4 HUMAN PAPILLOMAVIRUS TESTING FOR THE DETECTION OF HIGH-GRADE CERVICAL INTRAEPITHELIAL NEOPLASIA AND CANCER: FINAL RESULTS OF THE POBASCAM RANDOMISED CONTROLLED TRIAL

Dorien C. Rijkaart ■ Johannes Berkhof ■ Lawrence Rozendaal
Folkert J. van Kemenade ■ Nicole W. J. Bulkmans ■ Daniëlle A.M. Heideman
Gemma G. Kenter ■ Jack Cuzick ■ Peter J.F. Snijders
Chris J.L.M. Meijer ■

Lancet Oncology 2012; 1: 78-88

CHAPTER 4

ABSTRACT

Background

Human papillomavirus (HPV) testing is more sensitive for the detection of high-grade cervical lesions than is cytology, but detection of HPV by DNA screening in two screening rounds 5 years apart has not been assessed. The aim of this study was to assess whether HPV DNA testing in the first screen decreases detection of cervical intraepithelial neoplasia (CIN) grade 3 or worse, CIN grade 2 or worse, and cervical cancer in the second screening.

Methods

In this randomised trial, women aged 29–56 years participating in the cervical screening programme in the Netherlands were randomly assigned to receive HPV DNA (GP5+/6+-PCR method) and cytology co-testing or cytology testing alone, from January, 1999, to September, 2002. Randomisation (in a 1:1 ratio) was done with computer-generated random numbers after the cervical specimen had been taken. At the second screening 5 years later, HPV DNA and cytology co-testing was done in both groups; researchers were masked to the patient's assignment. The primary endpoint was the number of CIN grade 3 or worse detected. Analysis was done by intention to screen. The trial is now finished and is registered, number ISRCTN20781131.

Findings

22 420 women were randomly assigned to the intervention group and 22 518 to the control group; 19 999 in the intervention group and 20 106 in the control group were eligible for analysis at the first screen. At the second screen, 19 579 women in the intervention group and 19 731 in the control group were eligible, of whom 16 750 and 16 743, respectively, attended the second screen. In the second round, CIN grade 3 or worse was less common in the intervention group than in the control group (88 of 19 579 in the intervention group *vs* 122 of 19 731 in the control group; relative risk 0.73, 95% CI 0.55–0.96; p=0•023). Cervical cancer was also less common in the intervention group than in the control group (four of 19 579 in the intervention group *vs* 14 of 19 731; 0.29, 0.10–0.87; p=0.031). In the baseline round, detection of CIN grade 3 or worse did not differ significantly between groups (171 of 19 999 *vs* 150 of 20 106; 1.15, 0.92-1.43; p=0.239) but was significantly more common in women with normal cytology (34 of 19 286 *vs* 12 of 19 373; 2.85, 1.47–5.49; p=0.001). Furthermore, significantly more cases of CIN grade 2 or worse were detected in the intervention group than in the control group (267 of 19 999 *vs* 215 of 20 106; 1.25, 1.05-1.50; p=0.015). In the second screen, fewer HPV16-positive CIN grade 3 or worse were detected in the intervention group than in the control group (17 of 9481 *vs* 35 of 9354; 0.48, 0.27–0.85; p=0.012); detection of non-HPV16-positive CIN grade 3 or worse did not differ between groups (25 of 9481 *vs* 25 of 9354; 0.99, 0.57–1.72; p=1.00). The cumulative detection of CIN grade 3 or worse and CIN grade 2 or worse did not differ significantly between study arms, neither for the whole study group (CIN grade 3 or worse: 259 of 19 999 *vs* 272 of 20 106; 0.96, 0.81–1.14, p=0.631; CIN grade 2 or worse:

CHAPTER 4

427 of 19 999 *vs* 399 of 20 106; 1.08, 0.94-1.24; p=0.292), nor for subgroups of women invited for the first time (CIN grade 3 or worse in women aged 29–33 years: 102 of 3139 *vs* 105 of 3128; 0.97, 0.74–1.27; CIN grade 2 or worse in women aged 29–33 years: 153 of 3139 *vs* 151 of 3128; 1.01, 0.81–1.26; CIN grade 3 or worse in women aged 34–56 years: 157 of 16 860 *vs* 167 of 16 978; 0.95, 0.76–1.18; CIN grade 2 or worse in women aged 34–56 years: 274 of 16 860 *vs* 248 of 16 978; 1.11, 0.94–1.32).

Interpretation

Implementation of HPV DNA testing in cervical screening leads to earlier detection of clinically relevant CIN grade 2 or worse, which when adequately treated, improves protection against CIN grade 3 or worse and cervical cancer. Early detection of high-grade cervical legions caused by HPV16 was a major component of this benefit. Our results lend support to the use of HPV DNA testing for all women aged 29 years and older.

CHAPTER 4

Introduction

Infection with high-risk types of human papillomavirus (HPV) has a causal role in the development of cervical cancer.[1,2] This link has stimulated the development of HPV DNA testing, which might be useful in primary cervical screening.[3,4] Furthermore, prophylactic HPV16 and HPV18 vaccines have been developed and introduced in many countries as a primary prevention method.[5,6]

Randomised controlled screening trials of HPV DNA testing [7–11] have shown a decreased detection of high-grade cervical lesions at the second screening round compared with cytology alone. Although the screening protocols, study endpoints, and interval between screening rounds varied in these trials, the consistent results suggest that HPV DNA testing offers better protection against high-grade cervical lesions in second screening rounds than do cytology-based screening methods. Only one study10 was large enough to also show protection against cervical cancer in the second screening round.

We present the final results of the POpulation-Based SCreening study AMsterdam (POBASCAM) trial, a population-based, randomised controlled trial. Our main goal was to assess whether HPV DNA testing decreases detection of cervical intraepithelial neoplasia (CIN) grade 3 or worse, of CIN grade 2 or worse, and of cervical cancer, in the second screening round. Additionally, to assess the most appropriate age at which HPV DNA testing should start, we analysed women invited for the first time (aged 29–33 years) and older women (34–56 years) separately. We also assessed how detection of high-grade lesions in two screening rounds was associated with particular HPV genotypes.

Methods

Study design and participants

Patients were enrolled between January, 1999, and September, 2002, as part of the nationwide cervical screening programme. In the Netherlands, women are invited for cervical cancer screening at 5 year intervals starting in the year when they reach age 30 years and ending in the year when they reach age 60 years. The design, methods, and baseline results of the trial have been described.[7,12] Detection of neoplasia or cancer in the first 48 months was classed as detected in the first screening round and those detected during 48–108 months were classed as detected in the second screening round. The cutoff after 48 months was used because women in the Netherlands are invited for a new screen in the year they reach 30 years, 35 years, 40 years, and so on. Therefore, the actual screening interval between invited smears is between 4 and 6 years. Women who had a history of CIN grade 2 or worse, had abnormal cytology in the preceding 2 years, or who had had a hysterectomy were excluded. Women aged 57 years or older at baseline were also excluded because they would not routinely receive a smear in the second round. All participants provided written informed consent. The trial was approved by both the Medical Ethics Committee of VU University Medical Centre and the Ministry of Public Health (The Hague, Netherlands).

