72, p=0.001) lower than in the control group. The number of CIN3+ lesions detected over both

rounds was similar in both study groups (p=0.89). The time after baseline at which the CIN3+ cases were diagnosed are shown in figure 2. Most lesions in the baseline round were found in the first year. Few lesions were detected in years 3 and 4. The number of CIN3+ lesions peaked again in year 6, reflecting the 5-year screening interval.

Table 4 shows the number of CIN3+ lesions stratified for cytological and HPV DNA test results during the baseline and subsequent screening rounds. No significant differences between the groups were seen in the number of CIN3+ lesions detected over two rounds for women with normal cytological results, borderline or mild dyskaryosis, and moderate dyskaryosis or worse at baseline. For women with normal cytological results at baseline, the number of CIN3+ lesions detected was higher in the intervention group than in the control group during the baseline round (p=0.005) and lower during the subsequent round (p=0.001). For CIN3+ cases detected at the subsequent screening round and preceded by a normal smear at the baseline round, abnormal cytological rates were 85% (95% CI 62–97) in the intervention group and 83% (70–93) in the control group.

	CIN3+			CIN2+		
	Number	Risk		Number	Risk	
		Crude	Adjusted*		Crude	Adjusted*
<b>Intervention group</b>						
Normal cytological results and HPV DNA negative (n=7980)	22	0.1% (0.1-0.2)	0.1% (0.1-0.2)	10	0.3% (0.2-0.4)	0.4% (0.2-0.5)
HPV DNA negative (n=8113)	29	0.2% (0.1-0.3)	0.2% (0.1-0.3)	13	0.4% (0.3-0.5)	0.5% (0.3-0.6)
<b>Control group</b>						
Normal cytological results (n=8330)	70	0.6% (0.5-0.8)	0.8% (0.6-1.0)	51	0.8% (0.7-1.1)	1.1% (0.8-1.4)

Data are n or % (95% CI). \*Adjusted for women who did not attend the subsequent round and women who did not attend repeat testing during the subsequent round.

**Table 5: Cumulative number and risks of CIN3+ and CIN2+ detected over two screening rounds after a negative test at baseline**

	Referral		Biopsy		CIN3+		CIN2+	
	Number	Rate per woman screened	Number	Rate per woman referred	Number	Rate per woman referred	Number	Rate per woman referred
<b>Intervention group</b>								
Baseline round (n=8575)	201	2.3% (2.0-2.7)	136	68% (61-74)	66	33% (27-40)	94	47% (40-54)
Subsequent round (n=6887)*	87	1.3% (1.0-1.6)	59	68% (57-77)	22	25% (17-35)	35	40% (31-51)
<b>Control group</b>								
Baseline round (n=8580)	115	1.3% (1.1-1.6)	83	72% (63-80)	37	32% (24-41)	56	49% (40-58)
Subsequent round (n=6838)*	129	1.9% (1.6-2.2)†	90	70% (61-77)	51	40% (32-48)	67	52% (43-60)

Data are number or % per woman screened (95% CI). \*Women were only included when they attended the subsequent round. †Women in the control group were in the subsequent round referred on the basis of the HPV DNA testing result and cytological testing resulting in a higher referral rate than in the baseline round.

**Table 6: Referral rates and biopsy, CIN3+, and CIN2+ rates for women referred to colposcopy**

Of the CIN3+ cases in the control group identified at the subsequent round, 70% (56–82) were HPV DNA positive at the baseline round. 3% (2/68) of the CIN3+ cases in the intervention group and 8% (3/40) of the cases in the control group were found during the baseline round after negative DNA test result(s).

The 5-year cumulative risk of CIN3+ lesions per woman screened was 0.1% (95% CI 0.1–0.2, adjusted for loss to follow-up) after a combined negative HPV DNA and cytological result at baseline (table 5). This risk was lower than that for women found to be cytologically negative but who were not tested for HPV DNA (0.8%, 95% CI 0.6–1.0, adjusted for loss to follow-up). Post-hoc analyses showed that, after a negative HPV DNA result at baseline, the 5-year cumulative risk of CIN3+ was 0.2% (0.1–0.3, adjusted for loss to follow-up).