CHAPTER 4

Randomisation and masking

LR randomly assigned women to the intervention or control groups in a 1:1 ratio with computer-generated random numbers 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 so were unaware of a patient's assignment. Women in the intervention group were given their HPV DNA and cytology result and managed accordingly. For the control group, the patients and all medical personnel were masked to the HPV DNA test results. At the second screening round, women in both groups were managed according to their newly obtained HPV DNA and cytological test results. In both groups pathologists were aware of the cytology result but not of the HPV result. CIN (CIN grade 1 or worse) biopsies were reviewed by two experienced cervical pathologists (FJvK and LR) who were masked to the HPV and cytology results.

Procedures

Women were screened at baseline with either combined HPV DNA testing and cytology or cytology alone.[12] At the second screening round 5 years later, all women were screened with both HPV DNA testing and cytology so that we could detect high-grade CIN and cancer cases in the control group that we would otherwise have missed with cytology alone.

Conventional cytological smears were taken with a Cervex-Brush (Rovers, Oss, Netherlands) or a cytobrush. The brush was placed in a vial containing 5 mL phosphate-buffered saline for HPV DNA testing. Cytology results were read according to the CISOE-A classification,[13] which can be roughly converted to the 2001 Bethesda system.[14] Cytological results were grouped as normal, borderline or mild dyskaryosis, or moderate dyskaryosis or worse. In the 2001 Bethesda system, borderline

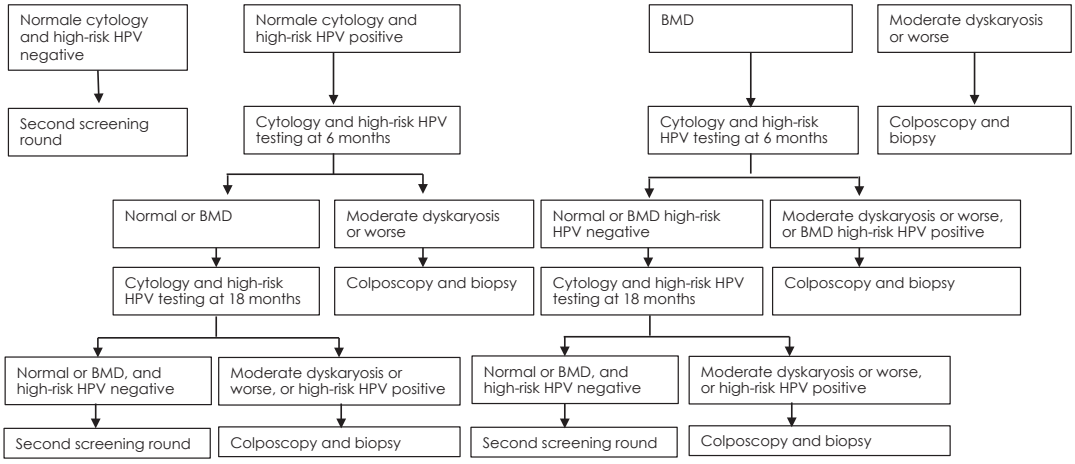

Figure 1 Management of women in the intervention group at the first screening round. In the second screening round women in both groups were managed in the same way as the intervention group were in the first screening round. BMD=borderline or mild dyskaryosis. HPV=human papillomavirus.

CHAPTER 4

or mild dyskaryosis corresponds to atypical squamous cells of unknown clinical significance, atypical squamous cells and cannot rule out high-grade squamous intraepithelial lesions, lowgrade squamous intraepithelial lesions, or atypical glandular cells. Moderate dyskaryosis or worse corresponds to both squamous and glandular highgrade intraepithelial lesions.

HPV DNA testing was done by general primer (GP5+/6+) PCR enzyme immunoassay,[15] which detects 14 high-risk types (types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68). Positive specimens were typed by reverse line blotting.[16]

Figure 1 shows how patients were managed, in accordance with Dutch guidelines,[13,17] and has been described in detail previously.[7] Of the women who were referred for colposcopy, colposcopy-directed biopsies of suspicious parts of the cervix were taken for histological examination, according to standard procedures in the Netherlands.[18] Colposcopies were not registered, therefore, data of registered biopsy rates were used to estimate the number of gynaecological tests.

Histology was examined locally and classified as normal, CIN grade 1, 2, 3, or invasive cancer, according to inter national criteria.[19,20] Glandular intraepithelial neoplasia grade 1, 2, and 3 were included in their respective CIN categories. In the analyses presented here, the original diagnoses were used. Cytology and histology results were retrieved from the nationwide registry of histopathology and cytopathology results and, when necessary, from individual laboratory records. The reviewed histological results for biopsies of CIN grade 1 or worse are shown in the appendix.

The primary outcome measure was histologically confirmed CIN grade 3 or worse, with a primary endpoint of the number of CIN grade 3 or worse detected. The secondary outcome measures were cervical cancer and CIN grade 2 or worse. Cervical cancer included squamous cell carcinoma, adenocarcinoma, and adenosquamous cell carcinoma; adenocarcinoma in situ were included in the CIN grade 3 group.

Statistical analysis

The main analyses included all randomly assigned women and were done by intention to screen. The overall number of CIN grade 3 or worse identified in each group was calculated for the first screening round, the second screening round, and the two rounds combined. For the second round, we included only those women who did not have CIN grade 2 or worse at baseline and who were eligible for subsequent screening, because those with CIN 2 or worse were managed according to current treatment protocols. When enrolment was complete all women had the opportunity of 108 months of follow-up. Events after 108 months were not included. Because HPV DNA prevalence decreases with age and CIN grade 2 or worse can spontaneously regress in young women, we analysed women invited for the first time (aged 29–33 years) and older women (34–56 years) separately to assess the most appropriate age at which HPV DNA testing should start.

Reason for smear test was not recorded in the nationwide registry of histopathology and cytopathology results. The contribution of opportunistic screening in women with a negative screening test at baseline was estimated by the proportion of women who had an additional smear before receiving a new invitation after 5 years.

CHAPTER 4

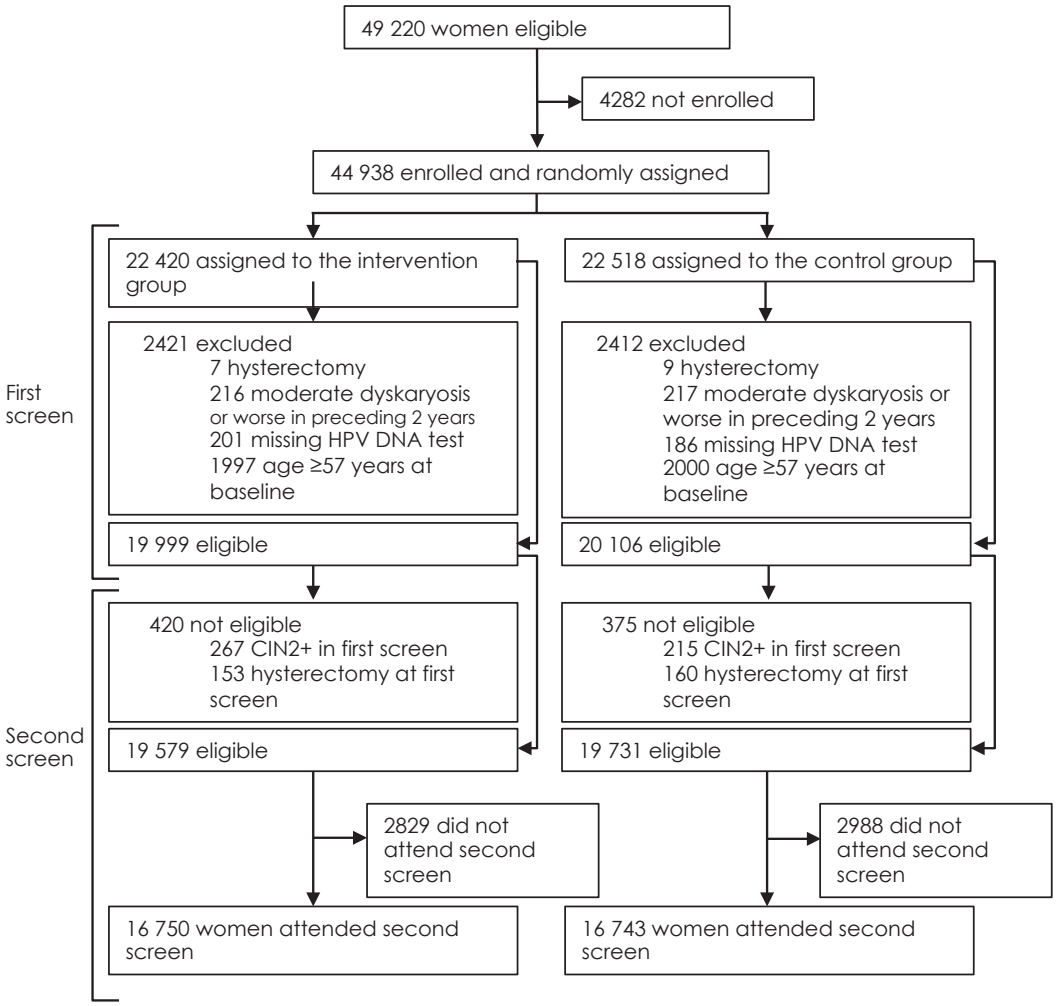

Figure 2 Trial profile. HPV=human papillomavirus. CIN2+=cervical intraepithelial neoplasia grade 2 or worse. *The major reason for women not to be enrolled in our study was lack of time for general practitioners to explain the objectives of the study.

Differences in detection between the groups were calculated with Fisher's exact test. All p values are two-sided. Analyses were done with SPSS version 12.0.

A study size of 44 000 was calculated to be sufficiently large to detect (with 80% power) a significant difference in the number of lesions CIN grade 3 or worse between groups at the second screen after borderline or mild dyskaryosis at baseline. The sample size was also sufficient to show a decrease at the second round of CIN grade 3 or worse lesions in women in the intervention group who had normal cytology at baseline compared with women in the control group who had normal cytology at baseline. For the power calculations, we assumed that 84.5% of the baseline smears were cytologically normal, that 14% of the baseline smears were

CHAPTER 4

borderline or mild dyskaryosis, and that 4% of the normal and 15% of the borderline or mild dyskaryosis smears were positive for HPV DNA. The prevalences of CIN grade 3 or worse in the second screen were assumed to be 0.4% and 0.9% for women with normal cytology and borderline or mild dyskaryosis at baseline, respectively. Furthermore, the relative risk of HPV DNA for CIN grade 3 or worse in the subsequent round was assumed to be 13, which is the lower bound of the 95% CI reported in a previous study,[21] which provided a conservative estimate for the required sample size. The study was not powered to detect a difference in CIN grade 3 or worse at baseline. This trial is registered as an International Standard Randomised Controlled Trial, ISRCTN20781131.

Table 1 Cervical intraepithelial neoplasia and cervical cancers at baseline screen and subsequent screen.

	Intervention group					Control group				
	Total	CIN0/1	CIN2	CIN3	Cervical cancer	Total	CIN0/1	CIN2	CIN3	Cervical cancer
Baseline screen										
Inadequate cytology										
HPV DNA positive	2	0	1	0	0	0	0	0	0	0
HPV DNA negative	31	1	0	0	0	27	2	0	0	0
Normal cytology										
HPV DNA positive	724	48	31	29	2	766	9	6	9	1
HPV DNA negative	18562	218	7	3	0	18607	233	4	2	0
Borderline or mild dyskaryosis										
HPV DNA positive	185	29	24	34	3	192	30	25	29	1
HPV DNA negative	330	28	3	3	1	335	22	2	5	0
Moderate dyskaryosis or worse										
HPV DNA positive	146	11	26	87	5	160	20	24	93	4
HPV DNA negative	19	6	4	3	1	19	4	4	6	0
Total	19999	341	96	159	12	20106	320	65	144	6
Subsequent round										
Inadequate cytology										
HPV DNA positive	0	0	0	0	0	2	0	0	0	0
HPV DNA negative	21	0	0	0	0	27	0	0	0	0
No HPV DNA test	21	2	0	0	0	23	0	0	0	0
Normal cytology										
HPV DNA positive	284	18	10	9	1	272	26	14	14	0
HPV DNA negative	8941	98	6	1	0	8811	107	3	1	0
No HPV DNA test	7025	134	14	1	1	7108	162	7	2	1
Borderline or mild dyskaryosis										
HPV DNA positive	67	20	9	12	0	77	16	12	15	1
HPV DNA negative	129	7	1	2	0	118	9	2	0	1
No HPV DNA test	164	23	14	7	0	200	22	9	11	1
Moderate dyskaryosis or worse										
HPV DNA positive	31	3	5	20	0	41	0	6	27	3
HPV DNA negative	8	2	3	1	0	6	0	0	4	0
No HPV DNA test	59	10	10	26	2	58	8	9	29	4
No screening test*	2829	52	0	5	0	2988	59	0	5	3
Total	19579	369	72	84	4	19731	409	62	108	14
Both rounds										
Total	19999	710	168	243	16	20106	729	127	252	20

Cases detected by opportunistic screening are also included. CIN3=cervical intraepithelial neoplasia grade 3. CIN2=cervical intraepithelial neoplasia grade 2. CIN0/1=cervical intraepithelial neoplasia grade 0 or 1.

*Women who had no second round smear recorded.