We compared the efficiency of the two screening strategies by calculating the number of referrals to colposcopy and the number of CIN3+ lesions for women referred to colposcopy during the baseline round (table 6). In the baseline round, the number of referrals was higher in the intervention group than in the control group ( $p < 0.0001$ ) but the number of CIN3+ lesions per referral was similar in both study groups ( $p = 0.90$ ). The biopsy rate was also similar in the two study groups ( $p = 0.40$ ). Furthermore, in the intervention group, the number of referrals was lower at the subsequent round than during the baseline

round ( $p < 0.0001$ ). In the subsequent round, the number of referrals in the intervention group was significantly lower than in the control group ( $p = 0.003$ ). The number of CIN3+ lesions per referral was slightly lower in the intervention group than in the control group ( $p = 0.03$ ).

We repeated the analyses with CIN2+ as the endpoint, and the results were similar to those with CIN3+ as the endpoint (tables 3–6). In particular, the number of detected CIN2+ lesions was 56% (95% CI 14–113,  $p = 0.006$ ) higher in the intervention group than in the control group and, at the subsequent round, the number of cases of CIN2+ in the intervention group was 47% (95% CI 22–64,  $p = 0.001$ ) lower than in the control group. In women with normal cytological results, the differences between groups in terms of the number of CIN2+ lesions detected were significant both at the baseline and subsequent round ( $p = 0.001$  and  $p = 0.002$ , respectively). Furthermore, no differences between study groups were found in the number of CIN2+ lesions over both screening rounds for women with normal cytological results, borderline or mild dyskaryosis, or moderate dyskaryosis or worse at baseline.

In the women who received HPV DNA testing at baseline, only two invasive cases of cervical cancer were seen in the subsequent round, compared with seven cases in those who only received cytological testing at baseline (table 3); however, this difference was not significant, since the number of cases was small.

## Discussion

Our data show that implementation of HPV DNA testing in cervical screening led to a substantial increase in the number of CIN3+ and CIN2+ lesions detected at the baseline screening round. At the subsequent round, combined HPV DNA and cytological testing was used in both study groups and significantly fewer CIN3+ and CIN2+ lesions were seen in the group of women that had also received HPV DNA testing at the baseline round than in the control group. The number of CIN3+ and CIN2+ over both screening rounds did not differ between the study groups. These data thus indicate that the high-grade lesions identified at the baseline round by HPV DNA testing are not a subset of regressive lesions, as has been suggested by some investigators.<sup>35</sup> Therefore, our results show that implementation of HPV DNA testing in cervical screening leads to earlier detection of clinically relevant cervical lesions. On the basis of these data, we suggest that the current screening interval of 5 years could be extended by at least 1 year. The extension will be advantageous to women because of a reduction in the lifetime number of screening tests and referrals.

Avoidance of unnecessary colposcopies is mandatory, and our data show that the management of HPV DNA positive smears and cytologically abnormal smears is equally efficient in terms of the number needed to refer to detect one case of CIN3+ (table 6). To achieve this, a conservative referral policy was used in which HPV DNA positive women without moderate or severe cytological abnormality were not immediately referred, but instead were rescreened after 6 and 18 months. Had we implemented a more aggressive referral policy in which all women who are HPV DNA positive, cytologically positive, or both, were referred, the rate of referral to colposcopy would have been 6·0% (compared with our rate of 2·3%), which would increase the burden on colposcopy clinics. In the subsequent round, the number of referrals was lower in the intervention group than in the control group. This result should be interpreted with caution because HPV DNA prevalence, and therefore the number of referrals, decreases with age,<sup>36</sup> and compliance with repeat testing was slightly better in the baseline round than in the subsequent round. However, the low number of referrals in the intervention group in the subsequent round also suggests that the increase in the number of medical procedures becomes small after one screening round. This idea is supported by the control group of women who, by contrast with the intervention group, were only screened for HPV DNA in the subsequent round, and showed an increase in the number of colposcopy referrals in that round. Thus, our study supports the idea that implementation of HPV DNA testing is possible with only a moderate increase in the number of colposcopies.

We found that the number of CIN3+ detected in the baseline round was about 70% higher in the intervention group than in the control group; by contrast, data from a

similar trial in Italy (NTCC) show increases of only 25%.<sup>18</sup> Although the increase in detection of CIN3+ was fairly large in our study, the 95% CI was wide and had a lower bound of only 15%. Additionally, the number of CIN3+ cases detected after moderate dyskaryosis or worse was, by chance, somewhat higher in the intervention than the control group (39 vs 28 cases). The increase in the number of CIN2+ lesions detected in the intervention group compared with the control group at baseline is consistent with NTCC trial data, which show an increase in CIN2+ of 47%.<sup>18</sup> Other similar trials (eg, the Finnish Randomised Public Health Trial, ARTISTIC in the UK, SWEDESCREEN in Sweden, CCCaST in Canada<sup>19–23</sup>) have yet to publish data for the number of histologically confirmed lesions found in the baseline round after repeat testing.

The rate of HPV DNA positivity at baseline in our study (4·5%) was low compared with other studies. Another trial with GP5+/6+ PCR testing for HPV DNA presence reported an HPV DNA positivity of 6·9%;<sup>22</sup> others with Hybrid Capture 2 testing reported figures of between 6·1% and 10·7%.<sup>18–21</sup> Previous population-based screening studies with Hybrid Capture 2 reported figures of 6·4–8·1%.<sup>10,15</sup> The differences in rates of HPV DNA positivity can be ascribed to geographical variation in prevalence of high-grade cervical lesions, differences in age distribution, and differences in HPV DNA testing. One should note that the GP5+/6+ PCR and Hybrid Capture 2 assays are the only two tests that have been extensively analysed previously for their clinical performance and can be considered clinically validated.<sup>37–42</sup> These assays have a similar sensitivity and specificity when used for the detection of CIN2+.<sup>38,43</sup> Previous data indicate that test sensitivity is not a sufficient requirement for use in cervical screening, since a test could detect transient infections with high-risk types of HPV characterised by low viral loads that do not develop into CIN3+, resulting in a poor clinical specificity.<sup>37,44</sup> Therefore, HPV DNA test requirements should be incorporated into the guidelines of the screening programme.<sup>45</sup>

This trial was done within the setting of the regular Dutch nationwide screening programme. Attendance rates at the second round were about 80% in both groups and were comparable with the coverage rate in the Netherlands.<sup>25</sup> The detection rate of 4·7 CIN3+ lesions per 1000 women in the control group was similar to that observed in the Dutch nationwide screening programme (4·3 CIN3+ per 1000 women).<sup>46</sup> Therefore, our results can be considered representative of the nationwide screening programme in the Netherlands. Complete adherence to the test repeats was only moderate (52–66%) and was 6% lower in the subsequent round than in the baseline round. A high proportion of women who received follow-up advice showed up for repeat testing at least once (78–94%), indicating that the current situation of two repeat smears at 6 and 18 months is not optimal and strategies with only one repeat smear need to be considered in the future. At

the subsequent round, 66% of the women who attended screening had an HPV DNA test result. Two important reasons for the absence of the HPV DNA test result were a change of general practitioner and a vial not having been sent for HPV DNA testing. An additional 1% of the vials were damaged during transport. The number of women with negative testing result(s) who repeated testing before the subsequent round was about 20%, and contributed 2% of the CIN3+ cases in the intervention group and 8% of the CIN3+ lesions in the control group. This shows that implementation of HPV DNA testing does not increase the degree of opportunistic screening and that most lesions can still be identified by the screening programme.

There is a continuing discussion about the role of HPV DNA testing in organised cervical screening. Meta-analyses<sup>11,47,48</sup> have shown that the sensitivity of HPV DNA testing is 23–43% higher than that for cytological testing for detecting high-grade cervical lesions, but the specificity is 5–8% lower. There is also debate as to whether combined HPV DNA and cytological testing is more effective in identifying CIN3+ lesions than HPV DNA testing alone. Post-hoc analyses showed that the risk of CIN3+ over two screening rounds was 0.2% for women who were HPV DNA negative at baseline and 0.1% for women who were both HPV DNA negative and cytologically negative at baseline; the cost-effectiveness of adding cytological testing to HPV DNA tests is thus doubtful. Furthermore, implementation of HPV DNA testing in cervical screening could lead to extra colposcopy referrals and treatments of lesions that would have regressed spontaneously otherwise.<sup>49</sup> Long-term data that show the effect of HPV DNA testing on the incidence of cancer and its precursor lesions at the next screening round(s) are required for us to be able to make an informed decision about HPV DNA testing in cervical screening. Several longitudinal cohort studies with a follow-up period of 3–10 years have already shown that a positive HPV DNA test at baseline confers a strongly increased risk for CIN3+ lesions in cytologically normal women,<sup>8,34,50–52</sup> but women in these studies were referred for colposcopy on the basis of cytological abnormality. Data from ongoing randomised clinical trials assessing the long-term effect of HPV DNA testing should provide conclusive evidence to determine whether the incidence of cervical lesions at the next screening round is sufficiently low in the HPV DNA testing arm to permit extension of the screening interval, and a full cost-effectiveness analysis will help to determine whether primary HPV DNA testing alone is the preferred strategy for primary cervical screening.