CHAPTER 4

Role of the funding source

The sponsors 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

Figure 2 shows the trial profile. The median age at recruitment was 40.0 years (IQR 34.0–49.0 years) in both groups. For women without CIN grade 2 or worse, the median time since last cytological results was 5.0 years (4.4–5.5). Compliance to follow-up testing (at least one repeat smear) was higher in the control group (477 of 527 patients [91%]) than in the intervention group (1007 of 1239 patients [81%]). The difference in compliance was related to baseline cytology. In the intervention group, 456 of 515 (89%) women with borderline or mild dyskaryosis at baseline, and 551 of 724 (76%) with normal cytology and a positive high risk HPV test complied with follow-up.

Attendance for the second screen was not significantly different between groups (16 750 of 19 579 [86%] patients in the intervention group *vs* 16 743 of 19 731 [85%] in the control group). Compliance to follow-up testing did not differ significantly between groups—539 (84%) of 644 patients in the intervention group and 564 (85%) of 667 in the control group complied. Attendance at the second screen of cytologically negative, HPV DNA positive women was much the same in the intervention and control groups (553 of 724 [76%] women *vs* 588 of 766 [77%] women). 2424 of 18 562 (13%) women who were negative at baseline in the intervention group and 2542 of 19 373 (13%) women who were negative at baseline in the control group had an additional smear before receiving a new invitation after 5 years (opportunistic screening). Table 1 shows the number of cervical lesions by study group, in relation to cytological and HPV DNA results at the first and second screens. Positive HPV DNA (masked in the control group) and abnormal cytology at baseline were much the same for the intervention and control groups.[12] The number of CIN grade 0 or 1 detected at the first and second screens was similar in the intervention and control groups. Most CIN grade 0 or 1 were detected after a negative first screen whereas most CIN grade 2 or 3, and cancer cases were detected after a positive first screen.

In the intervention group, 12 cancers (one adenocarcinoma and 11 squamous cell carcinomas) and five adenocarcinomas in situ were identified in the first screen; in the second screen, four cancers (one adenocarcinoma and three squamous cell carcinomas) and one adenocarcinoma in situ were identifi ed. In the control group, six cancers (two adenocarcinomas and four squamous cell carcinomas) and five adenocarcinomas in situ were identified at the first screen; 14 cancers (two adenocarcinoma and 12 squamous cell carcinomas) and four adenocarcinomas in situ were identified in the second screen.

Table 2 shows the number of high-grade cervical lesions in each group at first and second screens. For the first screen, detection of CIN grade 3 or worse did not differ significantly in the intervention group compared with the control group (p=0.239). Additionally, the number of cervical cancer cases did not diff er signifi cantly between the intervention and control groups (p=0.166). However, significantly more

CHAPTER 4

CIN grade 2 or worse were detected at baseline in the intervention group than in the control group (p=0.015). In the second screen, fewer CIN grade 3 or worse and cervical cancers were recorded in the intervention group than in the control group (p=0.023 and p=0.031, respectively). However, there was no significant difference in detection of CIN grade 2 or worse in the intervention group compared with the control group in the second screen (p=0.234). When combining both rounds, the detection rates in the intervention and control groups were much the same for CIN grade 2 or worse, CIN grade 3 or worse, and for cervical cancer (table 2). However, over two screening rounds, a third more CIN grade 2 were recorded in the intervention group than in the control group (table 2).

In the first screen, two cancers in the intervention group were missed by HPV DNA testing but had abnormal cytology (in patients aged 32 years with FIGO IB1 and aged 54 years with FIGO IA1). Three cervical cancers were missed by cytology testing (one patient aged 36 years and two patients aged 44 years, all three with FIGO IB1), but tested positive for HPV DNA. Five cervical cancers were diagnosed after borderline or mild dyskaryosis cytology (in patients aged 30 years with FIGO IB1, 32 years with FIGO IB1, 35 years with IB1, 40 years FIGO with 1B1, and 50 years with IA1), which could have been missed if patients were not followed up correctly. Only one woman who developed cervical cancer (aged 59 years with FIGO IB1) had a negative HPV DNA test at the second screen. She was also HPV negative in the first screen.

Table 2 Occurrences of high-grade disease

	Intervention group (n[%; 95% CI])	Control group (n[%; 95% CI])	Intervention vs control		
			Risk difference (95% CI)	Relative risk (95% CI)	p value
Baseline screen*					
Cervical cancer	12 (0.06%; 0.03-0.11)	6 (0.03%; 0.01-0.07)	0.03% (–0.01 to 0.07)	2.01 (0.76 to 5.36)	0.166
CIN3 or worse	171 (0.86%; 0.73-1.00)	150 (0.75%; 0.63-0.88)	0.11% (–0.07 to 0.28)	1.15 (0.92 to 1.43)	0.239
CIN3	159 (0.80%; 0.68-0.93)	144 (0.72%; 0.61-0.85)	0.08% (–0.09 to 0.25)	1.11 (0.89 to 1.39)	0.387
CIN2 or worse	267 (1.34%; 1.18-1.51)	215 (1.07%; 0.93-1.22)	0.27% (0.05 to 0.48)	1.25 (1.05 to 1.50)	0.015
CIN2	96 (0.48%; 0.39-0.59)	65 (0.32%; 0.25-0.41)	0.16% (0.03 to 0.28)	1.48 (1.09 to 2.04)	0.014
Subsequent screen †					
Cervical cancer	4 (0.02%; 0.01-0.06)	14 (0.07%; 0.04-0.12)	–0.05% (–0.09 to –0.01)	0.29 (0.10 to 0.87)	0.031
CIN3 or worse	88 (0.45%; 0.36-0.56)	122 (0.62%; 0.52-0.74)	–0.17% (–0.31 to –0.03)	0.73 (0.55 to 0.96)	0.023
CIN3	84 (0.43%; 0.34-0.53)	108 (0.55%; 0.45-0.66)	–0.12% (–0.26 to 0.02)	0.78 (0.59 to 1.04)	0.096
CIN2 or worse	160 (0.82%; 0.70-0.96)	184 (0.93%; 0.81-1.08)	–0.12% (–0.30 to 0.07)	0.88 (0.71 to 1.08)	0.234
CIN2	72 (0.37%; 0.29-0.47)	62 (0.31%; 0.24-0.41)	0.05% (–0.06 to 0.17)	1.17 (0.83 to 1.65)	0.387
Both screens*					
Cervical cancer	16 (0.08%; 0.05-0.13)	20 (0.10%; 0.06-0.16)	–0.02% (–0.08 to 0.04)	0.80 (0.42 to 1.55)	0.617
CIN3 or worse	259 (1.30%; 1.15-1.46)	272 (1.35%; 1.20-1.52)	–0.06% (–0.28 to 0.17)	0.96 (0.81 to 1.14)	0.631
CIN3	243 (1.22%; 1.07-1.38)	252 (1.25%; 1.11-1.42)	–0.04% (–0.25 to 0.18)	0.97 (0.81 to 1.16)	0.752
CIN2 or worse	427 (2.14%; 1.94-2.35)	399 (1.98%; 1.80-2.19)	0.15% (–0.13 to 0.43)	1.08 (0.94 to 1.24)	0.292
CIN2	168 (0.84%; 0.72-0.98)	127 (0.63%; 0.53-0.75)	0.21% (0.04 to 0.38)	1.33 (1.06 to 1.68)	0.017

*n=19 999 in the intervention group, n=20 106 in the control group. †n=19 579 in the intervention group, n=19 731 in the control group. CIN3=cervical intraepithelial neoplasia grade 3. CIN2=cervical intraepithelial neoplasia grade 2.