#### Contributors

CJLMM was the project leader, designed the study with MvB, FJV, LR, and had access to all data. NWJB, together with JB, CJLMM, LR, FjvK, SB, FJV, and PJFS, drafted the manuscript. NWJB and JB did the data analyses; JB was responsible for the data analyses. LR was responsible for database management. PJFS and CJLMM were responsible for HPV DNA testing. AJPB was responsible for communication with general practitioners. RV was responsible for communication with

gynaecologists. FJK, KvG, MEB, and WR were responsible for cytological testing at the individual laboratories. All authors critically reviewed the manuscript and approved the final version.

#### Conflict of interest statement

CJLMM has served as a consultant for Digene and has received lecture fees from GlaxoSmithKline. RHMV participated in the Humavac research group which does clinical trials with pharmaceutical companies such as GlaxoSmithKline and Merck Sharp & Dohme. All other authors declare that they have no conflict of interest.

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#### References

- Peto J, Gilham C, Fletcher O, Matthews FE. The cervical cancer epidemic that screening has prevented in the UK. *Lancet* 2004; **364**: 249–56.
- Sasieni P, Adams J, Cuzick J. Benefit of cervical screening at different ages: evidence from the UK audit of screening histories. *Br J Cancer* 2003; **89**: 88–93.
- Bulk S, Visser O, Rozendaal L, Verheijen RHM, Meijer CJLM. Incidence and survival rate of women with cervical cancer in the greater Amsterdam area. *Br J Cancer* 2003; **89**: 834–39.
- Aareleid T, Pukkala E, Thomson H, Hakama M. Cervical cancer incidence and mortality trends in Finland and Estonia: a screened vs an unscreened population. *Eur J Cancer* 1993; **29A**: 745–49.
- Nieminen P, Kallio M, Hakama M. The effect of mass screening on incidence and mortality of squamous and adenocarcinoma of cervix uteri. *Obstet Gynecol* 1995; **85**: 1017–21.
- van den Akker-van Marle ME, van Ballegooijen M, van Oortmarssen GJ, Boer R, Habbema JD. Cost-effectiveness of cervical cancer screening: comparison of screening policies. *J Natl Cancer Inst* 2002; **94**: 193–204.
- Sherman ME, Lorincz AT, Scott DR, et al. Baseline cytology, human papillomavirus testing, and risk for cervical neoplasia: a 10-year cohort analysis. *J Natl Cancer Inst* 2003; **95**: 46–52.
- Kjaer S, Hogdall E, Frederiksen K, et al. The absolute risk of cervical abnormalities in high-risk human papillomavirus-positive, cytologically normal women over a 10-year period. *Cancer Res* 2006; **66**: 10630–36.
- Rozendaal L, Westerga J, van der Linden JC, et al. PCR based high risk HPV testing is superior to neural network based screening for predicting incident CIN III in women with normal cytology and borderline changes. *J Clin Pathol* 2000; **53**: 606–11.
- Cuzick J, Szarewski A, Cubie H, et al. Management of women who test positive for high-risk types of human papillomavirus: the HART study. *Lancet* 2003; **362**: 1871–76.
- Cuzick J, Clavel C, Petry KU, et al. Overview of the European and North American studies on HPV testing in primary cervical cancer screening. *Int J Cancer* 2006; **119**: 1095–101.
- Clavel C, Masure M, Bory JP, et al. Human papillomavirus testing in primary screening for the detection of high-grade cervical lesions: a study of 7932 women. *Br J Cancer* 2001; **84**: 1616–23.
- Kulasingam SL, Hughes JP, Kiviat NB, et al. Evaluation of human papillomavirus testing in primary screening for cervical abnormalities: comparison of sensitivity, specificity, and frequency of referral. *JAMA* 2002; **288**: 1749–57.
- Ratnam S, Franco EL, Ferenczy A. Human papillomavirus testing for primary screening of cervical cancer precursors. *Cancer Epidemiol Biomarkers Prev* 2000; **9**: 945–51.
- Petry KU, Menton S, Menton M, et al. Inclusion of HPV testing in routine cervical cancer screening for women above 29 years in Germany: results for 8466 patients. *Br J Cancer* 2003; **88**: 1570–77.