CHAPTER 4

For women positive for high-risk HPV, with normal cytology at first screen, the number of high-grade lesions differed between the intervention and control group in both first and second screens (table 3). These women also have a substantial risk of CIN grade 3 or worse in the second screen in the intervention and control groups. At first screen, CIN grade 3 or worse was not significantly different between the intervention and control groups for women with moderate dyskaryosis or worse (96 of 165 women *vs* 103 of 179; RR 1.01, 95% CI 0.84–1.21; p=0.9) and for women with borderline or mild dyskaryosis (41 of 515 women *vs* 35 of 527; 1.20, 0.78–1.85; p=0.5). However, more CIN grade 3 or worse lesions were detected in women with normal cytology in the intervention group than in the control group (34 of 19 286 patients *vs* 12 of 19 373; 2.85, 1.47–5.49; p=0.001). At the second screen, excluding women who had CIN grade 2 or worse, or who had hysterectomy at baseline, numbers of CIN grade 3 or worse were not significantly different between study groups in women with

Table 3 Cervical cancers and high grade intraepithelial neoplasia by test results in the first and second screen rounds.

	First screen				Second screen		
	Total	Cervical cancer	CIN3	CIN2	Cervical cancer	CIN3	CIN2
Intervention group							
Inadequate cytology							
HPV positive	2	0	0	1	0	0	0
HPV negative	31	0	0	0	0	0	1
Normal cytology							
HPV positive	724	2	29	31	0	36	20
HPV negative	18562	0	3	7	2	36	40
Borderline or mild dyskaryosis							
HPV positive	185	3	34	24	1	9	8
HPV negative	330	1	3	3	0	1	2
Moderate dyskaryosis or worse							
HPV positive	146	5	87	26	1	2	1
HPV negative	19	1	3	4	0	0	0
Total	19999	12	159	96	4	84	72
Control group							
Inadequate cytology							
HPV positive	0	0	0	0	0	0	0
HPV negative	27	0	0	0	0	0	0
Normal cytology							
HPV positive	766	1	9	6	8	57	18
HPV negative	18607	0	2	4	4	32	36
Borderline or mild dyskaryosis							
HPV positive	192	1	29	25	0	13	5
HPV negative	335	0	5	2	0	2	2
Moderate dyskaryosis or worse							
HPV positive	160	4	93	24	2	3	1
HPV negative	19	0	6	4	0	1	0
Total	20106	6	144	65	14	108	62

CIN3=cervical intraepithelial neoplasia grade 3. CIN2=cervical intraepithelial neoplasia grade 2.

CHAPTER 4

baseline moderate dyskaryosis or worse (three of 39 *vs* six of 48; 0.61, 0.16–2.30; p=0.7) and in women with baseline borderline or mild dyskaryosis (11 of 443 *vs* 15 of 461; 0.75, 0.35–1.62; p=0.6). However, CIN grade 3 or worse differed between groups in women with normal cytology at the first screen (74 of 19 066 *vs* 101 of 19 196; 0.74, 0.55–0.99; p=0.049).

The cumulative number of CIN grade 3 or worse lesions detected over two screening rounds was much the same for both study groups in women with moderate dyskaryosis or worse at baseline (99 of 165 *vs* 109 of 179; 0.99, 0.83–1.17), women with borderline or mild dyskaryosis at baseline (52 of 515 *vs* 50 of 527; 1.06, 0.74–1.54), and women with normal cytology at baseline (108 of 19 286 *vs* 113 of 19 373; 0.96, 0.74–1.25). Furthermore, the cumulative number of CIN lesions of grade 3 or worse over two screening rounds was significantly lower for women who tested negative for HPV DNA at baseline in the intervention group than for those who had normal cytology at baseline in the control group (50 of 18 942 *vs* 113 of 19 373; 0.45, 0.32–0.63).

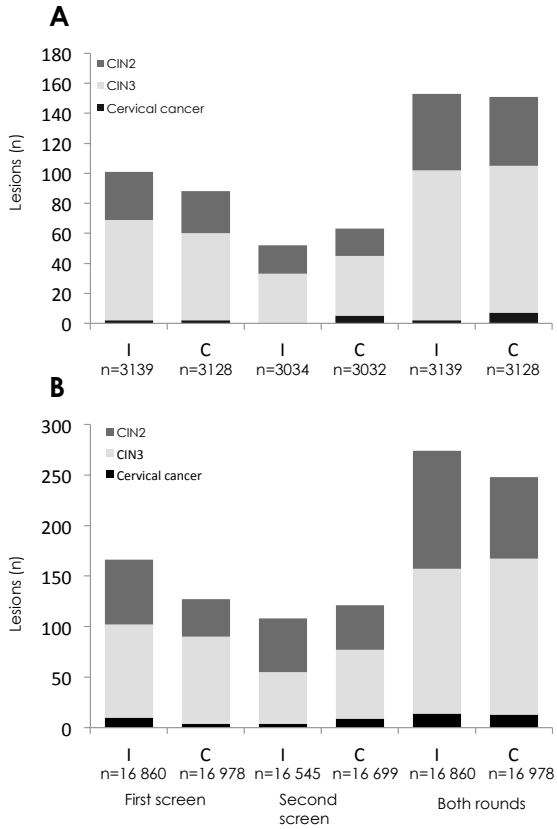

Figure 3 Histological results for women aged 29–33 years (A) and 34–56 years (B), by study group and screening round. CIN2=cervical intraepithelial neoplasia grade 2. CIN3=cervical intraepithelial neoplasia grade 3. I=intervention. C=control.

CHAPTER 4

Use of CIN grade 2 or worse as an outcome measure gave much the same result (107 of 18 942 *vs* 177 of 19 373; 0.62, 0.49–0.79). Use of reviewed histological results did not give different results (appendix).

373 of 3139 (12%) women aged 29–33 years in the intervention group and 379 of 3128 (12%) women aged 29–33 years in the control group had HPV DNA at baseline (p=0.786). By contrast, HPV at baseline was detected in 684 of 16 860 (4%) women in the intervention group aged 34–56 years and in 739 of 16 978 (4%) of women in the control group aged 34–56 years (p=0.176). 152 of 3139 (5%) women in the intervention group aged 29–33 years at baseline had biopsy samples taken, compared with 129 of 3128 (4%) women in the control group aged 29–33 years. In women aged 34–56 years, baseline screen biopsy samples were taken from 456 of 16 860 (3%) patients in the intervention group and 407 of 16 978 (2%) in the control group. 712 of 3136

Table 4 HPV types at baseline and subsequent screens and number of HPV-positive CIN grade 3 or worse and CIN grade 2 or worse detected.

	Total number of women	CIN3+	CIN2+
Intervention group			
First screen			
HPV16	333 (32%)	101 (63%)	135 (56%)
Inadequate	1 (0%)	0	1 (0%)
Normal	202 (19%)	23 (14%)	36 (15%)
BMD	55 (5%)	22 (14%)	30 (12%)
>BMD	75 (7%)	56 (35%)	68 (28%)
Other HPV type(s)	724 (68%)	59 (37%)	107 (44%)
Inadequate	1 (0%)	0	0
Normal	522 (49%)	8 (5%)	26 (11%)
BMD	130 (12%)	15 (9%)	31 (13%)
>BMD	71 (7%)	36 (23%)	50 (21%)
Second screen			
HPV16	100 (26%)	17 (40%)	23 (35%)
Normal	70 (18%)	6 (14%)	10 (15%)
BMD	19 (5%)	4 (10%)	5 (8%)
>BMD	11 (3%)	7 (17%)	8 (12%)
Other HPV type	282 (74%)	25 (60%)	43 (65%)
Normal	214 (56%)	4 (10%)	10 (15%)
BMD	48 (13%)	8 (19%)	16 (24%)
>BMD	20 (5%)	13 (31%)	17 (26%)
Control group			
Second round			
HPV16	99 (25%)	35 (58%)	42 (46%)
Normal	53 (14%)	7 (12%)	10 (11%)
BMD	24 (6%)	8 (13%)	11(12%)
>BMD	22 (6%)	20 (33%)	21 (23%)
Other HPV type	293 (75%)	25 (42%)	50 (54%)
Inadequate	2 (1%)	0	0
Normal	219 (56%)	7 (12%)	18 (20%)
BMD	53 (14%)	8 (13%)	17 (18%)
>BMD	19 (5%)	10 (17%)	15 (16%)

Data are n (%). CIN3+=cervical intraepithelial neoplasia grade 3 or worse. CIN2+=cervical intraepithelial neoplasia grade 2 or worse. BMD=borderline or mild dyskaryosis.

(23%) women aged 29–33 years in the intervention group had repeat screening test or histology, or both, after the baseline round compared with 554 of 3128 (18%) in the control group. In women aged 34–56 years, follow-up was 3100 of 16 860 (18%) in the intervention group and 2887 of 16 978 (17%) in the control group. If women with a negative first screen were not counted in the numerator, follow-up among women aged 29–33 years was 355 of 3139 (11%) in the intervention group and 172 of 3128 (5%) in the control group, for women aged 34–56 years follow-up was 843 of 16 860 (5%) in the intervention group and 497 of 16 978 (3%) in the control group. For the two screening rounds combined, the two study groups did not differ significantly for CIN grade 3 or worse and CIN grade 2 or worse for both women aged 29–33 and those aged 34–56 (figure 3). The ratio of CIN grade 3 or worse detection between the intervention and control groups was 0.97 (102 of 3139 *vs* 105 of 3128; 95% CI 0.74–1.27) for women aged 29–33 years and 0.95 (157 of 16 860 *vs* 167 of 16 978; 0.76–1.18) for women aged 34–56 years. Ratios for CIN grade 2 or worse were 1.01 (153 of 3139 *vs* 151 of 3128; 0.81–1.26) and 1.11 (274 of 16 860 *vs* 248 of 16 978; 0.94–1.32) for women aged 29–33 years and 34–56 years, respectively.

Finally, we compared the number of CIN grade 3 or worse and CIN grade 2 or worse at first and second screens for HPV16-positive and HPV16-negative women (table 4). We only included women with a valid HPV DNA test result. Individual HPV types other than HPV16 were not analysed separately because of their low prevalence in cervical lesions. For the first screen in the intervention group 101 (59%) of 171 of the CIN grade 3 worse and 135 (51%) of 267 of the CIN grade 2 or worse lesions were HPV16 positive. In women with normal cytology, 23 (68%) of 34 of CIN lesions of grade 3 or worse were HPV16 positive and in women with abnormal cytology, 78 (57%) of 137 of CIN grade 3 or worse lesions were HPV16 positive. In the second screen, fewer HPV16-positive CIN grade 3 or worse were detected in the intervention group than in the control group (17 of 9481 *vs* 35 of 9354; RR 0.48, 95% CI 0.27–0.85; p=0.012) whereas detection of non-HPV16-positive CIN grade 3 or worse did not differ (25 of 9481 *vs* 25 of 9354; 0.99, 0.57–1.72; p=1.00). The proportion of HPV16 in HPV-positive CIN grade 3 or worse and grade 2 or worse lesions was also lower in the intervention group than in the control group but the positivity ratio was not significantly different for either CIN grade 3 or worse (17 of 42 *vs* 35 of 60, 0.69; 0.45–1.06; p=0.08) or for CIN grade 2 or worse (23 of 66 *vs* 42 of 92; 0.76, 0.51–1.14; p=0.17).

CHAPTER 4

Panel: Research in context

Systematic review

This trial was designed in 1997, when no data about performance of HPV testing in population-based screening existed. The design was based on several findings. First, HPV prevalence data obtained by general primer PCR in women with normal cervix, cervical intraepithelial neoplasia (CIN) grade 1–3 and cervical cancer[26] led to the hypothesis that HPV testing by general primer PCR could be used for cervical cancer screening.[27] After improvement of our first generation general primer PCR by elongation of the primers and addition of an easy readout,[15] to allow high-throughput HPV detection, we applied this GP5+/6+ general primer PCR in case-control studies, showing that high-risk HPV was present in nearly all cervical carcinomas.[28] Analysis of normal cervical scrapings of women who later developed cervical cancer showed that the same HPV type as that in the carcinoma could be found in the preceding normal smear.[29] A subsequent prospective study on a gynaecological outpatient population showed that the presence of a persistent HPV infection in women with an abnormal smear was associated with progressive CIN disease.[30,31] For the design of the POBASCAM trial the Dutch health council, an independent advisory body of the government, required that the study be done in the setting of the nationwide screening programme, which has a screening interval of 5 years, which is why the results are only now available.

Interpretation

The final results accord with those of similar randomised controlled trials that used shorter screening intervals, such as Swedscreen,[8] ARTISTIC,[9] and NTCC,[10] as well as those of our interim analysis,[7] showing that HPV testing significantly reduces detection of CIN grade 3 or worse in the second screening round relative to conventional cytology. Additionally, as was the case in the NTCC trial, the final POBASCAM data also show that HPV screening protects against cervical cancer better than does cytology alone. By contrast with other studies, CIN was diagnosed by pathologists in daily routine practice rather than by a reviewed diagnosis, to study the performance of HPV screening in a routine population-based setting. Review of CIN diagnoses did not affect the results of the study. As such, the study can be classed as an implementation study. Furthermore, the long screening interval as used in POBASCAM allows assessment of whether a cervical lesion is persistent or regressive. Our study also lends support to the notion that HPV screening does not have to be postponed until patients reach age 35 years but can be implemented at age 30 years because the cumulative detection of CIN grade 3 or worse and CIN grade 2 or worse across two screening rounds does not differ between women aged 29–33 years and women 34 years or older.