- 16 Smith RA, Cokkinides V, Eyre HJ. American Cancer Society Guidelines for the early detection of cancer, 2005. *CA Cancer J Clin* 2005; **55**: 31–44.
- 17 Ronco G, Giorgi-Rossi P, Carozzi F, et al. Human papillomavirus testing and liquid-based cytology in primary screening of women younger than 35 years: results at recruitment for a randomised controlled trial. *Lancet Oncol* 2006; **7**: 547–55.
- 18 Ronco G, Segnan N, Giorgi-Rossi P, et al. Human papillomavirus testing and liquid-based cytology: results at recruitment from the new technologies for cervical cancer randomized controlled trial. *J Natl Cancer Inst* 2006; **98**: 765–74.
- 19 Mayrand MH, Duarte-Franco E, Coutlee F, et al. Randomized controlled trial of human papillomavirus testing versus Pap cytology in the primary screening for cervical cancer precursors: design, methods and preliminary accrual results of the Canadian cervical cancer screening trial (CCCaST). *Int J Cancer* 2006; **119**: 615–23.
- 20 Kotaniemi-Talonen L, Nieminen P, Anttila A, Hakama M. Routine cervical screening with primary HPV testing and cytology triage protocol in a randomised setting. *Br J Cancer* 2005; **93**: 862–67.
- 21 Kitchener HC, Almonte M, Wheeler P, et al. HPV testing in routine cervical screening: cross sectional data from the ARTISTIC trial. *Br J Cancer* 2006; **95**: 56–61.
- 22 Elfgrén K, Rylander E, Radberg T, et al. Colposcopic and histopathologic evaluation of women participating in population-based screening for human papillomavirus deoxyribonucleic acid persistence. *Am J Obstet Gynecol* 2005; **193**: 650–57.
- 23 Bulkman NWJ, Rozendaal L, Voorhorst FJ, et al. POBASCAM, a population-based randomised controlled trial for implementation of high-risk HPV testing in cervical screening. *Int J Cancer* 2004; **110**: 94–101.
- 24 Bulk S, Van Kemenade FJ, Rozendaal L, Meijer CJ. The Dutch CISOE-A framework for cytology reporting increases efficacy of screening upon standardisation since 1996. *J Clin Pathol* 2004; **57**: 388–93.
- 25 van Ballegoijen M, Hermens R. Cervical cancer screening in the Netherlands. *Eur J Cancer* 2000; **36**: 2244–46.
- 26 Jacobs MV, Snijders PJ, van den Brule AJ, Helmerhorst TJ, Meijer CJ, Walboomers JM. A general primer GP5+/GP6(+)-mediated PCR-enzyme immunoassay method for rapid detection of 14 high-risk and 6 low-risk human papillomavirus genotypes in cervical scrapings. *J Clin Microbiol* 1997; **35**: 791–95.
- 27 de Sanjose S, Bosch XF, Munoz N, et al. Screening for genital human papillomavirus: results from an international validation study on human papillomavirus sampling techniques. *Diagn Mol Pathol* 1999; **8**: 26–31.
- 28 Jacobs MV, Snijders PJ, Voorhorst FJ, et al. Reliable high risk HPV DNA testing by polymerase chain reaction: an intermethod and intramethod comparison. *J Clin Pathol* 1999; **52**: 498–503.
- 29 Hopman EH, Rozendaal L, Voorhorst FJ, Walboomers JM, Kenemans P, Helmerhorst TJ. High risk human papillomavirus in women with normal cervical cytology prior to the development of abnormal cytology and colposcopy. *BJOG* 2000; **107**: 600–04.
- 30 Hopman EH, Voorhorst FJ, Kenemans P, Meyer CJ, Helmerhorst TJ. Observer agreement on interpreting colposcopic images of CIN. *Gynecol Oncol* 1995; **58**: 206–09.
- 31 Anderson MC. Premalignant and malignant squamous lesions of the cervix. In: Fox H, Wells M, eds. *Obstetrical and gynaecological pathology*. 4th edn. New York: Churchill Livingstone, 1995: 292–97.
- 32 Wright TC. Precancerous lesions of the cervix. In: Kurman RJ, ed. *Blaustein's pathology of the female genital tract*. 4th edn. New York: Springer Verlag, 1995: 248–57.
- 33 Willard KE, Critchfield GC. Probabilistic analysis of decision trees using symbolic algebra. *Med Decis Making* 1986; **6**: 93–100.