CHAPTER 4

Discussion

Our results showed that fewer CIN lesions of grade 3 or worse and cervical cancer were detected during second screens in women who were screened for HPV DNA in combination with cytology at first screen than those who had cytology alone. Furthermore, the value of HPV DNA testing is lent support by the finding that women who tested negative for HPV DNA in the first screen had significantly fewer CINs of grade 3 or worse detected over two screening rounds than did women with normal cytology at baseline. These findings accord with previously reported data.[4,7–11] However, our study has longer follow-up than other studies did because the screening interval in the Netherlands is longer than that in other countries (5 years vs 3 years). This difference is important because it enables us to assess whether a cervical lesion is persistent or regressive. Our study also suggests that HPV screening does not have to be postponed until age 36 years or older but can be started at age 30 years because the cumulative two screen detection of CIN of grade 3 or worse and grade 2 or worse did not differ between women aged 29–33 years and women older than 33 years. Moreover, we showed that early detection of high-grade cervical lesions caused by HPV16 was a major component of the benefit of adding high-risk HPV testing to cytology.

The interim analysis of 17 155 women from this trial did not show a significant difference between the intervention and control groups in the number of cervical cancers detected at the second screening round.[7] Our final analysis of the whole population was large enough to identify a significant difference in the number of cervical cancers in the intervention group versus the control group at the second screen. Such an effect on cervical cancer in the second screening round because of HPV DNA testing has only been shown in one previous trial.[10] The investigators reported no cervical cancer in the second screen in 47 369 women screened by HPV DNA testing, whereas nine cervical cancers were recorded in 47 001 women in the control group. Reduced mortality from cervical cancer because of primary HPV DNA testing was also shown in a trial in India.[22] 34 deaths from cervical cancer occurred in the HPV DNA testing group (34 126 women) compared with 64 in the control group (31 488 women).

The decreased relative risks of CIN grade 3 or worse and cervical cancer in the second screening round occurred after an increase in relative detection of CIN grade 2 or worse in the baseline screen in the intervention group compared with the control group. By contrast with our interim findings,[7] we failed to show a significant difference in the detection of CIN grade 3 or worse in the first screen in the whole study population. However, our study was powered to detect a significant difference in the number of CIN grade 3 or worse at the second round rather than at the first screen. Also, in some other randomised trials no significant difference in detection of CIN grade 3 or worse at the first screen occurred after HPV DNA testing, despite decreased detection of CIN grade 3 or worse in the second screening round.[8,9] When we stratified our analysis on the basis of baseline cytology, we did not note a significant difference in the occurrence of CIN grade 3 or worse in women with borderline or mild dyskaryosis or moderate dyskaryosis or worse but report a significant difference

CHAPTER 4

in the number of CIN grade 3 or worse in women with normal cytology. The latter finding matches our expectations because no additional testing was recommended for women in the control group with baseline normal cytology. The extra CIN grade 3 or worse cases detected by HPV DNA testing compared with cytology are the main benefit of using HPV DNA testing for primary screening. The failure to detect a relative difference in the occurrence of CIN grade 3 or worse could be related to the decision to change the cytological classification criteria in 1996, whereas the study was powered by cytological results collected earlier. Notably, the use of more stringent criteria for borderline or mild dyskaryosis and moderate dyskaryosis or worse resulted in a decrease of borderline or mild dyskaryosis in the Netherlands, from 11% in 1999 to 3% in 2004.[13] This explains the low occurrence of CIN grade 3 or worse in women with borderline or mild dyskaryosis compared with other studies. The cumulative number of women with CIN grade 3 or worse and grade 2 or worse over both screening rounds did not differ substantially between study groups, which lends support to the idea that HPV DNA testing leads to earlier detection of clinically relevant high-grade lesions that, with the exception of a small number of CIN grade 2 lesions, do not regress.

An important issue is the age at which HPV DNA testing should be offered in primary screening. HPV DNA prevalence is age dependent,[23,24] therefore HPV DNA testing in young women might have a different risk-benefit ratio to HPV DNA testing in older women. Ronco and colleagues[10] reported that HPV DNA testing in women aged 25–34 years could lead to substantial overdiagnosis of regressive CIN grade 2 or worse lesions, particularly when HPV DNA positive women in this age group are directly referred for colposcopy without further triage testing. Our results suggest that with the triage algorithm used in this trial HPV DNA testing in women aged 29–33 years does not result in excessive diagnosis of lesions destined to regress and lends support to the implementation of HPV DNA testing in programmed cervical screening starting at age 30 years.

The protective effect against CIN grade 3 or worse in the second screen in the intervention group was largely attributable to HPV16. HPV16 is the main genotype present in cervical cancer, hence, the early detection of HPV16-associated CIN grade 3 lesions is expected to eventually have an effect on long-term outcomes such as cancer morbidity and mortality. However, our study was not large enough to provide detailed information about the effect of other, non-HPV16 types, in the first and second screening round. This investigation needs a pooled analysis of many screening studies, which is being done (pooling Swedscreen, NTCC, and data from this study).[8,10]

Variation in histological classification of cervical lesions between individual centres might be a limitation of our study. However, we believe that the use of the original diagnosis closely matches treatment guidance and mimics future implementation of HPV DNA testing in population-based screening programmes. Moreover, use of histological classification obtained after review by two experienced cervical pathologists, although leading to more CIN grade 2 or worse and CIN grade 3 or worse diagnoses, did not change our conclusions.

CHAPTER 4

A strength of our study is the large size of the trial and the longitudinal design. We included all cytology and histological follow-up data derived from the nation wide registry within 9 years after the first screen. The trial was done within the regular Dutch nationwide screening programme. Together with the HC2 assay, the GP5+/6+ PCR method is clinically validated and performs much the same as HC2 in sensitivity, specificity, and intra-laboratory and inter-laboratory agreement.[25] Therefore, our results can be considered representative for organised cervical screening.

Our results are from a population-based screening programme in which CIN diagnosis was made in routine pathology laboratories. Therefore, our study can be viewed as an implementation study, representative for organised screening and as such provides the strongest evidence to date in favour of implementation of HPV testing in nationwide cervical screening programmes (panel). On the basis of these data the Health Council of the Netherlands, an independent advisory body of the Dutch Government, has issued advice to the minister of health to convert the current cytology-based cervical screening programme to an HPV testing-based programme starting at 30 years. Additionally, offer of HPV testing using self-collected cervico-vaginal samples to women who do not respond to an invitation for cervical screening results in a response of about 30%,[32] showing that a proportion of women prefer self-sampling to a smear taken by a physician. Studies should investigate the acceptance of this alternative for HPV testing in regular attendees to the screening programme. Ultimately, this option will likely increase attendance to cervical screening programmes.