- 34 Rozendaal L, Walboomers JM, van der Linden JC, et al. PCR-based high-risk HPV test in cervical cancer screening gives objective risk assessment of women with cytologically normal cervical smears. *Int J Cancer* 1996; **68**: 766–69.
- 35 Crosbie EJ, Kitchener HC. Human papillomavirus in cervical screening and vaccination. *Clin Sci (Lond)* 2006; **110**: 543–52.
- 36 Jacobs MV, Walboomers JM, Snijders PJ, et al. Distribution of 37 mucosotropic HPV types in women with cytologically normal cervical smears: the age-related patterns for high-risk and low-risk types. *Int J Cancer* 2000; **87**: 221–27.
- 37 Snijders PJ, van den Brule AJ, Meijer CJ. The clinical relevance of human papillomavirus testing: relationship between analytical and clinical sensitivity. *J Pathol* 2003; **201**: 1–6.
- 38 Clavel C, Masure M, Putaud I, et al. Hybrid Capture II, a new sensitive test for human papillomavirus detection. Comparison with Hybrid Capture I and PCR results in cervical lesions. *J Clin Pathol*; **51**: 737–40.
- 39 Lorincz AT, Richart RM. Human papillomavirus DNA testing as an adjunct to cytology in cervical screening programs. *Arch Pathol Lab Med* 2003; **127**: 959–68.
- 40 Munoz N, Bosch FX, de Sanjose S, et al. Epidemiologic classification of human papillomavirus types associated with cervical cancer. *N Engl J Med* 2003; **348**: 518–27.
- 41 Nobbenhuis MA, Walboomers JM, Helmerhorst TJ, et al. Relation of human papillomavirus status to cervical lesions and consequences for cervical-cancer screening: a prospective study. *Lancet* 1999; **354**: 20–25.
- 42 Bulk S, Bulkman NW, Berkhof J, et al. Risk of high-grade cervical intra-epithelial neoplasia based on cytology and high-risk HPV testing at baseline and at 6-months. *Int J Cancer* 2007; **121**: 361–67.
- 43 Hesselink AT, Bulkman NW, Berkhof J, Lorincz AT, Meijer CJ, Snijders PJ. Cross-sectional comparison of an automated hybrid capture 2 assay and the consensus GP5+/6+ PCR method in a population-based cervical screening program. *J Clin Microbiol* 2006; **44**: 3680–85.
- 44 Brink AA, Zielinski GD, Steenberg RD, Snijders PJ, Meijer CJ. Clinical relevance of human papillomavirus testing in cytopathology. *Cytopathology* 2005; **16**: 7–12.
- 45 Stoler MH, Castle PE, Solomon D, Schiffman M. The expanded use of HPV testing in gynecologic practice per ASCCP-guided management requires the use of well-validated assays. *Am J Clin Pathol* 2007; **127**: 335–37.
- 46 Bos AB, van Ballegoijen M, van Oortmarssen GJ, Habbema JD. Women who participate in spontaneous screening are not at higher risk for cervical cancer than women who attend programme screening. *Eur J Cancer* 2002; **38**: 827–31.
- 47 Arbyn M, Sasieni P, Meijer CJ, Clavel C, Koliopoulos G, Dillner J. Chapter 9: Clinical applications of HPV testing: a summary of meta-analyses. *Vaccine* 2006; **24** (suppl 3): S78–89.
- 48 Franco EL. Chapter 13: Primary screening of cervical cancer with human papillomavirus tests. *J Natl Cancer Inst Monogr* 2003; **31**: 89–96.
- 49 Ostor AG. Natural history of cervical intraepithelial neoplasia: a critical review. *Int J Gynecol Pathol* 1993; **12**: 186–92.
- 50 Ho GY, Bierman R, Beardsley L, Chang CJ, Burk RD. Natural history of cervicovaginal papillomavirus infection in young women. *N Engl J Med* 1998; **338**: 423–28.
- 51 Khan MJ, Castle PE, Lorincz AT, et al. The elevated 10-year risk of cervical precancer and cancer in women with human papillomavirus (HPV) type 16 or 18 and the possible utility of type-specific HPV testing in clinical practice. *J Natl Cancer Inst* 2005; **97**: 1072–79.
- 52 Castle PE, Wacholder S, Sherman ME, et al. Absolute risk of a subsequent abnormal pap among oncogenic human papillomavirus DNA-positive, cytologically negative women. *Cancer* 2002; **95**: 2145–51.