Acknowledgments

The study was funded by Zorg Onderzoek Nederland (Netherlands Organisation for Health Research and Development). We thank the 242 family doctors and their assistants, the Municipal Health Service Southwest of Amsterdam, Medial, DHV Kennemerland-Haarlemmermeer EO, and the nationwide registry of histopathology and cytopathology. We also thank research staff and technicians of the Unit of Molecular Pathology, VU University Medical Centre, Amsterdam for HPV DNA testing, the cytotechnologists of the Spaarne Ziekenhuis (Hoofddorp), Kennemer Gasthuis (Haarlem), Leiden Cytology and Pathology Laboratory (Leiden), and VU University Medical Centre (Amsterdam) for cytological testing and logistics, and the administrative workers and the information technology team of the Department of Pathology, VU University Medical Centre, Amsterdam, for their supportive work. We also thank the Department of Public Health and Social Medicine of Erasmus University (Rotterdam) for help with the design of the trial, and the gynaecologists in the study region for their contribution.

CHAPTER 4

References

1. Walboomers JM, Jacobs MV, Manos MM, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol 1999; 189: 12–19.
2. Munoz N, Bosch FX, de Sanjose S, et al. Epidemiologic classifi cation of human papillomavirus types associated with cervical cancer. N Engl J Med 2003; 348: 518–27.
3. 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.
4. Cuzick J, Clavel C, Petry KU, et al. Overview of the European and North American studies on HPV testing in primary cervicalcancer screening. Int J Cancer 2006; 119: 1095–101.
5. Harper DM, Franco EL, Wheeler CM, et al. Sustained effi cacy up to 4.5 years of a bivalent L1 virus-like particle vaccine against human papillomavirus types 16 and 18: follow-up from a randomised control trial. Lancet 2006; 367: 1247–55.
6. Quadrivalent vaccine against human papillomavirus to prevent high-grade cervical lesions. N Engl J Med 2007; 356: 1915–27.
7. Bulkmans NWJ, Berkhof J, Rozendaal L, et al. Human papillomavirus DNA testing for the detection of cervical intraepithelial neoplasia grade 3 and cancer: 5-year follow-up of a randomised controlled implementation trial. Lancet 2007; 370: 1764–72.
8. Naucler P, Ryd W, Tornberg S, et al. Human papillomavirus and Papanicolaou tests to screen for cervical cancer. N Engl J Med 2007; 357: 1589–97.
9. Kitchener HC, Almonte M, Thomson C, et al. HPV testingin combination with liquid-based cytology in primary cervical screening (ARTISTIC): a randomised controlled trial. Lancet Oncol 2009; 10: 672–82.
10. Ronco G, Giorgi-Rossi P, Carozzi F, et al. Efficacy of human papillomavirus testing for the detection of invasive cervical cancers and cervical intraepithelial neoplasia: a randomized controlled trial. Lancet Oncol 2010, 11: 249–57.
11. Anttila A, Kotaniemi-Talonen L, Leinonen M, et al. Rate of cervical cancer, severe intraepithelial neoplasia, and adenocarcinoma in situ in primary HPV DNA screening with cytology triage: randomized study within organised screening programme. BMJ 2010; 340: c1804.
12. Bulkmans NW, Rozendaal L, Snijders PJ, et al. POBASCAM, a population-based randomized controlled trial for implementation of high-risk HPV testing in cervical screening: design, methods and baseline data of 44,102 women. Int J Cancer 2004; 110: 94–101.
13. 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.
14. Solomon D, Davey D, Kurman R, et al. The 2001 Bethesda system: terminology for reporting results of cervical cytology. JAMA 2002, 287: 2114–19.
15. 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.
16. van den Brule AJ, Pol R, Fransen-Daalmeijer N, Schouls LM, Meijer CJ, Snijders PJ. GP5+/6+ PCR followed by reverse line blot analysis enables rapid and high-throughput identification of human papillomavirus genotypes. J Clin Microbiol 2002; 40: 779–87.
17. van Ballegooijen M, Hermens R. Cervical cancer screening in the Netherlands. Eur J Cancer 2000; 36: 2244–46.
18. 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.
19. Anderson MC. Premalignant and malignant squamous lesions of the cervix. In: Fox H, Wells M, eds. Obstetrical and gynaecological pathology, 4th edn. New York: Chruchill Livingstone, 1995: 292–97.
20. Wright TC. Precancerous lesions of the cervix. In: Kurman RJ, ed. Blaustein's pathology of the female genital tract, 4th edn. NewYork: Springer Verslag, 1995: 248–57.
21. 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 cytomorphologically normal cervical smears. Int J Cancer 1996; 68: 766–69.
22. Sankaranarayanan R, Nene BM, Shastri SS, et al. HPV screening for cervical cancer in rural India. N Engl J Med 2009; 360: 1385–94.
23. Dillner J, Rebolj M, Birembaut P, et al. Long term predictive values of cytology and human papillomavirus testing in cervical cancer screening: joint European cohort study. BMJ 2008; 337: a1754.
24 Leinonen M, Nieminen P, Kotaniemi-Talonen L, et al. Age-specific evaluation of primary human papillomavirus screening vs conventional cytology in a randomized setting. J Natl Cancer Inst 2009; 101: 1612–23.
25. Meijer CJ, Berkhof J, Castle PE, et al. Guidelines for human papillomavirus DNA test requirements for primary cervical cancer screening in women 30 years and older. Int J Cancer 2009; 124: 516–20.
26. Van Den Brule AJ, Walboomers JM, Du Maine M, Kenemans P, Meijer CJ. Difference in prevalence of human papillomavirus genotypes in cyto-morphologically normal cervical smears is associated with a history of cervical intraepithelial neoplasia. Int J Cancer 1991; 48: 404–08.

CHAPTER 4

27. Meijer CJ, van den Brule AJ, Snijders PJ, Helmerhorst T, Kenemans P, Walboomers JM. Detection of human papillomavirus in cervical scrapes by the polymerase chain reaction in relation to cytology: possible implications for cervical cancer screening. IARC Sci Publ 1992; 119: 271–81.
28. Eluf-Neto J, Booth M, Mu.oz N, Bosch FX, Meijer CJ, Walboomers JM. Human papillomavirus and invasive cervical cancer in Brazil. Br J Cancer 1994; 69: 114–19.
29. Walboomers JM, de Roda Husman AM, Snijders PJ, et al. Human papillomavirus in false negative archival cervical smears: implications for screening for cervical cancer. J Clin Pathol 1995; 48: 728–32.
30. Remmink AJ, Walboomers JM, Helmerhorst TJ, et al. The presence of persistent high-risk HPV genotypes in dysplastic cervical lesions is associated with progressive disease: natural history up to 36 months. Int J Cancer 1995; 61: 306–11.
31. Nobbenhuis MAE, Walboomers JMM, Helmerhorst TJM, et al. Relation of human papillomavirus status to cervical lesions and consequences for cervical-cancer screening: a prospective study. Lancet 1999; 354: 20–25.
32. Gok M, Heideman DA, van Kemenade FJ, et al. HPV testing on self collected cervicovaginal lavage specimens as screening method for women who do not attend cervical screening: cohort study. BMJ 2010; 340: c1040.